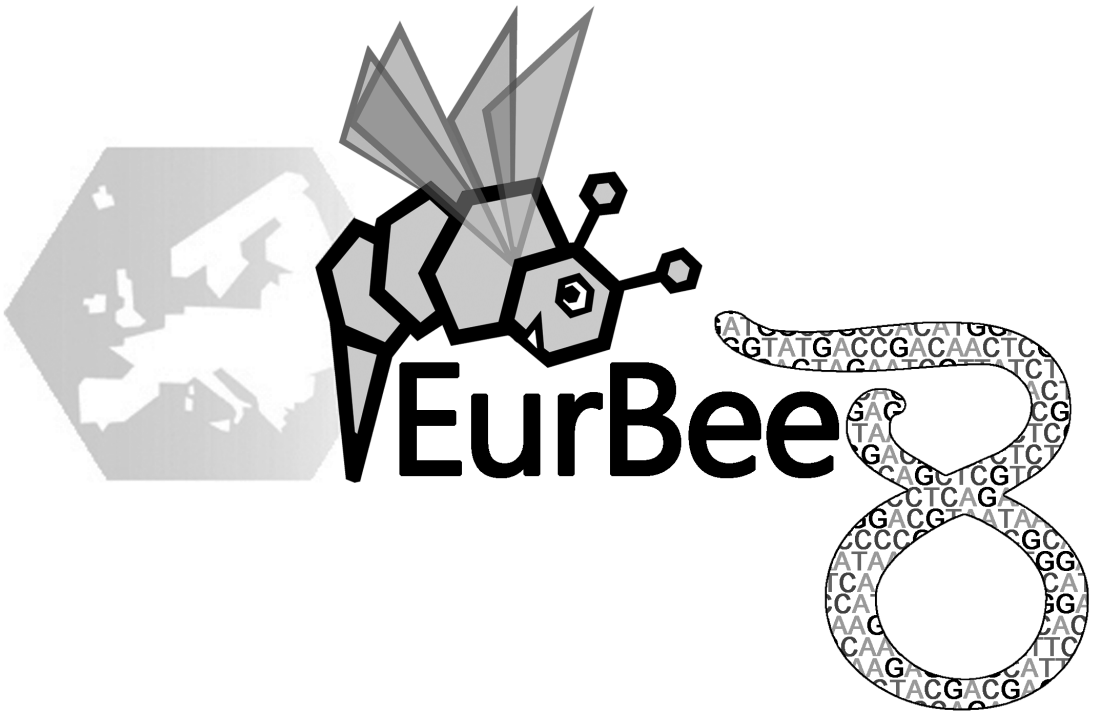
EurBee 8
8th Congress of Apidology

18-20 SEPTEMBER 2018
Ghent, Belgium
Program & Abstract Book

EurBee

Program & Abstract Book

CONTENT



HOSTED BY

GHENT UNIVERSITY

honeybee valley

Dear colleagues,
Dear friends,

It is with great honour and pleasure that we welcome you in Ghent for the 8th EurBee Congress of Apidology. The EurBee congresses are held every two years and are a major international forum for discussion of the latest and most important results in bee research. EurBee has become the premier event for researchers studying different aspects of wild and managed bees, and how they respond to environmental changes to address problems with species conservation, pollination services, beekeeping management and colony losses.

Experts from all over Europe and far beyond, have come to Ghent to exchange recent advancements and ideas that emerge from basic and applied research. We are particularly pleased with the program that we can offer you, with six invited talks and 157 selected talks on a wide variety of themes related to honeybees and wild bees. Three parallel sessions, in the morning supplemented with a thematic symposium, will run simultaneously. Needless to say that at a time when the ban on plant protection products is the subject of debate at all levels, we can offer strong sessions on ecotoxicological themes. However, for the first time ever, the program provides also sessions devoted to the theme 'resilience of the honeybee' and here too, the selection of oral presentations looks particularly interesting. Have we come to the turning point? Does the future of the bees look prosperous again? The future will tell. In any case, we were very pleased that we may hear here in Ghent so many uplifting stories as well.

May we hope that this congress in the medieval city of Ghent in the heart of Europe will meet all your expectations.

Dirk C. de Graaf, Ghent University
Local Organizer EurBee 8

COMMITTEES

Host Committee

Chaired by Dirk de Graaf

Koen Beeuwsaert
Marleen Brunain
Ellen Danneels
Katrien De Keukelaere
Jeroen Eerens
Mikalaï Khalenkow
Dries Laget
Pierre Rasmont
Bernadette Rotthier
Claude Saegerman
Karel Schoonvaere
Sara Vleminckx

Scientific Committee

Chaired by Robin Moritz, UHalle, Germany

Michel Asperges, UHasselt, Belgium
Etienne Bruneau, CARI, Belgium
Lina De Smet, UGent, Belgium
Roger Huybrechts, KULeuven, Belgium
Frans Jacobs, UGent, Belgium
Ivan Meeus, UGent, Belgium
Pierre Rasmont, UMons, Belgium
Wim Reybroeck, ILVO, Belgium
Claude Saegerman, ULg, Belgium
Guy Smagghe, UGent, Belgium
Octaaf Van Laere, UAntwerpen, Belgium

EurBee Board

Dirk de Graaf, President, UGent, Belgium
Robert Paxton, Secretary, UHalle, Germany

GENERAL INFORMATION

Venue

EurBee 8 takes place at the **Campus Ledeganck of Ghent University** (UGent).
Address: Karel Lodewijk Ledeganckstraat 35, 9000 Gent

House rules

Please wear your badge at all times
The venue is a public non-smoking building.
Switch off your mobile phone during the sessions.
It is not permitted to take cupes, glasses or plates into a room.

Use of wireless internet and access to e-mail at UGent

There are two options to connect to the internet:

1. If your home institution is part of the eduroam network, you can easily access the internet at the congress venue and other Ghent University buildings. Normally your device will identify a valid eduroam access point and log-in automatically. You can do so with your own login and password.
   The settings at UGent are the following:
   Network Name/SSID: eduroam (lower case!) (is broadcast)
   Security Type: WPA2-Enterprise
   Encryption Type: AES
   Authentication method: PEAP
   Authentication protocol: MSCHAP / Sub authentication method: EAP-MSCHAP V2

2. If you have no access via eduroam, follow the next steps:
   Step 1 – go to network connections
   Step 2 – select UGentGuest (If you have set up to request an IP address automatically, you will receive an IP address starting with 193.190.8x.)
   Step 3 – now you are connected, but not authenticated. You should start a web browser and you will be redirected to a login screen:
   Users name / login: **guestEurbee**
   Password: **5kc3mLNL**

Registration and Information Desk Opening Hours

Monday 17 September:	17h30-21h00	Aula Ghent University
Tuesday 18 September:	07h45-18h45	Campus Ledeganck
Wednesday 19 September:	08h00-15h00	Campus Ledeganck
Thursday 20 September:	08h00-18h30	Campus Ledeganck

Lost and Found

For lost and found personal belongings, please contact the Information desk.

First Aid

In case of emergency, please contact the Information Desk.

Meals

Coffee/tea and will be served at the McLeod Foyer of Campus Ledeganck of UGent.
Lunches will be served daily at the **Campus Ledeganck of HoGent** (HoGent).
Address: Karel Lodewijk Ledeganckstraat 8, 9000 Gent

Use of wireless internet and access to e-mail at HoGent:
Please follow the next steps:
Step 1 – go to network connections
Step 2 – select the network: **Eurbee8**
Password: **18092018**

Dress code

No specific dress code during the congress (typically casual / smart casual); for the banquet dinner typically casual/cocktail - still compatible with a dance party afterward.
We kindly advise you to wear comfortable shoes during the social activity Wednesday afternoon 19 September.

Badge

Your personal name badge is your entrance ticket to all sessions and other activities of the EurBee 8 Congress. Please remember to wear this badge at all times during the Congress and the social activities. At the back of your badge is a number that you can call during the Congress in case of emergencies.

Duplication / Recording / Photo Policy

Attendees or exhibitors are encouraged to network and enjoy the congress experience. As such, capturing memories of casual meeting activities and networking is permitted with the permission of those being prominently photographed. Photographing formal meeting presentations, posters, or displays is forbidden without permission of EurBee and the presenter.

Insurance and Liability

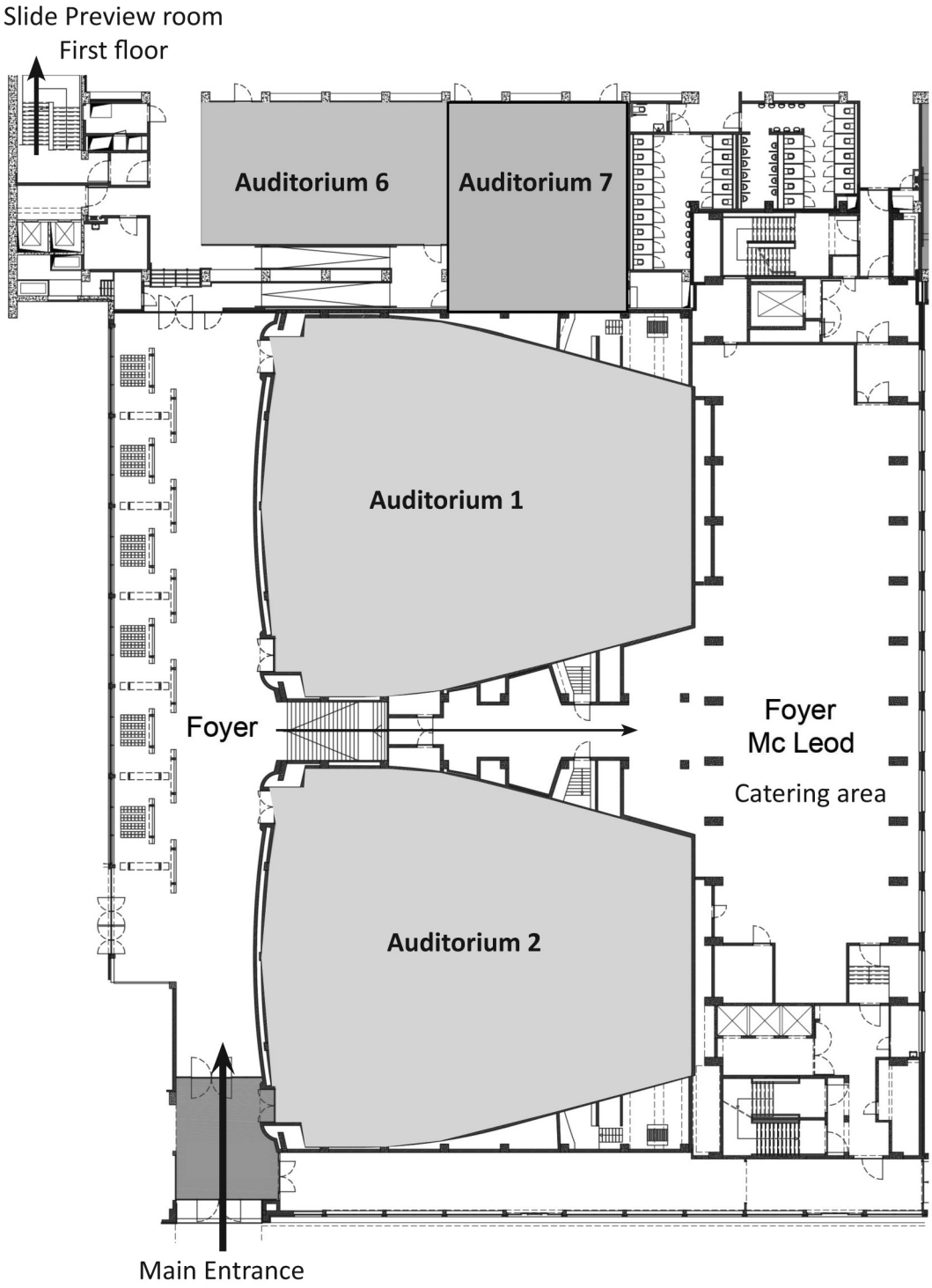
The Congress organising committee and Semico n.v. do not accept liability for personal medical expenses/ travel expenses/losses of whatever nature incurred by delegates.

Exhibition

An exhibition will be available during the meeting. We invite you to visit our exhibitors at the Campus Ledeganck UGent:

Tuesday 18 September:	09h30-18h00
Wednesday 19 September:	09h30-15h00
Thursday 20 September:	09h30-18h00

VENUE - Floorplan



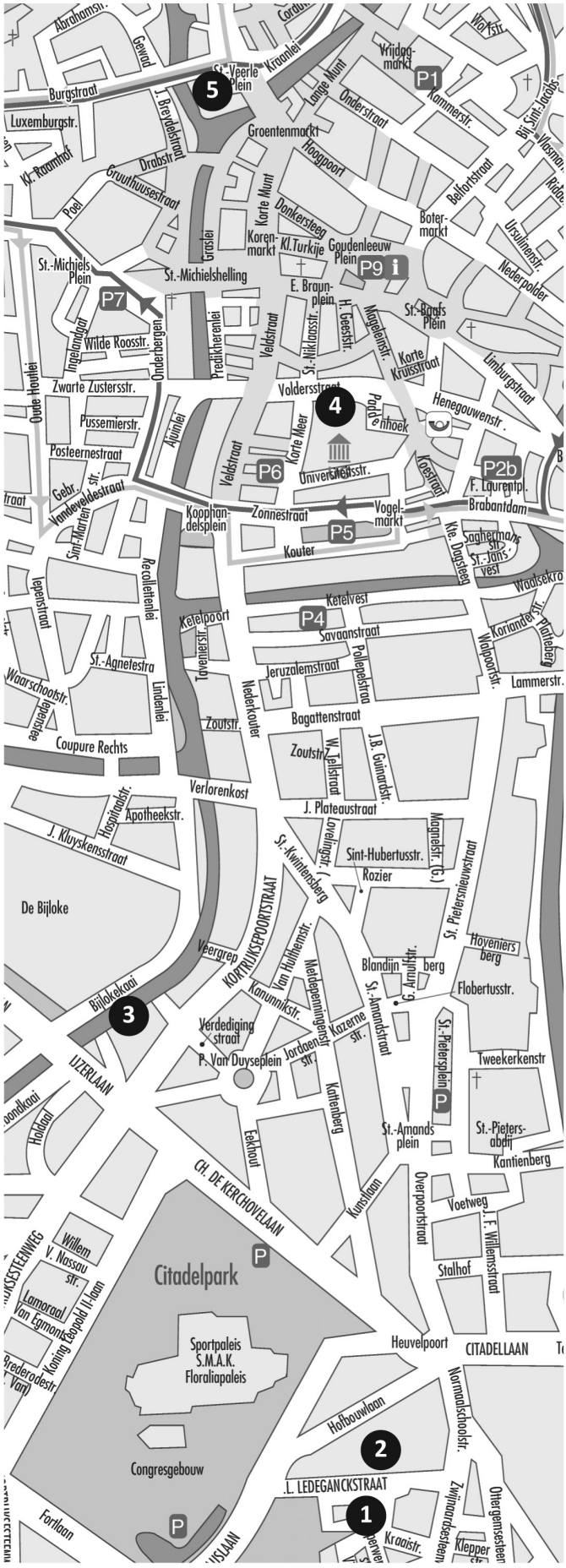
1 Campus Ledeganck UGent
Venue of EurBee 8
Address: Karel Lodewijk Ledeganckstraat 35

2 Campus Ledeganck HoGent
Lunches & Poster Sessions
Address: Karel Lodewijk Ledeganckstraat 8

3 Social Event- Discover Ghent
Departure point of boat trip
Address: Bijlokekaai 7

4 Aula Ghent University
Opening Cermony & Welcome Reception
Address: Volderstraat 9

5 Old Fish Market (Oude Vismijn)
Congress Banquet
Address: Rekelingestraat 5




PROGRAM OVERVIEW

Monday 17 September

17h30-21h00	Registration desk	*Aula Ghent University*
19h00-19h45	**Opening Ceremony**	*Aula Ghent University*
19h45-21h00	**Welcome Reception**	*Aula Ghent University*

Tuesday 18 September

08h15 - 08h30	Welcome Address	*Auditorium 1*
08h30 - 09h30	Keynote presentation by **Philipp Engel**	*Auditorium 1*
09h30 - 12h15	**Morning Sessions**	*Campus Ledeganck UGent*
09h30 - 11h00	**Microbiota**	*Auditorium 1*
11h15 - 12h15	**New Challenges in Bee-Microbe Community and Functional Ecology**	*Auditorium 1*
09h30 - 12h15	**Genetics 1 - Resilient Bees**	*Auditorium 2*
09h30 - 12h15	**Ecotoxicology 1**	*Auditorium 6*
09h30 - 12h15	**Where Have All the Wild Bees Gone, Long Time Passing? (BELBEES)**	*Auditorium 7*
10h00 - 10h30	Coffee & Tea	*Mc Leod Foyer*
12h15 - 14h30	Lunch	*Campus Ledeganck HoGent*
13h30 - 14h30	**Poster Discussion Session 1**	*Campus Ledeganck HoGent*
	Pathology 1 (P001 - P044)	
	Ecotoxicology (P045 - P055)	
	Behaviour and Colony Function (P056 - P076)	
14h45 - 17h15	**Afternoon Sessions**	*Campus Ledeganck UGent*
14h45 - 17h15	**Genetics 2**	*Auditorium 1*
14h45 - 17h15	**Bee Health**	*Auditorium 2*
14h45 - 17h15	**Ecology 1**	*Auditorium 6*
16h15 - 16h45	Coffee & Tea	*Mc Leod Foyer*
17h15 - 18h15	Keynote presentation by **Karen Kapheim**	*Auditorium 1*
18h15 - 18h45	Members' Meeting	*Auditorium 1*
10h00 - 17h00	Poster Viewing	*Campus Ledeganck HoGent*
07h45 - 18h45	Registration desk	*Campus Ledeganck UGent*

Wednesday 19 September

08h15 - 08h30	Report Members' Meeting	*Auditorium 1*
08h30 - 09h30	Keynote presentation by **Alison Mercer**	*Auditorium 1*
09h30 - 12h15	**Morning Sessions**	*Campus Ledeganck UGent*
09h30 - 11h00	**Neurobiology & Behaviour**	*Auditorium 1*
11h00 - 12h00	**Chemical Ecology**	*Auditorium 1*
09h30 - 12h00	**Genetics 3 - Resilient Bees**	*Auditorium 2*
09h30 - 12h15	**Pathology 1**	*Auditorium 6*
09h30 - 12h15	**Linking Foraging Patterns, Food Intake, Nutrition and Performance in Bees**	*Auditorium 7*
10h00 - 10h30	Coffee & Tea	*Mc Leod Foyer*
12h15 - 14h00	Lunch	*Campus Ledeganck HoGent*
13h00 - 13h50	**Poster Discussion Session 2**	*Campus Ledeganck HoGent*
	Pathology 2 (P077 - P116)	
	Genetics (P177 - P138)	
	BeeProducts (P139 - P151)	
14h00 - 15h00	Keynote presentation by **Yves Le Conte**	*Auditorium 1*
15h00 - 18h30	**Social Event - Discover Ghent**	
10h00 - 15h00	Poster Viewing	*Campus Ledeganck HoGent*
08h00 - 15h00	Registration desk	*Campus Ledeganck UGent*

Thursday 20 September

08h30 - 09h30	Keynote presentation by **Dan Hultmark**	*Auditorium 1*
09h30 - 12h15	**Morning Sessions**	*Campus Ledeganck UGent*
09h30 - 10h15	**Immunity**	*Auditorium 1*
10h45 - 12h15	**Bee Products**	*Auditorium 1*
09h30 - 12h15	**Ecotoxicology 2**	*Auditorium 2*
09h30 - 12h15	**Pathology 2 - Virus**	*Auditorium 6*
09h30 - 12h15	**Studies on Colony Multiple Enemies for Bee Health**	*Auditorium 7*
10h15 - 10h45	Coffee & Tea	*Mc Leod Foyer*
12h15 - 14h30	Lunch	*Campus Ledeganck HoGent*
13h15 - 14h15	**Poster Discussion Session 3** **Chemical Ecology** (P152 - P165) **Bee Health** (P166 - P202) **Ecology** (P203- P221)	*Campus Ledeganck HoGent*
14h30 - 17h30	**Afternoon Sessions**	*Campus Ledeganck UGent*
14h30 - 16h00	**Reproductive Biology**	*Auditorium 1*
16h30 - 17h15	**Evolutionary Biology**	*Auditorium 1*
14h30 - 17h15	**Ecology 2**	*Auditorium 2*
14h30 - 17h15	**Pathology 3 - Varroa**	*Auditorium 6*
16h00 - 16h30	Coffee & Tea	*Mc Leod Foyer*
17h30 - 18h30	Keynote presentation by **Tom Wenseleers**	*Auditorium 1*
20h00 -	**Congress Banquet**	*Old Fish Market*
10h00 - 17h00	Poster Viewing	*Campus Ledeganck HoGent*
08h00 - 18h30	Registration desk	*Campus Ledeganck UGent*

PROGRAM OVERVIEW

TUESDAY 18 SEPTEMBER

08h15	**AUDITORIUM 1** Welcome Address			
08h30	**GUT MICROBIOTA - Keynote lecture** **KN1** - Honey bee gut microbiota – a versatile model for understanding microbial community evolution and functioning - **Philipp Engel**			
09h30	**Parallel session 1** **MICROBIOTA**	**AUDITORIUM 2** **Parallel session 2** **GENETICS 1 - RESILIENT BEES**	**AUDITORIUM 6** **Parallel session 3** **ECOTOXICOLOGY 1**	**AUDITORIUM 7** **Symposium 1** **WHERE HAVE ALL THE WILD BEES GONE, LONG TIME PASSING? (BELBEES)**
09h30	**001** - Insights into the diversity and dynamics of microbes in stingless bees (Hasselman P.)	**010** - Field testing and selection on European honey bee populations (Smartbees project 2015-2018) (Büchler R.)	**019** - EU-wide restrictions on neonicotinoids have not entirely eliminated the risk for honeybees foraging on oilseed rape nectar (Wintermantel D.)	**028** - Introduction: Where have all the wild bees gone, long time passing? (Rasmont P.)
09h45	**002** - Exploring the microbiome's role in the biological invasion of the African honey bee genotype (Kowallik V.)	**011** - Mite adaptations in European *Apis mellifera* populations surviving *Varroa destructor* by means of natural selection? (Moro A.)	**020** - Time-to-death approach to reveal chronic and cumulative toxicity of a fungicide for honeybees not revealed with the standard ten-day test (Simon-Delso N.)	**029** - Declining distributions for BENELUX Bumblebees: climate and land use change interactions (Marshall L.)
10h00	***Coffee/Tea Break***			
10h30	**003** - Consequences of land-use for solitary bee microbiota composition and function (Peters B.)	**012** - Investigations into unmanaged honey bee colonies in Ireland (Browne K.A)	**021** - Effects of neonicotinoids on honey bee food glands (Williams G.)	**030** - Extreme shift in abundance and distribution of bumblebee composition in Belgium (Rollin O.)
10h45	**004** - Impact of medicaments and feed additives on the honeybee gut microbiota (Alberoni D.)	**013** - Black box selection leads to distinct traits of resistance to *Varroa destructor* in honeybees (Blacquière T.)	**022** - The sublethal effects of clothianidin on the honey bee gut microbiota (El Khoury S.)	**031** - Drift in distribution and quality of host-plant resources in common bumblebees (Michez D.)
11h00	**005** - Pollen diversity and the microbiome; implications for honey bee health (Ferrari R.)	**014** - The good, the bad and the ugly – dissecting *Apis mellifera* survivability to Varroa destructor (Beaurepaire A.)	**023** - Co-exposure to virus and pesticide (Deformed wing virus / neonicotinoid) alters bee behavioural performances (Dalmon A.)	**032** - The role of bees in interaction networks with plants as a conservation argument (Jacquemin F.)
11h15	**Symposium 2** **NEW CHALLENGES IN BEE-MICROBE COMMUNITY AND FUNCTIONAL ECOLOGY** **006** - Immune stimulation by the gut symbiont *Frischella perrara* in the honey bee (*Apis mellifera*) (Emery O.)	**015** - Breeding of *Apis cerana*: resistance of Sacbrood virus (Choi Y-S.)	**024** - The effects of neonicotinoids on circadian rhythms in honey bees (Sokolowski M.)	**033** - A century of temporal stability of genetic diversity in wild bumblebees (Maebe K.)
11h30	**007** - Lactic acid bacteria of the honey bee crop enhance adult longevity but do not provide specific defence against a microsporidian and a viral pathogen (Paxton R.)	**016** - Tolerance to Deformed wing virus at the individual and colony level in Swedish mite-resistant honey bees (Locke B.)	**025** - Chronic exposure to thiamethoxam can promote chronic bee paralysis virus infections in honey bees (Dubois E.)	**034** - Viruses in wild bees: to be included in future monitoring programs (Schoonvaere K.)
11h45	**008** - Characterisation of the honey bee *Apis mellifera* metagenome in Britain (Regan T.)	**017** - The Lord of the Rings: genotype-environment interactions for honey bee colonies surviving *Varroa destructor* by means of natural selection (Neumann P.)	**026** - Sublethal exposure to the neonicotinoid Insecticide thiacloprid impairs the immune defence in the solitary bee species *Osmia bicornis L.* (Brandt A.)	
12h00	**009**- Using contact networks and next-gen sequencing to reveal the community dynamics of the pollinator virome (Doublet V.)	**018** - BeeStrong: towards a genomic tool for the selection of *Varroa* resistant honey bees (Sann C.)	**027** - Effects of neonicotinoids on the behavior of foraging honey bees with artificial flower choices (Çakmak I.)	
12h15 - 14h30 13h30 - 14h30	**Lunch break** **Poster Discussion Session 1:** Pathology 1 (P001 - P044) Ecotoxicology (P045 - P055), Behaviour and Colony Function (P056 - P057)			

14h45	**AUDITORIUM 1** **Parallel session 4** **GENETICS 2**	**AUDITORIUM 2** **Parallel session 5** **BEE HEALTH**	**AUDITORIUM 6** **Parallel session 6** **ECOLOGY 1**
14h45	**035** - Distribution of complementary sex determiner alleles in *Apis mellifera* population (Cebrat M.)	**043** - Multi-stress effects on honey bee colonies (van Dooremalen C.)	**051** - Quantifying and modeling bee spatial strategies (Lihoreau M.)
15h00	**036** - De novo genome assembly of a western European *Apis mellifera mellifera* black bee (Vignal A.)	**044** - Impact of beekeeping management practices on honey bee mortality in Belgium (El Agrebi N.)	**052** - Design of a spatially explicit individual-based honeybee colony model (Wallis D.)
15h15	**037** - A comprehensive genomic and morphometric assessment of European honey bee diversity and identification of SNP markers for subspecies diagnosis (Parejo M.)	**045** - Classification and prediction of stress factors and bee health using ultra high performance liquid chromatography techniques with quadrupole and orbitrap high-resolution mass spectrometry-based metabolomics (Wang L.)	**053** - Where do wild bees want to live? Biodiversity in agricultural landscapes (Kratschmer S.)
15h30	**038** - Applying a SNP-based tool for conservation of wild and managed black bees in Ireland (McCormack G.P.)	**046** - MALDI mass spectrometry imaging: an *in situ* histoproteomic approach to monitor the response of pollinators to their stressors (Bulet P.)	**054** - Community structure of bees associated with different types of floral margins (Pérez-Marcos M.)
15h45	**039** - Genetic models for long-term simulation studies in honeybee breeding (Plate M.)	**047** - Assessment of honey bee cells using deep learning (Alves T.S.)	**055** - Not the perfect match? Biogeography and pollinator shifts rather than cospeciation dominate the evolutionary history of *Rediviva* bees and their *Diascia* host plants in the Cape biodiversity hotspot (Kahnt B.)
16h00	**040** - Unraveling patterns of population structure in *Bombus terrestris* from the Iberian Peninsula and North Africa (Silva S.E.)	**048**- Beneficial microorganisms and honeybees: colony level effects of lactic acid bacterial supplements (Forsgren E.)	**056** - Bumble-BEEHAVE: an agent-based population model for bumble bees and its application as a decision tool for land managers (Becher M.A.)
16h15	**Coffee/Tea Break**		
16h45	**041** - Signatures of selection revealed by population analyses of bumblebee genomes (Colgan T.J.)	**049**- COLOSS (prevention of honey bee Colony LOSSes): past forward (Neumann P.)	**057** - Mitogenome sequencing to study introgression events between close related subspecies of *Bombus terrestris* in the Iberian peninsula (Cejas D.)
17h00	**042** - Genomic signatures of introgression between commercial and native bumblebees, *Bombus terrestris*, in the Iberian Peninsula – implications for conservation and trade regulation (Seabra S.G.)	**050** - 10 years of coordinated study of honey bee colony loss rates – presentation of a COLOSS core project (Brodschneider R.)	**058** - Buzzing in the buckwheat – a diverse pollinator community brings more grains to the table (Korpela E-L.)
17h15	**GENETICS - Keynote lecture** **KN2** - Life outside the hive: What comparative genomics reveal about bee behavior, physiology, and evolution - **Karen Kapheim**		
18h15 - 18h45	**Members' Meeting**		

PROGRAM OVERVIEW

PROGRAM OVERVIEW

WEDNESDAY 19 SEPTEMBER

	AUDITORIUM 1	AUDITORIUM 2	AUDITORIUM 6	AUDITORIUM 7
08h15	Report Members' Meeting			
08h30	**NEUROBIOLOGY - Keynote lecture** **KN3** - Using associative learning to learn about brain health and bees - **Alison Mercer**			
09h30	**Parallel session 7** **NEUROBIOLOGY & BEHAVIOUR**	**Parallel session 8** **GENETICS 3 - RESILIENT BEES**	**Parallel session 9** **PATHOLOGY 1**	**Symposium 3** **LINKING FORAGING PATTERNS, FOOD INTAKE, NUTRITION AND PERFORMANCE IN BEES**
09h30	**059** - *In vivo* imaging of neonicotinoid influence on odour processing in the honeybee brain (Haase A.)	**067**- Comparison of hygienic behavior directed against *Varroa destructor* and *Tropilaelaps* sp. (Wegener J.)	**075** - Unbiased random mutagenesis contributes to a better understanding of the virulent behaviour of *Paenibacillus larvae* (De Smet L.)	**084** - Bees rely on their sense of taste to assess pollen nutritional composition (Ruedenauer F.A)
09h45	**060** - Honey bee waggle dance communication benefits pollen foraging (Nürnberger F.)	**068** - Explaining the resistance to *Varroa destructor* in the French honey bee population with VSH, SMR and colony dynamic data (Eynard S.E.)	**076** - Canada-wide surveillance of exotic pathogens: *Lotmaria passim* and *Crithidia mellificae* (Borba R.S.)	**085** - Sex-dependent nutrient demand is reflected in pollen supplied to larvae by mother of a generalist solitary bee *Osmia bicornis* (Filipiak M.)
10h00	***Coffee/Tea Break***			
10h30	**061** - Observations of honey bee wing movements with the use of a high-speed camera (Tofilski A.)	**069** - Preliminary researches regarding the efficiency of the formic acid treatment on *Varroa* (*Varroa destructor*) found in the artificially decapped bee brood (Siceanu A.)	**077** - *Nosema ceranae* and *Lotmaria passim*: partners in crime? (MacInnis C.I.)	**086** - Temporal disruption in flowers availability affects honeybee colony losses (Requier F.)
10h45	**062** - The effect of absolute and relative amounts of omega-3 and omega-6 essential fatty acids on associative learning in honey bees (Arien Y.)	**070** - Hygenic behaviour, *Varroa* and *Nosema* levels of Africanized honeybee colonies in Alagoas State, Northeastern Brazil (Beelen R.)	**078** - Factors influencing the development and the course of the *N. ceranae* infection in *Apis mellifera iberiensis* (Urbieta A.)	**087** - Pollen protein content drives bee community preference for an invasive thistle over five native plant species (Russo L.)
11h00	**Parallel session 10: CHEMICAL ECOLOGY** **063** - Chemical Communication and improved tools for hygienic selection in the honey bee, *Apis mellifera* (Wagoner K.)	**071**- Identification of the DNA variants associated with the hygienic trait of honey bees (Farajzadeh L.)	**079**- Effect of *Nosema ceranae* on dynamic communities of gut bacteria in native species of Thai honey bees (Naree S.)	**088** - How larval food quantities affect caste-fate in the stingless bee *Scaptotrigona depilis* (Buchholz J.)
11h15	**064** - Antibiotics, sex and social regulation - the role of C10 short chain fatty acids in honey bee biology (Crewe R.M.)	**072** - Combining elastic-net penalized regression and whole exome sequencing to identify phenotype-associated variants (Broeckx B.J.G.)	**080** - Tracking Asian hornets (*Vespa velutina*) to their nests with radio-telemetry (Kennedy P.)	**089** - Honeybees tolerate cyanogenic glucosides from clover nectar and flowers (Jensen A.B.)
11h30	**065** - Exposure of Nasonov gland by flying foragers of honey bee (Tofilski A.)	**073** - Progress in marker-assisted selection for honey bee breeding (Pernal S.F.)	**081** - Effect of *Nosema ceranae* on energetic stress of trehalose level in haemolymph and infection ratio of giant honey bee (Suwannapong G.)	**090** - The feeding with glandular secretions protects honeybee larvae from toxic pyrrolizidine alkaloids present in pollen (Kast C.)
11h45	**066** - Eat me or not- deciphering the molecular signals of diploid drone selection in the honey bee *Apis mellifera* (Gessler B.)	**074** - A Canadian perspective on marker assisted selection and growing the local queen supply (Guarna M.M.)	**082** - Oxidative stress increases in honey bees infected with *Lotmaria passim* (Stevanović J.)	**091** - New protocols to study nectar foraging in honey bees (Sokolowski M.)
12h00			**083** - Haploid males are more susceptible: spillover of *Crithidia mellificae* from managed honey bees to wild bees (*Osmia cornuta*) (Strobl V.)	**092** - Bees and resin, resin and bees: insights into an underrated resource (Leonhardt S.D.)
12h15 - 14h00 13h00 - 13h50	**Lunch break** **Poster Discussion Session 2:** Pathology 2, (P077 - P116) Genetics (P117 - P138) , Bee Products (P 139 - P151)			
14h00	**CHEMICAL ECOLOGY- Keynote lecture** **KN4** - State of the art in honeybee chemical ecology - **Yves Le Conte**			
15h00 - 18h30	**Social Event - Discover Ghent**			

THURSDAY 20 SEPTEMBER

08h30	**INNATE IMMUNITY - Keynote lecture** **KN5** - Nutrition and immunity – a systemic response to infection in the insect model, *Drosophila melanogaster*- **Dan Hultmark**			
09h30	**Parallel session 11** **IMMUNITY**	**AUDITORIUM 2** **Parallel session 12** **ECOTOXICOLOGY 2**	**AUDITORIUM 6** **Parallel session 13** **PATHOLOGY 2 - VIRUS**	**AUDITORIUM 7** **Symposium 4** **STUDIES ON COLONY MULTIPLE ENEMIES FOR BEE HEALTH**
09h30	**093** - Single von Willebrand factor C proteins and its role in insect immunity (Meeus I.)	**102** - Synergistic effects of stressors on bee health: pesticides, nutrition, and behaviour (Tosi S.)	**111** - Two deformed wing virus variants, genotypes A and B, cause low pupal mortality and a high frequency of wing deformities in metamorphosing honey bees (*Apis mellifera)* (Paxton R.J.)	**120** - European-wide analysis of *Paenibacillus larvae* genetic diversity: EuroPLarva (Sircoulomb F.)
09h45	**094** - Study of gene expression of immunologically important molecules in honey bees after application of probiotic preparation (Maruščáková I.)	**103** - Imidacloprid diffusion route: from apple orchard to the honey bee colony matrices (Fontana P.)	**112** - Using an in vitro feeding system to investigate *Varroa*-vectored DWV variant transmission (Bradford E.L.)	**121** - Health status and factors identified for winter losses of honey bee colonies by the Austrian surveillance study 2015/2016 (Morawetz L.)
10h00	***Coffee/Tea Break***			
10h30	**095** - On mechanisms of trans-generational immune priming in honeybees (Freitak D.)	**104** - Chronic toxicity of select pesticides to honey bee (*Apis mellifera* L.) larvae reared in vitro (Ellis J.D.)	**113** - Europe-wide diversity of Deformed wing virus variants (Sircoulomb F.)	**122** - Should Varroosis include honeybee viruses as causative agents? (Schäfer M.O.)
10h45	**Parallel session 14** **BEE PRODUCTS** **096** - Alarming situation on the EU beeswax market: the prevalence of adulterated beeswax material and related safety issues (Svečnjak L.)	**105** - The homing flight ring test: method to assess the effects of sublethal doses of pesticides on the honey bee in field conditions (Fourrier J.)	**114** - Healthy honey bees - analysis of the virus population to assess rational *Varroa* control (Woodford L.)	**123** - The yellow-legged hornet: its impact on European western honeybees and control methods (Pointeau S.)
11h00	**097** - Effect of beeswax adulterated with stearin on the development of worker bee brood: results of a field trial (Reybroeck W.)	**106** - Lazy Spring: pollen-bound pesticide mixtures make bees less efficient (Prado A.)	**115** - Virome analysis on Belgian honeybees reveals multiple undescribed viruses including a diverse bacteriophage population (Deboutte W.)	**124** - Insights into honey bee pathogen and parasite dynamics and interactions using high-throughput analysis (D'Alvise P.)
11h15	**098** - Effect of spatial allocation of nectar cells on storage and honey ripening dynamics in the honeybee *Apis mellifera* (Eyer M.)	**107** - Versatile stressor impact analysis by video observation of worker behavior and brood development within cells reveals neonicotinoid effects at field realistic concentrations (Siefert P.)	**116** - Apis Rhabdovirus-1, a negative-sense RNA virus present in populations of pollinators , replicates in *Apis mellifera* and *Varroa destructor* (Chejanovsky N.)	**125** - Small hive beetle invasion risk under current and future climate scenarios: a modelling approach to foster mitigation efforts (Cornelissen B.)
11h30	**099** - Culturing and conserving of stored pollen on the way into beebread (Božič J.)	**108** - Pharmacokinetic and molecular investigations providing insights into the honey bee-friendly profile of the butenolide insecticide flupyradifurone (SIVANTO prime®) (Zaworra M.)	**117** - A natural product inhibited the replication and expression of Israeli acute paralysis virus (Hou C.)	**126** - Survey of wild bee communities threatened by *Vespa velutina* (Manino A.)
11h45	**100** - Sugar, amino acids, and inorganic ion profiling of honeydew from aphid species hosting different tree species in Germany (Shaaban B.)	**109** - Pesticide residues in bee bread from the national honey bee disease survey (Traynor K.)	**118** - Constructing infectious Sacbrood virus clone with green fluorescence protein expression (Huang W-F.)	**127** - Patterns of *Vespa velutina* invasion in western Iberia and Italy as revealed by molecular markers (Quaresma A.)
12h00	**101** - Genuine Manuka honey!? - State of the art (Beitlich N.)	**110** - Gene expression profiling demonstrates that exposure to neonicotinoid pesticides affects multiple biological processes in bumblebees (*Bombus terrestris*) (Colgan T.J.)	**119** - Relevance of qualitative and quantitative changes in the virome to the survival of a Swedish *Varroa*-resistant honeybee population (de Miranda J.R.)	Discussion
12h15 - 14h30 13h15 - 14h15	**Lunch break** **Poster Discussion Session 3:** Chemical Ecology (P152 - P165), Bee Health (P166 - P202), Ecology (P203 - P221)			

PROGRAM OVERVIEW

14h30	**AUDITORIUM 1** **Parallel session 15** **REPRODUCTIVE BIOLOGY**	**AUDITORIUM 2** **Parallel session 16** **ECOLOGY 2**	**AUDITORIUM 6** **Parallel session 17** **PATHOLOGY 3- VARROA**
14h30	**128** - Reproductive success in European orchard bee *Osmia cornuta* (Hymenoptera: Megachilidae) influenced by the number of males (Stanisavljević L.)	**138** - Floral landscape enrichment and semi-natural habitats improve honeybee health, as evidenced by a 'Landscape physiology' approach (Alaux C.)	**148** - *Varroa destructor* feeds primarily on honey bee fat body tissue not hemolymph (Ramsey S.D.)
14h45	**129** - Differential circular RNAs expression in ovary during oviposition in honey bees (Shi W.)	**139** - Influence of major stresses on wild bees communities in an urban environment (Weekers T.)	**149** - *Varroa* mite saliva contains bioactive factors that aid mite feeding and manipulate the honeybee immune response (Campbell E.M.)
15h00	**130** - Influence of DMSO with dextran, PVP or PEG in freezing extender on queens (Çakmak I.)	**140** - The application of non-intrusive electronic bee hive monitoring to field studies (Evans H.A.)	**150** - Hormonal induction of in vitro egg production in the honeybee mite, *Varroa destructor* (Christie C.R.)
15h15	**131** - Queen temperature stress decreases sperm viability, queen performance, and colony productivity (Guarna M.M.)	**141** - Changes in bumble bee species diversity and abundance over 50 years on red clover fields in Estonia (Karise R.)	**151** - Transcriptome profiling of the parasite *Varroa destructor* provides new biological insights into the mite adult life cycle (Mondet F.)
15h30	**132** - Observations of the mating behaviour of *Apis melifera macedonica* and *Apis melifera cecropia* under natural conditions and under conditions of a control mating system (the Train of Virgin Queens) (Hatjina F)	**142** - European beech forests as a home for feral honey bee colonies (Rutschmann B.)	**152** - Early *Varroa* infection transcriptomics in the Asian honey bee (Routtu J.)
15h45	**133** - Preservation of domesticated honey bee (Hymenoptera: Apidae) drone semen (Giovenazzo P.)	**143** - Potential role of wetlands for honey bees diversity, population density and conservation in Sudan (Lattorff M.G.H.)	**153** - Lithium salts as varroacide - efficacy in the treatment of artificial swarms and side effects on bees and brood (Ziegelmann B.)
16h00	**Coffee/Tea Break**		
16h30	**Parallel session 18** **EVOLUTIONARY BIOLOGY** **134** - Experimental evolution of parasite virulence in a eusocial insect (Lattorff H.M.G.)	**144** - Do floral traits play a role in animal plant interaction? A study case with orchid bees (Boff S.)	**154** - Using RNAi to control *Varroa destructor* – a novel biopesticidal tool for effective control of the parasitic mite of honey bees (Verhaert J.)
16h45	**135** - Evolution of new gene functions by gene duplications - the case of major royal jelly proteins in the honey bee (Buttstedt A.)	**145** - Are non-native plants a valuable resource for wild bees? (Seitz N.)	**155** - Kairomones in the hive involves in *Varroa destructor* mite behavior (Pojar-Fenesan M)
17h00	**136** - Overview of the southwest Indian ocean honeybee: combining morphometry and genetics to investigate the original diversity of *Apis mellifera* spp. in Madagascar and surrounding archipelagos (Galataud J.)	**146** - Landscape related plant and resource diversity increases foraging and colony fitness in a tropical social bee (Kaluza B.)	**156** - Reproduction of the mite *Varroa destructor* in original and new honey bee hosts (Page P.)
17h15	**137** - Genetic basis for the evolution of non-reproduction of *Varroa destructor* in populations of *Apis mellifera* (Conlon B.H)	**147** - Response of wild bee diversity and functional traits to vineyard management and landscape diversity across Europe (Kratschmer S.)	**157** - Tracking genomic footprints of successful host switches in honey bee *Varroa* mites (Techer M.)
17h30 -18h30	**EVOLUTIONARY BIOLOGY - Keynote lecture** **KN6** - Social bees as model systems in the study of the evolution of cooperation and conflict - **Tom Wenseleers**		
20h00-	**Congress Banquet**		

SCIENTIFIC INFORMATION

Information for Oral Presenters

Oral / Symposium presenters are asked to load/check their presentation at least 4 hours prior to their session commencing to ensure the presentation is checked and tested. When you have to present on the first day, please load/check your presentation immediately upon your arrival.

The speakers' corner is located at the first floor of the venue and will be open on:
- ○ Tuesday 18 September: 07h45-18h00
- ○ Wednesday 19 September: 08h00-13h30
- ○ Thursday 20 September: 08h00-17h00

- Speakers are requested to come to the meeting room at least 5 minutes prior to the start of the session and identify themselves to the chair.
- Presenters must take place at seats in front of the room for the duration of the session.
- In the interests of fairness, please ensure that you keep to your allotted time frame. The chair will time your presentation and provide you with a warning at 2 minutes remaining and when time is up.

Oral presentations are allocated 13 minutes (10-minutes presentation time and 3 minutes question and answer time)

Instructions for Poster Presenters

Poster boards will be located in Campus Ledeganck of HoGent.
Posters will only be displayed for **ONE (1) day**. Please ensure that your poster is displayed on the correct poster board. Please ensure that you collect your poster at the conclusion of the day your poster was on display. Posters left behind at the conclusion of the congress will be discarded.

Posters are allocated into different sessions:
- ○ Poster session 1 is scheduled on Tuesday 18 September
  - Posters should be mounted on Tuesday (18 September) before 09h30 and left on display till 17h00 (posters must be removed at 18h00 at the latest)
- ○ Poster session 2 is scheduled on Wednesday 19 September
  - Posters should be mounted on Wednesday (19 September) before 09h30 and left on display till 17h00 (posters must be removed on Thursday 20 September at 09h00 at the latest)
- ○ Poster session 3 is scheduled on Thursday 20 September
  - Posters should be mounted on Thursday (20 September) between 09h00 and 09.h0 and left on display till 17h00 (posters must be removed at 18h00 at the latest)

Poster Discussion Sessions
Presenting authors are asked to attend their poster during the Poster Discussion Sessions, as indicated here:

For Poster Session 1 (P001-P076) on Tuesday 18 September	13h30 - 14h30
For Poster Session 2 (P077-P151) on Wednesday 19 September	13h00 - 13h50
For Poster Session 3 (P152-P221) on Thursday 20 September	13h15 - 14h15

SCIENTIFIC PROGRAM

Tuesday 18 September 2018

Plenary Session

AUDITORIUM 1

08h15 *Welcome Address*
Dirk de Graaf, Congress chair EurBee 8

08h30 *KN1* **Keynote 1**
Honey bee gut microbiota – a versatile model for understanding microbial community evolution and functioning
Philipp Engel

Morning Sessions

❒ **09h30 - 11h15 Parallel Session 1** **AUDITORIUM 1**
MICROBIOTA
Moderators: Ivan Meeus & Raquel Marin Hernandez

09h30 *001* *Insights into the diversity and dynamics of microbes in stingless bees*
Nunes-Silva C.G., Nacif-Marcal L., Fialho M.C.Q., D'Alvise P., Hasselmann M. (Stuttgart, Germany)

09h45 *002* *Exploring the microbiome's role in the biological invasion of the African honey bee genotype*
Kowallik V., Suenaga M., Rangel J., Mikheyev A.S. (Okinawa, Japan)

10h00 *Coffee & Tea*

10h30 *003* *Consequences of land-use for solitary bee microbiota composition and function*
Peters B., Leonhardt S.D., Keller A., Schloter M. (Würzburg, Germany)

10h45 *004* *Impact of medicaments and feed additives on the honeybee gut microbiota*
Alberoni D., Baffoni L., Gaggìa F., Stanton C., Ross P., Di Gioia D. (Bologna, Italy)

11h00 *005* *Pollen diversity and the microbiome; implications for honey bee health*
Ferrari R., Browne K.A., Voulgari-Kokota A., NIHBS, Keller A., McCormack G.P. (Galway, Ireland)

❒ **11h15 - 12h15 Symposium 2** **AUDITORIUM 1**
NEW CHALLENGES IN BEE-MICROBE COMMUNITY AND FUNCTIONAL ECOLOGY
Moderators: Benjamin Dainat & Vincent Doublet

11h15 *006* *Immune stimulation by the gut symbiont Frischella perrara in the honey bee (Apis mellifera)*
Emery O., Schmidt K., Engel P. (Lausanne, Switzerland)

11h30 *007* *Lactic acid bacteria of the honey bee crop enhance adult longevity but do not provide specific defence against a microsporidian and a viral pathogen*
Disayathanoowat T., Vásquez A., Olofsson T., Natsopoulou M., Doublet V., McMahon, D., Paxton R.J. (Halle, Germany)

11h45 *008* *Characterisation of the honey bee Apis mellifera metagenome in Britain*
Regan T., Barnett M.W., Laetsch D.R., Wragg D., Bush S.J., The BeeBiome Consortium, Blaxter M., Freeman T.C. (Edinburgh, UK)

12h00 *009* *Using contact networks and next-gen sequencing to reveal the community dynamics of the pollinator virome*
Doublet V., Doyle T., Brown M.J.F., Wilfert L. (Edinburgh and Penryn, UK)

❒ **09h30 - 12h15 Parallel Session 2** **AUDITORIUM 2**
GENETICS 1 - RESILIENT BEES
Moderators: Cédric Alaux & Samuel Boff
Eva Crane Trust

09h30 *010* *Field testing and selection on European honey bee populations (Smartbees project 2015-2018)*
Büchler R., Uzunov A., Hoppe A., Bienefeld K. (Kirchhain, Germany)

09h45 *011* *Mite adaptations in European Apis mellifera populations surviving Varroa destructor by means of natural selection?*
Moro A., Le Conte Y., Neumann P., de Miranda J.R., Locke-Grandér B., Dahle B., Blacquière T., Beaurepaire A. (Bern, Switzerland)

10h00 *Coffee & Tea*

10h30 *012* *Investigations into unmanaged honey bee colonies in Ireland*
Browne K.A., Henriques D., Hassett J., Geary M., Moore E., Pinto M.A., McCormack G.P. (Galway, Ireland)

10h45 *013* *Black box selection leads to distinct traits of resistance to Varroa destructor in honeybees*
Blacquière T., Boot W., Calis J., Van Stratum P. (Wageningen, The Netherlands)

11h00 *014* *The good, the bad and the ugly – dissecting Apis mellifera survivability to Varroa destructor*
Beaurepaire A., Alaux C., Crauser D., Dalmon A., Diévart V., Mondet F., Pioz M., Le Conte Y. (Avignon, France)

11h15 *015* *Breeding of Apis cerana: resistance of Sacbrood virus*
Choi Y-S., Vung N.N., Lee M-L., Lee M-Y., Kim H.K. ,Kim J.E. (Wanju-gun, South Korea)

11h30 *016* *Tolerance to Deformed wing virus at the individual and colony level in Swedish mite-resistant honey bees*
Locke B., Thaduri S., Stephan J., de Miranda J.R. (Uppsala, Sweden)

11h45 *017* *The Lord of the Rings: genotype-environment interactions for honey bee colonies surviving Varroa destructor by means of natural selection*
Neumann P., Oddie M.A.Y., Beaurepaire A., Blacquière T., Conlon B.H., Crauser D., Dahle B., Frey E., de Graaf D.C., Häußermann C., de Smet L. (Bern, Switzerland)

12h00 *018* *BeeStrong: towards a genomic tool for the selection of Varroa resistant honey bees*
Sann C., Poquet Y., Basso B., Mondet F., Eynard S., Servin B., Phocas F., Guillaume F., Bidanel J.P., Cluzeau-Moulay S. (Jouy-en-Josas, France)

❒ **09h30 - 12h15 Parallel Session 3** **AUDITORIUM 6**

ECOTOXICOLOGY 1

Moderators: Wim Reybroeck & Tjeerd Blacquière

09h30 *019* *EU-wide restrictions on neonicotinoids have not entirely eliminated the risk for honeybees foraging on oilseed rape nectar*
Wintermantel D., Odoux J-F., Decourtye A. , Henry M. , Allier F., Bretagnolle V. (Surgères and Villiers-en-Bois, France)

09h45 *020* *Time-to-death approach to reveal chronic and cumulative toxicity of a fungicide for honeybees not revealed with the standard ten-day test*
Simon-Delso N., San Martin G., Bruneau E., Hautier L. (Louvain la Neuve, Belgium)

10h00 *Coffee & Tea*

10h30 *021* *Effects of neonicotinoids on honey bee food glands*
Bruckner S., Straub L., Villamar-Bouza L., Neumann P., Williams G. (Auburn, USA)

10h45 *022* *The sublethal effects of clothianidin on the honey bee gut microbiota*
El Khoury S., Giovenazzo P., Derome N. (Québec, Canada)

11h00 *023* *Co-exposure to virus and pesticide (Deformed wing virus / neonicotinoid) alters bee behavioural performances*
Coulon M., Dalmon A., Prado A., Di Prisco G., Alaux C., Crauser D., Dubois E., Thiéry R., Ribière-Chabert M., Le Conte Y. (Avignon and Sophia-Antipolis, France)

11h15 *024* *The effects of neonicotinoids on circadian rhythms in honey bees*
Allain C., Sokolowski M. (Amiens, France)

11h30 *025* *Chronic exposure to thiamethoxam can promote chronic bee paralysis virus infections in honey bees*
Coulon M., Schurr F., Martel A-C., Di Prisco G., Dalmon A., Alaux C., Le Conte Y. , Thiéry R., Dubois E. (Sophia Antipolis, France)

11h45 *026* *Sublethal exposure to the neonicotinoid Insecticide thiacloprid impairs the immune defence in the solitary bee species Osmia bicornis L.*
Brandt A., Hohnheiser B., Sgolastra F., Bosch J., Meixner M.D., Büchler R. (Kirchhain, Germany)

12h00 *027* *Effects of neonicotinoids on the behavior of foraging honey bees with artificial flower choices*
Çakmak I., Hranitz J.M., Blatzheim L., Bower C.D., Polk T., Levinson B., Wells H. (Bursa, Turkey)

❒ **09h30 - 11h45 Symposium 1** **AUDITORIUM 7**

WHERE HAVE ALL THE WILD BEES GONE, LONG TIME PASSING? (BELBEES)

Moderators: Pierre Rasmont & Orianne Rollin

09h30 *028* *Where have all the wild bees gone, long time passing ?*
Rasmont P., Boevé J-L., Francis F., Dendoncker N., Dufrêne M., Smagghe G., Barbier Y., Brasero N., D'Haeseler J., Dekoninck W., Desmet L., Foschweiller M., Jacquemin F., Maebe K., Marshall L., Martinet B., Meeus I., Michez D., Moerman R., Pauly A., Roger N., Schoonvaere K., Vanderplanck M., Van Ormelingen P., Vray S., de Graaf D.C. (Mons, Belgium)

09h45 *029* *Declining distributions for BENELUX Bumblebees: climate and land use change interactions*
Marshall L., Biesmeijer J.C., Rasmont P., Vereecken N.J., Dvorak L., Fitzpatrick U., Francis F., Neumayer J., Ødegaard F., Paukkunen J.P.T.[1], Pawlikowski T., Reemer M., Roberts S.P.M., Straka J., Vray S., Dendoncker N. (Namur, Belgium and Leiden, The Netherlands)

10h00 *Coffee & Tea*

10h30 *030* *Extreme shift in abundance and distribution of bumblebee composition in Belgium*
Vray S., Rollin O., Michez D., Dendoncker N., Rasmont P. (Gembloux, Belgium)

10h45 *031* *Drift in distribution and quality of host-plant resources in common bumblebees*
Michez D., Roger N., Moerman R., Vanderplanck M. (Mons, Belgium)

11h00 *032* *The role of bees in interaction networks with plants as a conservation argument*
Jacquemin F., Violle C., Munoz F., Mahy G., Rasmont P., Michez D., Vereecken N.J., Roberts S.P.M., Vray S , Dufrêne M. (Gembloux, Belgium and Montpellier, France)

11h15 *033* *A century of temporal stability of genetic diversity in wild bumblebees*
Maebe K., Meeus I., Vray S., Dekoninck W., Boevé J-L., Rasmont P., Smagghe G. (Ghent, Belgium)

11h30 *034* *Viruses in wild bees: to be included in future monitoring programs*
Schoonvaere K., Francis F., de Graaf D.C. (Ghent, Belgium)

Afternoon Sessions

❒ **14h45 - 17h15** **Parallel Session 4** **AUDITORIUM 1**
GENETICS 2
Moderators: Guy Smagghe & Katarina Bilikova

14h45 *035* *Distribution of complementary sex determiner alleles in Apis mellifera population*
Zareba J., Blazej P., Laszkiewicz A., Sniezewski L., Majkowski M., Janik S., Cebrat M. (Wroclaw, Poland)

15h00 *036* *De novo genome assembly of a western European Apis mellifera mellifera black bee*
Vignal A., Eynard S.E., Klopp C., Canale-Tabet K., Marande W., Roulet A., Donnadieu C., Servin B. (Castanet Tolosan, France)

15h15 *037* *A comprehensive genomic and morphometric assessment of European honey bee diversity and identification of SNP markers for subspecies diagnosis*
Parejo M., Montes I., Bouga M., Estonba A., Papoutsis L., Nielsen R.O., Momeni J., Langa J., Vingborg R., Kryger P., Meixner M. (Leioa, Spain and Bern, Switzerland)

15h30 *038* *Applying a SNP-based tool for conservation of wild and managed black bees in Ireland*
Browne K.A., Henriques D., Pinto M.A., Native Irish Honeybee Society, McCormack G.P. (Bragança, Portugal)

15h45 *039* *Genetic models for long-term simulation studies in honeybee breeding*
Plate M., Bernstein R., Hoppe A., Bienefeld K. (Hohen Neuendorf, Germany)

16h00 *040* *Unraveling patterns of population structure in Bombus terrestris from the Iberian Peninsula and North Africa*
Silva S.E., Seabra S.G., Carvalheiro L.G., Nunes V.L., Marabuto E., Mendes R., Rodrigues A.S.B., Laurentino T.G., Paulo O.S. (Lisbon, Portugal)

16h15 *Coffee & Tea*

16h45 *041* *Signatures of selection revealed by population analyses of bumblebee genomes*
Colgan T.J., Arce A.N., Gill R.J., Ramos-Rodrigues A., Li L., Kanteh A., Wurm Y. (London, UK and Cork, Ireland)

17h00 *042* *Genomic signatures of introgression between commercial and native bumblebees, Bombus terrestris, in the Iberian Peninsula – implications for conservation and trade regulation*
Seabra S.G., Silva S.E., Nunes V.L., Sousa V.C., Martins J., Pina-Martins F., Rebelo M.T., Figueiredo E., Paulo O.S. (Lisbon, Portugal)

❐ **14h45 - 17h15 Parallel Session 5** **AUDITORIUM 2**
BEE HEALTH
Moderators: Anne Dalmon & Dennis VanEngelsdorp

14h45 *043* *Multi-stress effects on honey bee colonies*
van Dooremalen C., van Langevelde F., Blacquière T. (Wageningen, The Netherlands)

15h00 *044* *Impact of beekeeping management practices on honey bee mortality in Belgium*
El Agrebi N., Danneels E., de Graaf D.C., Saegerman C. (Liège, Belgium)

15h15 *045* *Classification and prediction of stress factors and bee health using ultra high performance liquid chromatography techniques with quadrupole and orbitrap high-resolution mass spectrometry-based metabolomics*
Wang L., Smagghe G., Meeus I. (Ghent, Belgium)

15h30 *046* *MALDI mass spectrometry imaging: an in situ histoproteomic approach to monitor the response of pollinators to their stressors*
Bulet P., Arafah K., Voisin S., Houdelet C., Bocquet M. (La Tronche and Archamps, France)

15h45 *047* *Assessment of honey bee cells using deep learning*
Alves T.S., Ventura P., Neves C.J., Candido Junior A., De Paula Filho P.L., Pinto M.A., Rodrigues P.J. *(Bragança, Portugal)*

16h00 *048* *Beneficial microorganisms and honeybees: colony level effects of lactic acid bacterial supplements*
Stephan J.G, Lamei S., Pettis J.S., Riesbeck K., de Miranda J.R., Forsgren E. (Uppsala, Sweden)

16h15 *Coffee & Tea*

16h45 *049* *COLOSS (prevention of honey bee Colony LOSSes): past forward*
Neumann P., Williams G., Chantawannakul P., The COLOSS consortium (Bern, Switzerland)

17h00 *050* *10 years of coordinated study of honey bee colony loss rates – presentation of a COLOSS core project*
Brodschneider R., Gray A.J., COLOSS C.P. (Graz, Austria)

❒ **14h45 - 17h15 Parallel Session 6** **AUDITORIUM 6**

ECOLOGY 1

Moderators: Robert Brodschneider & Norman Carreck

14h45 *051* *Quantifying and modeling bee spatial strategies*
Dubois T., Pasquaretta C., Gautrais J., Dore A., Aubert H., Lihoreau M. (Toulouse, France)

15h00 *052* *Design of a spatially explicit individual-based honeybee colony model*
Wallis D. Hatjina F. Jensen A., Simon-Delso N., Skov F., Topping C. (Rønde, Denmark)

15h15 *053* *Where do wild bees want to live? Biodiversity in agricultural landscapes*
Kratschmer S., Ockermüller E., Neumayer J., Hainz-Renetzeder C., Frank T., Pascher K., Pachinger B. (Vienna, Austria)

15h30 *054* *Community structure of bees associated with different types of floral margins*
Pérez-Marcos M., Ortiz-Sánchez F.J., López-Gallego E., Ramírez-Soria M.J., Sanchez J.A. (Murcia, Spain)

15h45 *055* *Not the perfect match? Biogeography and pollinator shifts rather than cospeciation dominate the evolutionary history of Rediviva bees and their Diascia host plants in the Cape biodiversity hotspot*
Kahnt B., Hattingh W.N., Theodorou P., Wieseke N., Kuhlmann M., Glennon K.L., van der Niet T., Paxton R.J., Cron G.V. (Halle and Leipzig, Germany)

16h00 *056* *Bumble-BEEHAVE: an agent-based population model for bumble bees and its application as a decision tool for land managers*
Becher M.A., Twiston-Davies G., Osborne J.L. (Toronto, Canada and Penryn, Cornwall, UK)

16h15 *Coffee & Tea*

16h45 *057* *Mitogenome sequencing to study introgression events between close related subspecies of Bombus terrestris in the Iberian peninsula*
Cejas D., López-López A., Ornosa C., De la Rúa P., Muñoz I. (Murcia, Spain)

17h00 *058* *Buzzing in the buckwheat – a diverse pollinator community brings more grains to the table*
Korpela E-L., Hokkanen H., Jaš S., Martikkala M., Toratti S. (Helsinki, Finland)

Plenary session

AUDITORIUM 1

17h15 *KN2* **Keynote 2**
Life outside the hive: What comparative genomics reveal about bee behavior, physiology, and evolution
Karen Kapheim

18h15 *Members' Meeting*

SCIENTIFIC PROGRAM

❒ PATHOLOGY 1 (P001 - P044)

P001 Deformed wing virus variant strains in the Indigenous and exotic honey bee in Saudi Arabia
P002 The German bee monitoring (DeBiMo): report 2016/ 2017
P003 Prevalence of protozoan parasites in *Vespa velutina* and in native European Hymenoptera (Vespoidea, Apoidea) from Galiza (NW-Iberian Peninsula)
P004 Impact of nutritional stress on colony strength and pathogen infection
P005 Does feeding pollen substitutes impact honey bee (*Apis mellifera*) colony strength parameters and Nosema infections?
P006 Toward a novel diagnostic tool for the selection of varroa-sensitive hygiene honey bee colonies
P007 Virus prevalence across sympatric field samples of *Apis mellifera* and *Bombus* species
P008 Transmission via hive products: globalization of the honey bee virosphere?
P009 Validation of three Real-Time PCR assays for the rapid differentiation of *Aethina tumida* from other *Nitidulidae* (Coleoptera) species
P010 Identification of honeybee colonies infected by *Paenibacillus larvae* through the powdered sugar examination
P011 Artificial brood interruption associated to the winter Varroa treatment
P012 *Nosema ceranae* found in the abdomens of small hive beetle (*Aethina tumida*) imagoes
P013 Histological features of the larvae of *Apis mellifera* on day 5 fed with toxic nectar in vitro
P014 Deformed wing virus variants in *Varroa destructor* susceptible and tolerant honey bee colonies
P015 Detection of viruses in virgin and mated queens of the honey bee *Apis mellifera L.*
P016 Inhibition of growth of *Paenibacillus larvae* by bacteriocin from *Brevibacillus laterosporus*
P017 Nosemosis control in European honey bees *Apis mellifera* by silencing the gene encoding *Nosema ceranae polar* tube protein 3
P018 Main pathogens in bumblebee species from Spain
P019 Comparative analysis of PCRprotocols to detect bee trypanosomatids
P020 Bee pathogens colonizing bee gums
P021 A survey of the different Varroa control methods used in Scotland and their efficacy
P022 Study of some pathogens in bumblebees and honeybees at four different locations in Slovenia
P023 BeeTyping™, an approach for monitoring bee health inspired from clinical microbiology biotyping
P024 Sanitary impacts and virus diversity in *Apis mellifera unicolor* population on Reunion Island, one year after *Varroa destructor* first detection
P025 The *Tropilaelaps* mites threat: an examination of the injuries inflicted on Apis mellifera host
P026 Field tests of the new varroacide VarroMed® in honey bee colonies with brood in late summer and autumn
P027 Biofilm formation of an Iranian isolate *Paenibacillius larvae*, the etiological agent of American FoulBrood Disease in honey bees larvae
P028 Chronic stress in honey bee colonies induced by acaricide residues and pathogens. A case study
P029 Trypanosimatids: a new threat for honey bee colonies?
P030 Tissue tropism of *Nosema apis* and *Nosema ceranae* in experimentally infected worker honey bees (*Apis mellifera*)
P031 Efficacy of Polyvar Yellow® for controlling *Varroa destructor* in Spanish honey bee colonies
P032 BEEHEAL: standardization of laboratory methods for sample processing, nucleic acids extraction and PCR for microsporidia and viruses analysis
P033 Infection levels of Nosema ceranae (fries et al., 1996) in honey bee colonies in Poland
P034 Does thiametoxan seed-treated oilseed rape have an impact on honey bee mortality?
P035 Antimicrobial activity of phytomolecules against *Paenibacillus larvae*
P036 Antimicrobial activity of phenolic extract of apple pomace against *Paenibacillus larvae* and its toxicity on *Apis mellifera*
P037 Honey bee feeding and its influence on the biochemical response to fluvalinate acaricide
P038 The Belgian national reference laboratory on bee diseases

P039 Detection of RNA viruses in bumblebees *Bombus atratus* in Uruguay
P040 Effect of different MRS-media on the growth of *Melissococcus plutonius*
P041 Diagnosis of the *Tropilaelaps* mites (Acari: Laelapidae) infestation: optimisation and standardisation of two identification methods based on morphological examination and PCR
P042 Prevalence of *Paenibacillus larvae* in central bohemian region: a case study during spring 2018
P043 Distribution of Deformed wing virus of honeybees (*Apis mellifera intermissa*) in the different regions of Algeria
P044 Honey bee colony losses and associated viruses in *Apis mellifera intermissa* in Algeria

❒ ECOTOXICOLOGY (P045 - P055)

P045 Neonicotinoids affect brain structural plasticity and olfactory memory in honey bees
P046 A new design for 10-day adult chronic toxicity tests: exposing honey bees (*Apis mellifera* L.) to active ingredients in pollen, sugar water, and wax matrices
P047 Synergistic effect of *Varroa destructor* and imidacloprid on immunity during honey bee development
P048 Survival and physiological impacts of pesticides combinations in the honeybee (*Apis mellifera*)
P049 Impact of imidacloprid, difenoconazole and glyphosate alone or in mixtures on honey bee
P050 Trinitrotoluene bioaccumulation in the honey bee hive
P051 Neonicotinoids decrease sucrose responsiveness of honey bees at first contact
P052 Effects of neonicotinoid insecticide exposure on nest-founding bumblebee queens
P053 Monitoring of honeybee colonies exposed to pesticide contamination in apple orchards and vineyards by means of Melixa systems
P054 Effects of pesticides on walking behavior of *Apis mellifera*
P055 Impacts of nutrition on the bumblebee's sensitivity to pesticides

❒ BEHAVIOUR AND COLONY FUNCTION (P056 - P076)

P056 Preliminary protocol to identify the decapped-recapped cells by worker bees, in order to estimate the hygienic behavior in connection with the capped brood
P057 The pheromones semiochemical effect study in a new solution for honey bee colony development
P058 Bumblebee foraging preferences and strawberry pollination effectivity according to surrounding biotopes
P059 Queen tracking inside the hive : a new methododology for the study of queen movement
P060 Apolipophorin III haemolymph content and weight as putative biomarkers of precocious foraging
P061 Hygienic behaviour in different lines of honey bee colonies
P062 Use of quadcopter drones to estimate honey bee population densities and define honey bee drone congregation locations
P063 The assessment of non-reproduction rate of *Varroa* (*Varroa destructor*) in a selection apiary in Romania – a comparative approach 2015-2016
P064 The behaviour of adult honey bees, *Apis mellifera* L., that were reared artificially
P065 Large scale monitoring of honey bee colony losses in Latin America
P066 Genetic analysis of royal jelly production and behavioural traits in *Apis mellifera*
P067 Beehive ventilation: a research agenda
P068 Screening of volatile organic compounds with semiochemical role from melliferous plants
P069 Extraction of sugar from vegetables and supplement carbohydrate sources to honeybee, *Apis mellifera*
P070 Optivar: Promoting the development of selection and breeding for honey bee colonies that are able to naturally limit the burden of *Varroa* infestations
P071 Are effective microorganisms possible supplement for honey bee colonies?
P072 Number of queen cells and reproductive swarms in the dwarf honey bee, *Apis* florea
P073 Evidence for short term evolutionary memory of genetic and environment interactions
P074 Finding Home: bumblebee homing in rural and urban environments
P075 *Nosema* infected bees increase protein intake to fight parasites
P076 Melliferous and polliniferous ressources of *Apis mellifera unicolor* in a tropical rainforest of Reunion Island

Wednesday 19 September 2018

Plenary session

AUDITORIUM 1

08h15 *Report Members' Meeting*
Robert Paxton

08h30 *KN3* **Keynote 3**
Using associative learning to learn about brain health and bees
Alison Mercer

Morning Sessions

❐ **09h30 - 11h00** **Parallel Session 7** **AUDITORIUM 1**
NEUROBIOLOGY & BEHAVIOUR
Moderators: Roger Huybrechts & Jozef Van der Steen

09h30 *059* *In vivo* imaging of neonicotinoid influence on odour processing in the honeybee brain
Andrione M., Cabirol A., Fayolle M., Zanini D., Zanon M., Vallortigara G., Antolini R., Haase A. (Rovereto, Italy)

09h45 *060* *Honey bee waggle dance communication benefits pollen foraging*
Nürnberger F., Härtel S., Keller A., Steffan-Dewenter I. (Würzburg, Germany)

10h00 *Coffee & Tea*

10h30 *061* *Observations of honey bee wing movements with the use of a high-speed camera*
Łopuch S., Tofilski A. (Krakow, Poland)

10h45 *062* *The effect of absolute and relative amounts of omega-3 and omega-6 essential fatty acids on associative learning in honey bees*
Arien Y., Dag A., Shafir S. (Rehovot, Israel)

❐ **11h00 - 12h00** **Parallel Session 10** **AUDITORIUM 1**
CHEMICAL ECOLOGY
Moderators: Pierre Rasmont & Adam Tofilski

11h00 *063* *Chemical Communication and improved tools for hygienic selection in the honey bee, Apis mellifera*
Wagoner K., Spivak M., Millar J., Schal C., Rueppell O. (Greensboro, NC, USA)

11h15 *064* *Antibiotics, sex and social regulation - the role of C10 short chain fatty acids in honey bee biology*
Crewe R.M., Moritz R.F.A. (Pretoria, South Africa)

11h30 *065* *Exposure of Nasonov gland by flying foragers of honey bee*
Tofilski A., Żmuda A. (Krakow, Poland)

11h45 *066* *Eat me or not- deciphering the molecular signals of diploid drone selection in the honey bee Apis mellifera*
Gessler B., Hasselmann M. (Stuttgart, Germany)

❒ **09h30 - 12h00 Parallel Session 8** **AUDITORIUM 2**
GENETICS 3 - RESILIENT BEES
Moderators: Tjeerd Blacquière & Peter Rosenkranz

09h30 *067* *Comparison of hygienic behavior directed against Varroa destructor and Tropilaelaps sp.*
Shrestha M., Wegener J., Gautam I., Bienefeld K. (Hohen Neuendorf, Germany)

09h45 *068* *Explaining the resistance to Varroa destructor in the French honey bee population with VSH, SMR and colony dynamic data*
Eynard S.E., Basso B., Guirao A-L., Mondet F., Vignal A., Servin B. (Castanet Tolosan, France)

10h00 *Coffee & Tea*

10h30 *069* *Preliminary researches regarding the efficiency of the formic acid treatment on varroa (Varroa destructor) found in the artificially decapped bee brood*
Siceanu A., Căuia E., Vișan G.O., Căuia D. (Bucharest, Romania)

10h45 *070* *Hygenic behaviour, Varroa and Nosema levels of Africanized honeybee colonies in Alagoas State, Northeastern Brazil*
Neto J.T.M., Almeida D.A., Lima E.G., Guimarães-Beelen P., Silva H.M., Beelen R. (Maceió, Brazil)

11h00 *071* *Identification of the DNA variants associated with the hygienic trait of honey bees*
Farajzadeh L., Momeni J., Nielsen R., Wegener J., Bienefeld K., Bendixen C. (Aarhus, Denmark)

11h15 *072* *Combining elastic-net penalized regression and whole exome sequencing to identify phenotype-associated variants*
Broeckx B.J.G., De Smet L., Blacquière T., Maebe K., Khalenkow M., Van Poucke M., Dahle B., Neumann P., Bach Nguyen K., Smagghe G., Deforce D., Van Nieuwerburgh F., Peelman L., de Graaf D.C. (Merelbeke, Belgium)

11h30 *073* *Progress in marker-assisted selection for honey bee breeding*
Pernal S.F., Borba, R., Hoover S.E., Currie R.W., Guarna M.M., Zayed A., Foster L.J. (Alberta, Canada)

11h45 *074* *A Canadian perspective on marker assisted selection and growing the local queen supply*
Bixby M., Guarna M.M., Hoover S., MacAfee A., Higo H., Pernal S.F., Foster L. (Vancouver, Canada)

❒ **09h30 - 12h15 Parallel Session 9** **AUDITORIUM 6**
PATHOLOGY 1
Moderators: Peter Neumann & Robert Paxton

09h30 *075* *Unbiased random mutagenesis contributes to a better understanding of the virulent behaviour of Paenibacillus larvae*
Descamps T., De Smet L., De Vos P., de Graaf D.C. (Ghent, Belgium)

09h45 076 *Canada-wide surveillance of exotic pathogens: Lotmaria passim and Crithidia mellificae*
Borba R.S, Wolf-Veiga P., Guarna M.M., Pernal S.F. (Beaverlodge and Vancouver, Canada)

10h00 *Coffee & Tea*

10h30 077 *Nosema ceranae and Lotmaria passim: partners in crime?*
MacInnis C.I., Luong L.T., Schwarz R.S., Guarna M.M., Pernal S.F. (Alberta and Beaverlodge, Canada)

10h45 078 *Factors influencing the development and the course of the N. ceranae infection in Apis mellifera iberiensis*
Urbieta A., Higes M., Meana A., Barrios L., Martín-Hernández R. (Marchamalo, Spain)

11h00 079 *Effect of Nosema ceranae on dynamic communities of gut bacteria in native species of Thai honey bees*
Naree S., Ellis J., Mayack C., Suwannapong G. (Chon Buri, Thailand)

11h15 080 *Tracking Asian hornets (Vespa velutina) to their nests with radio-telemetry*
Kennedy P., Ford S., Poidatz J., Thiery D., Osborne J. (Bordeaux-Aquitaine, France)

11h30 081 *Effect of Nosema ceranae on energetic stress of trehalose level in haemolymph and infection ratio of giant honey bee*
Ponkit R., Paxton R.J., Suwannapong G. (Chon Buri, Thailand)

11h45 082 *Oxidative stress increases in honey bees infected with Lotmaria passim*
Radaković M., Vejnović B., Glavinić U., Aleksić N., Mirilović M., Stanimirović Z., Stevanović J. (Belgrade, Serbia)

12h00 083 *Haploid males are more susceptible: spillover of Crithidia mellificae from managed honey bees to wild bees (Osmia cornuta)*
Strobl V., Yañez O., Straub L., Albrecht M., Neumann P. (Bern and Zürich, Switzerland)

❐ **09h30 - 12h15 Symposium 3** **AUDITORIUM 7**

LINKING FORAGING PATTERNS, FOOD INTAKE, NUTRITION AND PERFORMANCE IN BEES

Moderators: Sara Leonhardt & Cédric Alaux

09h30 084 *Bees rely on their sense of taste to assess pollen nutritional composition*
Ruedenauer F.A., Spaethe J., Strube-Bloss M.F., Leonhardt S.D. (Würzburg, Germany)

09h45 085 *Sex-dependent nutrient demand is reflected in pollen supplied to larvae by mother of a generalist solitary bee Osmia bicornis*
Filipiak M. (Kraków, Poland)

10h00 *Coffee & Tea*

10h30 086 *Temporal disruption in flowers availability affects honeybee colony losses*
Requier F., Odoux J-F., Henry M., Bretagnolle V. (Beauvoir sur Niort and Surgères, France)

10h45 *087* *Pollen protein content drives bee community preference for an invasive thistle over five native plant species*
Russo L., Vaudo A., Fisher C.J., Grozinger C., Shea K. (Dublin, Ireland)

11h00 *088* *How larval food quantities affect caste-fate in the stingless bee Scaptotrigona depilis*
Buchholz J., Hartfelder K. (Halle, Germany)

11h15 *089* *Honeybees tolerate cyanogenic glucosides from clover nectar and flowers*
Jensen A.B., Lecocq A., Green A.A., De Castro E.C.P., Olsen C.E., Zagrobelny M. (Copenhagen, Denmark)

11h30 *090* *The feeding with glandular secretions protects honeybee larvae from toxic pyrrolizidine alkaloids present in pollen*
Kast C., Kilchenmann V., Lucchetti M.A. (Bern, Switzerland)

11h45 *091* *New protocols to study nectar foraging in honey bees*
Sokolowski M. (Amiens, France)

12h00 *092* *Bees and resin, resin and bees: insights into an underrated resource*
Leonhardt S.D., Drescher N., Kaluza B.F., Neumann P., Klein A-M., Wallace H.M., Schmitt T. (Würzburg, Germany)

Plenary session

AUDITORIUM 1

14h00 *KN4* **Keynote 4**
State of the art in honeybee chemical ecology
Yves Le Conte

15h00-18h30 *Social Event - Discover Ghent*

❒ PATHOLOGY 2 (P077 - P116)

P077 Experimental infection of bumblebees (*Bombus terrestris*) with Deformed wing virus and Black queen cell virus
P078 Determining the efficacy of oxalic acid sublimation as a control for the honey bee pest *Varroa destructor* in Florida, USA
P079 A new multiplex PCR protocol to detect mixed trypanosomatid infections in species of *Apis* and *Bombus*
P080 Three years controlling the yellow-legged hornet (*Vespa velutina*), a new predator of honeybees in the Balearic Islands
P081 Black garden ants are alternative hosts of honey bee viruses
P082 Supplementation of *Apis mellifera* colonies with a beneficial microbes mixture based on *Lactobacillus kunkeei* strains
P083 Trapping *Vespa velutina* queens as a control method and its impact on honey bee colony strength
P084 Can sublethal pesticides exposure in honeybee colonies with subclinical infections by *Paenibacillus larvae* favour the development of American foulbrood in clinical form?
P085 Towards an electronic nose for American foulbrood: identifying volatile biomarkers for *Paenibacillus Larvae*
P086 Impact of essential oils at low doses on *Varroa destructor*
P087 Bee pathogen occurrence in commercial and traditional beekeeping
P088 Chronic bee paralysis: An emerging issue in honey bee health
P089 Multiple virus and microsporidian infections in individual honey bee workers (*Apis mellifera)*
P090 Comparative tests of in-hive traps for diagnosis and control of *Aethina tumida* infestations
P091 Prey (honeybee) predator (yellow-legged hornet) spill-over of the bee pathogen DWV
P092 Influence of *Nosema ceranae* infection on semen characteristics in honeybee drones
P093 Effect of winter stores on *Nosema Ceranae* infection and on colony fitness
P094 Dietary supplementation protects honey bee from immunosuppression caused by *Nosema ceranae*
P095 Evaluation of Varromed® performances in winter treatment of honey bee colonies (*Apis mellifera*) after brood interruption in a temperate area
P096 Use of traps containing biocides to diagnose and control SHB
P097 Monitoring of Small Hive Beetle (*Aethina tumida Murray*) in Calabria (Italy) from 2014 to 2016: practical identification methods
P098 Reactive oxygen species and nitric oxide in honey bee gut epithelial immunity
P099 Effect of protoporphyrin IX amide derivatives on *Nosema ceranae* development in *Apis Mellifera carnica*
P100 VarroMed - field trial data and lessons learned from the first centrally approved next-generation veterinary medicinal product against *Varroa*
P101 First report of the booklice *Liposcelis* spp (order: psocoptera) associated with honey bee hive
P102 Antimicrobial activity of *Bifidobacterium* spp. isolated from *Apis mellifera jemenitica* against drug multi-resistant human pathogens
P103 *Lactobacillus plantarum* from the gut of indigenous honeybee of Saudi Arabia inhibit the growth and biofilm formation of *Candida albicans*
P104 Antibacterial properties of Saudi Arabian Sidr honey against *Paenibacillus larvae*, the causal organism of American foulbrood
P105 The action of honeybee venom on drug multi-resistant human pathogens
P106 Bacterial strains of the *Lactobacillus* and *Fructobacillus* genera isolated from the gastrointestinal tract of honeybees for the use in the control and prevention of bee diseases and for probiotic preparations based on such bacterial strains (Patent application No. P.423363)
P107 European foulbrood disease: host-pathogen interactions and the impact of secondary invaders
P108 Effects of Deformed wing virus (DWV) on honey bee
P109 Sulforaphane is also present in bee pollen
P110 *Paenibacillus larvae*: genetic diversity and susceptibility to European and Asian honey bees and antagonistic activity of microflora in bee hives on American foulbrood pathogen

P111 Healthy bee: monitoring of Belgian honey bee health (2016-2017)
P112 Nosemosis in Estonian and Latvian apiaries: difference between countries, persistence over years and species distribution
P113 Ectoparasitic mites *Varroa underwoodi* in Eastern and Western honey bees
P114 Spatio-temporal variation of honeybee pathogens prevalence in wild bees in semiarid areas
P115 Cement honey - effects of the trisaccharide melezitose on honey bees
P116 Oxybee® (containing oxalic acid) in the treatment of varroosis in honey bees under field conditions in Germany

❒ GENETICS (P117 - P138)

P117 A study of local adaptation in the Iberian honeybee (*Apis mellifera iberiensis*) using a reciprocal translocation experiment
P118 Introgressive hybridization and latitudinal admixture clines in honeybees in East-Central Europe
P119 A comparative study of colony performance, hygienic behaviour and parasite and disease infection in the endemic honeybee *A. m. ruttneri* and the introduced *A. m. ligustica* in Malta
P120 Using exuviae as a non-destructive sampling method for population genetic analysis of bees
P121 Genetic characterization of the Italian *Vespa velutina nigrithorax* (*du Buysson*) population
P122 *Varroa* selection criteria: how can beekeepers use it?
P123 Smartbees - sustainable management of resilient bee population
P124 How to best control for differences in microsatellite loci variability when comparing genetic diversity across populations
P125 Genome-wide analysis of structural and single nucleotide variation at candidate loci for behavioural traits in Carniolan honeybee (*Apis mellifera carnica*)
P126 Applying reduce SNP assays for inferring C-lineage introgression patterns in Iberian honeybee populations of the Azores archipelago
P127 Developing reduced SNP assays from whole-genome sequence data to estimate C-lineage introgression in the Iberian honeybee (*Apis mellifera iberiensis*)
P128 Polymorphisms in cytochrome P450 versus cline distribution of evolutionary lineages in *Apis mellifera iberiensis*
P129 Contribution to the characterization of the genetic diversity of the honeybee *Apis mellifera*: case of the sex determination locus csd
P130 Applying molecular tools for conservation of wild and managed black bees in Ireland
P131 The transcription of ecdysteroids related genes in *Apis mellifera* workers and drones brood
P132 *Varroa* mite reproduction, hygienic and grooming behavior of Ethiopian honeybee (*Apis mellifera jementica*)
P133 High sample throughput genotyping for estimating C-lineage introgression in the dark honeybee: an accurate and cost-effective SNP-based tool
P134 A collection status of the world biogeography and population genomics of *Varroa destructor* project
P135 Identification of honeybee populations from the azores: insigths from wing geometric morphometrics
P136 Integrative approach apply to three Belgian species (*Thoracobombus*) involving DNA sequences and male marking secretions
P137 Genetic architecture of honey bee virus susceptibility
P138 The conservation of honey bee subspecies depends on beekeepers' involvement

• BEE PRODUCTS (P139 - P151)

P139 The impact of solid state fermentation on bee pollen out layer and phenolic compounds
P140 Alpha-mangostin and apigenin induced the death of BT474 breast cancer cells via necrosis involving with autophagy and inflammation
P141 Monocyclic aromatic hydrocarbons in urban honey bee larvae and pollen

P142 First scientific studies of beehive air composition
P143 Authentication of honeydew honeys by analyzing non-volatile components
P144 Quality and variety of Ukrainian honey
P145 Effect of honeybee race and season changes on propolis composition
P146 Microbeekeeping protocol to design a quantitative and qualitative properties of natural honey
P147 *Fallopia japonica* honey: an antimicrobial candidate against methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococus aureus*
P148 Bee products in Thailand: their medicinal properties
P149 Gbaya - beekeeping and honey hunting
P150 Physicochemical and sensory properties of different types of honey from Serbian market
P151 Development of liposome containing bee venom extract from *Apis dorsata* for anti-aging

Thursday 20 September 2018

Plenary session

AUDITORIUM 1

08h30 *KN5* **Keynote 5**
Nutrition and immunity – a systemic response to infection in the insect model, *Drosophila melanogaster*
Dan Hultmark

Morning Sessions

❒ **09h30 - 10h15** **Parallel Session 11** **AUDITORIUM 1**
IMMUNITY
Moderators: Roger Huybrechts & Robert Paxton

09h30 *093* *Single von Willebrand factor C proteins and its role in insect immunity*
Wang H., Niu J., Smagghe G., Meeus I. (Ghent, Belgium)

09h45 *094* *Study of gene expression of immunologically important molecules in honey bees after application of probiotic preparation*
Maruščáková I., Schusterová P., Bielik B., Toporčák J., Mudroňová D. (Košice, Slovakia)

10h00 *095* *On mechanisms of trans-generational immune priming in honeybees*
Freitak D., Salmela H., Harwood G., Amdam G. (Helsinki, Finland)

10h15 *Coffee & Tea*

❒ **10h45 - 12h15** **Parallel Session 14** **AUDITORIUM 1**
BEE PRODUCTS
Moderators: Etienne Bruneau & Janko Bozic

10h45 *096* *Alarming situation on the EU beeswax market: the prevalence of adulterated beeswax material and related safety issues*
Svečnjak L., Prđun S., Baranović G., Damić M., Rogina J. (Zagreb, Croatia)

11h00 *097* *Effect of beeswax adulterated with stearin on the development of worker bee brood: results of a field trial*
Reybroeck W., Van Nevel J. (Melle, Belgium)

11h15 *098* *Effect of spatial allocation of nectar cells on storage and honey ripening dynamics in the honeybee Apis mellifera*
Eyer M., Dietemann V. (Bern, Switzerland)

11h30 *099* *Culturing and conserving of stored pollen on the way into beebread*
Podrižnik B., Božič J. (Ljubljana, Slovenia)

11h45 *100* *Sugar, amino acids, and inorganic ion profiling of honeydew from aphid species hosting different tree species in Germany*
Shaaban B., Seeburger V., Schroeder A., Lohaus G. (Wuppertal, Germany)

12h00 *101* *Genuine Manuka honey!? - State of the art*
Beitlich N., Speer K. (Dresden, Germany)

09h30 - 12h15 Parallel Session 12 — AUDITORIUM 2

ECOTOXICOLOGY 2

Moderators: Claude Saegerman & Annely Brandt

09h30 *102* *Synergistic effects of stressors on bee health: pesticides, nutrition, and behaviour*
Tosi S. (San Diego, La Jolla, CA, USA)

09h45 *103* *Imidacloprid diffusion route: from apple orchard to the honey bee colony matrices*
Malagnini V., di Prisco G., Zanotelli L., Nazzi F., Annoscia D., Tonidandel L., Colombo R., Serra G., Boi M., Fontana P., Angeli G. (San Michele all'Adige, Italy)

10h00 *104* *Chronic toxicity of select pesticides to honey bee (Apis mellifera L.) larvae reared in vitro*
Dai P., Jack C.J., Mortensen A.N., Bustamante T.A., Bloomquist J.R., Ellis J.D. (Gainesville, Florida, USA)

10h15 *Coffee & Tea*

10h45 *105* *The homing flight ring test: method to assess the effects of sublethal doses of pesticides on the honey bee in field conditions*
Fourrier J., Rouzes A., Monchanin C., Henry M., Decourtye D., Aupinel P., Fortini D., Moreau C. (Avignon, France)

11h00 *106* *Lazy Spring: pollen-bound pesticide mixtures make bees less efficient*
Prado A., Pioz M., Vidau C., Brunet J.L., Jury, M., Crauser D., Requier F., Le Conte Y., Alaux C. (Avignon, France)

11h15 *107* *Versatile stressor impact analysis by video observation of worker behavior and brood development within cells reveals neonicotinoid effects at field realistic concentrations*
Siefert P., Hota R., Grünewald B. (Frankfurt am Main, Germany)

11h30 *108* *Pharmacokinetic and molecular investigations providing insights into the honey bee- friendly profile of the butenolide insecticide flupyradifurone (SIVANTO prime®)*
Zaworra M., Almanza M.T., Nauen R. (Monheim, Germany)

11h45 *109* *Pesticide residues in bee bread from the national honey bee disease survey*
Traynor K., vanEngelsdorp D., Rennich K., Rose R., Fahey R. (College Park, MD, USA)

12h00 *110* *Gene expression profiling demonstrates that exposure to neonicotinoid pesticides affects multiple biological processes in bumblebees (Bombus terrestris)*
Fletcher I.K., Colgan T.J., Arce A., Ramos-Rodrigues A., Stolle E., Gill R., Wurm Y. (London, UK)

09h30 - 12h15 Parallel Session 13 — AUDITORIUM 6

PATHOLOGY 2 - VIRUS

Moderators: Fanny Mondet & Joachim de Miranda

09h30 *111* *Two deformed wing virus variants, genotypes A and B, cause low pupal mortality and a high frequency of wing deformities in metamorphosing honey bees (Apis mellifera)*
Tehel A., Vu Q., Theodorou P., Paxton R.J. (Halle, Germany)

09h45 *112* *Using an in vitro feeding system to investigate Varroa-vectored DWV variant transmission*
Bradford E.L., Campbell E.M., Bowman A.S. (Aberdeen, Scotland, UK)

10h00 *113* *Europe-wide diversity of Deformed wing virus variants*
Sircoulomb F., Schurr F., Blanchard Y., Lucas P., Dubois E., Thiery R., Smartbees consortium partners (Sophia Antipolis, France)

10h15 *Coffee & Tea*

10h45 *114* *Healthy honey bees - analysis of the virus population to assess rational Varroa control*
Woodford L., Evans D.J., Bowman A., Highet F. (St Andrews, UK)

11h00 *115* *Virome analysis on Belgian honeybees reveals multiple undescribed viruses including a diverse bacteriophage population*
Deboutte W., Beller L., Yinda K.C., Conceição-Neto N., Maes P., de Graaf D.C., Matthijnssens J. (Leuven, Belgium)

11h15 *116* *Apis Rhabdovirus-1, a negative-sense RNA virus present in populations of pollinators , replicates in Apis mellifera and Varroa destructor*
Levin S., Galbraith D., Sela N., Erez T., Grozinger C.M., Chejanovsky N. (Israel)

11h30 *117* *A natural product inhibited the replication and expression of Israeli acute paralysis virus*
Yang S., Xu X., Zhao H., Deng S., Chu Y., Yang D., Wang X., Zhao D., Diao Q., Hou C. (Beijing, P.R. China)

11h45 *118* *Constructing infectious Sacbrood virus clone with green fluorescence protein expression*
Mehmood S., Jin T., Huang H., Huang W-F. (Fuzhou, P.R. China)

12h00 *119* *Relevance of qualitative and quantitative changes in the virome to the survival of a Swedish Varroa-resistant honeybee population*
Thaduri S., Locke B., Granberg F., de Miranda J.R. (Uppsala, Sweden)

❒ **09h30 - 12h15** **Symposium 4** **AUDITORIUM 7**
STUDIES ON COLONY MULTIPLE ENEMIES FOR BEE HEALTH
Moderators: Marie-Pierre Chauzat & Laurianne Paris

09h30 *120* *European-wide analysis of Paenibacillus larvae genetic diversity: EuroPLarva*
Sircoulomb F., Gaiani N., Paris L., Ribière-Chabert M., Rivière M.P. (Sophia Antipolis, France)

09h45 *121* *Health status and factors identified for winter losses of honey bee colonies by the Austrian surveillance study 2015/2016*
Morawetz L., Köglberger H., Derakhshifar I., Mayr J., Moosbeckhofer R., Crailsheim K. (Graz, Austria)

10h00 *122* *Should Varroosis include honeybee viruses as causative agents?*
Schäfer M.O. (Greifswald - Island Riems, Germany)

10h15 *Coffee & Tea*

10h45 *123* *The yellow-legged hornet: its impact on European western honeybees and control methods*
Pointeau S., Rome Q., Requier F., Fournier A., Decante D., Vallon J., Henry M., Decourtye A. (Avignon, France)

11h00 *124* *Insights into honey bee pathogen and parasite dynamics and interactions using high-throughput analysis*
D'Alvise P., Hasselmann M. (Stuttgart, Germany)

11h15 *125* *Small hive beetle invasion risk under current and future climate scenarios: a modelling approach to foster mitigation efforts*
Cornelissen B., Schweiger O., Neumann P. (Bern, Switzerland)

11h30 *126* *Survey of wild bee communities threatened by Vespa velutina*
Carisio L., Lioy S., Porporato M., Manino A. (Turin, Italy)

11h45 *127* *Patterns of Vespa velutina invasion in western Iberia and Italy as revealed by molecular markers*
Quaresma A., Henriques D., Godinho J., Maside X., Bortolotti L., Pinto M.A. (Bragança, Portugal)

12h00 Discussion time

Afternoon Sessions

❒ **14h30- 16h00** **Parallel Session 15** **AUDITORIUM 1**
REPRODUCTIVE BIOLOGY
Moderators: Fani Hatjina & Lina De Smet

14h30 *128* *Reproductive success in European orchard bee Osmia cornuta (Hymenoptera: Megachilidae) influenced by the number of males*
Stanisavljević L., Rašić S., Stanisavljević J. (Belgrade, Serbia)

14h45 *129* *Differential circular RNAs expression in ovary during oviposition in honey bees*
Chen X., Shi W., Chen C. (Beijing, P.R.China)

15h00 *130* *Influence of DMSO with dextran, PVP or PEG in freezing extender on queens*
Nur Z., Çakmak S.S., Çakmak I., Önder N.T., Gökçe E., Üstüner B., Alçay S., Toker B., Şen H., Soylu M.K. (Bursa, Turkey)

15h15 *131* *Queen temperature stress decreases sperm viability, queen performance, and colony productivity*
Guarna M.M., Pettis J.S., Pernal S.F. (Beaverlodge, AB, Canada)

15h30 *132* *Observations of the mating behaviour of Apis melifera macedonica and Apis melifera cecropia under natural conditions and under conditions of a control mating system (the Train of Virgin Queens)*
Hatjina F., Charistos L. (Nea Moudania, Greece)

15h45 *133* *Preservation of domesticated honey bee (Hymenoptera: Apidae) drone semen*
Paillard M., Rousseau A., Giovenazzo P., Bailey J.L. (Deschambault and Quebec, Canada)

16h00 *Coffee & Tea*

❒ **16h30- 17h30** **Parallel Session 18** **AUDITORIUM 1**
EVOLUTIONARY BIOLOGY
Moderators: Guy Smagghe & Anja Buttstedt

16h30 *134* *Experimental evolution of parasite virulence in a eusocial insect*
Lattorff H.M.G. (Nairobi, Kenya and Halle-Jena-Leipzig, Germany)

16h45 135 *Evolution of new gene functions by gene duplications - the case of major royal jelly proteins in the honey bee*
Buttstedt A., Mureșan C.I., Helbing S., Moritz R.F.A. (Halle and Dresden, Germany)

17h00 136 *Overview of the southwest Indian ocean honeybee: combining morphometry and genetics to investigate the original diversity of Apis mellifera spp. in Madagascar and surrounding archipelagos*
Galataud J., Delatte H., Bernet C., Techer M., Reynaud B., Clémencet J. (Saint Denis, France)

17h15 137 *Genetic basis for the evolution of non-reproduction of Varroa destructor in populations of Apis mellifera*
Conlon B.H., Moritz R.F.A., Routtu J. (Halle, Germany)

❒ **14h30- 17h30 Parallel Session 16** **AUDITORIUM 2**
ECOLOGY 2
Moderators: Ivan Meeus & Andrzej Oleksa

14h30 138 *Floral landscape enrichment and semi-natural habitats improve honeybee health, as evidenced by a 'Landscape physiology' approach*
Alaux C., Allier F., Decourtye A., Odoux J-F., Le Conte Y., Henry M. (Avignon, France)

14h45 139 *Influence of major stresses on wild bees communities in an urban environment*
Weekers T., Leclercq N., Hainaut H., Caruso G., Molenberg J-M., Vereecken N.J. (Brussels, Belgium)

15h00 140 *The application of non-intrusive electronic bee hive monitoring to field studies*
Evans H.A., Evans S.K. (Newcastle upon Tyne, UK)

15h15 141 *Changes in bumble bee species diversity and abundance over 50 years on red clover fields in Estonia*
Karise R., Mänd M., Viik E. (Harjumaa, Estonia)

15h30 142 *European beech forests as a home for feral honey bee colonies*
Rutschmann B., Kohl P.L. (Würzburg, Germany)

15h45 143 *Potential role of wetlands for honey bees diversity, population density and conservation in Sudan*
El-Niweiri M.A.A., Lattorff M.G.H. (Nairobi, Kenya and Halle-Jena-Leipzig, Germany)

16h00 *Coffee & Tea*

16h30 144 *Do floral traits play a role in animal plant interaction? A study case with orchid bees*
Boff S., da Luz C.F.P., Raizer J., Lima L.C.P. (São Paulo, Brazil)

16h45 145 *Are non-native plants a valuable resource for wild bees?*
Seitz N., vanEngelsdorp D., Leonhardt S.D. (Würzburg, Germany)

17h00 146 *Landscape related plant and resource diversity increases foraging and colony fitness in a tropical social bee*
Kaluza B., Wallace H., Heard T., Leonhardt S. (Würzburg, Germany and Maroochydore QLD, Australia)

17h15 147 *Response of wild bee diversity and functional traits to vineyard management and landscape diversity across Europe*
Kratschmer S., Pachinger B., Paredes D., Macavei L., Guzmán G., Guernion M., Nicolai A., Fertil A., Popescu D., Bunea C., Zaller J., Winter S. (Vienna, Austria)

❒ **14h30- 17h30 Parallel Session 17** **AUDITORIUM 6**

PATHOLOGY 3 - VARROA

Moderators: Marina Meixner & Frans Jacobs

Véto-pharma
Committed to apiculture

14h30 *148* *Varroa destructor feeds primarily on honey bee fat body tissue not hemolymph*
Ramsey S.D., Ochoa R., Bauchan G., Gulbronson C., Mowery J., Cohen A., Lim D., Joklik J., Cicero J.M., Ellis J.D., Hawthorne D., vanEngelsdorp D. (College Park, Maryland, USA)

14h45 *149* *Varroa mite saliva contains bioactive factors that aid mite feeding and manipulate the honeybee immune response*
Campbell E.M., Bowman A.S. (Aberdeen, Scotland, UK)

15h00 *150* *Hormonal induction of in vitro egg production in the honeybee mite, Varroa destructor*
Christie C.R., Budge G.E., Campbell E.M., Bowman A.S. (Aberdeen, Scotland, UK)

15h15 *151* *Transcriptome profiling of the parasite Varroa destructor provides new biological insights into the mite adult life cycle*
Mondet F., Rau A., Klopp C., Rohmer M., Severac D., Le Conte Y., Alaux C. (Avignon, France)

15h30 *152* *Early Varroa infection transcriptomics in the Asian honey bee*
Routtu J., Conlon B.H., Devaraju S., Brockmann A., Moritz R.F.A. (Halle-Wittenberg, Germany)

15h45 *153* *Lithium salts as varroacide - efficacy in the treatment of artificial swarms and side effects on bees and brood*
Ziegelmann B., Makosch M., Hannus S., Rosenkranz P. (Stuttgart, Germany)

16h00 *Coffee & Tea*

16h30 *154* *Using RNAi to control Varroa destructor – a novel biopesticidal tool for effective control of the parasitic mite of honey bees*
Gleit Kielmanowicz M., Verhaert J., Inberg A., Masucci J.D., Avni D., Cooper S., Durnel N., Back S. (Brussels, Belgium)

16h45 *155* *Kairomones in the hive involves in Varroa destructor mite behavior*
Pojar-Fenesan M., Balea A., Ciotlaus I. (Cluj-Napoca, Romania)

17h00 *156* *Reproduction of the mite Varroa destructor in original and new honey bee hosts*
Lin Z., Qin Y., Page P., Wang S., Li L., Wen Z., Hu F., Neumann P., Zheng H., Dietemann V. (Bern, Switzerland and Chiang Mai, Thailand)

17h15 *157* *Tracking genomic footprints of successful host switches in honey bee Varroa mites*
Techer M., Roberts J.M.K, Mikheyev A.S. (Okinawa-ken, Japan)

Plenary session

AUDITORIUM 1

17h30 *KN6* **Keynote 6**
Social bees as model systems in the study of the evolution of cooperation and conflict
Tom Wenseleers

❒ CHEMICAL ECOLOGY (P152 - P165)

P152 Increased fluctuating asymmetry in honey bee drones exposed to neonicotinoid pesticides
P153 Drone's endophallus pigment: comparative study of absorption spectra of four *Apis* species
P154 Honeybees (*Apis mellifera*) and bee pollen as bio-indicators of heavy metal pollution in different geographic areas
P155 Multi-stress approach for the assessment of decline causes for honeybee
P156 The brood survival rate of a colony predicts the adult emergence rate of its larvae reared in vitro
P157 The adverse effects of nanosilver on *Apis mellifera carnica*
P158 Effects of a potential natural acaricide carvacrol on carnolian honeybee (*Apis melifera carnica*)
P159 Hemolytic activity of pathogenic bacteria, erythrocyte membrane protection, and immunostimulatory effects of Saudi honeys
P160 Field effects of two neonicotinoid pesticides on honey bee drones, *Apis mellifera*
P161 The ups and downs of hops (*Humulus lupulis*) beta acids in *Varroa* control
P162 The impact of in hive pesticide contaminations on honey bee mortality
P163 Enhancement of chronic bee paralysis virus levels in honeybees acute exposed to imidacloprid: a Chinese case study
P164 The effects of tau-fluvalinate and tebuconazole on honeybee (*Apis mellifera*) queens
P165 Thyme nectar and pollen terpenes potentially improving honey bee health

❒ BEE HEALTH (P166 - P202)

P166 The Norwegian bumblebees in the face of climate change
P167 BPRACTICES and Hivelog web application for honey bee products traceability
P168 Possible side effects of sugar supplementary nutrition on honey bee health
P169 Fat body evaluation of wintering bees with NIR (near infrared spectroscopy)
P170 Influence of pH on stability and structure of major royal jelly proteins
P171 Molecular mechanism study of honeybee caste differentiation regulated by the comformation change of major royal jelly protein 1
P172 Colonies and queen replacement strategies: a systemic experiment
P173 Sustainability of beekeping farms: a definition proposal
P174 Laurel (*Laurus nobilis*) extract induces gene expression of antioxidative enzymes in honeybees
P175 New probiotic preparation for honey bees
P176 Pollen as a key factor for the response of honey bees to mite parasitization
P177 Opportunities and challenges for sustainable apiculture on the mountain Avala and surrounding region (Belgrade area, Serbia)
P178 The Scientific Veterinary Medical Association for Apiculture (SVETAP)
P179 BPRACTICES: first attempt of definition of Good Beekeeping Practices (GBPS)
P180 Three species of native Thai honey bees exploit overlapping pollen resources: Identification of bee flora from pollen loads and midguts from *Apis cerana*, *A. dorsata* and *A. florea*
P181 Pollen consumption by adult solitary bees: amount, frequency, and species composition
P182 BDA (beekeeping database) in Italy: an achievement in the management of bee health
P183 Quality of honeybee nutrition originating from proteins is key factor of honeybee health
P184 Heavy metal pollution levels in honey bee larvae depending on pollen and environmental pollution
P185 Sustainable beekeeping and advisory service: a critical review
P186 Comprehensive study of midgut/pyloric bacterial population in European and Japanese honey bees
P187 The influence of beekeepers' management practices and pesticides on bee health in Chile's Central Valley
P188 Proline enriched diet effects on haemolymph amino acid composition in *Osmia bicornis* (L.) females and males

P189 Reducing the risk of honey bee colony loss through beekeeping management practices
P190 Perception of risk factors affecting bee colonies (*Apis mellifera*) health and mortality in Belgium
P191 Natural comb honeybee management in frame hives for professional beekeepinga
P192 First European study of honey production in beehives under an annual "Vita Feed" nutritional protocol
P193 Validation of reference genes for RT-qPCR analysis of thermal stress gene expression in *Bombus terrestris*
P194 BeeHeal: promoting bee health for sustainable agriculture
P195 Bioactive compouds that could be found in bee pollen
P196 SAMS - International partnership on innovation in Smart Apiculture Management Services
P197 Bee world is celebrating its 100th Anniversary in 2019
P198 Study regarding the content of the various nutrients in the *Crataegus monogyna*, *Brassica* spp. and *Prunus* spp. bee pollen
P199 A multi-stressor analysis of spatio-temporal shifts in Belgian bee community
P200 Colony performance of *Apis mellifera* feeded with *Lactobacillus johnsonii* AJ5 metabolites
P201 Bee *Varroa* scanner
P202 Creating overwintered nucleus colonies for early spring research

❒ ECOLOGY (P203- P221)

P203 An assessment of the Belgian *Halictids* species, with an overview of the endangered species in other countries in Europe
P204 Improvement of the almond production using bumblebees
P205 Ensuring access to high quality flower resources can reduce impacts of climate change on bumblebee colony development
P206 Hyperthermic stress resistance of bumblebees: what about of sub-boreal Belgian species
P207 Foraging in the cities: ecological niche breadth and overlap of Euglossini bees
P208 Foraging activity of honey bees and wild pollinators on fruit trees and berry shrubs
P209 Effects of some behavior characteristics of honey bee (*Apis mellifera l.)* and bumble bee (*Bombus terrestris*) on cherry pollination (production, quality, phenology and yield) and climatic temperature change
P210 Genetic analysis on recently found *B. veteranus* specimens in Belgium, does the supposed extinct species returned or just never left?
P211 Spatio-temporal floral resource shifts in Belgium
P212 Exotic and native plant species and their role attracting native pollinators
P213 Pollination efficiency of thirteen bee species visiting *Cajanus cajan* in Cameroon
P214 Status and trends of wild pollinators in Belgium and North of France
P215 Adaptation to a changing world: how wild bees cope with climate change
P216 May regulations against thistles threaten bumblebees?
P217 Beekeeping potential in the red dwarf honeybees, *Apis florea*
P218 Honey bees (*Apis mellifera* L.) use pollen from the biogas crop Sorghum bicolor and increase the seed-set
P219 Impacts of honey bee density on crop yield: a meta-analysis
P220 Effects of landscape structure and floral border on pollination services provided by honeybees and native bees in avocado orchards of central Chile
P221 The effect of pest management in orchards on honey bee welfare


SOCIAL PROGRAM

EurBee 8 Social Program

Belgium fires one's imagination. Those with a craving for art and culture or architectural beauty, will be pampered in our city of art. Those who like a taste of gastronomy, cultural events and nightlife will also have every wish satisfied. Throughout the EurBee 8 social program you will discover in the city of Ghent all the above!

Monday evening 17th, 19h00 – 21h00
Opening Ceremony and Welcome Reception at the Aula of Ghent University

Volderstraat 9, Ghent

When Ghent University was founded, the city decided to design and build a "celebration palace", the Aula. The foundation stone of this neoclassical building with a Greek-looking porch, spacious peristilium, and round Hall with a panelled ceiling was laid in 1819. The building was opened in 1826. Nowadays, the Aula is still the place for official ceremonies, honorary doctorates, proclamations and so on.

The EurBee 8 host committee would like to welcome you at 19h00 at the Aula of Ghent University for the EurBee 8 Opening Ceremony , which will be followed by the Welcome Reception, where you can catch up with colleagues.

Wednesday afternoon 19th, 15h00 – 18h30
Social event - Discover Ghent

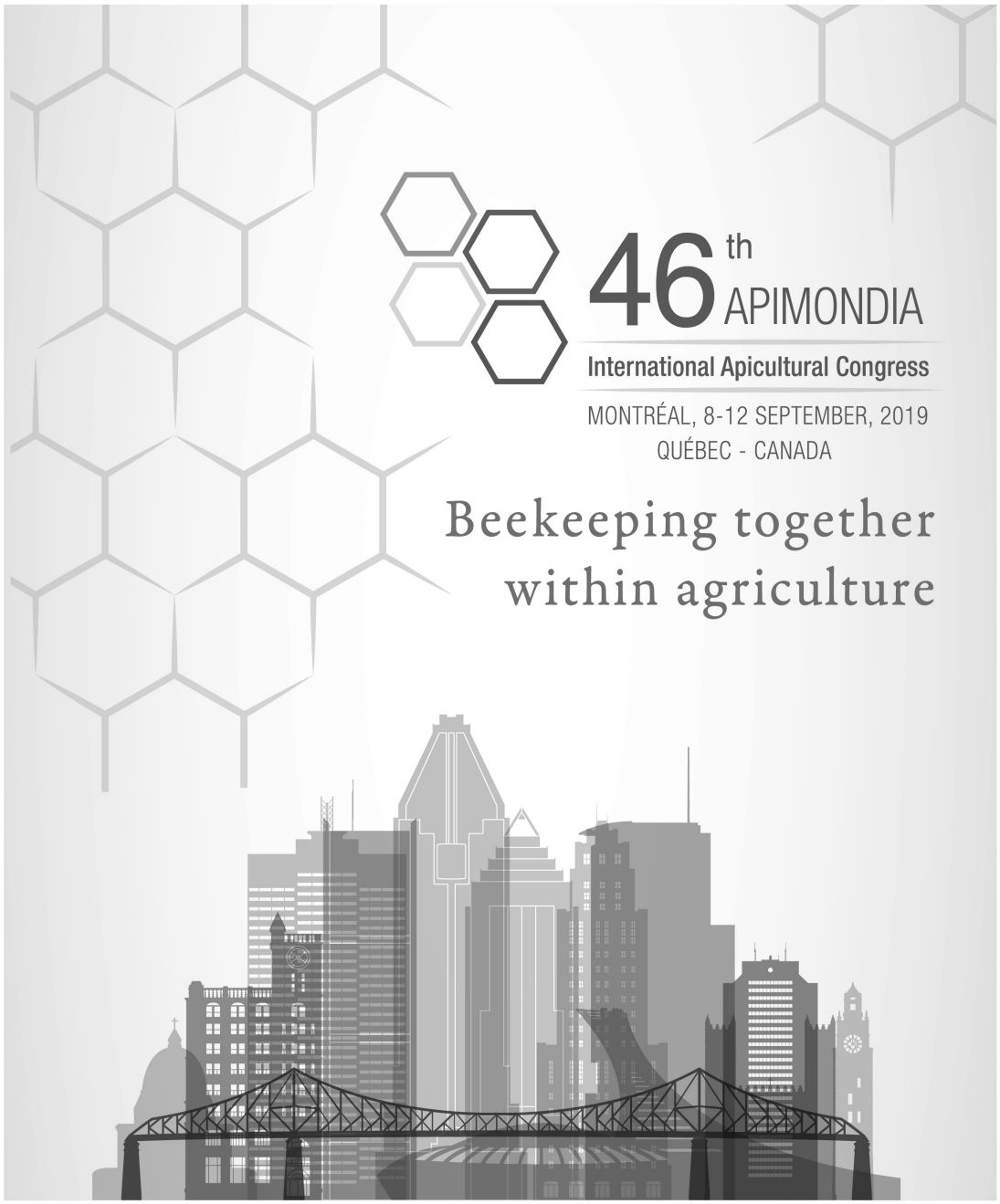
We will leave immediately after the afternoon plenary session from the congress venue and walk through the Citadelpark to the Bijlokekaai. A boat will bring you to the historical inner city centre of Ghent, where the guides will take you along to discover the city. The tour will last approximately 2 hours. Beware of the cobblestones and wear comfortable shoes!

Thursday evening 20th, 20.00 – Congress Banquet at the Old Fish Market (Oude Vismijn)

Rekelingestraat 5

The Oude Vismijn used to be the fish, meat and vegetable market of Ghent and is located in the most historical heart of the city of Ghent. This cultural historical patrimony has recently been renovated.
Here centuries-old history and high-tech facilities go hand in hand. Opposite the Castle of the Counts lies the monumental gateway (1689) to the Old Fish Market. Neptune keeps watch over the Scheldt (male) and the Lys (female). Time is foreseen for awards and speeches and of course after the dessert we make place to install the dancing floor. Drinks will be offered during the banquet but will be on delegates account afterwards.

With the kind support of the 46th Apimondia



KEYNOTE SPEAKERS

KEYNOTE SPEAKERS

Theme: GUT MICROBIOTA

The animal gut is colonized by complex and highly specialized bacterial communities, which impact health and disease of their host in manifold ways. The honey bee (Apis mellifera) has recently emerged as a relevant model to study gut microbiota-host interactions. Bees harbor a simple gut microbiota with striking parallels to the mammalian system and importance for bee health. The research group of Dr. **PHILIPP ENGEL** at the University of Lausanne, Switzerland, employs experimental and genomic approaches to study bee gut bacteria. His team is interested in understanding their evolution, metabolic functions and symbiotic interactions. The researchers believe that the bee gut microbiota is a powerful and versatile model for addressing key questions about microbial symbiosis, gut microbiology and bee health.

KN1 Honey bee gut microbiota – a versatile model for understanding microbial community evolution and functioning

Specialized bacterial communities colonize the animal gut and impact health and disease of the host in manifold ways. However, their complex composition presents a veritable challenge for elucidating fundamental aspects of gut microbiota functions, ecology, and evolution. In my lab, we study the honey bee gut microbiota, a surprisingly simple, yet conserved gut community that is experimentally amenable and shares striking parallels to the mammalian system. Our overall goal is to combine experimental and genomic approaches to systematically understand functioning and evolution of this specialized, host-associated community.
In my talk, I will summarize two recent projects, in which we applied metabolomics and shotgun metagenomics, respectively, to disentangle metabolic capacities of individual community members and identify the community's population genomic structure. Our results show distinct roles of bee gut symbionts in the conversion of major pollen wall constituents, but also suggest potential cross-feeding activities between them. Moreover, the genome-level information obtained from shotgun metagenomic data revealed the existence of discrete evolutionary lineages within previously defined species (as defined per 16S community profiling). These lineages are characterized by a remarkably high level of divergence and functional gene content variation suggesting diversification by adaptation to different metabolic niches.

Tuesday 18 September - 08h30-09h30

Theme: GENETICS

The research of Dr. **KAREN KAPHEIM** at Utah State University, USA, addresses the evolutionary processes responsible for the diversity and plasticity of social behavior in bees. She combines comparative genomics with behavioral and physiological ecology to investigate the developmental, social, and genomic mechanisms underpinning social evolution. By bridging these mechanisms with individual fitness consequences, Kapheim seeks to understand the ways in which genomic architecture influences social behavior, and in turn, how social
evolution shapes the genome.

KN2 Life outside the hive: what comparative genomics reveal about bee behavior, physiology, and evolution

Some of the most fascinating examples of socially-mediated behavior and physiology can be found inside a honey bee hive, but how this sensitivity to the social environment evolves is unknown. My collaborators and I use the comparative method to identify mechanisms that regulate behavioral and physiological plasticity in bee species from across the social spectrum. We focus primarily on a facultatively eusocial bee (*Megalopta genalis*) and the closely related solitary alkali bee (*Nomia melanderi*). Here I will describe the results of experiments designed to understand endocrine, sensory, and social influences on behavior, reproductive physiology, and brain gene expression profiles in each of these species. We find that solitary alkali bees are regulated by many of the same endocrine and reproductive mechanisms as social bees. Unlike for facultatively eusocial bees, however, many of these regulatory pathways are not influenced by social cues. We used comparative genomics to investigate the mechanisms underlying these differences. We find that many of the genes involved in social plasticity in *M. genalis* are highly conserved among species, and have undergone adaptive regulatory shifts in expression. One potential source of these regulatory shifts are lineage-specific microRNAs that alter conserved gene networks. These results begin to provide insight into the evolutionary processes that have produced socially-mediated plasticity among eusocial species.

Tuesday 18 September - 17h15-18h15

Theme: NEUROBIOLOGY

ALISON MERCER is Professor of Zoology at the University of Otago in Dunedin, New Zealand. Her research focuses on cellular and molecular mechanisms that underpin learning and memory. For many years, she has used the honey bee, Apis mellifera, as a model to investigate how the brain enables animals to learn from experience. However, the arrival in New Zealand of the parasitic mite, Varroa destructor, drew her attention to the need also for a better understanding of stress reactivity in honey bees. This has led to the establishment of collaborative efforts exploring the impacts of acute and chronic stressors on honey bee brain function and behaviour.

KN3 Using associative learning to learn about brain health and bees

"EurBee has become the premier event for researchers studying different aspects of wild and managed bees, and how they respond to environmental changes to address problems with species conservation, pollination services, beekeeping management and colony losses" (de Graaf, 2018).

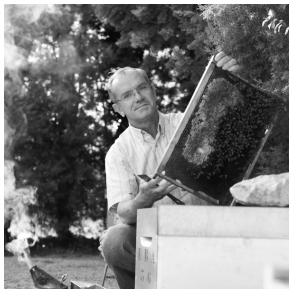
Where does neurobiology fit in to this picture?

Neurobiology seeks to understand the neural mechanisms that underpin behaviour, and how nervous systems respond to environmental challenges. Pathogens, parasites, pesticides and poor nutrition are all examples of environmental stressors that threaten bees. All can have significant impacts on neural function, and consequently also on bee behaviour. Dance communication, navigation to a food source, flower recognition, threat avoidance, the ability to find home, all of these behaviours and many more, rely on learning and memory. This talk will discuss how bees learn from experience, what bee biology can tell us about learning mechanisms, and how learning behaviour can be used to evaluate the impact of environmental stressors on brain function and behaviour.
Because honey bees learn rapidly, and display robust memories, they have served for decades as a model for exploring cellular and molecular mechanisms that support learning and memory formation. These studies suggest brain health is as important to honey bees as it is to humans. However, learning and memory recall are critical to the success and survival of all bee species, not only honey bees. Given the economic significance of bees as a group, stronger linkages between neurobiologists and others in the bee research community would seem desirable. What needs to be done to facilitate fruitful dialogue between researchers with applied vs fundamental knowledge and expertise, and what questions could neurobiologists be addressing to help resolve problems associated, for example, with species conservation, pollination services and colony survival? Attempts will be made in this talk to address these important questions.

Grants from the Royal Society of New Zealand Marsden Fund supporting the work discussed are gratefully acknowledged.

Wednesday 19 September - 08h30-09h30

Theme: CHEMICAL ECOLOGY



Dr. **YVES LE CONTE** is Research Director at the I.N.R.A. (Institut National de la Recherche Agronomique, Avignon, France) in charge of programs dealing with behavioral, physiological, genetical aspects of the honey bee biology and pathology. Since 1983, his research focuses on the biology and chemical ecology of honey bee colonies. With his team and collaborators, they have discovered a few pheromones from the brood and the adult bees which are at the center of social regulations in the honey bee colony. Those are primer and releaser pheromones. The primer effect had been studied at the molecular and physiological level. With regard to the varroa mite, his team is also very much involved in research dealing with the host parasite relationships and also applied research to control the mite. Since the recent honey bee losses in Europe, his team studies the effects of different pathogens and parasites on bee health and focus on the interactions with pesticides to understand honey bee decline from the molecular and socio-genomic level to colony level.

KN4 State of the art in honeybee chemical ecology

Chemical communication is one of the most fascinating aspects of social insect biology as they use primer and releaser pheromones both strongly involved in development and social regulations of the colony. When many releaser pheromones were discovered in the animal kingdom, only a few primer pheromones, modulating the physiology of the recipient, have been identified, most of them in the honeybee *Apis mellifera*. The honeybee is probably one of the most extensively studied models in chemical ecology. Recent studies on honeybee pheromones suggest that chemical communication is much richer than we thought and deeply involved in social regulations. More than 50 chemical compounds have been identified having pheromonal effects on the honeybee. I will present a review of findings on releaser pheromones produced by the colony giving a special emphasis on the different primer pheromones and their interactions on social regulations between the different actors of the colony.

The same pheromonal compound can be produced by different actors of the colony and triggers both releaser and primer effects. Honeybee pheromone signals can be described as complexity, synergy, and context dependency in which they are deployed, mediated through both temporal and spatial distribution.

The importance of chemical communication will be describe in the framework of honey bee losses worldwide as stresses related to honeybee losses can act on chemical communication processes, modulating production or reception of the pheromonal compounds. Moreover, there are examples of the same chemical compound being used by both the host (honeybee) and the parasite (varroa).

Finally, the major challenges for future research in the field of chemical communication in the honeybee will be presented for discussion.

Wednesday 19 September - 14h00-15h00

Theme: INNATE IMMUNITY

Professor **DAN HULTMARK** of the Department of Molecular Biology at Umeå University in Sweden, is studying the immune response in insects, using Drosophila as a model. He was involved in the discovery of the first antimicrobial peptides, the cecropins, in the Cecropia moth, and later he studied the induction mechanisms of the humoral immune response in Drosophila. His present research is focused on the fly's cellular immune response; investigating how blood cells respond to parasite infection and studying the genes that are involved in this response. Of special interest are the interactions between different tissues in infected animals, redirecting nutrient resources towards the needs of the immune cells.

KN5

Nutrition and immunity – a systemic response to infection in the insect model, *Drosophila melanogaster*

We have discovered a systemic signaling network that controls blood cell activation and proliferation in parasitized *Drosophila* larvae. The blood cells interact with the fat body, neurosecretory cells in the brain and, more surprisingly, the skeletal musculature. These interactions result in a systemic response that redirects the flow of nutrients, presumably according to the demands of the immune cells. This response is an integrated part of the immune defense and is vital for a successful elimination of the parasite.

Thursday 20 September - 08h30-09h30

Theme: EVOLUTIONARY BIOLOGY

TOM WENSELEERS is a professor at KU Leuven, Belgium, who combines theory with empirical research to study the fundamental factors that drive cooperative social behaviour and other complex traits in nature. To this end he uses a combination of theoretical modelling approaches and empirical research on diverse organisms, including social insects (ants, bees and wasps), microorganisms and humans. He also uses digital evolution with swarms of simulated robots to gain insight into possible routes towards complex sociality. Furthermore, with the advent of next-gen omics techniques and modern high-throughput mass-spectrometry, his lab is also committed to obtain a better mechanistic understanding of social traits in diverse organisms, including their (epi)genetic and genomic basis, as well as in unravelling the advanced chemical communication systems that are part of the complex sociality in insects."

KN6 Social bees as model systems in the study of the evolution of cooperation and conflict

Social behaviour is ubiquitous in nature, and lies at the heart of the so-called "major transitions in evolution", which resulted in the progressive evolution of cells, organisms and animal societies. Throughout my career I have studied the question of how conflicts among lower-level units is suppressed in the creation of integrated higher units of selection. For much of this work, I have used social bees, including honeybees, stingless bees and bumblebees, as model systems, and the insights obtained from them have yielded surprisingly similar conclusions. For example, my work on honeybees and stingless bees has shown that both in the context of preventing worker reproduction and in regulating caste development, various social control mechanisms appear to neutralize costly internal conflicts, thereby allowing group-beneficial majority interests to prevail. In addition, I have found that these social control mechanisms help to align the queen and the workers' evolutionary interests and that in this way they strongly stabilize queen-worker chemical signalling systems. Finally, in a recent upcoming study we suggest that collective social control mechanisms also play a role in suppressing intragenomic conflict within individual organisms. In particular, we showed that even though parent-of-origin specific gene expression (genomic imprinting) occurs and has the scope to cause intragenomic conflicts within individual worker insects, the "parliament of the genes" typically ensures that the majority interests of non-imprinted genes prevail. This conclusion followed from the fact that the imprinting states of genes in social insects appears to evolve extremely quickly, as there was virtually no overlap in which genes were found to be imprinted in the honeybee and which ones we found to be imprinted in bumblebees, presumably due to the successive invasion of imprinting modifiers. Altogether, these studies all point to collective social control mechanisms playing a key role in stabilizing major transitions in evolution.

Thursday 20 September - 17h30-18h30

Abstracts

Oral presentations

001 Insights into the diversity and dynamics of microbes in stingless bees

Nunes-Silva C.G.[1], Nacif-Marcal L.[1], Fialho M.C.Q.[2], D'Alvise P.[3], Hasselmann M.[3]
[1] Department of Genetics, [2] Department of Morphology, Federal University of Amazonas, Manaus, Brazil; [3] University of Hohenheim, Institute of Animal Science, Department of Livestock Population Genomics, Stuttgart, Germany

Social bees harbor distinct microbial communities in their intestines, usually consisting of few symbiotic resident species that constitute the bulk of the community and a small proportion of diverse transient microbes acquired from the environment. There is growing evidence that the microbiota of social bee species has co-evolved with the respective hosts, therefore every species-specific resident microbiota constitutes an individually evolved answer to the similar symbiotic host demands, such as degradation of recalcitrant food components, production of essential nutrients, and antagonism against pathogens and transient microbes. The honey bee *Apis mellifera* is established as model system for basic microbiota research. In this context, the >300 stingless bee species represent an underexplored reservoir of phylogenetically distant but functionally similar microbial communities. Since the food spectra (mostly nectar and pollen) are very similar, the metabolic and inhibitory properties of the symbiotic microorganisms can be directly compared and may thereby offer new insights into underlying mechanisms of microbial symbiosis. In this study we explore the intestinal bacterial communities of two sympatric, but distantly related species of Amazonian stingless bees, *Melipona seminigra* and *Duckeola ghilliani*. We study the microbial communities in the different gut compartments, as well as in fresh and stored pollen and in the derived larval food by 16S rRNA and metatranscriptome analysis. Thereby we obtain for the first time an overview of the microbiota and the microbial succession from pollen to larval food in these stingless bees, providing novel insights into the diversity and dynamics of their microbial community. In addition, our study will contribute to a better understanding of the interaction of social insects with their environment.

002 Exploring the microbiome's role in the biological invasion of the African honey bee genotype

Kowallik V.[1], Suenaga M.[1], Rangel J.[2], Mikheyev A.S.[1]
[1] Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan; [2] Agriculture and Life Sciences Texas A&M University, Texas, US

The host-associated microbiota can provide its host with flexibility beyond that encoded in host genomes, as rapid changes in the microbiome can directly affect important host life history traits and therefore, may enable rapid acclimation to new environments and resistance to environmental disturbances. One of the most rapid and successful biological invasion known is the spread of African honey bees throughout the New World. In parallel, asymmetrical hybridization with pre-existent European honey bee strains occurred which has been described as Africanization. This process can be viewed as a giant natural experiment to explore the consequences of changes in host genetic backgrounds on the associated microbiome with a potential link to host fitness. As Africanized hybrids show higher resistance towards the same stress factors than the European-like colonies, it would be highly interesting to identify if microbiome differences exist which could play a key role. We take advantage of a historical dataset consisting of more than 1000 honey bees sampled over a decade along the Mexican-American eastern coast. This unique sample set will allow to generate a detailed picture of the internal bee microbiome before, during and after the hybridization event using total RNA sequencing. This method will make it possible to determine changes in the microbial community composition, including bacteria, fungi and viruses, as well as to get deeper insights into strain-level variation of symbionts and their active roles. While pathogens and toxins are main stressors on honey bee health, we will also screen the samples for the presence of pesticides and other types of toxins as well as search for pathogen information in our sequence output. We hope to explore the complex interplay between biotic and abiotic factors that affect honey bee health, and how changes in the genetic background in the hosts affect microbial and viral communities.

003 Consequences of land-use for solitary bee microbiota composition and function

Peters B.[1], Leonhardt S.D.[1], Keller A.[2], Schloter M.[3]
[1] Department of Animal Ecology and Tropical Biology, [2] Department of Bioinformatics, Biocenter, Würzburg, Germany; [3] Helmholtz Center Munich, School of Life Sciences, Technical University of Munich, Munich, Germany

When examining effects of intensively managed agricultural landscapes on pollinators, one important player has been mostly ignored: microorganisms.
Floral resources, like pollen and nectar, but also nesting materials, are strongly influenced by land-use. They further define the microbiota of solitary bee adults, larvae and nests, which are supposed to play a significant role in bee host health and thus fitness. We examined whether bees harbor a core microbiome, which fulfills mutualistic functions and should be consistent within a bee species across the entire land-use gradient. We further investigated variation and specialization in the non-core microbiota and how they were affected by floral / pollen resources.
To investigate the structure of mutualistic plant-bee and bee-microbe associations we used DNA- metabarcoding of bacteria and pollen. We further performed chemical analyses of floral resources, and we conducted bacterial growth bioassays with supplemented plant compounds to explore functional associations between plant biochemistry and larval microbiomes. We present our preliminary results on the impact of land-use on solitary bee microbiota, their potential functions in floral resource use as well as the spectra of flower resources visited for pollen collection.

004 Impact of medicaments and feed additives on the honeybee gut microbiota

Alberoni D.[1], Baffoni L.[1], Gaggìa F.[1], Stanton C.[2], Ross P.[2], Di Gioia D.[1]
[1] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy; [2] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

Honeybees are exposed to diseases whose virulence and spread are often enhanced by beekeeping management practices. Overcrowded apiaries, nomadism and international honeybees' trade are some of the anthropic factors contributing to honeybees weakening. In the past, antibiotics were widely used to counteract diseases and to promote colony growth and productivity. However, in 2001 the use of antibiotics in Europe was substantially prohibited by revoking commercial licenses for therapeutic use in beekeeping, while in other extra EU countries they are still widely used. Lately beekeepers demand of alternative medicaments fulfilling organic farming directives has stimulated both researchers and private companies in finding innovative solutions. A number of researches in feed additives have produced new commercial products capable to stimulate animals' immuno system or showing slight atimicrobial activity. Several feed aditives have been proposed on the veterinary market, some based on natural oils (Thymol), other containing proteins, vitamins, or beneficial bacteria. Gut microbial community has an important role in nutrient intake and immuno defence in honeybees. The core microbiome is composed by 9 bacterial genus potentially perturbable by medicament or feed additives. In our study we aimed to unveil the impact of medicaments (Tylosin, Tetracycline and Sulfaquinoxaline) and feed additives (Thymol and a beneficial bacteria mixture). Metagenomics approaches (16S rRNA sequencing and whole genome shotgun sequencing) where used to investigate changes in the gut microbial community. The results show an unexpected stability of the gut microbial community with a non-significant shift of the core microbiome in most experimental theses. Only the antibiotic Tylosin led to a microbial population shift, with eradication of bifidobacteria core species together with a strong reduction of Lactobacilli and niches replacement with bacterial genus that are usually in a lower abundance.

005 Pollen diversity and the microbiome; implications for honey bee health

Ferrari R.[1], Browne K.A.[1], Voulgari-Kokota A.[3], NIHBS[4], Keller A.[3], McCormack G.P.[1]
[1] Department of Zoology, National University of Ireland, Galway, Ireland; [3] Department of Bioinformatics, Center for Computational and Theoretical Biology, University of Würzburg, Würzburg, Germnay; [4] Native Irish Honey Bee Society (NIHBS)

Pollen collected by honeybees in Ireland varies, as expected, throughout the course of the year with its benefits to colony health including the contribution of intestinal and other biochemicals, amino acids and microorganisms, however there is no detailed information on pollen diversity or its relationship to gut flora & colony health. In honeybees the gut microbiota is remarkably stable and relatively species poor with the same major bacterial phylotypes reported from different studies/localities. However, the specific strains of the major bacterial phyla present can vary from location to location and little is yet known about the extent of this variability with no information available at all from the pure *Apis mellifera mellifera* (*Amm*) population in Ireland.
As bacterial symbionts in arthropods are credited variously with resistance to viruses, limiting plasmodial infection and affecting reproductive fitness this is a worthy area of investigation for honey bee health. The microbiome of honeybees is important in nutrition via contributions of intestinal enzymes, conversion and preservation of pollen, and also in disease resistance. *Bacillus* spp, from the bee intestinal microflora inhibit chalkbrood while gut lactic acid bacteria inhibit *Paenibacilus larvae*, the causative agent of American foulbrood.
There is very little data on pollen usage of pure *Amm* in Ireland and no data at all on the gut microflora of Irish bees. Any information on fitness of the pure *Amm* population in Ireland is important on a much wider scale given that this subspecies is almost extinct in the rest of Europe. Here we set out to provide a baseline of information in this regard. We are employing next generation sequencing data to investigate pollen and gut microbiome diversity in eight *Amm* apiaries in the South East (6) and the West (2) of Ireland over the course of one year. We will present our analyses on the associations between pollen and bacterial diversity with respect to the health and fitness of the colonies.

006 Immune stimulation by the gut symbiont *Frischella perrara* in the honey bee (*Apis mellifera*)

Emery O., Schmidt K., Engel P.
Department of Fundamental Microbiology, University of Lausanne, Switzerland

Honey bees possess a simple yet specific gut microbiota mostly composed of only eight species that are horizontally acquired, providing a promising model to study fundamental mechanisms in host-symbiont interactions which could also unravel new perspectives relevant to bee health. Microbiota-free bees can be generated and subsequently colonized with selected gut microbiota members cultured in vitro, thereby allowing to study the host response to defined gut microbiota communities.
Frischella perrara is a gammaproteobacterial gut symbiont present in most bees specifically causing the so-called "scab" phenotype that corresponds to a dark band restricted to the pylorus region. The scab is hypothesized to result from melanization, a well-known immune response of insects to pathogens or wounding. Using cage experiments and RNAseq we show that monocolonization with *F. perrara* leads to a higher number of differentially expressed host genes in the pylorus in comparison to monocolonization with *Snodgrassella alvi*, another symbiont not causing the phenotype. Interestingly, the most highly upregulated genes by *F. perrara* correspond to immune responsive genes and we detected upregulation of genes belonging to the melanization cascade.
Our transcriptome analysis provides first insights of this particular host-symbiont interaction, providing evidence that the scab phenotype indeed corresponds to melanization and that *F. perrara* leads to a potent host immune response and possibly changes in gut homeostasis. We postulate that this response may keep the number of *F. perrara* bacteria in check but also change gut homeostasis thereby possibly preventing colonization by pathogens.

007 Lactic acid bacteria of the honey bee crop enhance adult longevity but do not provide specific defence against a microsporidian and a viral pathogen

Disayathanoowat T.[1], Vásquez A.[2], Olofsson T.[2], Natsopoulou M.[3], Doublet V.[4], McMahon, D.[5], Paxton R.J.[6]
[1] Department of Biology, Chiang Mai University, Chiang Mai, Thailand; [2] Department of Laboratory Medicine, Medical Microbiology, Lund University, Lund, Sweden; [3] Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark; [4] Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom; [5] Institute of Biology, Free University Berlin, Berlin, Germany; [6] Institute for Biology, Martin Luther University Halle-Wittenberg, Halle, Germany

Animal gut microbiomes, including those of the honey bee (*Apis mellifera*), comprise a rich diversity of symbiotic bacteria that are thought to confer health benefits on their hosts. Adult honey bees additionally support a specialised community of lactic acid bacteria (LAB) in their crops that, when fed to larvae, provide defence against larval pathogens. Taking advantage of the fact that freshly eclosed honey bees are aposymbiotic (devoid of a symbiotic gut microbiome), we experimentally tested whether LAB enhanced adult longevity and protected them from two widespread gut pathogens: *Nosema ceranae* and Deformed wing virus. We found clear support for the role of LAB in enhancing adult longevity, independent of pathogen challenge. Pathogens were pathogenic even for LAB-fed honey bees, reducing host survival. Also, feeding LAB did not reduce pathogen loads but rather increased them, suggesting that LAB do not provide specific defence against adult diseases. In the light of elevated rates of honey bee colony mortality, attention should nevertheless be paid to management practices that sustain the life-enhancing microbiota of the honey bee's alimentary canal.

008 Characterisation of the honey bee *Apis mellifera* metagenome in Britain

Regan T.[1], Barnett M.W.[1], Laetsch D.R.[2], Wragg D. [1], Bush S.J. [1], The BeeBiome Consortium, Blaxter M.[2], Freeman T.C.[1]
[1] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Edinburgh, UK; [2] The Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

Honey bee health is a complex product of host genetics, the microbes with which they are associated (commensal, opportunistic and pathogenic), and their environment. Improved understanding of the genetics of bee populations and their microbiome has the potential to help manage modern challenges to bee health and production. We have sequenced and analyzed the metagenomes of 19 colonies across Britain. Microbiome composition appeared to be more driven by geography and forage rather than host genetics. Apiaries had a high level of diversity in the composition and relative abundance of individual microbiome taxa. While most non-bee sequences derived from known honey bee commensal bacteria, we also detected DNA from plants (food sources), and numerous additional bacterial, protozoan and metazoan organisms, as well as known pathogens such as *Varroa* (Arthropoda), *Nosema* (Microsporidia), *Lotmaria passim* (Trypanasomatida), and potentially pathogenic cobionts, such as an Apicomplexan. These infections were confirmed using custom PCR assays. Colonies which suffered from *Nosema* or *Lotmaria passim* infections tended to exhibit extreme dysbiosis, with few reads of commensal origin such as Firm-4 and Firm-5 relative to healthy colonies. Sets of co-occurring taxa which may reflect metastable communities were identified, containing core commensal species. Using a novel network analysis approach, we have used data from the BeeBiome to classify data derived from previously uncharacterised organisms through clustering orphan contigs. Our analysis demonstrates the power of high-throughput, directed metagenomics, and identifies potential additional threats to honey bees present in their microbiota.

009 Using contact networks and next-gen sequencing to reveal the community dynamics of the pollinator virome

Doublet V.[1,2], Doyle T.[2], Brown M.J.F.[3], Wilfert L.[2]
[1] Institute of Evolutionary Biology, University of Edinburgh, UK; [2] College of Life and Environmental Sciences, University of Exeter, Penryn Campus, UK; [3] School of Biological Sciences, Royal Holloway University of London, Egham, UK

Horizontal transmission of infectious diseases is strongly determined by the contact network between hosts. Environmental and ecological variables that define the probability of contacts between individuals have been largely neglected in models studying the dynamics and epidemiology of wildlife diseases. Here, we use pollinators to study the role of contact networks in a real-life multi-host pathogen community. In agricultural landscapes, wild flowers are the most important food resources of insect pollinators such as bees and flies. To boost yields of agricultural crops and ensure pollinator conservation, the UK Environmental Stewardship scheme prompted farmers to grow pollinator-friendly wild flower margins along their fields. We constructed high-resolution plant-insect visitor networks from flower visitation data collected in ten farms in Southern England (five farms participating in the scheme vs. five control farms), and sampled the most abundant pollinators to characterize their virome by deep transcriptome sequencing. We found that agri-environmental scheme and wild flower margins increase bee density and clusterize plant-pollinator networks, generating a so called 'small-world' network. We combined environmental data and sequencing to reveal the impact of the agri-environmental scheme and test if virus transmission is likely to be host density dependent. Ultimately, we want to know if virus transmission is enhanced in farms enriched with wild flowers. Overall, we aim to identify environmental (flower density, agricultural practices) and ecological factors (plant taxa, insect community assemblage) that significantly enhance the transmission of plant and pollinator viral diseases within our model to eventually improve agricultural practises and wildlife management.

010 Field testing and selection on European honey bee populations (Smartbees project 2015-2018)

Büchler R.[1], Uzunov A.[1,2], Hoppe A.[3], Bienefeld K.[3]
[1]Landesbetrieb Landwirtschaft Hessen, Bee Institute, Kirchhain, Germany; [2] University Ss. Cyril and Methodius in Skopje, Faculty of Agricultural Sciences and Food, Republic of Macedonia; [3] Institute for Bee Research Hohen Neuendorf, Germany

One central goal of the EU funded SMARTBEES project was to initiate breeding activities for the genetic improvement of different European *Apis mellifera* populations. Selective breeding, by performance testing combined with controlled queen rearing and mating and genetic evaluation are key elements towards the preservation of local populations, some of which are highly endangered. Only the availability of attractive (healthy, productive and gentle) local stock will discourage beekeepers from using queens and colonies of non-local origin.
Since 2015, we implemented an intensive extension program (field training, seminars, smartphone app development etc.) in more than 20 countries for enhancement of beekeepers' understanding of systematic breeding concerning important economic traits, such as colony development, swarming, gentleness, honey production and, in particular, resistance to Varroa destructor. Thus, a network of testing apiaries was established and significantly expanded, including all 9 European honey bee subspecies. Performance tests have been completed in 15 countries, and BLUP breeding values have been estimated (www.beebreed.eu) for the testing years 2016 and 2017. Till now, we have published (www.testbees.eu) breeding values for 1236 queens, which were already used to select queens for the next generation. In addition, colonies from the second generation are under intensive testing.
Furthermore, intensive testing activities were performed for studying regional *Varroa* threshold values. To this end, colonies (n=130) from Germany, Poland, Moldova, Croatia and Romania were systematically monitored for natural mite fall, bee and brood infestation from 2015 to 2017, and threshold values estimated for each of the regions and populations. Meanwhile, several breeding groups took on the responsibility for expanding the network on a regional and local scale and we encourage them to cooperate towards the establishment of a Europe-wide honey bee breeding network.

011 Mite adaptations in European *Apis mellifera* populations surviving *Varroa destructor* by means of natural selection?

Moro A.[1], Le Conte Y.[2], Neumann P.[1], de Miranda J.R.[3], Locke-Grandér B.[3], Dahle B.[4,5], Blacquière T.[6], Beaurepaire A.[2]
[1] Institute of Bee Health, University of Bern, Bern, Switzerland; [2] INRA, Unité 406 - Abeilles et Environnement, Avignon, France; [3] Swedish University of Agricultural Sciences, Uppsala, Sweden; [4] Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway; [5] Norwegian Beekeepers Association, Kløfta, Norway; [6] Wageningen University & Research, Wageningen, Netherlands

The ectoparasitic mite *Varroa destructor* is a key factor driving colony losses of Western honey bees, *Apis mellifera*. However, some populations of European *A. mellifera* subspecies have survived by means of natural selection >10 years without mite treatments. This could result from adaptations for host resistance and/or adaptations of the parasite. Given that mites have adapted one would expect genetic differentiation between mites from local surviving and susceptible host colonies. Here, we estimated possible genetic differentiation among mites from four surviving and local susceptible populations (Norway, Sweden, France and the Netherlands) using 9 polymorphic DNA microsatellite markers (N=1270 mites from 44 colonies). Significant but low levels of genetic differentiation between mites infesting surviving and susceptible colonies were detected in the Netherlands, France and Norway. In addition, the comparison of mites from the four countries revealed genetic differences reflecting the geographical location of the apiaries. Our results suggest that colonies from these surviving populations do not survive because of parasite adaptations. However, the levels of genetic differentiation among the four locations indicate that *V. destructor* is more diversified than previously recognized in Europe. This finding suggests that surviving honey bee populations may be locally adapted to their sympatric mite populations. Altogether, this work is of strong relevance for the utilization of surviving honey bee colonies in their non-natural environment.

012 Investigations into unmanaged honey bee colonies in Ireland

Browne K.A.[1], Henriques D.[2,3], Hassett J.[4], Geary M.[4], Moore E.[4], Pinto M.A.[2], McCormack G.P.[1]
[1] Department of zoology, National University of Ireland, Galway, Ireland; [2] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal; [3] Centre of Molecular and Environmental Biology (CBMA), University of Minho, Braga, Portugal; [4] Department of Applied Science, Limerick Institute of Technology, Limerick, Ireland

Unmanaged honey bee colonies of local ecotype surviving without human intervention are likely to form a valuable genetic resource for the sustainability of managed apiaries as well conservation of threatened subspecies. In Ireland, following the Isle of Wight disease (which devastated honey bee colonies at the beginning of the 20th century) and subsequent hybridisation with C lineage bees, there has been a general acceptance by government agencies, scientists, and many beekeepers that no *Apis mellifera mellifera* (*Amm*) colonies persisted in the wild.
However, sporadic reports were received in 2014/2015 of the existence of unmanaged honey bee colonies. Given that Ireland's human population is low in density with only 32 persons per square km in some rural areas and only approximately 3000 registered beekeepers, many of whom are reported to not favour purchasing imported bees, it is feasible that honeybees could have naturally adapted to introduced pathogens such as *Varroa destructor*. We initiated an investigation into the state of unmanaged honey bee colonies and in 2016 we launched a nationwide request through press and social media seeking locations of unmanaged colonies which realised over 170 replies in a short time period.
We found that unmanaged colonies have utilised a wide variety of both natural and artificial cavities and survived unaided for periods reported to be from three to over 20 years. Given the difficulty in confirming the authenticity of these timings the survival of individual colonies has been monitored since 2016. Sixty-two of the colonies were sampled and a combined approach using mitochondrial, microsatellite and single nucleotide polymorphism (SNP) genotyping has shown the majority to be pure *Apis mellifera mellifera* and forming an integral part of the previously described pure *Amm* population in Ireland. This data, along with survival records for >2 years, and details of surrounding habitat and health of the unmanaged colonies, will be presented.

013 Black box selection leads to distinct traits of resistance to *Varroa destructor* in honeybees

Blacquière T.[1], Boot W.[2], Calis J.[2], Van Stratum P.[2]
[1] Wageningen University & Research, Wageningen, The Netherlands; [2] Inbuzz v.o.f. Beekeeping company, Laren, The Netherlands

Honeybee populations left uncontrolled for the ubiquitous parasitic mite *Varroa destructor* have shown to be able to survive for many years. Here we present three populations of honeybees in the Netherlands that have been intentionally left untreated for *Varroa* for 9-11 years now. Part of one of the initial populations has been treated twice per year to control *Varroa*, to serve as a reference population. Apart from *Varroa* control these colonies were managed alike the selection populations. The protocol provides in yearly splitting of all fit colonies into 4 nukes with own young queens each. Queens were within population mated on remote places.
After severe losses in early years the populations have become stable. Mite infestation of the colonies, measured twice per year is also stable and does not reach lethal rates (as it does in the reference colonies if left uncontrolled for *Varroa* mites).
In two of the three populations traits contributing to the resistance have been investigated: mite fertility in worker brood was reduced, as well as the fecundity of the mites. None of the populations had become hygienic (pin-killed brood test as well as freeze-killed brood test), nor had auto- and allo-grooming increased. One of the two selections had increased *Varroa* sensitive hygienic behaviour. The latter difference between selections may have been caused by genetics as well as interactions with (slightly) different environments. Black box selection with local bees may therefore be the road to resilient and well adapted bees. Applying this approach on a global scale on many locations may help us find the crucial adaptations allowing honeybees to survive *Varroa* destructor infestation.

014 The good, the bad and the ugly – dissecting *Apis mellifera* survivability to *Varroa destructor*

Beaurepaire A., Alaux C., Crauser D., Dalmon A., Diévart V., Mondet F., Pioz M., Le Conte Y.
Abeilles et Environnement, INRA, Avignon, France

Finding sustainable solutions against *Varroa destructor* has been one of the greatest challenge for beekeeping in the past decades. A promising alternative to chemical treatments against the mite is to use *A. mellifera* populations that have naturally adapted and survived to the parasite. Understanding which mechanisms allow these populations to survive can help implementing future control strategies to mitigate the deleterious impact of *V. destructor*. We investigated a diverse set of resistance and tolerance mechanisms in parallel in two surviving French honeybee populations located in Vaucluse and Sarthe that have survived for over 15 years without treatment. To do so, we evaluated and compared the respective role of these mechanisms in our surviving colonies and compared them to control colonies located in the same apiaries. In particular, this presentation will focus on the interplay among bees ("the good"), *Varroa* ("the bad") and viruses ("the ugly") in our surviving populations. Our results shed light on the complex interactions among these organisms and help understanding how to optimize sustainable control strategies against *Varroa*.

015 Breeding of *Apis cerana*: resistance of Sacbrood virus

Choi Y-S., Vung N.N., Lee M-L., Lee M-Y., Kim H.K. ,Kim J.E.
Agricultural Biology, Rural Development Administration, National Agricultural Acadeamy Science, Wanju-gun, South Korea

South Korea has over 0.38 million of managed honey bee (*Apis cerana*) colonies before 2009 years ago, which produce the highest quantity of honey in the Korea; however, almost colony (90%) were collapsed by Korean Sacbrood Virus (KSBV) in South Korea. Korean Sacbrood Virus (KSBV) is the pathogen of *A. cerana* Sacbrood disease, which poses a serious threat to honeybee *A. cerana*, and tends to cause bee colony and even the whole apiary collapse. Colony collapse of *A. cerana* was first reported on the Pyeong-Chang of the South Korea in 2009. Several scientists and governments has been tried research for cure the sacbrood disease in *A. cerana* colony by medicines and management techniques. Unfortunately, The sacbrood disease dosen`t improve. So, we were developed a better breed of *A. cerana* for resistance of sacbrood virus by selection and than artificial insemination. *A. cerana* breeding technique was first successful applied with *A. cerana* in Korean. Queens was grafted from sacbrood resistance line and than it were growing in sacbrood disease colony that was survived 100%. Altogether selected 18 queens were artificially inseminated and 2,000 drones *of A. cerana* in Korea was used to evaluate amount of semen collection. We are select two scabrood resistance *A. cerana* line (R and H). R line be used for rearing the Queen. Drone was reared in H line colony. The RH hybrid were not infected sacbrood virus even spread sacbrood virus (2*f*106~2018). RH colonies has very excellent hygienic behavior, brood, and sacbrood disease resistance activity.

016 Tolerance to Deformed wing virus at the individual and colony level in Swedish mite-resistant honey bees

Locke B., Thaduri S., Stephan J., de Miranda J.R.
Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

The ectoparasitic mite, *Varroa destructor*, is probably the most significant major threat world-wide to the health status of the European honey bee, *Apis mellifera*. However, it is important to distinguish that *Varroa*-induced colony mortality is most often caused by honey bee viruses that are effectively vectored by the mite, in particular Deformed wing virus (DWV). Uncontrolled mite populations will grow at an exponential rate, rapidly causing a DWV epidemic that results in colony mortality. Nevertheless, a few sub-populations of *A. mellifera* can survive for extened periods (over 15 years) without mite control. One well-studied population, on the island of Gotland, Sweden, has adapted mite-resistant traits supporting their long-term survival. In 2014 our research group published a study demonstrating colony-level tolerance to DWV in the Swedish mite-resistant bees. Despite having equally high DWV infection levels, the mite-resistant colonies survived winter while mite-susceptible colonies all died. To examine DWV tolerance at the individual level we infected adult bees in cage experiments and laboratory reared larvae with both DWV and Acute bee paralysis virus (ABPV) and compared the infection dynamics as well as the mortality rate between mite-resistant and mite-susceptible individuals. While infection dynamics were nearly equal in both adult bees and larvae between the two groups, the mite-susceptible adults had significantly higher mortality. These results imply that tolerance defence mechanisms are an important component of disease interactions in the Gotland mite resistant population supporting their long-term survival.

017 The Lord of the Rings: genotype-environment interactions for honey bee colonies surviving *Varroa destructor* by means of natural selection

Neumann P.[1], Oddie M.A.Y.[1], Beaurepaire A.[1], Blacquière T.[1], Conlon B.H.[1], Crauser D.[1], Dahle B.[1], Frey E.[1], de Graaf D.C.[2], Häußermann C.[1], de Smet L.[2]
[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium

The ectoparasitic mite, *Varroa destructor*, is the main threat to colony survival for European honeybee, *Apis mellifera*, sub-species. However, European *A. mellifera* populations can clearly survive >10 years without mite treatments by means of natural selection. Since the surviving colony phenotype inevitably results from both bee genetics and environment, it appears crucial to test whether the bees are also able to survive without mite treatment in a new environment. Given that the bees are able to survive, strong heritable traits are at the basis of colony survival and future breeding should use them. If, however, the relocated colonies do not survive, then local bees should always be used by beekeepers for breeding programs. Here, we conducted a large-scale Ring Test over a two-year-period involving eight institutes. At each institute, colonies were established using mated queens from naturally surviving populations (Ås/Kløfta (Norway), Avignon (France), Uppsala (Sweden), Lelystad / Tiengemeten (Netherlands)) and from respective local susceptible controls. Then, colonies were maintained without mite treatments. Data on colony development and mortality will be presented.

018 BeeStrong: towards a genomic tool for the selection of *Varroa* resistant honey bees

Sann C.[1], Poquet Y.[1], Basso B.[2], Mondet F.[3], Eynard S.[4], Servin B.[4], Phocas F.[5], Guillaume F.[6], Bidanel J.P.[5], Cluzeau-Moulay S.[2]
[1] Labogena, Jouy-en-Josas, France; [2] ITSAP, Avignon, France; [3] INRA PACA, Abeilles et Environnement, Avignon, France; [4] INRA, GenPhySe, Toulouse, France; [5] INRA, Department of Animal Genetics, Jouy-en-Josas, France; [6] Evolution, Noyal sur Vilaine, France

The mite *Varroa destructor* is known for causing devastating colony losses in the western honey bee *Apis mellifera* populations. So far, efforts to fight *Varroa* have been thwarted by the parasite's ability to adapt quickly and develop resistances. Yet, alternative sustainable solutions can be implemented by selecting and breeding naturally *Varroa* resistant honey bees. However, the large scale development of such lines is currently restricted by the difficulty to evaluate the ability of colonies to resist to the mite.
The BeeStrong project aims at developing a diagnosis tool for *Varroa* resistance that will simplify selection for beekeepers and research facilities.
To achieve this goal, phenotypic data have been collected for over 1500 colonies between 2016 and 2018 mainly in France but also in Switzerland, USA, New Zealand, Luxembourg, Sweden and the Netherlands. Phenotypic data consisting of colony performance (ColEval method), phoretic Varroa infestation and suppressed mite reproduction (SMR) trait have been measured. In a second phase of the project, whole genomes from these colonies will be sequenced and pool and genome wide association studies will be performed to detect genetic markers associated with the resistance trait.
In addition to bringing new understanding on the genetic basis of *Varroa* resistance in *A. mellifera*, the information on these markers will be used to develop a genotyping service to evaluate varroa resistance in honey bee colonies that can be used by anyone in an easy and affordable way.

019 EU-wide restrictions on neonicotinoids have not entirely eliminated the risk for honeybees foraging on oilseed rape nectar

Wintermantel D.[1,2], Odoux J-F.[1], Decourtye A. [3], Henry M. , Allier F.[3], Bretagnolle V.[2,5]
[1] INRA UE Entomologie, Surgères, France; [2] Centre d'Etudes Biologiques de Chizé, UMR 7372, CNRS & Université de La Rochelle, Villiers-en-Bois, France; [3] ITSAP–Institut de l'Abeille, Site Agroparc, Avignon, France; [4] INRA, UR406 Abeilles et Environnement, Avignon, France; [5] LTSER Zone Atelier Plaine & Val de Sèvre, CNRS, Villiers-en-Bois, France

Chronic exposure of bees to sublethal dosages of neonicotinoids in pollen and nectar has been implicated in recent declines of bees, which led the European Commission to ban the use of three neonicotinoids in bee-attractive crops. Nonetheless, several studies detected substantial levels of the neonicotinoid imidacloprid in oilseed rape, despite being only permitted for use on winter cereals and sugar beets. Neonicotinoids are highly persistent and relatively water-soluble. These properties ensure systemic protection of treated crops, but also facilitate transport by water and wind as well as the uptake by succeeding crops. It remains, however, elusive what conditions favor carryover from treated crops to insect-pollinated plants. Further studies assessing neonicotinoid levels in mass-flowering crops and their potential effects on bees are needed to inform the debate on a ban of all neonicotinoids and the pending decision on the approval of the currently restricted neonicotinoids, imidacloprid, clothianidin and thiamethoxam. Therefore, we repeatedly quantified neonicotinoid residues in nectar of oilseed rape flowers from 264 fields within a Long-Term Socio-Ecological Research (LTSER) site with documented land use. We detected four out of the five neonicotinoids that are approved for plant protection in the EU. Imidacloprid and thiacloprid were present in all four years of the study. Particularly imidacloprid varied widely in concentrations within and among years and showed large inter-annual differences in prevalence with about 5% of fields being positive in 2015 and over 90% in 2016. We found, however, little relation between neonicotinoid contamination and environmental factors, such as weather conditions, soil type or the cultivation of winter cereals in previous years, suggesting a diffuse contamination of the environment. Based on literature values on the acute and chronic toxicity of neonicotinoids as well as the foraging behavior of nectar foragers, we estimated the mortality risk of honeybees foraging predominantly on oilseed rape nectar and found a considerable threat for foragers at several of the sampled fields. We conclude that the EU moratorium has not entirely eliminated the risk for bees foraging on oilseed rape and that there is an urgent need to better understand the pathways of neonicotinoid spread in the environment.

020 Time-to-death approach to reveal chronic and cumulative toxicity of a fungicide for honeybees not revealed with the standard ten-day test

Simon-Delso N.[1], San Martin G.[2], Bruneau E.[1], Hautier L.[2]
[1] Beekeeping Research and Information Centre (CARI), Louvain la Neuve, Belgium; [2] Walloon Agricultural Research Centre, Life Sciences Department, Plant Protection and Ecotoxicology Unit, Gembloux, Belgium

Synthetic fungicides are pesticides widely used in agriculture to control phytopathogenic fungi. The systemicity, persistency and intense application of some of these fungicides, such as boscalid, leads to long periods (even months) of exposure for honeybees via contaminated water, pollen and nectar. Our aim was to test the impact of such a long-term exposure on the survival of bees by using a time-to-death approach.
We exposed adult honeybees in the lab to food contaminated with boscalid at field application rates levels for 33 days instead of the standard 10-day test. Given the low toxicity of boscalid in acute terms, most of the toxic effects were observed after 10 days. The median time to death (LT50) ranged from 24.9 days (lowest concentration) to 7.1 days (highest concentration) and was significantly shorter in all cases than with the control (32.0 days). The concentration and dietary doses of boscalid inducing 50% mortality (LC50and LDD50, respectively) decreased strongly with the time of exposure: LC50 = 14,729 and 1,174 mg/l and LDD50 = 0.318 and 0.0301 mg bee-1 day-1 at days 8 and 25, respectively. We found evidence of reinforced toxicity when exposure is prolonged, but with an unusual pattern: no cumulative toxicity is observed until 17-18 days, when a point of inflexion appears that suggests a reduced capacity of bees to deal with the toxicant.
Our results show the importance of time-to-death experiments rather than fixed-duration studies for evaluating chronic toxicity of pesticides.

021 Effects of neonicotinoids on honey bee food glands

Bruckner S.[1], Straub L.[2], Villamar-Bouza L.[3], Neumann P.[2], Williams G.[1]
[1] Department of Entomology and Plant Pathology, Auburn University, Auburn, USA; [2] Institute of Bee Health, University of Bern, Bern, Switzerland; [3] European Food Safety Authority, Parma, Italy

The hypopharyngeal glands (HPGs) of adult nursing honey bees, *Apis mellifera*, are responsible for producing food for developing individuals within the colony. Previous studies have demonstrated that neonicotinoid insecticides can affect HPG development. However, effects of timing of exposure are not known. Based on previous literature, we expected that field realistic concentrations of neonicotinoids would negatively affect HPGs, especially in individuals both developing (i.e. as brood) and residing (i.e. as young adults) under neonicotinoid exposure. To explore this, we employed a cross-foster experimental approach. Workers were obtained from 14 colonies previously fed a pollen paste for 49 days. Half the colonies received paste that contained 4.9 ppb and 2.1 ppb of the neonicotinoids thiamethoxam and clothianidin, respectively (Neonicotinoid); the other half received paste containing no neonicotinoids (Control). At emergence, workers from each colony were either transferred to another colony within the same treatment (Control to Control or Neonicotinoid to Neonicotinoid), or to another colony of the opposite treatment (Control to Neonicotinoid or Neonicotinoid to Control). At the typical age of nursing, experimental workers were recaptured for HPG examination. We found that neonicotinoids negatively affected the size of HPGs. The smallest HPGs were observed in individuals both developing and residing under neonicotinoid exposure. These results suggest that HPG development may be an important marker for the sub-lethal effects of neonicotinoids. Given their importance to colony health, pesticide risk assessment schemes should consider quality of HPGs in the future.

022 The sublethal effects of clothianidin on the honey bee gut microbiota

El Khoury S.[1], Giovenazzo P.[1,2], Derome N.[1]
[1] Université Laval, Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Québec, Canada; [2] CRSAD, Centre de recherche en sciences animales, de Deschambault, Québec, Canada

Honey bees (*Apis mellifera*) are facing synergistically interacting stress factors affecting their health and productivity. Neonicotinoid, as Clothianidin, act as a neurotoxin targeting the nicotinic acetylcholine receptors in bees inducing adverse impact on immunity and behavior enhancing diverse pathogens. Moreover, in insects, functions associated with immune response and behavior are controlled by the intestinal flora (i.e. gut microbiota). Given that clothianidin is persistent in the environment and stable to hydrolysis, there is an urgent need to develop alternative strategies to mitigate its toxic effects on bee health. The first objective of our project was to identify the host microbiota functional interactions impacted by clothianidin exposure. In vivo studies were conducted to test the impact of clothianidin on bee survival, behavior, syrup consumption and homeostasis of the gut microbiota. Then, metatranscriptomic analyses (16S rRNA SSU and RNA-seq) were performed to identify which bee gut microbiota strains were impacted in terms of functional activity by sublethal doses of clothianidin, and assess the functions that were impaired. Three concentrations (0.1; 1 and 10 ppb) have been tested. Strikingly, the lowest concentration (0.1 ppb) exerted the most negative impact on bees, showing the highest mortality rate compared to 1 and 10 ppb experimental groups. Moreover, salient phenotypes changes were observed on bees in all clothianidin exposed groups, who became non-bright mat black with no abdominal hair on the day 10. No differences in the syrup consumption were observed in all the groups. The second objective of this project is to select endogenous probiotic candidates being able to grow in contact with clothianidin and degrate it. Preliminary in vitro results highlighted that bacterial strains are promising probiotic candidates to develop a probiotic formulation mitigating the negative impact of neonicotinoid exposure on honey bee colonies.

023 Co-exposure to virus and pesticide (Deformed wing virus / neonicotinoid) alters bee behavioural performances

Coulon M.[1,2], Dalmon A.[1], Prado A.[1], Di Prisco G.[3], Alaux C.[1], Crauser D.[1], Dubois E.[2], Thiéry R.[2], Ribière-Chabert M.[2], Le Conte Y.[1]
[1] INRA, Unité de recherche Abeilles et environnement, UR406, Site Agroparc, Avignon, France; [2] Anses, Laboratoire de Sophia Antipolis, Unité de pathologie de l'abeille, Sophia-Antipolis, France; [3] Laboratorio di Entomologia E. Tremblay, Università degli Studi di Napoli "Federico II", Portici, Italy

Most of the honeybee colonies (*Apis mellifera*) show asymptomatic virus infections. They can also be co-exposed to others stress factors, like pesticides. The combination of both is suspected to severely weaken honeybees, but little is known about how co-exposure to stress factors can alter bee survival and behavioural performances in natural conditions. We therefore investigated the impact of a virus/pesticide co-exposure on foraging behaviour, survival and viral infection in the colony by inoculating newly emerged bees with Deformed wing virus (DWV) and orally exposing them to field-relevant doses of a neonicotinoid (thiamethoxam). Bee flight activity was recorded with optical bee counters.
A precocious onset of foraging was observed in DWV-injected bees and was associated to a reduction in the vitellogenin expression level. DWV injection resulted in increased DWV loads and reduced bee survival. Combined exposure to DWV and thiamethoxam did not result in higher DWV loads compared to bees exposed to DWV alone, but induced precocious foraging after 5 to 10 days (compared to 12 days for mock-injected honeybees and 21 days for non-exposed honeybees). More, co-exposure increased the risk of not returning to the hive after the first exit, up to 65% for 1ng thiametoxam exposure, compared to 41% for mock-injected control. Hence co-exposure decreased survival when compared to thiamethoxam or DWV exposure. Finally, DWV-treated bees spent significantly more time outside the hive than control bees, and co-exposure to thiametoxam increased this phenomenon, as a synergistic effect.
This is the first evidence of deleterious interaction between DWV and thiamethoxam in natural conditions.

024 The effects of neonicotinoids on circadian rhythms in honey bees

Allain C., Sokolowski M.
Université de Picardie - Jules Verne, Amiens, France

We propose here new device and new automatized protocols to measure the consumption of caged honey bees. Contrary to the standard lab protocol using free access to food, in our conditioning chambers, honey bees receive only a small amount of syrup when they visit the feeder. When exposed to light/dark cycles, we observe cyclic feeding patterns highly correlated with light exposure.
We tested the chronic exposure to neonicotinoids at several sublethal concentrations. We show that such pesticides can highly disrupt the feeding patterns and we show also that the non lethal toxicity can be increased when food availability is reduced.
Because our protocol offer many advantages related to the standard laboratory cage, we suggest our device and protocols could be used systematically to test the lethal and non lethal effects of pesticides.

025 Chronic exposure to thiamethoxam can promote chronic bee paralysis virus infections in honey bees

Coulon M.[1], Schurr F.[1], Martel A-C.[1], Di Prisco G.[2], Dalmon A.[3], Alaux C.[3], Le Conte Y. [3], Thiéry R.[1], Dubois E.[1]
[1] ANSES, Sophia Antipolis Laboratory, Honey Bee Pathology Unit, Sophia Antipolis, France; [2] Laboratorio di Entomologia E. Tremblay, Università degli Studi di Napoli Federico II, Portici, Italy; [3] INRA, UR 406 Abeilles et Environnement, Avignon, France

Co-exposure to pesticides and viruses is likely to occur in honeybee colonies. Pesticides can be present in pollen, nectar, and persist in stored food (honey and bee-bread). Viruses can spread between honeybees by contact or trophallaxis, or may be vectorised by the mite Varroa destructor. However, the outcome of pesticide/virus co-exposure remains largely unexplored. We therefore studied the effect on honeybee health of chronic co-exposure to thiamethoxam (to daily doses of 0.25, 2.5 and 5.0 ng/bee) and to Chronic bee paralysis virus (CBPV). No synergistic effect of co-exposure was observed on bee survival, nor on the ability of bees to metabolize the neonicotinoid pesticide into clothianidin. However, we found that co-exposure caused an increase in CBPV loads to about 8 log10 copies per bee, viral levels which are usually found in overt infections. The effect of co-exposure on CBPV replication was associated with down-regulation of vitellogenin and dorsal-1a gene transcription. These results could explain CBPV-related mortality peaks in colonies exposed to both stress factors.

026 Sublethal exposure to the neonicotinoid Insecticide thiacloprid impairs the immune defence in the solitary bee species *Osmia bicornis* L.

Brandt A.[1], Hohnheiser B.[1], Sgolastra F.[2], Bosch J.[3], Meixner M.D.[1], Büchler R.[1]
[1] LLH Bee Institute, Kirchhain, Germany; [2] Dipartimento di Scienze Agrarie, Università di Bologna, Bologna, Italy; [3] CREAF, Bellaterra, Spain

Solitary bees are frequently exposed to neonicotinoid pesticides, which are discussed as one of the stress factors that may lead to population declines. A strong immune defence is vital for the fitness of bees, however, the immune system can be weakened by environmental factors that may render bees more vulnerable to parasites and pathogens.
Here we demonstrate for the first time that exposure to field-realistic concentrations of the neonicotinoid insecticide thiacloprid can severely affect the immunocompetence of male *Osmia bicornis* L.. In contrast to the females, where thiacloprid exposure (100 or 200 µg/kg sucrose solution ad libitum for 3 days) had no effect on all tested immune parameters, males exposed to 200 µg/kg thiacloprid showed a severe reduction in the total haemocyte number. Moreover, functional aspects of the immune defence namely the antimicrobial activity of the haemolymph were impaired in males. In contrast to honeybees, dietary exposure to thiacloprid did not affect melanisation/wound healing in male or female *O. bicornis*. Intriguingly, we observed that males consumed 1.77 times more thiacloprid spiked sugar solution than females, which might explain the immunosuppressive effects observed in males.
In conclusion, our results demonstrate that neonicotinoid insecticides can negatively affect the immunocompetence of *O. bicornis*, possibly leading to an impaired disease resistance capacity.

027 Effects of neonicotinoids on the behavior of foraging honey bees with artificial flower choices

Çakmak I.[1], Hranitz J.M.[2], Blatzheim L.[3], Bower C.D.[2], Polk T.[4], Levinson B.[5], Wells H.[6]
[1] Uludag University, Beekeeping Development-Application and Research Center, MKP Vocational School, Bursa, Turkey; [2] Bloomsburg University, Department of Biological and Allied Health Sciences, Bloomsburg, PA, USA; [3] Southwestern Oklahoma State University, OK, USA; [4] Southern Nazarene University, OK, USA; [5] University of California, San Diego, CA, USA; [6] University of Tulsa, OK, USA

Effects of neonicotinoids were studied on the foraging behavior of free-flying bees (*Apis mellifera anatoliaca*) visiting artificial flower patches of blue and white flowers. Neonicotinoids doses from 2 % to 40 % of the reported LD50 value were given to bees. The study consisted of three experimental parts performed sequentially without interruption. In part 1, we offered bees 6 μL of a 1 M sucrose reward in both flower colors. In part 2, we offered bees 6 μL of 1.5 M sucrose solution in blue flowers and 6 μL of 0.5 M sucrose solution in white flowers. In part 3, we reversed the sucrose solution rewards values with respect to flower color. Each experiment began 30 min after administration of the insecticide. The number of bees foraged was recorded, as was flower patch visitation rate, number of flowers visited and flower choices of the bees that did return. The forager return rate declined linearly with increasing neonicotinoids dose and number of foraging trips of returning bees was also affected adversely. Out of 96 bees, the majority of unreturned (50) bees belonged to higher dosages of neonicotinoids groups. However, flower fidelity was not affected by neonicotinoids dose. Foragers visited both blue and white flowers extensively in experimental part 1 and showed greater fidelity for the flower color offering the higher molarity reward in parts 2 but there were less visits to flowers offering the higher molarity reward in part 3 indicating that the bees failed to learn what were the flowers with higher reward. Our study showed that neonicotinoids affected: the number of returning bees, the number of foraging trips and reward re-learning.

028 Where have all the wild bees gone, long time passing ?

Rasmont P.[1], Boevé J-L.[2], Francis F.[3], Dendoncker N.[4], Dufrêne M.[3], Smagghe G.[5], Barbier Y.[6], Brasero N.[1], D'Haeseler J.[7], Dekoninck W.[2], Desmet L.[5], Foschweiller M.[1], Jacquemin F.[3], Maebe K.[5], Marshall L.[4], Martinet B.[1], Meeus I.[5], Michez D.[1], Moerman R.[1], Pauly A.[2], Roger N.[1], Schoonvaere K.[5], Vanderplanck M.[1], Van Ormelingen P.[7], Vray S.[1,4], de Graaf D.C.[5]
[1] University of Mons, Mons, Belgium; [2] Royal Belgian Institute of Natural History, Brussels, Belgium; [3] Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium; [4] University of Namur, Namur, Belgium; [5] Ghent University, Ghent, Belgium; [6] DEMNA, Service Public de Wallonie, Gembloux, Belgium; [7] Natuurpunt, Mechelen, Belgium; [8] Université Libre de Bruxelles, Brussels, Belgium

It is now well known that many of our wild bees are gone. Many hypotheses have been proposed to explain their massive regression. It was missing however a quantification of the respective roles played by the different triggers. The BELBEES project aimed to gather all information about the vanishing of wild bees in Belgium, where the regression phenomenon is dramatic. Thanks to long lasting efforts, our country shows an exceptional monitoring cover since more than one century. We assessed the fate of wild bee species since the beginning of observations. The respective roles of the next triggers have been integrated in our evaluation: constrains in flower association, food quality and availability, landscape changes, climates changes, genetic bottleneck, pathogenes, pesticides. We managed to evaluate the risks of pollination shortage for agriculture. We also prepared predictive assessments' of the distribution of bumblebees along the present century following different scenarios. Mitigation proposal and policy changes have been provided.

029 Declining distributions for BENELUX Bumblebees: climate and land use change interactions

Marshall L.[1,2], Biesmeijer J.C.[2,3], Rasmont P.[4], Vereecken N.J.[5], Dvorak L.[6], Fitzpatrick U.[7], Francis F.[8], Neumayer J.[9], Ødegaard F.[10], Paukkunen J.P.T.[11], Pawlikowski T.[12], Reemer M.[13], Roberts S.P.M.[14], Straka J.[15], Vray S.[1,4], Dendoncker N.[1]

[1] Department of Geography, University of Namur, Namur, Belgium; [2] Naturalis Biodiversity Center, Leiden, The Netherlands; [3] Institute of Environmental Sciences (CML), Leiden University, Leiden, The Netherlands; [4] Laboratoire de Zoologie, Research institute of Biosciences, University of Mons, Mons, Belgium; [5] Agroecology & Pollination Group, Landscape Ecology & Plant Production Systems (LEPPS/EIB), Campus de la Plaine CP 264/2, Université Libre de Bruxelles (ULB), Brussels, Belgium; [6] Municipal Museum Mariánské Lázně, Mariánské Lázně, Czech Republic; [7] National Biodiversity Data Centre, Beechfield house, Carriganore WIT West Campus, County Waterford, Ireland; [8] Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium; [9] Elixhausen, Austria; [10] Norwegian Institute for Nature Research – NINA, Trondheim, Norway; [11] Finnish Museum of Natural History, Zoology Unit, University of Helsinki, Finland; [12] Ecology and Biogeography, Nicolaus Copernicus University, Toruń, Poland; [13] European Invertebrate Survey (EIS), Naturalis Biodiversity Center, Leiden, The Netherlands*; [14] Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, Reading, UK; [15] Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic*

Bumblebees represent a group which has demonstrated considerable range contractions in Europe during the last 100 years. This decline is expected to continue in the foreseeable future with projected climate representing a significant threat. However, climate change is only one of the potential drivers of bee decline but is often studied in isolation. Here we aimed to combine projections of land use change for 2050 and 2100 with climate change projections to see how they interact and how the distribution of bumblebees is expected to change in Belgium, Netherlands and Luxembourg (BENELUX) and Europe in the future. Using species distribution models we compared and contrasted the projected range loss for 48 bumblebees across Europe, with a focus on two key model differences; (1) climate change only models vs. models with models which incorporate land use changes as well, and (2) climate and land use changes models where one model assumes unchanging, static land use change and the other changing, dynamic land use. We made this comparison for three future change scenarios; extreme growth strategy (GRAS); business as usual (BAMBU); and sustainable development goals (SEDG). We found a significant difference between the models for the predicted current distributions and the percentage of projected range loss for bumblebee species, both at the BENLUX and European scale. Overall, climate only models projected a greater present range and greater range loss, whereas models incorporating land use change estimated a significantly smaller current range and less percentage range loss. However, the land use change models showed a significantly restricted distribution for bumblebees in the future. We observed a clear interaction between land use and climate change models and suggest that when available land use change projections should be incorporated into biodiversity scenarios. However, we show that more detailed projections with a finer resolution and greater focus on land use management are necessary to improve our understanding of future losses in biodiversity.

030 Extreme shift in abundance and distribution of bumblebee composition in Belgium

Vray S.[1,2], Rollin O.[3], Michez D.[1], Dendoncker N. [2], Rasmont P.[1]

[1] Research Institute for Biosciences, University of Mons - UMONS, Mons, Belgium; [2] Department of Geography, University of Namur, Namur, Belgium; [3] BIOSystem Engineering Department, University of Liège - Gembloux AgroBioTech, Gembloux, Belgium

Bumblebees (*Bombus* genus), which play a widely recognized and essential role on the pollination ecosystem service, have seen their populations drastically decreasing for decades around the world. The causes advanced include the reduction of floral resources, the degradation and fragmentation of natural habitats through urbanization and agricultural intensification, and more recently climate change. In order to project and mitigate the consequences of these environmental changes on future assemblages of bumblebees, it is important to understand how these changes have influ-

enced their assemblages in the past. Belgium is a particularly suitable country for this purpose, as it represents a typical example of the environmental changes observed in Western Europe, and its bumblebee fauna has been studied for more than 100 years. The present work aims to determine what changes in Belgian bumblebee assemblages occurred during the last century and to assess the impact of environmental changes on these modifications. Two main questions were addressed here: (1) What changes in bumblebee assemblages can be observed over the last century, and which species are the most affected by the decline? (2) What importance do thistles (i.e. Cardueae) have in the diet of bumblebees, and could the legislations against thistles be a threat for their conservation? The modifications of Belgian bumblebee assemblages were studied between three time periods: 1910-1930, 1970-1989, and 1990-2016. Various indicators of species richness and diversity were evaluated and all show a global regression over the century, more or less accentuated depending on the regions. Population trend analyses of each bumblebee species, based on abundance and range size, showed that between 68 and 88% of species have been declining over the last century. Only few species tend to increase their relative abundance and dominate the others, leading to a homogenization of the assemblages. These differences in population trends are correlated with the species ecological traits. Regarding thistles, our results reveal the great importance of these plants in the diet of male bumblebees. We show that a high number of species, many of which are rare in Belgium and Europe, depend largely on the four thistle species for which the destruction is legislatively mandatory in several European countries including Belgium. Such laws could therefore negatively affect bumblebee populations, already greatly weakened by global environmental changes. We argue for the abolishment of these legislations in favor of alternative measures that reconcile the conservation of biodiversity and agricultural needs.

ORAL PRESENTATIONS

031 Drift in distribution and quality of host-plant resources in common bumblebees

Michez D.[1], Roger N.[1], Moerman R.[1,2], Vanderplanck M.[1]
[1] Research Institute for Biosciences, University of Mons - UMONS, Mons, Belgium; [2] Evolutionary Biology & Ecology, Université Libre de Bruxelles (ULB), Brussels, Belgium

As nearly all bee species exclusively rely on pollen for development and survival, it has been proposed that the decline of some species could be related to the shift in the abundance and the quality of their host-plants. Generalist species are globally less affected than specialist species as they are able to shift on alternative resources. However some species in generalist group like bumblebees are declining while other species are indeed stable. We aim in the present study to investigate whether common bumblebees share, or not, stability in the distribution and chemical quality of their pollen resources. We selected five species of common bumblebees (*i.e. B. hortorum, B. lapidarius, B. pascuorum, B. pratorum* and *B. terrestris*) in NW Europe for which we had a precise description of the shift in their pollen diet between two periods of time (past and recent). First we analyzed the evolution of the potential distribution of these host-plant resources based on Species Distribution Models for the two periods of time to detect, or not, a relation between shift in the host plant distribution and shift in bumble bee host plant choices. Second we compared the chemical composition (i.e. amino acids and sterols) of pollen from the past plant community with pollen from the recent plant community to evaluate the stability in the chemical quality of the bumblebee diets. Third we experimentally tested the impact of new pollen diet and non-host pollen diet on the development of *Bombus terrestris* micro-colony. Our results clearly show that responses to drifts in host-plant distributions vary between the five bumblebee species. Some species (i.e. *B. terrestris* and *B. lapidarius*) are able to take advantage of increasing pollen resources with suitable chemical composition (i.e. high essential amino acid concentration and suitable sterol composition) for colony development, but other abundant species are not (i.e. *B. hortorum*). We did not find a significant difference in the chemical composition between the two plant communities (i.e. past and recent). Diversity of plant community seems an important element to allow all the bumblebees to forage on a balanced diet as the chemical similarity was not found at species level. Development of micro-colonies was normal with the new pollen host and deeply affected with non-host-pollen.

032 The role of bees in interaction networks with plants as a conservation argument

Jacquemin F.[1, 2], Violle C.[2], Munoz F.[3], Mahy G.[1], Rasmont P.[4], Michez D.[4], Vereecken N.J.[5], Roberts S.P.M.[6], Vray S.[4, 7], Dufrêne M.[1]
[1] UR Biodiversité et Paysage, Université de Liège - Gembloux Agro-Bio Tech, Gembloux, Belgium; [2] CEFE, Univ. Paul Valéry Montpellier 3, Université de Montpellier, EPHE, CNRS, IRD, Montpellier, France; [3] Laboratoire d'Ecologie Alpine, Université Grenoble Alpes, Grenoble, France; *[4] Laboratoire de Zoologie, Université de Mons-Hainaut, Mons, Belgium; [5] Agroecology Lab, Université libre de Bruxelles (ULB), Bruxelles, Belgium; [6] Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, Reading, UK; [7] Department of Geography, University of Namur, Namur, Belgium*

In a context of major pollinator decline, mutualist interactions between plants and pollinators have been widely studied last years. Anthropogenic disturbances are known to threaten these interaction networks. Our objective was therefore to identify the species on which the structure of the network mainly depend to target conservation measures. Some studies have shown that the structure of plant-pollinator networks is modular and that modules consist of few highly connected species and many weakly connected species around them. However, due to a lack of historical data, we have little information about the temporal variability of the identity of these species. To figure out if they are the same species that structure networks over time, we compared two networks (before and after 1970) built from a unique century-old database of wild bee specimens identified on plants. We compared their structure through their modularity and grouped bee species into modules based on shared plants. Depending on their number of interactions intra- and inter-modules, we calculated for each bee species a connectivity and participation coefficient, respectively. It was then possible to assign them a role: module hubs, highly connected to species of their module; connectors, highly connected to species of other modules; and network hubs that are both. All other species were classified as peripherals. We observed a high variability of the network structure, especially in terms of species composition. During each period, we identified several hubs and connectors but only four species had such a role over time (*Andrena bicolor*, *A. haemorrhoa*, *Bombus terrestris* and *B. pascuorum*). Species identified as hubs and connectors tend be abundant and to present particular traits: they are large generalists flying over a longer period during the year than peripheral species. The presence of these species being essential to the network functionality, we advise to focus the conservation measures on them.

033 A century of temporal stability of genetic diversity in wild bumblebees

Maebe K.[1], Meeus I.[1], Vray S.[2,3], Dekoninck W.[4], Boevé J-L.[4], Rasmont P.[2], Smagghe G.[1]
[1] Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium; [2] Laboratoire de Zoologie, University of Mons, Mons, Belgium; [3] Department of Geography, University of Namur, Namur, Belgium; [4] Royal Belgian Institute of Natural Sciences, Brussels, Belgium

Since the 1950s, bumblebee (*Bombus*) species are showing a clear decline worldwide. Although many plausible drivers have been hypothesized, the cause(s) of this phenomenon remain debated. Here, genetic diversity in recent *versus* historical populations of bumblebee species was investigated by selecting four currently restricted and four currently widespread species. Specimens from five locations in Belgium were genotyped at 16 microsatellite loci, comparing historical specimens (1913–1915) with recent ones (2013–2015). Surprisingly, our results showed temporal stability of genetic diversity in the restricted species. Furthermore, both historical and recent populations of restricted species showed a significantly lower genetic diversity than found in populations of co-occurring widespread species. These results suggest that the genetic pauperization took place well before the agricultural revolution started with a massive use of pesticides and fertilizers around 1950-1960s. A future sampling in the entire distribution range of these species will infer if the observed link between low genetic diversity and population distribution on the Belgium scale correlates with species decline on a global scale.

034 Viruses in wild bees: to be included in future monitoring programs

Schoonvaere K.[1,2], Francis F.[3], de Graaf D.C.[1,2]
[1] Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium; [2] Honeybee Valley, Ghent University, Ghent, Belgium; [3] Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium

Bee viruses are numerous and omnipresent in plant-pollinator environments. At least the majority of known viruses that can exploit the honey bee as a replication hub are well-established pathogens and are often included in bee health monitoring programs. In contrast, the possibility that wild bees come with their own set of viruses has received little attention. As a part of the multidisciplinary BELBEES project, we finalized two metagenomics surveys wherein an extensive and diverse collection of novel viruses was identified in wild bees. The viruses can be roughly broken down in three groups based on taxonomy or biological role. The first group includes viruses with a clear relationship to insect pathogens although their pathology in wild bees was not extensively demonstrated. The orders *Picornavirales* and *Tymovirales*, which comprise many of the honey bee pathogens, were well presented and included relatives to Deformed wing virus, Bee macula-like virus and plant viruses. Furthermore, two DNA viruses of the family *Nudiviridae* and *Parvoviridae* with active replication in mason bees (*Osmia*) and bumble bees (*Bombus*) are related to serious insect pathogens of beetles and crickets. The second group comprises of insect-specific viruses distantly related to plant viruses (nege-like viruses, sobemo-like viruses, toti-like viruses). They were abundantly present and in case of the nege-like viruses the relation was host specific. The occurrence of these insect-specific viruses in bees is in agreement with global studies that report this group in virtually all insect orders but their biological role remains unclear. Finally, the third group includes the mysterious negative-stranded RNA viruses. We reported a mononegavirus, Scaldis River bee virus, and a bunyavirus, Ganda bee virus, related to the recently discovered rhabdoviruses in social bees. Together, the range of viruses found in wild bees greatly exceeds the traditional honey bee viruses. Their biological role in the bee host is currently vague and requires further study. A good start lies in the inclusion of these viruses in current bee monitoring programs to probe their prevalence, shared hosts and transmission routes.

035 Distribution of complementary sex determiner alleles in *Apis mellifera* population

Zareba J.[1], Blazej P.[2], Laszkiewicz A.[1], Sniezewski L.[1], Majkowski M.[1], Janik S.[1], Cebrat M.[1]
[1] Laboratory of Molecular and Cellular Immunology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland; [2] Department of Genomics, Faculty of Biotechnology, Wroclaw University, Wroclaw, Poland

The complementary sex determiner (csd) gene determines the sex of the western honey bee (*Apis mellifera* L.). Bees that are heterozygous at the csd locus develop into females; whereas hemizygous bees develop into males. The co-occurrence of two identical csd alleles in a single diploid genome leads to the genetic death of the bee. Thus, the maintenance of csd diversity in the population is favoured. The number and distribution of csd alleles is particularly interesting in light of the recent decline in the honey bee population. In this study, we analysed the distribution of csd alleles in two Polish populations separated by about 100 km. We analysed the maternal alleles of 193 colonies and found 121 different alleles. We also analysed the distribution and frequency of the alleles, and found that they are distributed unevenly. We show that the methods that have been used so far to estimate the total worldwide number of csd alleles have significantly underestimated their diversity. We also show that the uneven distribution of csd alleles is caused by a large number of infrequent alleles, which most likely results from the fact that these alleles are generated very frequently.

036 De novo genome assembly of a western European *Apis mellifera mellifera* black bee

Vignal A.[1], Eynard S.E.[1], Klopp C.[2], Canale-Tabet K.[1], Marande W.[3], Roulet A.[3], Donnadieu C.[3], Servin B.[1]
[1] GenPhySE, INRA, Castanet Tolosan, France; [2] MIAT, INRA, Castanet Tolosan, France; [3] CNRGV, INRA, Castanet Tolosan, France; [4] GeT-PlaGe, Genotoul, INRA, Castanet Tolosan, France

The current honeybee genome sequence, Amel4.5, was produced from a commercial strain and although it was recently updated, it still suffers from imperfections, having numerous gaps in the assembly. In order to construct a new genome assembly with improved continuity, we used the Pacific Biosciences long-read technology and produced all sequence reads from a single haploid drone to avoid assembly problems due to polymorphism. The sample is an *A. m. mellifera* M-type from a conservatory on the island of Ouessant in Brittany, France. As this population has been closed for 30 years, it represents well the subspecies originally present in western Europe, that is still used by some beekeepers.

A total of 200 contigs (gap-free sequence tract) were obtained. The longest contig is 11.6 Mb and the N50 contig size (a measure of sequence contiguity in which 50% of the assembly is contained in contigs of size larger than N50) is 5.1 Mb. This is a great improvement in comparison to the 46 kb N50 contig of Amel4.5.
Alignment of our assembly with Amel4.5 agreed on the chromosomal assignment of the contigs but revealed many disagreements of large chromosome segments internal ordering. To order and orient our contigs along the chromosomes, we used published sequencing reads to build a genetic map. As a result, the total genetic map is 50 Morgans long and the average recombination rate in the genome is 23 cM/Mb, closer to the first estimations based on the microsatellite genetic map, than to the most recent ones based on SNPs derived from sequence data. This latter difference is explained by recombination hotspots detected on Amel4.5 at the breakpoint positions between the two assemblies, that have disappeared when the sequence order and orientation are corrected in our assembly.
Our results will allow detailed analyses of structural rearrangements between the genomes of C-type or hybrid honeybees used by the majority of beekeepers and the M-type subspecies *A. m. mellifera* black bee.

037 A comprehensive genomic and morphometric assessment of European honey bee diversity and identification of SNP markers for subspecies diagnosis

Parejo M.[1,2], Montes I.[1], Bouga M.[3], Estonba A.[1], Papoutsis L.[3], Nielsen R.O.[4], Momeni J.[4], Langa J.[1], Vingborg R.[4], Kryger P.[5], Meixner M.[5]
[1] Lab. Genetics, University of the Basque Country (UPV/EHU), Leioa, Spain; [2] Swiss Bee Research Center, Agroscope, Bern, Switzerland; [3] Lab of Agricultural Zoology and Entomology, Agricultural University of Athens, Athens, Greece; [4] GenoSkan A/S, Aarhus, Denmark; [5] Department of Agroecology, Aarhus University, Slagelse, Denmark; [6] LLH Bee Institute Kirchhain, Kirchhain, Germany

With numerous endemic subspecies representing three evolutionary lineages, Europe holds a large fraction of honey bee genetic diversity. Using both morphometric and genomic markers, our study aims to provide a comprehensive characterisation of this diversity, and to identify SNP markers for subspecies diagnosis.
To this end, 23 populations belonging to 13 subspecies (*A. m. iberiensis*, *A. m. mellifera*, *A. m. carnica*, *A. m. caucasica*, *A. m. ligustica*, *A. m. siciliana*, *A. m. macedonica*, *A. m. cecropia*, *A. m. cypria*, *A. m. adami*, *A. m. ruttneri*, *A. m. anatoliaca*, *A. m. armeniaca*) have been sampled throughout Europe and from adjacent regions in Asia and Africa. For each population, worker bees from ~100 colonies, each one from a different apiary, have been collected (in total >2200 colonies).
Representative subsets of each population (between 25 and 50 colonies, 10 bees per colony) were subjected to morphometric analysis to provide data compatible with the historic subspecies descriptions.
In order to analyse the genetic variation within and among subspecies with whole-genome sequencing (Pool-Seq), pools of 86-100 workers (each individual representing one apiary) for 22 populations have been sequenced (50-200X coverage). After bioinformatic processing, population differentiation was estimated using FST distance, and private SNPs were identified. The most informative markers for subspecies diagnosis were selected based on the highest pairwise FST. The subspecies allocation of the samples was either determined based on previous studies, or confirmed by morpho-

metric analysis at population level. The diagnostic SNP markers distinguish with high accuracy between evolutionary lineages, and also between major subspecies, while it is more challenging to differentiate closely related subspecies. This study represents a comprehensive survey of *A. mellifera* genetic diversity in Europe, combining genomic and morphometric data for several hitherto unexamined populations. The identified SNP markers permit a fast and cost-effective subspecies identification, and thus are a valuable tool to be applied for conservation purposes.

038 Applying a SNP-based tool for conservation of wild and managed black bees in Ireland

Browne K.A.[1], Henriques D.[2], Pinto M.A.[2], Native Irish Honeybee Society, McCormack G.P.[1]
[1] *Department of Zoology, Ryan Institute, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland;*
[2] *Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal*

Apis mellifera mellifera (black bees) is threatened over much of its natural range. However, in Ireland microsatellite and mitochondrial data have shown that a significant population of this subspecies exists in pure form and spread over a large geographical region on the Island. Black bees have been managed and protected by beekeepers on the island, some of who formed the Native Irish Honeybee Society in 2012. The application of a SNP panel that detects hybridization between M and C lineages clearly supports other data in that the majority of beekeepers included who purported to keep black bees indeed have bees that show very low to no introgression from the C lineage. Furthermore, SNP data has also been applied to the first feral bee colonies located in Ireland subsequent to the introduction of Varroa. Long considered extinct, feral bees sampled to date show high levels of *A. m. mellifera* purity using SNPs. Here we will present this data and also discuss the use of this SNP panel to elucidate patterns in colour variation and honeybee subspecies purity in wild and managed bees towards improving conservation approaches in the face of potential hybridization threat.

039 Genetic models for long-term simulation studies in honeybee breeding

Plate M., Bernstein R., Hoppe A., Bienefeld K.
Institute for Bee Research Hohen Neuendorf, Hohen Neuendorf, Germany

The EU project SMARTBEES (www.smartbees.eu) will implement breeding programs for various European honeybee races to improve their resilience. For these breeding programs, two conflicting interests have to be met. While striving for gain in the genetic quality of the bees, it is imperative to maintain genetic variability within the population.
In animal breeding, Monte Carlo simulations is a widely used tool to predict the effects of different breeding strategies on the genetic gain, the average inbreeding and the loss of genetic variance in a population. The simulation studies are vital for reliable long-term predictions on the evolution of genetic variability. Thus a suitable model must be chosen in respect of the biological features of honeybees.
There are two main options to model animal genetics in such simulations. In finite locus models (FLM), a trait is genetically controlled by a finite number of gene loci with differing influences. These models yield complex and time intensive simulations. The infinitesimal model (IM) on the other hand assumes infinitely many loci, each of which has the same infinitesimal influence on the trait. This leads to simplifications in the implementation and reduction of the simulation run-times.
We compared the behavior of these genetic models in simulations of honeybee breeding schemes and found major differences in simulations that exceeded the time scale of 20 years. In a simulation setting of a population with 300 colonies per year and intense selection over 100 years, the FLM showed a realistic decrease of genetic variance of 79-92%, whereas the variance in the IM only halved (47%).
We therefore conclude that long-term simulation studies in honeybee breeding should rely on FLM as the IM underestimates the dangers of losing genetic variety in the population.
With the FLM, we undertook further investigations, amongst other things on the importance of safe mating control for breeding success.

040 Unraveling patterns of population structure in *Bombus terrestris* from the Iberian Peninsula and North Africa

Silva S.E.[1], Seabra S.G.[1], Carvalheiro L.G.[1,2], Nunes V.L.[1], Marabuto E.[1], Mendes R.[1], Rodrigues A.S.B.[3], Laurentino T.G.[4], Paulo O.S.[1]
[1] Centre for Ecology, Evolution and Environmental Changes (cE3c), Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; [2] Department of Ecology, Universidade Federal de Goiás, Goiânia, Brazil; [3] Centre for Ecology, Evolution and Environmental Changes (cE3c), Natural History and Systematics (NHS) Research Group /MUHNAC - Museu Nacional de História Natural e da Ciência, Universidade de Lisboa, Lisbon, Portugal; [4] Zoological Institute, University of Basel, Basel, Switzerland

Bumblebees (*Bombus* spp.) are key pollinators with high ecological and economic importance, and some species show accentuated population declines. With new genomic approaches and the complete bumblebee genome already available, there is an opportunity to improve information on bumblebee biology. Coupled with knowledge on species' ecology, this information has the potential to revolutionize our understanding of bumblebee adaptation and resilience. We examined population genetic structure in *Bombus terrestris* from the Iberian Peninsula (subspecies *Bombus terrestris lusitanicus*) and northern Africa (subspecies *Bombus terrestris africanus*) using the mitochondrial DNA Cytochrome Oxidase I (COI) marker and several thousands of genome-wide Restriction-site Associated DNA (RAD) markers. We analyzed a total of 205 individuals sampled across several altitudinal and latitudinal clines in the Iberian Peninsula, and from Morocco. Lack of population structure was observed on *B. t. lusitanicus* across the Iberian Peninsula but a clear differentiation was observed between *B. t. lusitanicus* and *B. t. africanus*. This suggests a panmictic pattern across this European region but limited dispersion between the Iberian Peninsula and northern Africa, despite the close proximity of these two continents at the Strait of Gibraltar and the good dispersal capability of these insects. Also, a greater genetic diversity was found in the Iberian Peninsula when compared to values obtained in previous studies for the rest of *B. terrestris'* European continental distribution and for other *Bombus* species. The existence of loci with potential roles in local adaptation and associated to environmental variables will be further explored. By combining data on genetic markers with ecological niche modelling (ENM) we are evaluating future responses of these bumblebees to ongoing environmental changes.

041 Signatures of selection revealed by population analyses of bumblebee genomes

Colgan T.J.[1,2], Arce A.N.[3], Gill R.J.[3], Ramos-Rodrigues A.[3], Li L.[1], Kanteh A.[1], Wurm Y.[1]
[1] Queen Mary, University of London, London, United Kingdom; [2] University College Cork, Cork, Ireland; [3] Imperial College London, London, United Kingdom

Understanding the ability of an organism to adapt to a changing environment is a fundamental question within the field of evolutionary biology. Insect pollinators, including social bees, are key to ecosystem stability, as well as agricultural yields. However, recent studies have highlighted population declines with a number of contributing factors suggested, including land-use change, pesticide exposure, climate change, pathogens and newly emerging diseases. At present, our understanding of the potential effects of such stressors on wild pollinator species at the molecular and genomic level is limited. To help understand the relative impacts of these competing challenges, we conducted a population genomic study on the buff-tailed bumblebee, *Bombus terrestris*, a common Eurasian species and important ecological pollinator. From 28 sites across the island of Great Britain, we sampled wild individuals and performed whole-genome sequencing. We identify the presence of recent selective sweeps and copy number variation within the British population affecting genes involved in important biological processes, such as immunity, neurology, as well as detoxification processes. Furthermore, we identify evolutionarily conserved gene-rich regions under selection within other social bees. Taken collectively, the results provide a novel insight into ongoing selection events, as well the recent evolutionary history of this important ecological insect pollinator.

042 Genomic signatures of introgression between commercial and native bumblebees, Bombus terrestris, in the Iberian Peninsula – implications for conservation and trade regulation

Seabra S.G.[1], Silva S.E.[1], Nunes V.L.[1], Sousa V.C.[1], Martins J.[2], Pina-Martins F.[1], Rebelo M.T.[3], Figueiredo E.[2], Paulo O.S.[1]
[1] Centre for Ecology, Evolution and Environmental Changes (cE3c), Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Portugal; [2] Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Lisboa, Portugal; [3] Centre for Environmental and Marine Studies (CESAM), Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Bumblebees have been deliberately introduced across the world for crop pollination with known negative impacts on native pollinators. In this study we analysed two of those potential impacts: hybridization between commercial and native species; and spillover of parasites or pathogens from commercial to native bumblebees. We sampled *Bombus terrestris lusitanicus* in two areas in the western Iberian Peninsula near and far from greenhouses where commercial bumblebees are used for pollination. We examined signatures of introgression from commercial bumblebees (likely *B. t. terrestris* and *B. t. dalmatinus*) using thousands of genome-wide Restriction site associated DNA (RAD) markers. In natural populations sampled near greenhouses, we detected hybrids between native and commercial lineages, as well as potential escaped commercial bumblebees (some of them males), strengthening the idea that commercial hives are not male-free and these bumblebees are able to escape from greenhouses and genetically interfere with local populations, namely through introgression of maladaptive alleles. These results suggest that the risk of using commercial bumblebee stocks should be better evaluated, to avoid impacts on local bumblebee populations. We are also performing a molecular screening with parasite specific PCR primers to assess parasite prevalence in commercial and native bumblebees. We have so far been able to amplify the gut parasites *Apicystis bombi* (Apicomplexa: Neogregarinorida) and *Crithidia bombi* (Kinetoplastea: Trypanosomatidae) in both commercial and native *B. terrestris*.

043 Multi-stress effects on honey bee colonies

van Dooremalen C.[1], van Langevelde F.[2], Blacquière T.[1]
[1] Bees@wur, Wageningen University & Research, Wageningen, The Netherlands; [2] Resource Ecology group, Wageningen University & Research, Wageningen, The Netherlands

High losses of honey bee colonies in recent decades are of great societal and economical concern and has been experienced as sign of vulnerability of agriculture, including the service of crop pollination, and of beekeeping. *Varroa destructor* infestation is acknowledged as an important cause of these losses and often suggested to act in concert with contributing stressors, such as low or monotonous food availability, infestation by *Nosema* spp. or exposure to insecticides. In several different field experiments, we studied the relative and interactive effects of *V. destructor* infestation and these stressors at field-realistic exposures on the performance and survival of honey bee colonies. In one study we found that ample food could not compensate negative effects by *V. destructor* on colony size or survival. In another study, colonies infested by V. destructor were 13% smaller in size and were 59.1 times more likely to die than colonies infested with low levels of V. destructor, but in contrast to the expectations no interactions with *Nosema* spp. or imidacloprid were found for colony size or survival. At individual level however, pollen foragers from colonies exposed to *V. destructo*r in combination with imidacloprid flew less far (in a flight mill) compared to colonies exposed to a single stressor. Colonies as a superorganism may well be able to compensate at the colony level for negative effects of stressors on their individuals. In all of our experimental studies under field realistic exposure to stressors, *V. destructor* was by far the most lethal stressor for honeybee colonies.

044 Impact of beekeeping management practices on honey bee mortality in Belgium

El Agrebi N.[1], Danneels E.[2], de Graaf D.C.[2], Saegerman C.[1]
[1] Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine of Liège, University of Liege, Belgium; [2] Laboratory of Molecular Entomology and Bee Pathology, Department of Biochemistry and Microbiology, University of Gent, Ghent, Belgium

Europe and particularly Belgium are strongly impacted by honey bee mortality. Colony success depends also on management of the ectoparasitic bee mite, *Varroa destructor.* Despite honey bees being managed as a domestic pollinator, the impacts of beekeeping management practices has often been overlooked. In an attempt to understand the impact of bee management practices on bee mortality in Belgium, we correlated the variables obtained from a face to face questionnaire interview, to winter mortality rates. A logistic regression model was built in Stata SE 14.1® using colony losses rate as dependent variable (threshold value of 10% of mortality rate, which is considered acceptable) and questionnaire answers as explanatory variables. *A univariate* and a multivariate analysis was conducted (odds ratio's with 95% confidence intervals (CI 95%)) completed by a Classification and Regression Tree (CART) Analysis. The sample of Belgian beekeepers was representative, randomized (n=200) and stratified. In this study, we present the first evidence of a relationship between beekeeping management practices and bee mortality. The results show that the main factors protecting honey bee colonies are the resilience of the beekeepers, the hive type, the equipment use, wintering in proper condition that includes the use of partition, the colony strength, winter check and last but not least, an appropriate integrated pest management. Proper diagnosis of *Varroa* infestation rates should be generalised before using acaricides with parsimony. More efforts are needed in beekeeper training programs to promote good beekeeping practices and achieve early identification of clinical signs of disease.

045 Classification and prediction of stress factors and bee health using ultra high performance liquid chromatography techniques with quadrupole and orbitrap high-resolution mass spectrometry-based metabolomics

Wang L., Smagghe G., Meeus I.
Ghent University, Department Plants and crops, Ghent, Belgium

Objective: Bees are exposed to multiple stressors of natural and anthropogenic origin. Understanding the effects of single drivers, within the complex environmental context of antagonistic or synergistic interaction effects, is key to setup effective mitigation measures to restore bee population. Here we assess if metabolomic profiling can work as a categorizing tool that identifies stressors to which bees are exposed and its impact.
Methods: We first built a training set, a metabolomics dataset of *B. terrestris* on different diets (normal sugar water and 25% reduced sugar water), with bees of variable age (day 6 and 12), and hierarchy (worker and pseudo queen). We employed liquid chromatography and Q-ExactiveTM Orbitrap mass spectrometry, following both targeted and nontargeted approaches. The discrimination capability of the training set was further assessed by k-Nearest Neighbor (k-NN) classification algorithms.
Results: Hemolymph profiling comprised up to 3877 features whereby multivariate discriminant analysis was able to point out significant metabolome differences between age (Q2=0.69) and diet (Q2=0.544), using the validated models, a total of 11 and 14 metabolites were assigned marker potential of age and diets, respectively. The predictive model could identify the treatment diet stress in the validation set with an accuracy of 87.5%.
Conclusions: We demonstrate the classification power of hemolymph metabolomics within a setup of standardized bumble bees, and discuss the potential of this technique as an assessment tool of bee health status in the real environments.

046 MALDI mass spectrometry imaging: an in situ histoproteomic approach to monitor the response of pollinators to their stressors

Bulet P.[1,2], Arafah K.[2], Voisin S.[2], Houdelet C.[1,2], Bocquet M.[2,3]
[1] University Grenoble Alpes, La Tronche, France; [2] Plateforme BioPark d'Archamps, Archamps, France; [3] Michel Bocquet, Annecy, France

Facing many environmental stress factors, pollinator populations continue to decline precipitously, placing increasing pressure on managed pollinators like honeybees to fulfil crop pollination. Those factors do not operate only individually, but and more often so, in combination with each other. How deeply these stressors (biotic and abiotic; individually or combined) impact honeybee colonies is not well understood and remains a major challenge for the beekeeping industry.
We have been developing a protocol investigating in situ the bee physiological status, by monitoring the protein signatures with respect to bee histology using MALDI Mass Spectrometry Imaging (MSI) or MALDI histoproteomics. MALDI-MSI is a cutting-edge imaging technology that allows to map the distribution of hundreds of biomolecules within organs, tissues and even whole body sections in a single experiment (Arafah et al., 2012; Bulet & Arafah, 2013, 2014a,b; Schwamborn et al., 2016; Lagarrigue et al., 2016). MALDI-MSI does not require labeling biomolecules prior to detection; from its initial biological application for a better understanding of biological organisms, symbiosis and host-response to infection, this technology is now used for biomarker discovery in clinical settings. MALDI-MSI has been applied to study the relationships between host, pathogens and other stressors and to follow some already known immune related peptides within sections of whole bees and on biological features (surface epithelia…) involved in the response to stressors. We have designed and tested an experimental procedure for imaging the molecular fingerprints of both the bee whole body and its isolated digestive tract. Our preliminary data are suggesting that MALDI MSI which result in spatio-temporal images of a physiological status of a whole organism is a useful innovative approach for monitoring the honeybee health. Moreover, we have very recently successfully applied this approach to other pollinators.

047 Assessment of honey bee cells using deep learning

Alves T.S.[1], Ventura P.[2], Neves C.J.[3], Candido Junior A.[4], De Paula Filho P.L.[4], Pinto M.A.[3], Rodrigues P.J.[1]
[1] Polytechnic Institute of Bragança, CeDRI Research Centre in Digitalization and Intelligent Robotics, Bragança, Portugal; [2] Apis Ventura S.U. Lda, Bragança, Portugal; [3] Polytechnic Institute of Bragança, CIMO Mountain Research Centre, Bragança, Portugal; [4] Federal University of Technology - Paraná, DACOM Computer Science Department, Medianeira, Brazil

Temporal assessment of honey bee colony strength is required for different applications in many research projects. This task often requires counting the number of cells with brood and food reserves multiple times a year from images taken in the apiary. There are thousands of cells in each frame, which makes manual counting a time-consuming and tedious activity. Thus, the assessment of frames has been frequently been performed in the apiary in an approximate way by using methods such as the Liebefeld. The automation of this process using modern imaging processing techniques represents a major advance. The objective of this work was to develop a software capable of extracting each cell from frame images, classify its content and display the results to the researcher in a simple way. The cells' contents display a high variation of patterns which added to light variation make their classification by software a challenging endeavor. To address this challenge, we used Deep Neural Networks (DNNs) for image processing. DNNs are known by achieving the state-of-art in many fields of study including image classification, because they can learn features that best describe the content being classified, such as the interior of frame cells. Our DNN model was trained with over 60,000 manually labeled images whose cells were classified into seven classes: egg, larvae, capped larvae, honey, nectar, pollen, and empty. Our contribution is an end-to-end software capable of doing automatic background removal, cell detection, and classification of its content based on an input image. With this software the researcher is able to achieve an average accuracy of 94% over all classes and get better results compared with approximation methods and previous techniques that used handmade features like color and texture.
This research was funded through the 2013-2014 BiodivERsA/FACCE-JPI Joint call for research proposals, with the national funders FCT(Portugal), CNRS(France), and MEC(Spain).

048 Beneficial microorganisms and honeybees: colony level effects of lactic acid bacterial supplements

Stephan J.G.[1], Lamei S.[1,2], Pettis J.S.[3], Riesbeck K.[2], de Miranda J.R.[1], Forsgren E.[1]
[1] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2] Department of Translational Medicine, Lund University, Malmö, Sweden; [3] USDA ARS, Beltsville Agricultural Research Center-East, Beltsville, MD, USA

Paenibacillus larvae, causative agent of the lethal American Foulbrood (AFB) disease, is the primary bacterial pathogen affecting honeybees and beekeeping. The main current methods for controlling AFB diseased colonies are either enforced incineration or prophylactic antibiotic treatment, neither of which is fully satisfactory. The search for superior means for controlling AFB has led to an increased interest in the natural relationships between the pathogenic and mutualistic microorganisms of the honeybee microbiome, and in particular the antagonistic effects of Honeybee-Specific Lactic Acid Bacteria (hbs-LAB) against honeybee pathogens including *P. larvae*. These effects have so far only been demonstrated on individual larvae in controlled laboratory bioassays. Here we investigated whether supplemental administration of these bacteria had a similar beneficial effect on *P. larvae* infection at the colony level in two different experimental set-ups.
First, we monitored treated and untreated colonies in AFB-affected and unaffected apiaries in Sweden throughout a season. The results showed that, over the entire season, the hbs-LAB supplements did not affect either colony-level hbs-LAB composition or P. larvae spore levels. Hbs-LAB composition was, however, more diverse in apiaries with a history of clinical AFB, although again this was unrelated to colony-level *P. larvae* spore levels.
A second colony-level experiment investigated whether supplemental administration of hbs-LAB had a beneficial effect on *P. larvae* infection by comparing experimentally AFB-infected colonies treated with bacterial supplements to untreated colonies and antibiotic (tylosin)-treated colonies, recording AFB symptoms, bacterial spore levels and various measures of colony health and performance. The results showed that tylosin mitigated AFB disease symptoms but did not affect *P. larvae* spore levels while the hbs-LAB supplements had no effect on AFB symptoms, *P. larvae* spore levels or colony strength.
These results do not contradict the antagonistic effects from hbs-LAB observed at the individual level but rather suggest that supplementary administration of live bacteria may not be the most effective way to harness such effects in a useful application.

049 COLOSS (prevention of honey bee Colony LOSSes): past forward

Neumann P.[1,2], Williams G.[3], Chantawannakul P.[4], The COLOSS consortium[5]
[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Agroscope, Swiss Bee Research Centre, Bern, Switzerland; [3] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA; [4] Bee Protection Center, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; [5] 1068 scientists from 95 countries (13.03.2018, www.coloss.org)

COLOSS (prevention of honey bee Colony LOSSes) emerged in 2008 as a COST Action and has now developed into a global, non-profit scientist association. COLOSS is dedicated to improving the well-being of honey bees by:
1) development of standard research methods;
2) coordinating and conducting large-scale honey bee monitoring and research projects;
3) disseminating knowledge and providing training related to the needs of bees and;
4) advocating for honey bees, and their conservation, especially to government legislators and administrators.
COLOSS addresses key factors affecting honey bee well-being, including: pests & pathogens (e.g. mites *Varroa destructor* and viruses); environment (e.g. pesticides, nutrition) as well as breeding & conservation (e.g. genetic diversity, disease resistance).
Core Projects and initiatives involving the entire network include international monitoring of colony losses, production of the BEEBOOK (manual of standard research methods) and improvement of beekeeping sustainability through dedicated research and targeted extension (B-RAP: Bridging Research and Practice).

The talk will provide an overview on our COLOSS achievements, as well as ongoing activities and future plans of our Core Projects and Task Forces (e.g. Apitox, CSI [Citizen Science Investigation] Pollen, Small Hive Beetles, Survivors, Sustainable Bee Breeding, *Varroa* Control, *Vespa velutina*, and viruses).

050 10 years of coordinated study of honey bee colony loss rates – presentation of a COLOSS core project

Brodschneider R.[1], Gray A.J.[2], COLOSS C.P.[3]
[1] University of Graz, Institute of Biology, Graz, Austria; [2] University of Strathclyde, Department of Mathematics and Statistics, Glasgow, UK; [3] International consortium of more than 30 researchers active in the last few years

The COLOSS history of collecting and analyzing data on honey bee colony losses extends back to 2009, when a coordinated approach involving twelve European countries was undertaken to better understand colony losses. This group developed a standardized questionnaire to ask beekeepers about their hive management and overwintering success. This crowd-sourcing approach expanded to more countries over time and as of recent years is now regularly applied through annual surveys in about 30 countries, including some from outside Europe. The response rate varies greatly between countries, being more than ten percent of beekeepers in several countries. For winter 2016/17, for example, 27 European countries plus Algeria, Israel and Mexico collected data from 14,813 beekeepers who collectively wintered 425,762 colonies. Colony losses can be divided into colonies lost as live colonies with unsolvable queen problems, colonies lost due to natural disaster and dead colonies (or empty hives) after winter. The sum of these three categories suggests an overall loss rate of 20.9% (95% confidence interval: 20.6-21.3%) of honey bee colonies during winter 2016/17, with marked differences among countries. The data obtained is used in single factor quasi-binomial GzLMs to model probability of loss. In several years we identified operation size as a risk factor for winter colony losses, with smaller operations experiencing higher losses than larger ones ($p<0.001$). On the other hand, overall analysis of the 2016/17 data showed that migratory beekeeping had no significant effect on the risk of winter loss, though there was an effect in several countries. At the conference first results for winter 2017/18 will be presented, from data which to a great extent were collected via a common online survey. These results will include the effects of several different forage sources.

051 Quantifying and modeling bee spatial strategies

Dubois T.[1], Pasquaretta C.[1], Gautrais J.[1], Dore A.[1,2], Aubert H.[2], Lihoreau M.[1]
[1] Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier, Toulouse, France; [2] LAAS-CNRS, University Paul Sabatier, Toulouse, France

For a bee, foraging on flowers represents a complex challenge that necessitates to achieve several spatial operations. A forager needs first to locate flowers, then to find solutions to efficiently move across a constellation of flower locations, and finally to accurately return to the nest location for unloading its food crop into the colony. Despite more than a century of research on the behaviour and cognition of these insects, how bees achieve these spatial feats remain poorly understood. This is primarily because it remains difficult to measure and analyse the foraging patterns of small flying animals that can often visit hundreds of different flowers and travel several kilometres within a single foraging event. Here, we will present experimental and simulated data on the spatial foraging strategies of a model species: the buff-tailed bumblebee. Based on manipulative experiments in the lab and in the filed, we will show how one or multiple bees tend to establish predictable foraging routes as they gain experience with their environments based on spatial learning and memory. We will then illustrate how experimental data obtained by optical and radar technologies can be used to develop models and run simulations that can help unravel the cognitive mechanisms guiding bee complex spatial behaviour. Finally we will discuss how this fundamental research on the basic spatial strategies of bees can ultimately bring new insights about pollination and community ecology.

052 Design of a spatially explicit individual-based honeybee colony model

Wallis D.[1] Hatjina F.[2] Jensen A.[3], Simon-Delso N.[3], Skov F.[1], Topping C.[1]
[1]Institute for Bioscience, Aarhus University, Rønde, Denmark; [2] Division of Apiculture, Institute of Animal Science, Hellenic Agricultural Organisation 'DEMETER', Nea Moudania, Greece; [3] Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

We introduce a design concept for a honeybee colony model that is being developed for the European Food Safety Authority's MUST-B project, which contributes towards an integrated environmental risk assessment of multiple stressors on bees. Our model uses a spatially explicit individual-based approach. Overall behaviour of the colony system emerges from the physical and biological processes within the colony. Bee behaviour is not controlled at a system level. In our model, individual bees respond to their own needs, instincts, and to their immediate environment, both within the hive and in the wider landscape. The behaviour of the system as a whole is governed, therefore, by the decisions made by individuals within the colony. Parasites and infections are also modelled at a spatially explicit individual-based level.
Many of the physical processes within the hive (evaporation of nectar for example), and the decisions made by individual bees, depend on temperature. Accounting for the honey budget over an annual cycle has a strong temperature dependency also. We calculate the temperature of each bee within the hive, and the temperature of each cell in the honeycomb at each model timestep. We extend the individual-based approach to the thermodynamics model. Temperatures are calculated by modelling a coupled thermal network linking each honeycomb cell and honeybee. The system is analogous to an electrical circuit, where capacitors represent thermal units, and resistors represent the thermal connections between them. This generates a system of equations which is solved to find the temperature of each node within the network. A similar system is used to model trophallaxis in the food distribution calculation.
Our model will track pesticide levels from application, to individual storage cells within the hive, and then to the effects on individual bees and colony behaviour. There is a large variation in pesticide levels between storage cells in the hive. Pooling the pesticide level in a single system variable fails to predict observable adverse effects. Much more realistic behaviour is observed, however, with a spatially explicit individual-based bee model and discrete storage cells.

053 Where do wild bees want to live? Biodiversity in agricultural landscapes

Kratschmer S.[1], Ockermüller E.[1], Neumayer J.[1], Hainz-Renetzeder C.[2], Frank T.[3], Pascher K.[3], Pachinger B.[1]
[1] Institute of Integrative Nature Conservation Research, University of Natural Resources and Life Science (BOKU), Vienna, Austria; [2] Institute of Landscape Development, Recreation and Conservation Planning (ILEN), University of Natural Resources and Life Sciences (BOKU), Vienna, Austria; [3] Institute for Zoology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Wild bees provide a valuable ecosystem service by pollinating crops and wild plants in agricultural landscapes. Agricultural intensification is known to be a major driver for the decline of wild bees. The project BINATS 2 (BIodiversity–NATure–Safety) assesses the biodiversity of representative indicators in Austrian agrarian regions. Wild bees are recorded additionally to the indicators habitat structure, vascular plants, grasshoppers, and butterflies in 100 test areas.
During two consecutive years, these indicators are sampled in 50 test areas in maize (2017) and 50 test areas in oil-seed rape cultivation regions (2018). Within each of the 625x625 m test areas habitat structures are mapped. Plant and animal indicators are recorded in ten randomly selected test circles (40 m diameter) per test area. Wild bees are sampled by a semi-quantitative method on two center crossing 40x2 m transects in each test circle.
The wild bee fauna is assessed once per test area, which enables a snapshot of the species inventory during maize and oil seed rape florescence, respectively. Additionally, 30 of the 100 test areas are sampled three more times between April and August during the survey year to evaluate the situation of wild bees in the Austrian agrarian region.
In 2017, we recorded 192 wild bee species in the Austrian maize cultivation region. Common species were *Andrena flavipes*, *Bombus lapidarius*, *B. lucorum/terrestris*, *Halictus simplex* and *Lasioglossum pauxillum*. We also observed rare

species like *Pseudapis diversipes*, *Halictus tectus* and *Lasioglossum pressithorax*, a new species for Austria. Our data will further identify relevant habitat structures which enhance wild bee diversity in agro-environments and their occurrence in different agricultural land-use systems. Furthermore, selected measures implemented under the Austrian Agri-environmental Scheme (e.g. biodiversity areas) which should enhance wild bee diversity in agricultural areas will be evaluated.

054 Community structure of bees associated with different types of floral margins

Pérez-Marcos M.[1], Ortiz-Sánchez F.J.[2], López-Gallego E.[1], Ramírez-Soria M.J.[1], Sanchez J.A.[1]
[1] Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Murcia, Spain; [2] Grupo de Investigación "Transferencia de I+D en el Área de Recursos Naturales, Universidad de Almería, La Cañada de San Urbano, Almería, Spain

Bee communities in areas of intensive agriculture have experienced a great reduction in abundance and diversity due to habitat destruction. It has been shown that the addition of plant edges around crop fields may improve pollinators. So, the present study focuses on the composition of the bee communities in different types of floral margins in order to find which ones enhance abundance and diversity of this entomological group. This work was conducted in four farms located in south-eastern Spain during 2014 and 2015. Two kinds of field margins were used: arbustive (mainly plants of the family *Lamiaceae*) and herbaceous (mixture of herbaceous plants belonging to the *Apiaceae*, *Asteraceae*, *Boraginaceae*, *Brassicaceae*, *Caryophyllaceae*, *Fabaceae* and *Lamiaceae* families). Controls were established in no revegetated edges. The margins were sampled periodically from February to July to determine the abundance and diversity of bees visiting each one. The global bee assemblage studied was constituted by five families, being the most abundant Apidae (62.86%), followed by *Andrenidae* (13.90%), *Megachilidae* (13.18%), *Halictidae* (6.14%), and finally, *Colletidae* (3.82%). The hedge with the highest number of bees was the herbaceous one with 4,356 individuals, followed by the arbustive margin with 3,312 individuals and the control, where 1,049 individuals were observed. The bee community structure was significantly different among hedges. Moreover, some bee families were found to be closely associated to particular plant families. This work emphasises the interest and the importance of choosing the right plants composition for field edges to provide resources for the conservation of bee communities and pollinators in general.

055 *Not the perfect match? Biogeography and pollinator shifts rather than cospeciation dominate the evolutionary history of Rediviva bees and their Diascia host plants in the Cape biodiversity hotspot*

Kahnt B.[1,2], Hattingh W.N.[3], Theodorou P.[1,2], Wieseke N.[4], Kuhlmann M.[5], Glennon K.L.[3], van der Niet T.[6], Paxton R.J.[1,2], Cron G.V.[3]
[1] Martin-Luther-University Halle-Wittenberg, General Zoology, Halle (Saale), Germany; [2] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany; [3] University of the Witwatersrand, School of Animal, Plant and Environmental Sciences, Gauteng, South Africa; [4] University of Leipzig, Institute for Informatics, Leipzig, Germany; [5] Kiel University, Zoological Museum, Kiel, Germany; [6] University of Kwazulu-Natal, School of Life Sciences, Pietermaritzburg, South Africa

So far we still know little about the distinct processes that shape the evolution and diversification of biological interactions between pollinators and their host plants. Understanding the evolutionary processes underlying plant-pollinator interactions is particularly interesting in biodiversity hotspots such as the Greater Cape Floristic Region (GCFR) due to the increased richness and endemism of plant and pollinator species. We studied a bizarre and putatively coevolved pollination system in the GCFR biodiversity hotspot: long-legged *Rediviva* bees and their long-spurred *Diascia* hosts. To do so, we reconstructed the phylogenies for *Rediviva* and *Diascia* and computed interaction network modules. We then assessed the strength of cophylogenetic signal between *Rediviva* and *Diascia* considering either all interactions within network modules (nm data) or only interactions with the main interaction partner (th data). Distance-based analyses (Parafit, PACo) indicated significant phylogenetic congruence ($P < 0.05$) for the nm dataset but yielded mixed results for

the th dataset. However, phylogenetic congruence was not correlated with ecological dissimilarity for either *Rediviva* or *Diascia* (Mantel test $P > 0.05$), as would be expected under cospeciation. Congruence might therefore result from alternative processes such as vicariance. Furthermore, event-based reconciliation (CoRe-PA, Jane, CoRe-ILP) using the nm or th dataset also revealed only few (≤ 8) cospeciation events. In contrast, sorting events (≥ 12) and host switching events (≥ 5) were frequently inferred. Hence, our study suggests that host switching and biogeographic events in combination with the unique climate of the region are the key to understand the evolution of *Rediviva-Diascia* interactions, and that co-evolution may be less important than previously thought.

056 Bumble-BEEHAVE: an agent-based population model for bumble bees and its application as a decision tool for land managers

Becher M.A.[1,2], Twiston-Davies G.[2], Osborne J.L. [2]
[1] The Fields Institute for Research in Mathematical Sciences, Toronto, Canada; [2] Environment & Sustainability Institute, University of Exeter, Penryn Campus, Penryn, Cornwall, UK

Objectives: The decline in abundance and species-richness of bumblebees raises serious concerns as they are important pollinators for crops and wild flowers. While honeybees have been studied for a long time, resulting in a plethora of data as well as models on colony dynamics, foraging behaviour, and host-parasite interactions data on bumblebees is comparatively scarce and few models are available.
Nevertheless, a bumblebee population model that is based on individual behaviour, and resulting in colony dynamics could be immensely useful to improve our understanding of the threats and stressors bumblebees face in the wild.
Material and Methods: Here we present our new bumblebee population model Bumble-BEEHAVE. The model simulates multiple bumblebee species in a spatially-explicit landscape. Bees, represented as individuals or as cohorts, decide about their activity, using a stimulus-threshold approach. Activities include egg laying, foraging for nectar and pollen, and brood care. Successful colonies will produce new queens and males. Stressors such as parasites, predators and pesticides, can, to some degree, be included.
Results and Conclusions: Bumble-BEEHAVE can be used to predict and identify the variables associated with bumblebee colony success. Beesteward, a modified version of Bumble-BEEHAVE, was then designed to be specifically used by farmers, land managers and land advisors as a management tool for the conservation of pollinators in agricultural landscapes. The model will be freely available to download from http://beehave-model.net/.

057 Mitogenome sequencing to study introgression events between close related subspecies of *Bombus terrestris* in the Iberian peninsula

Cejas D.[1], López-López A.[1,2], Ornosa C.[3], De la Rúa P.[1], Muñoz I.[1]
[1] Universidad de Murcia, Departamento de Zoología y Antropología Física, Murcia, Spain; [2] Universidad Miguel Hernández, departamento de Agroquímica y Medio Ambiente, Elche, Spain; [3] Universidad Complutense de Madrid, departamento de Biodiversidad, Ecología y Evolución, Madrid, Spain

Two of the nine *Bombus terrestris* (Linnaeus, 1758) subspecies present in Europe can be found in the Iberian Peninsula: the endemic *B. t. lusitanicus Krüger*, 1956 and *B. t. terrestris*, which is naturally distributed from centre Europe to the Pyrenees. However, *B. t. terrestris* can also be found in the southern half of the peninsula due to escapes from greenhouses. The taxonomic identity of these two subspecies is based on morphological traits, and traditional molecular approaches such as DNA barcoding do not discriminate between them. Here we present their mitogenome sequences as a molecular framework to test possible introgression events between both subspecies that may affect the biodiversity of this important pollinator.
B. t. terrestris individuals were sampled in northern France (Normandy) and *B. t. lusitanicus* individuals were from centre and northern Spain (Burgos, Soria and Vizcaya). Morphologically identified individuals of each subspecies were grouped into subspecific pools and their mitogenomes were sequenced and assembled. The sequence of the 13 protein-coding genes (PCGs), the two ribosomal RNAs and the 22 transfer RNA genes were obtained for the two subspecies. Only 20

point-mutations were detected between both subspecies from the sequenced mitochondrial genomes (~17.000 bp). According to this information, a set of mitogenomic fragments were selected to be tested as diagnostic features to either subspecies in individuals from Spain and France. Two different 16S haplotypes discriminating *B. t. terrestris* and *B. t. lusitanicus* were observed in the Iberian populations, albeit both haplotypes were present in both morphological subspecies. Our results suggest introgression events in the intergradation area between both subspecies, and also from commercial bumblebees. This molecular approach provides a tool to study the introduction of non-endemic subspecies and the spread of reared populations into wild Iberian ones.

058 Buzzing in the buckwheat – a diverse pollinator community brings more grains to the table

Korpela E-L.[1], Hokkanen H.[2], Jaš S.[1], Martikkala M.[1], Toratti S.[2]
[1] Finnish Beekeepers´Association, Helsinki, Finland; [2] Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland

The main producers of buckwheat (*Fagopyrum esculentum*) are China and Russia. In Finland this grain crop is cultivated on 2000+ hectares. Its importance is likely to grow, however, as consumers are becoming more and more conscious about healthy food choices. Buckwheat seeds are rich in proteins, dietary fibre and antioxidants. Moreover, they are gluten-free and thus suitable for people with celiac disease.
We studied buckwheat pollination in southern Finland in summer 2017. The study plot (30 m x 150 m) was sown in mid-June and there were four experimental treatments: FP = free pollination (no pollination cage), OC = open pollination cage, CC = closed pollination cage (excluding all pollinators) and HC = closed cage with a honeybee colony inside (forced honeybee pollination). The study set-up was a randomized block design with four study blocks. The pollination cages were pitched and honeybee colonies for HC brought in just before flowering started in late July. In addition, one colony was placed at field edge to perform FP. Here, we wanted to see, whether the pollination outcome differs between the "honeybee as the sole pollinator" -situation and the "naturally occurring pollinators together with honeybees" -situtation. Pollinators were monitored by transect counts six times during the experiment.
In buckwheat, a major part of the yield is formed during early flowering. During this period 59 % of observed pollinator individuals in transect counts were honeybees. Other groups were bumblebees (18 %), syprhid flies (5 %), other *Diptera* (12 %) and butterflies (6 %).
Buckwheat yield varied between the treatments as follows: 82,2 g/m2 in FP, 71,8 g/m2 in OC, 3,7 g/m2 in CC and 42,7 g/m2 in HC. Yield was significantly higher in free pollination (FP) than in forced honey bee pollination (HC). The OC treatment seemed to slightly interfere with yield formation, but the difference with FP was non-significant. The CC treatment produced the poorest result, which is to expected in an insect-pollinated crop.
Our results highlight the importance of maintaining and supporting diverse pollinator communities in buckwheat production areas. We would also like to encourage paid pollination agreements between farmers and beekeepers.

059 *In vivo* imaging of neonicotinoid influence on odour processing in the honeybee brain

Andrione M.[1], Cabirol A.[1], Fayolle M.[1], Zanini D.[1], Zanon M.[2], Vallortigara G.[1], Antolini R.[1,2], Haase A.[2]
[1] University of Trento, Center for Mind/Brain Sciences, Rovereto, Italy; [2] University of Trento, Department of Physics, Trento, Italy

Exposure to neonicotinoids is considered one of the possible causes of honeybee population decline. At sublethal doses, these pesticides have been shown to negatively affect a number of behaviours, many of those involve the olfactory system. One of the most studied behaviours is associative odour learning using the Proboscis extension reflex (PER) paradigm. Studies show consistently an impairment of animals' performance after neonicotinoid exposure. This leads to the general conclusion that odour memory is impaired by these pesticides. Here we present a study that aims at a

more precise identification of the mechanisms causing the observed changes in honeybee behaviour. We, therefore, follow the neuronal responses to odour stimuli through the honeybee brain by in vivo two-photon imaging. This allows identifying the bottleneck along the odour information processing pathway which is most sensitive to neonicotinoid exposure. We can thus differentiate whether the behavioural changes are indeed caused by an impairment of learning or memory retrieval or whether already further upstream processes like odour reception, odour coding, or odour evaluation are the principal cause for the altered performance.

060 Honey bee waggle dance communication benefits pollen foraging

Nürnberger F.[1], Härtel S.[1], Keller A.[2], Steffan-Dewenter I.[1]
[1] Department of Animal Ecology and Tropical Biology, [2] Department of Bioinformatics, Würzburg, Germany

The waggle dance communication is a unique behaviour in honey bees that conveys information on the spatial position of rewarding resource patches. Although it is widely assumed to play a major role in honey bee foraging success, its actual relevance under natural conditions is still not well understood. Particularly little is known on the role of dance communication in pollen foraging. We investigated the benefits of instructive dance communication for nectar and pollen foraging in *Apis mellifera* under different natural foraging conditions within a temperate zone environment.
24 *Apis mellifera* colonies with either disrupted or unimpaired instructive component of dance communication were placed into eight Central European agricultural landscapes that differed in heterogeneity and resource availability. We monitored colony weight change and pollen harvest as measure of foraging success. Next generation sequencing was used to identify pollen species collected by foragers.
Irrespective of landscape characteristics, dance disruption did not alter colony weight change, but decreased pollen harvest compared to communicating colonies by 40%. Further, instructive dance communication stabilized diversity of collected pollen independent of local resource distribution.
Our results indicate that in temperate regions the dance communication benefits pollen foraging more than nectar foraging and might play an important role in ensuring a diverse pollen diet. We conclude that the so far largely unexplored role of dance communication in pollen foraging requires further consideration as pollen is a crucial resource and a diverse pollen diet is important for colony development and health.

061 Observations of honey bee wing movements with the use of a high-speed camera

Łopuch S., Tofilski A.
Department of Pomology and Apiculture, Agricultural University, Krakow, Poland

Honey bees use acoustic signals to communicate and coordinate different activities in the nest. Those signals play an important role in some bee behaviours including queen and worker piping or waggle dance. The acoustic signals are generated by the thoracic muscles producing vibrations transmitted to substrate or transferred by moving wings as airborne sounds. Detection of vibrations or sounds using microphones can be difficult because of noise in the nest.
The aim of this study was to describe wing movements occurring during social interactions between bees. High-speed video recording was used for observation of wing movements. Behaviours of three colonies of honey bees housed in observation hives were recorded using a high-speed camera.
Wing beating was observed in queens, workers and drones in many social contexts. Some of behaviours, for instance wing beating of young queen during swarming, were reported earlier. However, many of behaviours were observed for the first time including: drones moving their wings while leaving the nest for mating flights or in a colony with drone-laying queen, and workers vibrating their wings in the presence of a queen outside of the process of swarming, in turns with a vibration signal or while evicting drones from the nest. Importantly, wing beating significantly differed among castes and behaviours. Queens moved their wings with higher frequency, performing longer wing-beating puls-

es than drones and workers ($p<0.05$). Drones vibrated their wings with a mean frequency similar to workers ($p>0.05$), but they performed shorter pulses of wing beating ($p<0.05$). In workers, the mean frequency of wing beating and the mean duration of wing-beating pulses were different between various behaviours ($p<0.05$).
High-speed video recording revealed that wing beating was performed by bees in many different social contexts. Wing beating differed in frequency and pulse duration, which indicates that it may be used for information transfer.

062 The effect of absolute and relative amounts of omega-3 and omega-6 essential fatty acids on associative learning in honey bees

Arien Y.[1], Dag A.[2], Shafir S.[1]
[1] B.Triwaks Bee Research Center, Department of Entomology, Robert H. Smith Faculty of Agriculture, Food & Environment, The Hebrew University of Jerusalem, Rehovot, Israel; [2] Gilat Research Center, Institute of Plant Sciences, Agricultural Research Organization, Negev, Israel

Pollen is an essential component in honey bee nutrition. It is the main source for protein but also provides other nutrients, including fatty acids. Pollens of different plants differ in fatty acid content and composition. In modern agriculture, bees are placed in large areas of monoculture (single-species crops), where bees forage on an unbalanced pollen diet that could lead to malnutrition.
In mammals, the importance of essential omega-3 fatty acids is well known, and deficiency of omega-3 fatty acids, mainly long-chain polyunsaturated fatty acids (PUFAs), is associated with several mental and cognitive disorders. In insects, on the other hand, the effect of deficiency in omega-3 has not yet been studied. We are studying the importance of essential omega-3 fatty acids in honey bee nutrition. We have recently shown, via proboscis extension response (PER) assays, that omega-3-poor diets impair honey bee olfactory and tactile associative conditioning. In one experiment, bees were fed artificial diets enriched with vegetable oils that were either rich or poor in omega-3, or mixed bee-collected pollen pellets, rich in omega-3. In another experiment, bees were fed Eucalyptus pollen pellets, which are poor in omega-3, or a pollen mixture rich in omega-3.
In further work, we investigated whether the cognitive impairment is due to low absolute amounts of omega- 3 or to a high omega-6:3 ratio in the diet. We fed bees one of 12 diets, which had total lipid concentration of 1%, 2%, 4% or 8%, with omega-6:3 ratios of 0.3, 1, or 5. When tested in a PER conditioning assay, bees fed an omega-6:3 ratio of 5 showed consistently low levels of performance. Best performance was achieved by bees fed an omega-6:3 ratio of 1, with 4% total lipid concentration. Our results with honey bees are consistent with those with mammals, showing that proper cognition requires sufficient absolute amounts of omega-3 in the diet, but also a sufficiently low omega-6:3 ratio.

063 Chemical Communication and improved tools for hygienic selection in the honey bee, *Apis mellifera*

Wagoner K.[1], Spivak M.[2], Millar J.[3], Schal C.[4], Rueppell O.[1]
[1] Department of Biology, University of North Carolina at Greensboro, Greensboro, NC, USA; [2] Department of Entomology, University of Minnesota, St. Paul, MN, USA; [3] Department of Entomology, University of California at Riverside, Riverside, CA, USA; [4] Department of Entomology & Plant Pathology, North Carolina State University, Raleigh, NC, USA

The health of the honey bee *Apis mellifera* is currently being challenged by several natural and anthropogenic threats. Among these are the ectoparasitic mite *Varroa destructor*, and the numerous harmful pathogens it vectors to its honey bee host. While many *Varroa* control methods have been developed, constraints such as limited uptake and the evolution of resistance to chemical interventions have resulted in little progress, such that *Varroa* remain a critical threat to honey bee health today. One promising avenue for improved intervention is the breeding of "hygienic" honey bees, capable of detecting and removing brood that is parasitized or otherwise unhealthy. In an effort to improve hygienic selection methods and expand our understanding of the role of brood in hygienic behavior, we investigated honey bee cuticular chemicals associated with unhealthy brood. We identified multiple hydrocarbons that are elevated in unhealthy brood, as well as in brood targeted for hygienic removal. In bioassays with synthesized hydrocarbon standards,

we demonstrated that specific compounds could be applied to wax caps to induce hygienic removal. There was a significant positive correlation between hygienic responses in our assays and the traditional liquid nitrogen-killed brood assay. These findings expand our understanding of honey bee chemical communication and suggest that an assay based on natural honey bee brood chemicals may serve as an improved indicator of colony hygiene level. The goal of this work is to develop improved tools for colony monitoring and hygienic selection, and thus improve resistance of honey bees to *Varroa* and associated pathogens.

064 Antibiotics, sex and social regulation - the role of C10 short chain fatty acids in honey bee biology

Crewe R.M.[1], Moritz R.F.A.[1,2]
[1] University of Pretoria, Social Insects Research Group, Pretoria, South Africa; [2] Martin-Luther-University Halle-Wittenberg, Molecular Ecology, Halle, Germany

A suite of C10 short chain fatty acids have been identified in the mandibular glands of the female castes of honey bees. The particular blend of these fatty acids is expressed in a caste specific manner and the components of these blends have specific functions related to honey bee social organization.
The role of the 10-hydroxy C10 acids in workers will be described in relation to their use as an antibiotic in larval food and as well as a key factor in determining the viscosity of royal jelly and its role in the rearing of queen larvae. The role of the 9-hydroxy C10 acids in queens will be explored in relation to their functions as a sex pheromone and social regulator of worker reproductive capacity. Furthermore, we will explore the ability of workers to switch the biosynthesis of the fatty acids in their mandibular glands from 10-hydroxy to 9-hydroxy C10 acids with the associated ability to act as false queens and intraspecific social parasites.
This exploration of the roles of the C10 short chain fatty acids will lead us to a consideration of the evolution of sex pheromones and social regulatory pheromones in honey bees.

065 Exposure of Nasonov gland by flying foragers of honey bee

Tofilski A., Żmuda A.
Department of Pomology and Apiculture, Agricultural University, Krakow, Poland

Honey bee workers expose Nasonov gland while standing next to an attractive food source. It was suggested that workers expose this gland also during flight, however, so far this was not convincingly confirmed. Observations of flying bees is difficult because of relatively high flight speed. Conventional cameras record less than 30 frames per second and in many circumstances image quality is not good enough for determination if the Nasonov gland was exposed in flight or not. In order to solve this problem we have used high speed camera which records 1200 frames per second at resolution 800x600 pixels.
Honey bee workers were trained to visit a food source by transferring them from the nest entrance to the food source. If the worker returned to the food source it was marked with white pain and other workers visiting the food source were collected and stored in a cage until the end of day. The food source consisted of diluted honey At the time of the observations natural sources of food were scarce and the experimental food source was very attractive to bees. The food source was located at the end of a tunnel. In order to reach the food workers were flying through the tunnel because there were obstacles on the walls which discouraged them from walking. We have recorded behaviour of bee in the tunnel at distance of one meter from the food source.
We have made 41 recordings of 13 workers during their flight to the food source. In 36 of those recording Nasonov gland was exposed. We have also made 23 recordings of workers during their flight back to the nest. In none of these recordings Nasonov gland was exposed. The results presented here are the first direct evidence that honey bee workers expose Nasonov gland during flight to a food source. This behaviour can be an important element of honey bee recruitment system.

066 Eat me or not- deciphering the molecular signals of diploid drone selection in the honey bee *Apis mellifera*

Gessler B., Hasselmann M.
Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany

Social insects have gained many novel morphological and behavioral traits relative to their solitary ancestors. One remarkable behavior of the western honey bee *Apis mellifera* is the cannibalism of diploid drone larvae at early developmental stages. Diploid drones differ in their homozygous genotype for the gene complementary sex determiner (csd) compared to diploid females (heterozygous) and haploid drones (hemizygous). Quantitative differences of larval cuticular substances are proposed to be recognized by worker bees; however the molecular signals and mechanisms are still unknown.
We want to test the hypothesis that the regulatory signaling of the sex determination pathway affects the synthesis of cuticular substances in honey bee larvae. We expect to detect differences in gene expression and cuticular substances among developmental stages in diploid females, haploid males and diploid males.
Sex specific cuticular extracts of honey bee larvae have been detected in preliminary Bioassays. Analysis of cuticular extracts via mass spectrometry showed differences between the sexes of interest. Finally the results of transcriptome analysis using RNA-seq of diploid females and diploid males detect novel expression pattern at young larval stages in Apis mellifera.
Consequently, our study combine for the first time molecular analysis and biochemical analysis to investigate the differences between the sexes in young larvae to elucidate the cannibalism behaviour of diploid drones in *Apis mellifera*.

067 Comparison of hygienic behavior directed against *Varroa destructor* and *Tropilaelaps* sp.

Shrestha M.[1,2], Wegener J.[1], Gautam I.[2], Bienefeld K.[1]
[1] Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Hohen Neuendorf, Germany; [2] *Natural History Museum of Nepal, Swayambhu, Manjushree Bazaar, Kathmandu, Nepal*

While the European beekeeping industry is still struggling to sustainably manage *Varroa destructor*, beekeepers in parts of Asia are additionally confronted with mites of the genus *Tropilaelaps*, which also reproduce on the brood and can infest both *Apis cerana* and *Apis mellifera*. Their spread into Europe is regarded as possible. Brood hygiene is at the center of breeding programs for Varroa resistance. Within the EU-project SmartBees, we asked whether this approach could also confer resistance to Tropilaelaps, by comparing the frequency of hygienic behavior directed against the two mites in *A. mellifera*. Additionally, we compared *A. mellifera* and *A. cerana* with regard to their removal of *Tropilaelaps*-infested brood. The experiment was carried out in Nepal, where both mites are autochthonous. Groups of 1.800 - 2.000 individually-marked workers were placed on brood that was artificially infested with the two types of mites. They were observed with an infrared camera to identify the bees opening infested cells. The experiment was repeated 5x with *Mellifera*-bees, 3x with *Cerana*-bees, and 5x with a mix of both. *A. mellifera* showed capable also of detecting *Tropilaelaps*-infested brood, albeit at a lower rate compared to *Varroa*-infested brood (20% vs. 51% of opened cells; $P < 0.01$). *A. cerana*-workers removed *Tropilaelaps*-infested brood significantly more efficiently than *A. mellifera*-workers (36% vs. 20%; $P < 0.01$) when observed in monospecific groups. In mixed groups however, hygienic behavior was almost exclusively performed by *A. mellifera*. Our results show that selection of Asian and Western honeybees for hygienic behavior could be a promising strategy also with regard to the threat by *Tropilaelaps*. As the same specialist individuals often carried out hygienic behavior on brood infested by both types of mites, the genetic basis might be similar in both cases. Our results also throw an interesting light on task specialization within inter-specific groups of worker bees.

068 Explaining the resistance to *Varroa destructor* in the French honey bee population with VSH, SMR and colony dynamic data

Eynard S.E.[1], Basso B.[2], Guirao A-L.[2], Mondet F.[3], Vignal A.[1], Servin B.[1]
[1] GenPhySE, INRA, Castanet Tolosan, France; [2] ITSAP, Avignon, France; [3] Abeilles et Environnement, INRA, Avignon, France

Honey bees in Europe, *Apis mellifera*, are under the threat of *Varroa destructor* infestation. Three main phenotypes can be measured on the colony to inform on its resistance toward such infestation: *Varroa*-sensitive hygiene (VSH), suppressed mite reproduction (SMR) and *Varroa* mite infestation dynamic. The first, VSH, is related to a behaviour involving the detection and the cleaning of infested brood cell by worker bees. The second, SMR, informs on the colony load in reproductive *Varroa* mites within brood cells. The last one, estimates changes in *Varroa* mite infestation level through time. For the present study 60 colonies were followed in 2016 and 60 more in 2017. The dynamics of the colonies were estimated throughout the year by eight evaluations of the number of bees in the colony, its resource status in honey and pollen as well as growth success by counting the number of occupied brood cells. Up to three SMR scores were assigned to each colony, giving an estimation of reproductive versus non reproductive varroa mite load in the brood cells. Moreover, VSH was tested using the classical protocol: counting the number of open infested brood cells after placing an infested frame within the tested colony for seven days. Finally, phoretic *Varroa* mite load, *Varroa* mite infestation of brood cells and *Varroa* mite fall were measured multiple times throughout the experimental period as a proxy for total varroa mite infestation. Complementary phenotypes, relevant for beekeeping, such as gentleness, swarming tendency, hygienic behaviour, and production were also measured. The aim of such study is to correlate dynamics of the colonies and varroa mite resistance phenotypes to improve models of *Varroa* mite infestation and help predict the success of colonies through time. Ultimately, our goal is to support the design of selection criteria that can be easily measured and therefore would be applicable in practice by beekeepers.

069 Preliminary researches regarding the efficiency of the formic acid treatment on varroa (*Varroa destructor*) found in the artificially decapped bee brood

Siceanu A., Căuia E., Vișan G.O., Căuia D.
Institute for Beekeeping Research and Development, Bucharest, Romania

The objective of the study was to establish the effect of formic acid on *Varroa*, found inside the capped brood cells, which were artificially decapped, based on a specific method, by scrapping, using a decapping fork, the treatment being done without affecting the brood. The method of decapping was described in a specific video film which can be found at the following YouTube address:
https://www.youtube.com/watch?v=qHq2woncbN4. The experiments were carried out in the autumn 2017 - spring 2018, on honeybee colonies infested with *Varroa* (*Varroa destructor*). The treatments were done with formic acid, impregnated in special card boards (150 mm X 170 mm X 4 mm). The method was applied on honeybee colonies as a whole as well as on bee brood combs, without bees, put in a special treatment box. The treatment in colonies was done with doses between 25 ml and 60 ml formic acid, of 60-65% concentration, the exposing time being between 14h and 36 h. The nocturnal temperatures and the evaporated quantity of formic acid were also registered during the experiments. The treatment applied in special boxes for bee brood was done with doses between 60 and 100ml formic acid of 66 and 85% concentration, the exposing time being between 10 min and 30 h. By this method the bees in the colonies were treated separately, in order to have a full treatment. The researches were focused on establishing the mortality level of *Varroa* in brood and the effect of formic acid on viability of capped bee brood, artificially decapped. The results show a high mortality of *Varroa*, especially for protonimfa and deutonimfa and the main conclusion is that the brood decapping method combined with formic acid could be a useful technique to control varroa infestation, both in brood and bees. The experiments were done in the frame of Smartbees project, grant 613960, funded by European Commission.

070 Hygenic behaviour, *Varroa* and *Nosema* levels of Africanized honeybee colonies in Alagoas State, Northeastern Brazil

Neto J.T.M., Almeida D.A., Lima E.G., Guimarães-Beelen P., Silva H.M., Beelen R.
Alagoas Federal University, Animal Science Department, Honeybee Lab, Maceió, Brazil

Honeybees play an important economic role in Northeastern Brazil. Beekeeping became a very promising activity in Alagoas State reducing the shortage of income and playing an important role in social inclusion. In the State's semiarid backcountry beekeeping is focused on honey production and on the coastline the main product is the newly discovered red propolis. Apiculture has been practiced locally with ecologically friendly techniques. Colonies do not receive any kind of treatment against parasites or diseases. So far, mortalities or pathologies have not been noticed. But on the other hand, apiaries are not officially registered, there is no accurate records on colony numbers and there are no governmental programs promoting surveys on bee health. The present study aimed at evaluating the hygienic behavior, *Varroa* and *Nosema* infestation in honeybee colonies in Alagoas State. Twenty-four colonies from six apiaries were sampled (3 in the semiarid region and 3 on the coast). Hygienic behavior (HB) was assessed using the pierced brood method. Bees (300 per colony) were collected and placed in vials containing alcohol at 70% and shipped to the lab for parasite evaluation. A piece of comb with capped brood was taken from each colony to screen *Varroa* on brood. HB ranged from 76.2 to 97.5%. Only two colonies presented a HB under 80%. *Varroa* mites were found in all colonies sampled. The degree of infestation varied from 0.2 to 14%, with an average infestation rate of 5.11%. These levels are very acceptable. *Nosema* spores were found in bees sampled in two apiaries only, but at low levels (330 spores/bee). It was not found any statistical difference in infestation between regions, but a significant difference was found between apiaries ($p<0,05$). Management practices might be associated to the higher levels of *Varroa* observed in some apiaries. In those apiaries beekeepers did not replace old combs nor supplemented their colonies. No significant correlation was found between HB and *Varroa* infestation of adult bees ($r2 = 0.0921$), but a negative significant correlation was found between HB and brood infestation ($r2 = - 0.8187$). The rusticity and HB of the Africanized honeybee seems to keep parasite levels under control.

071 Identification of the DNA variants associated with the hygienic trait of honey bees

Farajzadeh L.[1], Momeni J.[2], Nielsen R.[2], Wegener J.[3], Bienefeld K.[3], Bendixen C.[1]
[1] Department of Molecular Biology and Genetic, Aarhus University, Aarhus, Denmark; [2] GenoSkan A/S, Aarhus, Denmark; [3] Institute for Bee Research Hohen Neuendorf, Hohen Neuendorf, Germany

Varroa destructor is considered a worldwide threat to honeybee's health. The mite weakens infested individuals due to feeding on honeybee haemolymph but importantly acts as a vector of a range of honeybee viruses such as Deformed Wing Virus (DWV). Hygienic behaviour is the most promising defence behaviour against *Varroa* and can result in uncapping and removal of diseased or parasitized brood. The main objective of the study is to identify genomic loci regulating hygienic behaviour against *Varroa* and characterize genetic variants affecting the trait. To achieve this goal, worker bees were phenotyped for their ability to uncap *Varroa*–parasitized brood using a well-established bioassay (Marked individuals on combs containing artificially infested brood cells are observed for their behaviour). Honey bee families from 6 subspecies and crosses including *carnica*, *Caucasica*, *Macedonica*, *mellifera*, *macedonicaXcarnica*, and *melliferaXcarnica* were phenotyped. Pools of phenotyped worker bees were subjected to genetic analysis using a pool-sequencing approach. The pooling-scheme were as follows: Pools of Beginners (those bees initiating uncapping of infested cells), Helpers (continuers), super-beginners (involved in uncapping of more than one infested cell) and controls (observed at infested cells, but not involved in uncapping of *Varroa* parasitized brood cells). Analysis of the genomic sequences of the pools from each family successfully identified DNA variants including SNPs, CNVs, and INDELs, significantly associated with hygienic behaviour. Altogether, 93 genomic intervals with high association with hygienic behaviour were detected. Our work demonstrates the existence of genomic regions with a significant effect upon hygienic behaviour against *Varroa*. These data will provide new insight into the biology of *Varroa*-resistance as well as it will be instrumental in breeding efforts towards *Varroa*-resistant honey bees.

072 Combining elastic-net penalized regression and whole exome sequencing to identify phenotype-associated variants

Broeckx B.J.G.[1,], De Smet L.2, Blacquière T.[3], Maebe K.[4], Khalenkow M.[5], Van Poucke M.[1], Dahle B.[6,7], Neumann P.[8], Bach Nguyen K.[9], Smagghe G.[4], Deforce D.[10], Van Nieuwerburgh F.[10], Peelman L.[1,], de Graaf D.C.[2,5]
[1]Department of Nutrition, Genetics and Ethology, Ghent University, Merelbeke, Belgium; [2]Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium; [3]Wageningen University & Research, Wageningen, The Netherlands; [4]Department of Plants and Crops, Ghent University, Ghent, Belgium; [5]Honeybee Valley, Ghent University, Ghent, Belgium; [6]Norwegian Beekeepers Association, Kløfta, Norway; [7]Department of Animal and Apicultural Sciences, Norwegian University of Science and Technology, Ås, Norway; [8]Institute of Bee Health, University of Bern, Bern, Switzerland; [9]Department of Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium; [10]Department of Pharmaceutics, Ghent University, Ghent, Belgium

An often-used approach to identify phenotype-associated variants is the genome-wide association study (GWAS) that usually combines Single Nucleotide Polymorphism (SNP) arrays with single-variant marker tests. In this combined approach, it is usually only the marginal effect between a phenotype and a variant that is analyzed and, as such, it potentially ignores important information when multiple variants are associated with complex phenotypes while at the same time, with SNP arrays, generally not the causal mutations are genotyped directly, but tagSNPs that are in linkage disequilibrium with the causal SNP.
Here, we propose an alternative approach that combines targeted sequencing of all protein-coding regions, i.e. whole exome sequencing (WES), with elastic-net penalized regression. While WES allows the direct identification of phenotype-associated variants if they fall within the target regions, elastic-net penalized regression allows joint modelling of variants and combines the strengths of "least absolute shrinkage and selection operator" (LASSO) regression in terms of parameter selection and ridge regression to deal with correlated polymorphisms.
Based on the Amel.4.5 annotation, a WES design targeting 26,184,643 base pairs divided over 81,571 regions was developed and used to sequence 64 drones (Illumina NextSeq 500 PE75). Performance-wise, >97% of the 26Mb target base pairs were covered at ≥10x, more than 140 000 variants were discovered and the sequencing reproducibility was found to be high. Elastic-net penalized regression was used next to identify variants associated with reduced mite reproduction in drone brood. With the associated SNPs, we were able to predict close to 90% of the phenotypes correctly. Overall, we conclude that the newly developed WES design excels in terms of performance characteristics and have demonstrated its practical use by identifying variants associated with reduced mite reproduction in drone brood.

073 Progress in marker-assisted selection for honey bee breeding

Pernal S.F.[1], Borba, R.[1,5], Hoover S.E.[2], Currie R.W.[3], Guarna M.M.[1], Zayed A.[4], Foster L.J.[5]
[1] Agriculture and Agri-Food Canada, Beaverlodge Research Farm, Beaverlodge, Alberta, Canada; [2] Alberta Agriculture and Forestry, Lethbridge Agriculture Centre, Lethbridge, Alberta, Canada; [3] Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada; [4] Department of Biology, York University, Toronto, Ontario, Canada; [5] Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada

Most economically-desirable traits in honey bees show considerable levels of heritability and thus can be improved via artificial selection. The honey bee's high recombination rate requires that new approaches for identifying stable markers for selective breeding be considered. To that end, our team previously developed a novel approach to marker identification, notably the discovery of protein expression patterns that were highly correlated with specific behavioural traits, which were then used to enrich hygienic behaviour across several hundred colonies in Western Canada. This produced stock with improved disease resistance, Varroa tolerance, economic performance and winter survival.
Based on our previous success, we have now embarked on a large-scale study to combine proteomics and genome-wide association for identifying highly discriminant markers for bee breeding. The aim of our project is to measure 12 economically-valuable traits of honey bees (colony phenotypes) and develop genomic and proteomic markers that will enable beekeepers to rapidly select and breed healthy and productive colonies, well adapted to the Canadian climate.

In the first year of our study, 1025 colonies from across Canada were phenotyped for the following colony-level traits: Varroa mite population growth, grooming, hygienic behaviour, defensiveness, honey production, brood area, pathogen abundance, innate immunity, gut microbiota and overwintering success. As anticipated, significant correlations were found among similar productivity phenotypes such as fall and spring colony weights ($r2 = 0.8374$; $P < 0.001$), as well as between instantaneous and total honey production ($r2 = 0.6905$; $P < 0.001$). We are continuing to model and analyze these data to determine predictive relationships, which will be further discussed. Progress in identifying proteomic and SNP markers for economically-desirable traits will be reviewed along with implications for improved methods for trait selection.

074 A Canadian perspective on marker assisted selection and growing the local queen supply

Bixby M.[1], Guarna M.M.[1,2], Hoover S.[3], MacAfee A.[1], Higo H.[1], Pernal S.F.[2], Foster L.J.[1]
[1] Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada; [2] Agriculture & Agri-Food Canada, Beaverlodge Research Farm, Beaverlodge, AB, Canada; [3] Alberta Agriculture and Forestry, Lethbridge Agriculture Centre, Lethbridge, AB, Canada

Pathogen and parasites continue to affect honey bee health and are important factors in colony losses in North America and Europe. One method to reduce colony loss is through selective breeding of queens to produce disease resistant stock. Historically, the only method for identifying desirable traits in honey bees was through field evaluations. In a previous study, we identified proteins in bee antennae that can be used as markers of disease resistance and can be tested for in a laboratory. We also demonstrated that marker-assisted selection (MAS) based on protein markers can be used to produce hygienic, pathogen-resistant honey bee colonies.
Based on this research, we modeled the adoption of MAS in a bio-economic case study where a beekeeper's profit function and preliminary MAS cost and performance data was used to evaluate the economic impact of adopting colonies selected for hygienic behavior into an apiary. Our results showed a range of net profit gains for the beekeeper depending on parasite load and treatment. In our current Beeomics project, we are evaluating the efficacy of MAS for several economically-important traits including honey production. We are also examining the opportunities and challenges of increasing queen breeding and production in Canada. In two recent surveys of Canadian beekeepers and breeders we collected data to compare the demand and supply sectors for breeding in Canada. The survey responses highlight which traits are most desirable for selection, the willingness of beekeepers to purchase selected stock at a premium price and the barriers to increasing domestic queen production including the disparity between the needs of beekeepers for early spring queens and breeders ability to deliver given the long Canadian winters.
We will discuss the results of these investigations and present a Canadian perspective of real and perceived hurdles to stock selection and to the expansion of our queen breeding and production industry.

075 Unbiased random mutagenesis contributes to a better understanding of the virulent behaviour of *Paenibacillus larvae*

Descamps T.[1], De Smet L.[1], De Vos P.[2], de Graaf D.C.[1]
[1] Laboratory of Molecular Entomology and Bee Pathology, Faculty of Sciences, Ghent University, Ghent, Belgium; [2] Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium

American foulbrood, caused by the Gram-positive bacteria, *Paenibacillus larvae*, is one of the most severe bacterial diseases of the European honey bee. The bacterium has been known for long, but only the last decade the mechanisms used by the pathogen to cause disease in its host are starting to unravel. In this study, the knowledge of this virulent behaviour is expanded and several possible virulence factors are suggested.
Identification of possible virulence factors has been done by random mutagenesis to ensure an unbiased approach. A library of mutants was tested for a significant difference in virulence using in vitro exposure assays.

Different techniques were used to identify the disrupted genes but finally whole genome sequencing showed that the EZ-Tn5kanRR6Kl transposon was not present in the genome of any of our mutants. It is possible that the detected mutations are due to an unstable insertion of the EZTn5kanRR6Kl transposon or to spontaneous mutations. The affected loci were characterized and their potential to contribute in virulence of the pathogen was assessed.
The identified mutated loci dacB, dnaK, metN, ywqD, lysC, serC and gbpA are known to encode for virulence factors in other bacteria and are suggested to play a similar role in *P. larvae*.
The study identified new possible virulence factors for *P. larvae* genotype ERIC I in an unbiased way. This contributes to the knowledge and understanding of the possible mechanisms used by this pathogen to colonize and kill its host.

076 Canada-wide surveillance of exotic pathogens: *Lotmaria passim* and *Crithidia mellificae*

Borba R.S [1,2], Wolf-Veiga P.[3], Guarna M.M.[2], Pernal S.F.[2]
[1] Agriculture and Agri-Food Canada, Beaverlodge Research Farm, Beaverlodge, Canada; [2] University of British Columbia, Center for High-Throughput Biology, Vancouver, Canada; [3] Grande Prairie Regional College, National Bee Diagnostic Center, Beaverlodge, Canada

The presence of high levels of pathogens and/or parasites in honey bee colonies is one of the most prominent factors affecting bee health and contributing to colony losses. Every year, Canadian beekeepers import about 250,000 queens, mainly from the US, as well as package bees from Australia and New Zealand. The importations of foreign queens and bees have the potential to introduce new pathogens into the Canadian honey bee population, such as the recently characterized trypanosomatids *Crithidia mellificae* and *Lotmaria passim*.
The aims of this study are to: 1) investigate whether *C. mellificae* and *L. passim* are widespread in managed honey bee populations in Canada, as well as the relative abundance of each species; and 2) establish a trypanosomatid cell culture isolated from Canadian honey bees.
A total of 263 colonies were sampled from nine provinces and one territory. Fifty bees per colony were collected in late August, before the application of any disease treatment to the colony. All bees from the same colony were pooled and DNA was extracted from the pooled sample. The prevalence of *C. mellificae* and *L. passim* was quantified via real-time PCR using published primers and protocols. Additionally, a third generic primer ('Universal' primer) was also used to quantify the presence of all members of the trypanosomatid group. Cell culture was established by using dissected ileum and hindgut from individual bees collected from trypanosomatid-positive colonies. After dissection, samples were incubated in Drosophila medium at room temperature for a week to promote trypanosomatid cells growth.
Our results show that *L. passim* is more prevalent than *C. mellificae* in the Canadian honey bee population. Every province sampled had at least one colony infected with *L. passim*, while only two provinces had colonies infected with *C. mellificae*. We have successfully isolated *L. passim* and we currently maintain a healthy culture at Agriculture Agri-food Canada's Beaverlodge Research Farm. Prior to this survey, the prevalence of these two trypanosomatid species in the Canadian honey bee population was unknown. This first national survey conducted for trypanosomatids sheds light on their prevalence as well as their regional, provincial and national distribution.

077 *Nosema ceranae* and *Lotmaria passim*: partners in crime?

MacInnis C.I.[1,2], Luong L.T.[2], Schwarz R.S.[3], Guarna M.M.[2], Pernal S.F.[2]
[1] Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada; [2] Agriculture and Agri-Food Canada, Beaverlodge Research Farm, Beaverlodge, Canada; [3] Department of Biology, Fort Lewis College, Durango, USA

Nosema ceranae and *Lotmaria passim* are two commonly encountered eukaryotic parasites of the honey bee (*Apis mellifera*) digestive tract. In recent years, the microsporidian parasite *N. ceranae* has been shown to negatively impact the honey bee at both the individual and colony level, and is commonly cited by Canadian beekeepers as a factor contributing to winter colony mortality. *Lotmaria passim* is a recently characterized trypanosomatid parasite that is

globally prevalent. Given the emergent nature of *L. passim*, little is known about how the parasite impacts its honey bee host. Unfortunately, even less is known regarding the impact mixed infections of *N. ceranae* and *L. passim* have on honey bees despite the prevalence of co-infections. Here we present data from some of our ongoing work where, using real-time (RT) PCR and hoarding cage bioassays, we follow the infection progress of *L. passim* in individually-inoculated bees over time, and investigate the impacts of both parasites (single and mixed infections) on honey bee mortality. Preliminary data suggest that *L. passim* does not significantly impact honey bee mortality, even at moderate to high doses (>5.0×105 promastigotes/bee). We will also describe the humoral defense responses of honey bees individually inoculated with one or both parasite species after short and prolonged exposure by quantifying antimicrobial peptide gene expression via RT-PCR. This study is the first to examine the pathological impacts of both N. ceranae and L. passim on the honey bee.

078 Factors influencing the development and the course of the *N. ceranae* infection in *Apis mellifera iberiensis*

Urbieta A.[1], Higes M.[1], Meana A.[2], Barrios L.[3], Martín-Hernández R.[1,4]
[1] Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF-JCCM), Marchamalo, Spain; [2] Universidad Complutense de Madrid, Faculty of Veterinary Medicine, Department of Animal Health, Spain; [3] Computing Center SGAI-CSIC, Statistics Department, Madrid, Spain; [4] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Ciencia y la Tecnología (INCRECYT), Albacete, Spain

The microsporidian parasite *Nosema ceranae* is a honey bee pathogen with a high prevalence globally. Apis mellifera is considered to be a recent host for this *Microsporidia* and it raises questions related to its effects on host physiology, behavior, and longevity at individual and colony level. Many experimental factors can influence the development of *N. ceranae* infection in honey bees such as bee genetic origin (*A. mellifera* strain), caste, age, diet and beekeeping practices, dose of infection or source of spores, among others. Some factors have been previously addressed, however other had been neglected in many studies.
The aim of this study was to evaluate under experimental conditions the effect of three important factors in the development and course of the *N. ceranae* infection: the spore dose, the age of the bees at the time of infection and the method of infection. To do this, three different groups of experiments were developed.
Our results show that inoculum´s spore dose, the age of the bee at the infection time and the method of infection (individual or collective) have a direct influence in the parasite load developed (determined by qPCR) and in the mortality rates of infected bees. Infected-bees significantly reduced the survival time when compared with uninfected control. Infection doses of 104 fresh-spores per bee or higher, significantly reduced the survival time of the 50% of the bees (Kaplan-Maier test). There were also significant differences related to the age of infection: the youngest bees (newborn and 1-day-old bees) developed a higher parasite load at 7 days post-infection and this drastically changed in 2-days-old bees and older, although some differences were found also between 4-7 and 9-days-old bees. Additionally, the method of infection showed variations in the parasite load of bees infected at the same age, with the bees infected individually developing more loads than those infected collectively. The results highlight the importance of bee age, infective dose and way of inoculation since all they influence the development and the course of the N. ceranae infection in honey bees. These factors should be considered when comparing between published reports.
Funded by: INIA-CCAA RTA2012-00076-C02-01 and RTA2015-00013-C03-01 (INIA, FEDER).

079 Effect of *Nosema ceranae* on dynamic communities of gut bacteria in native species of Thai honey bees

Naree S.[1], Ellis J.[2], Mayack C.[3], Suwannapong G.[1]
[1] Department of Biology, Burapha University, Chon Buri, Thailand; [2] Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; [3] Martin-Luther-Universität Halle-Wittenberg, Institute for Biology/General Zoology, Halle (Saale), Germany

Nosema disease is caused by a group of microsporidia, *N. apis*, *N. ceranae* and *N. neumanni*. According to the infection of *Nosema* spores in midgut of honeybees cause a massive host ventricular cell lyses that caused many negative effects on physiological systems of its host in particular digestive system where it contains many species of gut bacteria, however there is no report about the community changing of gut bacteria in honeybees after getting infection by *N. ceranae*. We have investigated the communities of gut bacteria in four native species of honeybees in Thailand infected by *N. ceranae* with sub-lethal doses rearing at 33 ±1˚C compared to those of control bees. We also investigated the use of propolis extract of giant honey bee, *Apis dorsata* to control *N. ceranae* infected bees and investigated how propolis extract impact on the dynamic communities of gut bacteria in native species of Thai honeybees. The finding showed that *N. ceranae* affected the community changing of gut bacteria of infected bees.

080 Tracking Asian hornets (*Vespa velutina*) to their nests with radio-telemetry

Kennedy P.[1], Ford S.[1], Poidatz J.[2], Thiery D.[2], Osborne J.[1]
[1] Environment & Sustainability Institute, University of Exeter, Penryn, UK; [2] SAVE, INRA Bordeaux-Aquitaine, France

Asian hornets (*Vespa velutina*) are voracious predators of bees, and are the latest emerging threat to managed and wild pollinator populations in Europe. They are having a serious impact on honeybee colonies in France, preying on bees and shutting down foraging. The likely impact on wild pollinators is of great concern but so far unquantified. To prevent establishment or reduce the rate of spread of *V. velutina*, early detection and destruction of nests is considered the only option. Detection is difficult as their large nests are well hidden and flying hornets are difficult to follow over long distances. To meet this challenge we tracked individual *V. velutina* workers flying back to their nests using radio telemetry for the first time. We describe conditions under which tagged hornets could be tracked, leading us to five previously undiscovered nests in areas of differing terrain. This fast, effective method offers a step-change in managing this emerging threat to beleaguered pollinator populations in areas where Asian hornets have recently become established.

081 Effect of *Nosema ceranae* on energetic stress of trehalose level in haemolymph and infection ratio of giant honey bee

Ponkit R.[1], Paxton R.J. [2], Suwannapong G.[1]
[1] Department of Biology, Burapha University, Chon Buri, Thailand; [2] General Zoology, Martin-Luther-University Halle-Wittenberg, Germany

Nosema ceranae cause many negative effects in honey bees result in the reduction of honey bee life span and colony population. They were reported as pathogens originally described from *Apis cerana*. Nowadays, they have jumped host to *A. mellifera*, and other honeybee species including native honey bee species of Thailand.
Materials and methods: Here, we studied the experimental infection of *A. dorsata* workers by *N. ceranae* isolated from heavily infected *A. florea* workers and propagated in *A. mellifera* workers at 33˚C for 14 days in three different doses; 100,000, 300,000 and 500,000 spores per bee; trehalose level in haemolymph was investigated on day 6, 10 and 14 post infection (p.i.) compared to those of 50% propolis extract in ethanol (v/v) treated bees and the control bees.

Moreover, the infection ratio, the proportion of infected cells to non-infected cells of infected cells was evaluated. Results and conclusion: Trehalose levels in haemolymph of *N. ceranae* infected *A. dorsata* workers on day 6 showed the lowest of trehalose levels in haemolymph compared on days 10 and 14 p.i. ($F2=5.71$, $P=0.0039$). Trehalose level of control propolis was significantly different higher than those of others ($F3=4.12$, $P=0.0073$). However, there were not significantly different between bees infected with *N. ceranae* at the dosage of 500,000 spores per bee and bees infected with *N. ceranae* and treated with 50% propolis ($F3=4.12$, $P=0.0073$). In Nosema infected bees group, trehalose level was increased on day 14 p.i. compared on day 6 p.i. Thus, *N. ceranae* isolated from *A. florea* is capable of infecting another bee species and may contribute to their shortened life span when *A. dorsata* infected with *N. ceranae* over 500,000 spores.

082 Oxidative stress increases in honey bees infected with *Lotmaria passim*

Radaković M.[1], Vejnović B.[2], Glavinić U.[1], Aleksić N.[1], Mirilović M.[2], Stanimirović Z.[1], Stevanović J.[1]
[1] Department of Economics and Statistics, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; [2] Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

The effects of the trypanosomatid *Lotmaria passim* on honey bees are unknown. The aim of this study was to investigate if it induces oxidative stress in parasitised honey bees. During March 2017, in an apiary situated in the northwest of Serbia, 68 honey bee colonies of similar strength were checked for the presence on L. passim by the analysis of 60 adult bees per colony using PCR assay. Forty colonies were selected and processed: 20 *L. passim*-infected and *L. passim*-free. Twenty forager bees were sampled from each colony, macerated and the resulting homogenate used for confirmation of *L. passim* presence/absence and 10 for estimating the markers of oxidative stress: superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities and malondialdehyde (MDA) concentration. The activities of the enzymes and the level of MDA were determined by spectrophotometry. The results revealed significant differences in all markers of oxidative stress between *L. passim*-infected and *L. passim*-free bees. The activity of SOD was significantly lower, while the other markers - the activities of CAT and GST, and the concentration of MDA - were significantly higher in *L. passim*-infected bees in comparison to *L. passim*-free bees. The results obtained indicate that oxidative stress was significantly greater in *L. passim*-infected bees than in *L. passim*-free bees. Oxidative stress-induced cellular damage in *L. passim*–infected bees should be considered at least as one of the pathogenicity mechanisms of this protozoan. It is essential to conduct further investigation into the detrimental effects of this trypanosomatid on host defence mechanisms

083 Haploid males are more susceptible: spillover of *Crithidia mellificae* from managed honey bees to wild bees (*Osmia cornuta*)

Strobl V.[1,2], Yañez O.[1], Straub L.[1], Albrecht M.[2], Neumann P.[1,3]
[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Agroecology and Environment, Agroscope, Zürich, Switzerland; [3] Swiss Bee Research Centre, Agroscope, Bern, Switzerland

The haploid susceptibility hypothesis predicts that male bees should be more susceptible and pathogens from managed honey bees (*Apis mellifera*) might contribute to the decline of wild bees. However, detection of novel pathogens in wild bees does not necessarily imply that they serve as hosts. Here, we test the haploid susceptibility hypothesis by challenging male and female wild bees (*Osmia cornuta*) with the honey bee trypanosome *Crithidia mellificae*. *O. cornuta* and honey bee workers were maintained in the laboratory and fed ad libitum for 24 h with a sucrose solution containing live *C. mellificae* or not. Then, individual bees were dissected every 4-6 days (40 male and 51 female *O. cornuta*; 100 A. mellifera workers) and cell counts were conducted using light microscopy and an improved Neubauer counting chamber. *C. mellificae* cell numbers increased 4.8 times in honey bees, 2.6 times in female, and 2.1 times in male *O. cornuta* between day 6 and 15 post infection (all $p<0.001$). No *C. mellificae* were found in any negative control. Infected honey bees showed a decreased longevity compared to non-infected ones (36.3% and 24.5% mortality, respectively; $p<0.05$). Male *O. cornuta* showed higher cell counts than honey bees (day 6: $p<0.05$, day 10: $p<0.001$, day 15: $p<0.05$). None

of the infected male *O. cornuta* bees, survived the whole experiment, thereby showing a significantly reduced longevity compared to all other groups ($p<0.001$), whereas *C. mellificae* infection only marginally reduced longevity in female *O. cornuta* (control 19.3% and infected 32% mortality; $p=0.075$). The results provide empirical support for the haploid susceptibility theory because male *O. cornuta* showed higher *C. mellificae* cell counts and a reduced longevity. In principle, *O. cornuta* can serve as a novel host of *C. mellificae*, thereby possibly impacting this solitary bee species. Field studies are now required to quantify potential spillover and impact *of C. mellificae* on wild *O. cornuta* populations.

084 Bees rely on their sense of taste to assess pollen nutritional composition

Ruedenauer F.A.[1], Spaethe J.[2], Strube-Bloss M.F.[2], Leonhardt S.D.[1]
[1] Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany; [2] Department of Behavioral Physiology and Sociobiology, University of Würzburg, Würzburg, Germany

Over- or undereating nutrients can be detrimental to bees. Their main source of most macronutrients is pollen. Bees would therefore benefit from being able to taste specific nutrients in pollen and hence assess its nutritional composition in order to differentiate between different pollen species and forage on the most appropriate ones. The sensory mechanisms underlying pollen nutrient assessment are however still largely unexplored.
We used different behavioral and electrophysiological experiments, i.e. (1) chemotactile conditioning of the proboscis extension response (PER), (2) a new technique for measuring electroantennogram (EAG) activity with chemotactile stimulation and (3) feeding assays, to test whether bumblebees (Bombus terrestris) are able to receive, perceive and differentiate amino acids and fatty acids in pollen.
Bombus terrestris workers were principally able to taste both amino acids and fatty acids. However, when assessing pollen nutritional quality they seemed to focus on fatty acids and ignore other nutritional cues. This behavior nicely fits to the observation, that bumblebees usually prefer pollen with high protein to lipid ratios and their survival was strongly reduced on fat-rich pollen diets.

085 Sex-dependent nutrient demand is reflected in pollen supplied to larvae by mother of a generalist solitary bee *Osmia bicornis*

Filipiak M.
Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

The fitness of an organism depends on the nutritional quality of available resources, and sexes may have different nutritional demands. Unbalanced nutritional composition of pollen limits the growth and development of its consumer. The ecological stoichiometry framework was used to study the differences in the demand and supply of nutrients and the stoichiometric balance of the diet of an important pollinator – mason bee *Osmia bicornis* L. The concentrations and stoichiometric ratios of C, N, S, P, K, Na, Ca, Mg, Fe, Zn, Mn, and Cu were investigated in bee production (body and cocoon) of both sexes and pollen supplied by the mother, i.e., the only food eaten during larval development. Females had higher demands for and were supplied with pollen richer in P, Cu and Zn than males. Female fitness may be particularly related to a high P proportion and low *C:P* ratio in their diet. Additionally, males had higher demands for Na and lower demands for K than females, but these elements were similarly concentrated in the pollen supply for both sexes. These tendencies were reflected in conversion efficiencies of elements from food to bodies and cocoons, various for different elements and sexes. Bee production, growth and development may be limited by the availability of P, Na, Mn, Mg, K, Fe Ca, Zn and Cu, i.e., elements that show high taxonomical concentration variabilities in pollen. Females provide their daughters and sons with different pollen mixtures that better fulfil sex-specific nutritional demands. Key plant species might allow for nutritional balancing.

086 Temporal disruption in flowers availability affects honeybee colony losses

Requier F.[1,2], Odoux J-F.[2], Henry M.[3], Bretagnolle V.[1]
[1] Centre d'Etudes Biologiques de Chizé, CNRS & Université de La Rochelle, UMR 7372, Beauvoir sur Niort, France; [2] INRA, UE 1255, UE Entomologie, Surgères, France; [3] INRA, UR 406 Abeilles & Environnement, Site Agroparc, Avignon, France

A third to a half of managed honeybee colonies are lost every winter in Europe and North America. This decline in managed honeybees threatens honey production and crop pollination services in many countries, leading to concerns for negative social, economic and ecological effects. Among the list of stresses known to affect honeybee health, the lack of alternative food resources to mass-flowering crops is only suggested as a potential stress factor in farmlands. However, intensive cereal farmland habitats are characterized by a temporally fragmented succession of mass-flowering crops with a two-month scarcity of flowers in spring. The concept of carry-over effect was used to test the possible causal link between scarcity of flowers in spring and honeybee colony losses in winter. Carry-over effects are mostly based on some kind of food shortage, which has a detrimental effect at a later life history stage and affects the fitness or reproductive value of individuals. Given that insects have several life-history stages (egg, larvae, pupae and adult), a food shortage at an early life-history stage, e.g. larvae, could result in detrimental carry-over effects on subsequent life-history stages, e.g. adult. By monitoring honeybee colony dynamics, this study showed that a spring shortage in pollen, the main source of protein used to feed the larvae had a direct limiting effect on brood production, leading to a reduction in the size of the adult colony population later in the season and lower honey reserves before the onset of winter. As a final cascading cost of this carry-over effect, the spring pollen shortage weakened the colony health, with higher *Varroa* mite load and high seasonal and winter colony losses. These results suggest that pollen shortage – as a proxy of the limited flower availability – may have been overlooked as a cause of honeybee colony losses in farmlands.

087 Pollen protein content drives bee community preference for an invasive thistle over five native plant species

Russo L.[1], Vaudo A.[2], Fisher C.J. [3], Grozinger C.[2], Shea K.[3]
[1] Botany Department, Trinity College Dublin, Dublin, Ireland; [2] Entomology Department, [3] Biology Department, Penn State University, University Park, USA

Our previous work showed an invasive thistle, *Carduus acanthoides*, is strongly preferred by bee communities of agroecosystems in central Pennsylvania. Our objective was to determine what traits of this plant allowed it to preferentially attract pollinators compared to four confamilial native species and one native legume. We established 30 2x2m experimental plots with a controlled background density of five native annuals, then divided these plots into six blocks, composed of five experimental treatments. These treatments involved the invasion of the thistle at two different timings (early and late in the summer) and intensities (high and low abundance), as well as a control plot which was not invaded and comprised only the five native plant species. We measured bee visitation to each plant species throughout the summer, as well as plant traits including: above-ground biomass, number and size of flowers, and pollen protein, sugar, and lipid content.
Although all plots with the thistle had more bee visits than control plots, we found no effect of treatment on the visitation rate (bees per flower per min) to the native plant species. Within a given plant species, the number of flowers was a significant predictor of bee visitation; however, this trend was no longer significant when we looked across different plant species. In other words, the number of flowers did not predict bee preference for different plant species. The biomass, floral display, pollen sugar and lipid content were also not significant predictors of bee preference. We found a significant and strong ($R2 = 0.9$) correlation between the protein content of each plant species' pollen and visitation rate. The high protein content of the thistle pollen may allow it to compete for pollinators. In addition, the thistle has a similar protein:lipid ratio to that of the legume, previously found to be a strong predictor of bumblebee visitation, while providing a higher protein concentration.

088 How larval food quantities affect caste-fate in the stingless bee *Scaptotrigona depilis*

Buchholz J., Hartfelder K.
[1] Department of Zoology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), German; [2] Department of Cell and Molecular Biology and Pathogenic Bioagents, University of São Paulo, Ribeirão Preto, Brazil

The honey bee *Apis mellifera* is the best studied species concerning caste development, in which differential nutrition and juvenile hormone (JH) have been known to act as the main drivers of caste determination. In order to explore the mechanistic details of the interaction between nutrition and JH signalling, many studies have focused on two ancestral nutrient-sensing cascades: IIS (Insulin/Insulin-like Signaling) and TOR (Target of Rapamycin), with growing evidence for their role in caste determination in the honey bee.
In the tropics, members of the tribe *Meliponini* form the main social bee species, whose mechanisms of caste determination are largely unknown. When compared to honey bees that practice progressive larval food provisioning, stingless bees are very different in utilising a mass provisioning strategy. In this study, an *in vitro* rearing method was used to examine expression patterns in workers and queens of *Scaprotrigona depilis* of two genes: TOR (TOR cascade) and AKT (IIS cascade), associated with nutrient sensing. Furthermore, expression of these genes was studied in JH-induced queen phenotypes. In aiming to explain the connection between JH and nutrient signalling, JH-titres were also measured in worker phenotypes induced by Rapamycin (specific TOR-inhibitor).
No relationship of the ancestral nutrient sensing pathways (TOR and IIS) and caste determination was found in *S. depilis*. Nevertheless, it could be shown that JH has the same queen-inducing effect in *S. depilis*, as previously shown for other social Hymenoptera. The JH titres observed in Rapamycin treated larvae revealed more questions than answers. Nonetheless, the findings of this experiment might provide an interesting foundation for further investigations. Future studies on caste determination could focus on different target genes or signalling pathways using the characterisation of larval instars, as defined here for the first time for a stingless bee species.

089 Honeybees tolerate cyanogenic glucosides from clover nectar and flowers

Jensen A.B., Lecocq A., Green A.A., De Castro E.C.P., Olsen C.E., Zagrobelny M.
Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Honeybees (*Apis mellifera*) pollinate flowers and collect nectar from many important crops. Clover (*Trifolium* sp) is widely grown as a temperate forage crop, and requires honeybee pollination for seed set. The objectives of this study was to examine whether two clover species, *T. repens* and *T. abbiguum*, have insecticidal secondary compounds in the nectar and pollen and if so how these coumpounds will affect honeybees. Using a quantitative LC-MS (Liquid Chromatography-Mass Spectrometry) assay, we show that the cyanogenic glucosides linamarin and lotaustralin are present in the leaves, sepals, petals, anthers, and nectar of both *T. repens* and *T. abbiguum*. Cyanogenic glucosides are generally thought to be plant defense compounds, releasing toxic hydrogen cyanide upon degradation. However, increasing evidence indicates that plant secondary metabolites found in nectar may protect pollinators from disease or predators. In a laboratory survival study with chronic feeding of plant secondary compounds, we show that honeybees can ingest the cyanogenic glucosides linamarin and amygdalin at naturally occurring concentrations with no ill effects. This even though we showed in an HCN emission assay and a BLAST search in the honeybee genome that honeybees have β-glucosidase enzymes and enzyme activity towards degradation of cyanogenic glucosides. No HCN emission was shown in the crop which suggests that honeybees can ingest and tolerate cyanogenic glucosides from flower nectar by enzyme compartmentalization.

090 The feeding with glandular secretions protects honeybee larvae from toxic pyrrolizidine alkaloids present in pollen

Kast C.[1], Kilchenmann V.[1], Lucchetti M.A.[1,2]
[1] Agroscope, Swiss Bee Research Centre, Bern, Switzerland; [2] Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

The production of nursing secretions in honeybees has previously been associated with several benefits in terms of better brood hygiene and faster larval maturation. Here, we propose that larval nursing also protects honeybee larvae from secondary metabolites that can be found in floral rewards. To test this hypothesis, we first studied the impact of toxic pyrrolizidine alkaloids (PAs) that are present at high concentrations in pollen of *Echium vulgare* on honeybee larvae. Second, we investigated whether these PAs are transferred into glandular secretions (jelly) produced by nurses to feed larvae.
Echimidine and echivulgarine were isolated from inflorescences and leaves of *E. vulgare* plants and added to diets used to feed in vitro reared larvae. Larvae were very sensitive to PAs. The median lethal dose (LD50) recorded on day 21 (adult emergence) was 3.8 µg for echimidine and 12.5 µg for echivulgarine, corresponding to dietary PA concentrations of 21.8 and 70.9 µg/g, respectively. Chronic larval exposure tests also showed that concentrations of echimidine below 15.0 µg per gram of diet were non-lethal.
Adult nursing bees consume substantial quantities of bee bread to produce jelly for feeding larvae. We added echimidine to bee bread (2000 µg/g) and subsequently quantified the echimidine levels found in the royal jelly produced by nurses that previously fed on the PA- supplemented bee bread. Our study shows that only a very small fraction of the echimidine was transferred from the bee bread into the royal jelly. The mean echimidine concentration measured in royal jelly was 2.1 µg/g, which is an echimidine level that is well below the toxicity threshold of 15.0 µg/g for larvae. Hence, apart from the known benefits of nursing, the feeding with glandular secretions may decrease exposure of honeybee larvae to toxins present in pollen and allow honeybees to exploit a wide pollen spectrum.

091 New protocols to study nectar foraging in honey bees

Sokolowski M.
Université de Picardie - Jules Verne, Amiens, France

Based on new technological developments, we propose new automatized protocols whose purpose is to control the exposure to the nectar resource and to measure in detail the nectar foraging behavior of honey bees.
Free flying honey bees are trained to come to visit a computer controlled artificial flower that gives access to small amounts of syrup. First, we show that the nectar flow entering the colony is constant over time and decreases when nectar resources decrease over time. Then, we show that pesticides may affect this nectar flow. Finally, we show that toxicity of nectar may increase when resources become more scarce in the environment.
As nectar flow entering the colony is a dependent variable highly linked to colony fitness and beekeeper economic health, we suggest to assess this flow when testing the non lethal effects of pesticides.

092 Bees and resin, resin and bees: insights into an underrated resource

Leonhardt S.D.[1], Drescher N.[2], Kaluza B.F.[1,3], Neumann P.[4], Klein A-M.[5], Wallace H.M.[3], Schmitt T.[1]
[1] University of Würzburg, Germany; [2] University of Lüneburg, Germany; [3] University of the Sunshine Coast, Australia; [4] University of Bern, Switzerland; [5] University of Freiburg, Germany

For centuries, bees have been known for collecting sticky resins from various plants and using it for nest construction. The mixture resulting from resin and wax is typically referred to as propolis. Although largely disliked by beekeepers, propolis has been used by humans for its antimicrobial, antiviral and wound healing properties for decades. Surprisingly, however, the role of resin/propolis in the bees' very own nest has not been studied in more details until only very recently.
Using chemical analyses, field observations and manipulations as well as laboratory assays with resin extracts, we studied a) the precise resin source plants of honeybees (*Apis mellifera*) in Germany and stingless bees (*Meliponini*) in Australasia, b) inter –colony and –apiary variation in the amount and spectra of resins collected, and c) the importance of resin and resin diversity as a natural defense of bee colonies.
We show that both honeybees and stingless bees collect resin from several plant species with clear preferences for specific plant species, but often colony-specific resin spectra. While resin showed no effect against *Varroa* mites, titers of the *Varroa*-associated deformed wing virus (DWV) increased less in colonies with additional propolis. Notably, the defensive properties of resin/propolis were enhanced when bees had access to a diversity of different resin sources, indicating that bees strongly benefit from access to diverse resin sources.

093 Single von Willebrand factor C proteins and its role in insect immunity

Wang H., Niu J., Smagghe G., Meeus I.
Department of Plants and Crops, Ghent University, Ghent, Belgium

A newly discovered protein family within arthropods, the single von Willebrand factor C (SVCs) proteins, are responsive in relation to environmental ques like the nutritional status, bacterial and viral infections. Most arthropods contain multiple proteins which contain the defining cysteine motif (i.e. 8 of the 10 cysteine of the conserved canonical von Willebrand factor type C domain); while in hymenopteran species, we noticed a reduced repertoire of SVC protein family members. For instance in bumble bees only one SVC protein is found in the genome. This allows to perform functional studies after gene silencing experiments. Here we talk about the potential immune modulator role of BtSVC (the SVC of *Bombus terrestris*) in relation to viral infections. We showed that silencing of BtSVC resulted in increased viral titers (Israeli acute paralysis virus (IAPV)) in the fat body. Aside from its viral activity we also found relations with other immune pathways related to bacterial infections. The single SVC seems to be multi-functional; like others suggested, an insect cytokine acting similary to mammalian interferons.

094 Study of gene expression of immunologically important molecules in honey bees after application of probiotic preparation

Maruščáková I.[1], Schusterová P.[1], Bielik B.[2], Toporčák J.[2], Mudroňová D.[1]
[1] Department of microbiology and immunology, [2] Clinic for birds and exotic animals, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

The aim of our work was to determine the expression of genes encoding immunologically important molecules in the samples from honey bee intestines (*Apis mellifera*). In the experiment, hives were divided into three groups: control group, which was not feeding; group, which was feeding a pollen suspension; group, which was feeding a pollen

suspension together with the autochthonous probiotic *Lactobacillus brevis* B50 Biocenol ™ (CCM 8618) isolated from healthy adult honey bees. Pollen suspension and pollen suspension with *L. brevis* were applied three times from the beginning with one week distance. The schedule of sampling was follows: zero collection of samples was performed before feeding, first collection one week after first feeding, second collection one week after second feeding, and third collection two weeks after third feeding pollen suspension and pollen suspension with *L. brevis*. For the study of gene expression of immunologically important molecules were selected primers encoding genes for: peptidoglycan recognition proteins (PGRP), Toll-like receptors, Cactus, Dorsal, Abaecin, Defensin-1. Our results show that feeding of pollen suspension with L. brevis increased expression of genes encoding antimicrobial peptides, toll-like receptors, Cactus and PGRP, mainly in the first and second samplings. However, expression of Dorsal (NF-κB) was reduced, so Toll Pathway was suppressed. The expression results of genes encoding above mentioned molecules correspond with the microbiological analyses of gastrointestinal tracts, where numbers of lactic acid bacteria were increased and enterobacteria were reduced. Finally, we can assume that pollen suspension with *L. brevis* stimulates gene expression of antimicrobial peptides but not through the Toll pathway. In future, we want to study IMD pathway.
Acknowledgement: This work was supported by VEGA project 1/0358/16.

ORAL PRESENTATIONS

095 On mechanisms of trans-generational immune priming in honeybees

Freitak D.[1], Salmela H.[1], Harwood G.[2], Amdam G.[2]
[1] University of Helsinki, Department of Life Sciences, Helsinki, Finland; [2] Arizona State University, School of Life Sciences, Tempe, USA

The mechanism behind the antibody-free trans-generational immunity in invertebrates has been an enigma for decades. We have established that egg-yolk protein is responsible for binding to immune elicitors and then carrying these to eggs. These immune elicitors can then act as specific signals for immune system to trigger defense against infection. It could be a long-sought answer to the question, how are invertebrates priming their offspring. It seems, that pathogens encountered via food are digested, disarmed and in some way transported via midgut tissue to the hemocoel, there they are further incorporated into the developing eggs.
Here we show evidence of both in vivo and in vitro experiments, how TGIP against honeybee diseases takes place and that honeybee queens orally exposed to pathogens can enhance the immunity of their offspring by altering the physiology of the larvae. We also propose a mechanism, how this type of transfer of immune priming could take place on the level of the beehive.

096 Alarming situation on the EU beeswax market: the prevalence of adulterated beeswax material and related safety issues

Svečnjak L.[1], Prđun S.[1], Baranović G.[2], Damić M.[1], Rogina J.[1]
[1] University of Zagreb Faculty of Agriculture, Department of Fisheries, Beekeeping, Game Management and Special Zoology, Zagreb, Croatia; [2] Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia

The control of beeswax used in pharmaceutical and food industry (pharmaceutical grade beeswax and food additive E901, respectively) includes strict EU regulations defining beeswax quality criteria and standardized analytical methods for its testing. Contrary, beeswax used in the apiculture sector, where it is classified as animal by-product (ABP) not intended for human consumption and primarily used in the form of comb foundations, is not subjected to obligatory quality control prior to its placement on the market. According to the Reg. (EC) 1069/2009, beeswax and its products are categorized as category 3 material which includes ABPs that do not present a potential risk for the food chain as they must not contain residues of other substances and environmental contaminants. However, beeswax is frequently marketed as "safe" category 3 even when it contains substances of questionable origin and chemical background (such as most commonly used adulterants, paraffin and stearic acid), due to the lack of obligatory legal regulations. In this way, contaminated beeswax is regularly re-entering beekeeping technology and honey production process via uncontrolled

comb foundation production and trade. The aim of this study was to investigate the quality of beeswax present on the EU market. In total 137 samples of comb foundations and wax blocks used for their production were collected from 15 European countries during the period 2016-2018. Samples were analysed by FTIR-ATR spectroscopy based analytical procedure developed for qualitative and quantitative detection of adulterants in beeswax. The results have revealed that >65% of analysed beeswax samples were adulterated with various share of paraffin (5 to 93%), while stearic acid was detected sporadically in samples from Western European countries. In addition to the violation of EU regulations in force (incorrect beeswax categorization, false advertising, deception of consumers), adulteration of beeswax also raises the question of food safety and public health issue given that honey (food that enters the global food chain) is ripened and stored in the honeycombs constructed on comb foundations containing foreign and potentially hazardous substances. Therefore, there is an urgent need for effective legislation related to the quality control of beeswax in the EU apiculture sector.

097 Effect of beeswax adulterated with stearin on the development of worker bee brood: results of a field trial

Reybroeck W.[1], Van Nevel J.[2]
[1] *Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Technology and Food Science unit, Melle, Belgium;*
[2] *Federal Public Service Health, Food chain safety and Environment, DG Animals, Plants and Food, Brussels, Belgium*

In 2016, beekeepers alerted problems related to the poor development and dying-off of the bee brood after the insertion of new wax foundations which had been produced on an industrial scale. Following analysis, it appeared that the abnormal beeswax was adulterated with some 20 to 30% of stearin. In literature no data were available on the effects of stearin on the bee brood.
To examine whether the addition of stearin to beeswax leads to mortality of part of the worker bee brood, experiments were conducted at ILVO. Stearin was added to selected cast beeswax (reference) in increasing weight concentrations, namely 15, 20, 25, 30, 35 and 40% and wax foundations were cast. Four openings of 8x8 cm (= 64 cm2) were applied to reference wax foundation sheets, into which a piece of the cast wax foundation to be tested could fit. Each test frame was hung separately in a bee colony in the super, to enable the wax honeycomb to be built up by the worker bees. After 2 to 3 days, the queen was enclosed on the test frame with built-up honeycomb, by placing a frame with a queen excluder on both sides. Two days later, after checking whether an egg had been laid in each cell, the queen was removed from the frame and released into the brood area below, under a queen excluder. The further brood development was monitored and the survival percentage calculated.
The addition of 15% stearin to beeswax resulted in significant mortality of the worker bee brood in the cells built up on wax foundations made of this kind of wax. Higher additions (up to 40%) resulted in an increase in mortality. Related to the mortality of worker bee brood in the reference wax, the average mortality in the wax with added stearin was at least 49.0% (with 15% stearin). The highest average mortality amounted to 71.0% (with 35% stearin).
The results show that beeswax with added stearin (in the tested percentages from 15% onwards) is not suitable for the production of wax foundations for use in apiculture.

098 Effect of spatial allocation of nectar cells on storage and honey ripening dynamics in the honeybee *Apis mellifera*

Eyer M., Dietemann V.
Agroscope, Swiss Bee Research Centre, Bern, Switzerland

Honeybees store nectar they collect in wax cells of their combs. This carbohydrate source is transformed into honey, providing the colonies with energy stores to be consumed during dearth periods or winter. Honeybees could optimise production of honey based on the sugar concentration of collected nectar. Spatial clustering of cells with content of similar concentration could facilitate ripening since humidity conditions could be adapted locally to promote the process. Due to methodological limitations, the ripening process and the storage strategies that underlie it are difficult to

investigate and were, so far, studied without considering their dynamics. Diagnostic radioentomology is an appropriate non-invasive method to study these processes over time, without interfering with worker behaviour or cell content. We demonstrated, using spatial analyses, that early in the nectar ripening and storage processes, spatial distribution of cells is not optimized based on similar concentration of cell content. Areas of combs with similarly concentrated content are generated during the ripening process, when concentration converges towards that of honey.

099 Culturing and conserving of stored pollen on the way into beebread

Podrižnik B., Božič J.
Department of Biology, University of Ljubljana, Biotechnical faculty, Ljubljana, Slovenia

Bee bread is known as main source of proteins for larvae breeding and as food of young bees. It is processed collected pollen, stored in wax comb and at the end of processing covered with honey and wax cap. We tried to get insight in the roll of the bees in these process. Collection and packing of pollen was monitored during chestnut pasture. Comb cells of stored pollen were obtained at different phases of processing into bee bread. Opened, before covering with honey, freshly covered cells and additionally aged comb cells were selected for analysis. Each cell were separated on 3 layers of bee bread and additionally layer of honey in case of covered cells. Detailed pollen analysis were obtained to check for the purity of chestnut source and potential effects of existence of some diversity in the samples. Main objective was to test for antimicrobial activity and how that might be depended on phenolic compounds, lactic acid and processing by bees using gluconic acid as an indicator. Proportion of honey added during processing was evaluated by measuring main honey sugars glucose and fructose. Phenolic compounds did not correlate to antibacterial activity in a bee bread as it is usual for honey and were the highest at the bottom of the honeycomb cell. Lactic acid, as well as gluconic acid separately contribute (directly or indirectly) to the antimicrobial activity, which was the highest in upper layers and showed that both together might act stronger then each one alone. Gluconic acid was 90 – 370 times higher concentration then lactic acid and was the main acidity factor in bee bread. Main honey sugars content in bee bread indicates that bees add as much honey to fill empty space between pollen grains, when packed them in a beebread. When our results were compared to some other research of bee bread in last decade we were able to propose a model for bee bread preparation which put much stronger role of worker bees contribution then to their allied microorganisms.

100 Sugar, amino acids, and inorganic ion profiling of honeydew from aphid species hosting different tree species in Germany

Shaaban B.[1], Seeburger V.[2], Schroeder A.[2], Lohaus G.[1]
[1] Molecular Plant Research/ Plant biochemistry, University of Wuppertal, Wuppertal, Germany; [2] Apicultural State Institute, University of Hohenheim, Stuttgart, Germany

Honeydew honey is considered to be one of the most popular honey types in Germany. Various hemipterous insects feed on plants and after consuming leaf or stem phloem they excrete most of the sugars as honeydew. Different L*achnidae* and *Coccidae* species are the most important honeydew producers in Germany. For honeybees (*Apis mellifera* L.) honeydew is a relevant source for honey production in summer.
The composition of honeydew is influenced by aphid species, host plants, and other environmental conditions. To date, there is no chemical analysis method to distinguish between different honeydew honey types. Therefore, the aim of the project BoogIH (please see https://boogih.uni-hohenheim.de) is to develop such methods to identify honey according to its compositional variation. In order to identity the zoological and botanical origin of honeydew honey, honeydew of different host plants (e.g. *Picea*, *Abies*, *Quercus*) and aphid species (*Cinara*, *Physokermes*, *Thelaxes*, *Phyllaphis* and *Eucallipterus*) were collected and sugar alcohols, mono-, di- and oligosaccharides, amino acids, organic acids and inorganic ions were measured using different HPLC approaches. Furthermore, tissues and phloem exudates from different host plants were analyzed.

Differences in sugar composition of honeydew among aphid species as well as for different host plants were found. This is particularly important for trisaccharides such as melezitose or erlose. However, there is a considerable difference in the presence and the proportion of sugars between honeydew and host plants and some sugars (e.g. melezitose) are exclusively produced by the aphids. In contrast, differences in amino acid or inorganic ion composition of honeydew were less pronounced between aphid species.
As a conclusion, honeydew compositions are mainly influenced by aphid species and to some degree also by the host plant, thus zoological and botanical origin do affect the honeydew honey composition. However, it is still important to investigate the impact of the honeybees on the honeydew composition during honeydew honey production.

101 Genuine Manuka honey!? - State of the art

Beitlich N., Speer K.
Technische Universität Dresden, Chair of Food Chemistry and Food Production, Dresden, Germany

Manuka honey is one of the most adulterated monofloral honeys in the world since it is the major medical grade honey currently approved for clinical application, especially for wound healing. The antibacterial activity of manuka honey is mainly caused by methylglyoxal (MGO), aside of other as yet unknown compounds. This has led to more so called manuka honey being sold on the market than actually produced. For this reason the blending and adulteration of manuka honey has come into focus everywhere. Therefore, the New Zealand Government and the UMFHA have requested robust and clear parameters for the identification of genuine manuka honey.
As a result, our classification system named HAHSUS (Honey Authentication by HS-SPME-GC/MS and UHPLC-PDA-MS/MS combined with Statistics) was developed which is capable of differentiating and classifying manuka honey from other honeys, especially from the pollen-identical kanuka honey. It is also possible to estimate the percentage of manuka honey in manuka-kanuka honey mixtures. Furthermore, the classification of more than 80 New Zealand honeys using HAHSUS will be compared to the five attributes test proposed by the New Zealand Government.

102 Synergistic effects of stressors on bee health: pesticides, nutrition, and behaviour

Tosi S.
Division of Biological Sciences, Section of Ecology, Behavior, and Evolution, University of California, San Diego, La Jolla, CA, USA

Bees health is influenced by the impact multiple stressors, such as diseases, pesticides, and nutrition deficiencies. Combined exposure to stressors can cause amplified effects if two agents (i.e. two pesticides) interact synergistically, reducing bee health. Most of the research focused on the interactive effects of combined pesticide-pesticide and pesticide-disease exposure on bee survival. Although bees can be simultaneously exposed to pesticides and nutritional deficiencies, and bee health depends on bee behaviour, little is known about the synergistic effects caused by the pesticide-nutrition deficiency interaction, and about the adverse synergistic effects elicited on bee behaviour. A major Risk Assessment (RA) agency (the European Food Safety Authority) has identified chemical mixtures as one of its current priority tasks, but synergistic effects on animal behaviour, as well as synergies caused by non-pesticide stressors, are still currently not taken into account. The complexity of performing experiments testing interactive effects contributed to the scarcity of scientific methodologies and valuable results. This difference between the complexity of real world situations, involving exposure to multiple stressors affecting survival and behaviour, and simplified scientific scenarios, could lead to uncertainties in the assessment of bee health. Thus, we appositely developed the methods and tested the synergistic effects of new pesticide-pesticide combinations, and of a novel pesticide-nutrition deficiency combination, on bee health, addressing both survival and behavioural effects. The experiments demonstrated, for the first time, that animal health can be synergistically impaired by the combination of two major common stressors, pesticides and poor

nutrition, at field-realistic exposures. We also demonstrated that combined chemical stresses can synergistically impair bee behaviour (e.g. locomotion, coordination). Because each specific stressor alone, at the same level, did not cause any significant effect, our results suggest that studying these stressors individually could lead to an underestimation of risks for bees. The consequences for bee health and RA are discussed, as well as possible future directions towards an holistic approach integrating multiple stressors.

103 Imidacloprid diffusion route: from apple orchard to the honey bee colony matrices

Malagnini V.[1], di Prisco G.[2], Zanotelli L.[1], Nazzi F.[3], Annoscia D.[3], Tonidandel L.[1], Colombo R.[4], Serra G.[4], Boi M.[4], Fontana P.[1], Angeli G.[1]
[1]Centro Trasferimento tecnologico, Fondazione Edmund mach, San Michele all'Adige, Italy; [2]Università degli Studi di Napoli "Federico II", Dipartimento di Agraria, Laboratorio di Entomologia E. Tremblay, Napoli, Italy; [3]Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italy; [4]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria-Centro di Ricerca Agricoltura e Ambiente (CREA-AA) Bologna, Italia

Honeybees play a pivotal role in natural and rural ecosystems by enhancing human and animal food production through pollination services. However, in the context of cultivated areas, bees can be exposed to chemicals used for crop protection. In particular, neonicotinoid insecticides can adversely affect honeybee colonies due to negative effects on immunity, behavior and ultimately survival at sublethal and lethal concentrations. However, despite these insecticides are considered as possible contributors to widespread colony losses, not enough is yet known about the way of entry and diffusion of these pesticides into the hive. Here we wanted to fill this gap by studying the diffusion route of the pesticide Imidacloprid and its metabolites in the hive by analyzing different materials collected in bee colonies used for apple orchard pollination in the framework of Integrate Pest Management strategy. The results show that the first way of entrance of Imidacloprid is the pollen loads transported by foragers; then the pesticide and its metabolites accumulates in bee bread, honey and wax for at least 3 months. This finding should be considered in light of the repeated feeding of bees on bee bread keeping into account that, in this case, damage thresholds may be exceeded contributing to colony losses.

104 Chronic toxicity of select pesticides to honey bee (*Apis mellifera L.*) larvae reared in vitro

Dai P.[1,2], Jack C.J.[2], Mortensen A.N.[2 3], Bustamante T.A.[2], Bloomquist J.R.[4], Ellis J.D.[2]
[1] Key Laboratory of Pollinating Insect Biology, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; [2] Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; [3] Plant & Food Research, Hamilton, New Zealand; [4] Emerging Pathogens Institute, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

The effects of chronic exposure to two neonicotinoids (clothianidin and imidacloprid), two organophosphates (chlorpyrifos and dimethoate), one fungicide (chlorothalonil) and one insect growth regulator (diflubenzuron) on the survival to adulthood, developmental rate and larval weight of honey bee larvae reared in vitro were determined. Diets containing the chemicals were fed to larvae with the range of concentrations for each compound based on published acute toxicity experiments and residues found in pollen and nectar. Four concentrations of each compound were tested. The controls included a positive control: dimethoate (45 mg/L); solvent control: acetone or methanol; and a negative control: no addition of compounds or solvents to larval diet. Negative control and solvent control survival to adulthood was >80% while positive control survival was <30%, thus validating the experimental design. A significant decrease in survival to adulthood occurred in the 0.8, 1.2 and 8 mg/L chlorpyrifos, 0.4, 2 and 10 mg/L clothianidin, 30 or 100 mg/L chlorothalonil, 0.8, 1.3 or 2 mg/L diflubenzuron, and 45 mg/L dimethoate diets, but not the imidacloprid diets. We were able to use the no observable adverse effect level to calculate risk quotients for the test compounds using the

U.S. Environmental Protection Agency's BeeREX program. The results suggest that none of the test compounds pose a significant risk to immature bee survival at the test concentrations. Nevertheless, our data do not preclude any sublethal effects that chronic exposure to either compound may cause. None of the test compounds affected any developmental rate or larval weight predictably at field relevant exposure concentrations. In our study, weight and developmental rate were uninformative end-points and failed to contribute any toxicological insights beyond those provided by mortality. Overall, our results are valuable in evaluating the chronic toxicity of these pesticides to developing honey bees.

105 The homing flight ring test: method to assess the effects of sublethal doses of pesticides on the honey bee in field conditions

Fourrier J.[1,2], Rouzes A.[1,2], Monchanin C.[1,2], Henry M.[2,3], Decourtye D.[1,2,4], Aupinel P.[5], Fortini D.[5], Moreau C.[5]
[1] ITSAP-Institut de l'Abeille, INRA Domaine Saint Paul, Avignon, France; [2] UMT PrADE, INRA Domaine Saint Paul, Avignon, France; [3] INRA UR 406 Abeilles et Environnement, Domaine Saint Paul, Avignon, France; [4] ACTA, INRA Domaine Saint Paul, Avignon, France; [5] INRA UE 1255 APIS, Centre de recherche de Poitou Charentes, Surgères, France

In the framework of the current revision of plant protection product risk assessment on the honeybee by European authority (EFSA, 2013), a European ring test is conducted from 2015 with 11 voluntary laboratories to test a methodology assessing the effects of subletal doses of a plant protection product administered in controlled conditions on the homing capacity of forager bees in the field. The objective is to validate the method in different contexts in order to propose it in the OECD international guidelines for risk assessment performed before pesticide homologation.
Homing success is measured by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology. To do so, we capture at the hive entrance, foragers coming from a known site located at 1 km (+/- 100 m) away from the experimental colony, to ensure that the foragers have a prior knowledge of the pathway back to the colony. RFID-tagged bees are orally exposed to 3 sublethal dosing solutions (0.1, 0.3 and 1 ng/bee) of the reference item, thiamethoxam, or to a control in laboratory. The dosing solutions are collectively administered to the honeybees with 20 µl per bee of a 30% sucrose solution (w/v). Then foragers are released on the known site and the homing success is recorded at the hive entrance with RFID system for 24 hours after release. The test endpoint is defined as the determination of a No-Observed Effect Dose (NOED) on the homing success.
In 2015, 7 laboratories out of 10 conducted the test and found a common NOED of 0.3 ng per bee. Methodological improvements have been proposed for the standardization of the method. From 2016, all the laboratories could conduct the test. The factors of variability modulating the effects of the insecticide on homing success such as infestation in varroa mites, satiety level of the bees and climatic conditions will be discussed.

106 Lazy Spring: pollen-bound pesticide mixtures make bees less efficient

Prado A.[1,2], Pioz M.[1,2], Vidau C.[2,3], Brunet J.L.[1,2], Jury, M.[1], Crauser D.[1,2], Requier F.[1,4], Le Conte Y.[1,2], Alaux C.[1,2]
[1] INRA, UR406 Abeilles & Environnement, Site Agroparc, Avignon, France; [2] UMT PrADE, Site Agroparc, Avignon, France; [3] ITSAP-Institut de l'abeille, Site Agroparc, Avignon, France; [4] IRNAD, Universidad Nacional de Río Negro, San Carlos de Bariloche, Argentina

While foraging in agro-environments honey bees are exposed to a wide range of agrochemicals used as fungicides, herbicides, insecticides and growth regulators. Most eco-toxicological studies have focused on the effects of single pesticides and report decreases in bee longevity. However, bees rarely encounter single pesticides in agro-environments, as mixtures of different contaminants is the norm. Studies that combine the identification of potentially hazardous pesticides mixtures with manipulative experiments are therefore critical for improving risk assessments. Here, we developed a method for identifying field-relevant pesticide mixtures present in pollen, based on molecules prevalence, mode of action, co-occurrence and association to lower brood production. We then tested their toxicity on worker honey bees by recording their flight activity and foraging efficiency. We identified two pesticide mixtures that significantly changed

bee behavior and life expectancy. Two mixtures consisting of 3-4 fungicides and 1 insecticide at very low concentrations, induced a delay in the onset of foraging and a slower foraging activity. As bee longevity is strongly influenced by the amount of time spent foraging, exposed bees outlived control bees. Furthermore, one of these pesticide mixtures hampers pollen foraging. Physiological analysis revealed that the altered behavior was preceded by perturbations of the energetic metabolism. Simulations of colony dynamics revealed that a prolonged exposition late in the foraging season would prevent exposed colonies from recovery and lead to their death in the following spring. In conclusion, we found for the first time that pesticide exposure can translate into longer-lived but slower bees. Under certain circumstances, these behavioral changes could lead to colony decline. These findings contrast with the commonly reported increase in bee mortality and are probably cryptic to the current risk assessment methods.

107 Versatile stressor impact analysis by video observation of worker behavior and brood development within cells reveals neonicotinoid effects at field realistic concentrations

Siefert P.[1], Hota R.[2], Grünewald B.[1]
[1] Goethe-University, Bee Research Institute, Frankfurt am Main, Germany; [2] Goethe-University, Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany

Risk evaluation of chemicals used in agriculture and beekeeping is necessary to preserve health and populations of honey bee colonies. To monitor effects, experiments are most commonly designed either to take snapshots of a colony's state in field experiments, or with a couple of bees in laboratory setups. While the former lacks in detecting behavioral impacts, the latter is hardly comparable with field conditions, also because greater bee numbers have various compensation possibilities. Non-disturbing long-term experiment studies of within-hive effects on behavior are largely missing, most probable due to several issues in visibility, tracking and disturbance.
Here we show our digital video recording method to detect stressor effects on worker behavior and brood development, using an observation hive that enables within-cell vision. In this context, we present exclusive video footage of various colony processes that have been rarely visualized in the past: deployment, modification and consumption of pollen, how bees use mandibles and wax for comb construction, or how nest temperature is preserved - we show why eggs are descending over time, how inspections, feedings and cannibalization of larvae look like, and demonstrate worker and queen ontogenesis in detail, including larval cocoon spinning and metamorphosis. We furthermore present commonly unseen pests behaviors, for example of developing wax moths or Varroa destructor, and show the honey bees' hygienic reactions to them. To evaluate the function of our application, we fed sublethal dosages of the neonicotinoids clothianidin (1 and 10 ppb) and thiacloprid (200 ppb) in syrup over three weeks, and were able to detect significant alterations in nursing behavior throughout larval development days one to four. With our presented method of video analysis, including state of the art deep learning algorithms, we believe our setup can be versatilely applied for impact analysis of chemicals, pests, and other stressors.

108 Pharmacokinetic and molecular investigations providing insights into the honey bee-friendly profile of the butenolide insecticide flupyradifurone (SIVANTO prime®)

Zaworra M., Almanza M.T., Nauen R.
Bayer AG, Crop Science Division R&D, Monheim, Germany

Flupyradifurone (SIVANTO prime®) is a novel butenolide insecticide that received its first EU registration very recently in the Netherlands. The discovery of flupyradifurone was inspired by the insecticidal activity of stemofoline, a botanical compound isolated from the Asian medicinal plant Stemona japonica.
Flupyradifurone shows reversible, agonistic binding to the insect nicotinic acetylcholine receptor (nAChR) with a novel pharmacophore system not found in any other insecticide. Due to its safety profile it already received in 2013 approval as a reduced risk insecticide by the Environmental Protection Agency of the USA. Meanwhile it has been launched in many regions of the world and is applied to control a broad range of sucking key pest species while displaying a favorable ecotoxicological profile.

Its acute toxicity (LD50 (48h)) to adult honey bees upon contact and oral exposure is >100 µg a.i./bee and 1.2 µg a.i./bee, respectively. SIVANTO prime® poses no unacceptable side-effects on foraging honey bees, the brood or the colony when it is used according to recommended label rates, even when the product is applied during flowering as recently demonstrated by numerous field studies.
Here we present pharmacokinetic, biochemical and molecular data explaining the honey bee-friendly profile of flupyradifurone. Pharmacokinetic studies with radiolabeled flupyradifurone revealed that it is only slowly taken up by the integument and readily metabolised when compared to other radiolabeled market standards such as neonicotinoids. Furthermore we investigated its metabolism in vitro by recombinantly expressing all clade 3 cytochrome P450´s of the honey bee genome in insect cell lines. We identified three cytochrome-P450 enzymes metabolising flupyradifurone to non-toxic metabolites as shown by nAChR receptor binding studies. Our study provides a molecular explanation for the observed honey bee-friendly profile of flupyradifurone under applied conditions.

109 Pesticide residues in bee bread from the national honey bee disease survey

Traynor K.[1], vanEngelsdorp D.[1], Rennich K.[1], Rose R.[2], Fahey R.[1]
[1] University of Maryland, Entomology, College Park, MD, USA; [2] USDA Animal and Plant Health Inspection Service

As part of the National Honey Bee Disease Survey, samples of stored pollen (bee bread) were collected from over 35 US States over six consecutive years. For each sample, the bee bread was collected from 8 colonies in the same apiary, pooled for an apiary-wide sample and analyzed for a suite of approximately 175 different pesticide residues. Preliminary analysis revealed that approximately 20% of all apiary samples were free of pesticide residues. Approximately 15% of all bee bread samples had residue levels equal to 10% of an adult honey bee's LD50, if consumed cumulatively over the 10-day nursing phase and not detoxified. The number of residues detected was highest in samples collected in the early spring. Miticides were the predominant pesticide residue detected, but typically these contributed very little to the hazard quotient risk. When a threshold of 0.5% of a honey bee's LD50 was used to eliminate low level residues, the most predominant class of pesticide detected was insecticides. Three insecticides (chlorpyrifos, fenpropathrin, and pyridaben) and two fungicides (chlorothalonil and propiconazole) were frequently detected residues. The maximum number of fungicide residues detected per sample increased over time.

110 Gene expression profiling demonstrates that exposure to neonicotinoid pesticides affects multiple biological processes in bumblebees (*Bombus terrestris*)

Fletcher I.K.[1], Colgan T.J.[1], Arce A.[2], Ramos-Rodrigues A.[2], Stolle E.[1], Gill R.[2], Wurm Y.[1]
[1] Queen Mary University of London, School of Biological and Chemical Sciences, UK; [2] Imperial College London, Department of Life Sciences, Silwood Park, UK

Insect pollinators including social bees are key to ecosystem stability as well as agricultural yields. There have been recent concerns about declines in social bees worldwide and one of the factors implicated in these declines is the use of pesticides on agricultural crops. These are intended to control pest species, but pesticides can also negatively affect non-target wild social bees. In particular, behavioural and field studies have clearly demonstrated that exposure to neonicotinoid pesticides negatively affects learning and memory abilities, foraging behaviour and colony survival of social bees. We know relatively little however about the molecular mechanisms by which pesticide exposure affects bees. To address this gap in our understanding, we exposed *Bombus terrestris* bumblebee workers to sublethal concentrations of two commonly used neonicotinoid pesticides, clothianidin and imidacloprid. We found widespread effects of pesticide exposure on gene expression in heads of *B. terrestris* workers. Some biological processes, including cellular transport and muscle contraction, were affected by both pesticides, clothianidin had a greater overall impact on gene expression. These findings provide novel insight into the molecular response of bumblebees to neonicotinoids, identifying candidate pathways for further experimentation. Our work provides a novel manner of understanding and of quantifying the effects of pesticide exposure and informs the debate on the costs of pesticide use.

111 Two deformed wing virus variants, genotypes A and B, cause low pupal mortality and a high frequency of wing deformities in metamorphosing honey bees (*Apis mellifera)*

Tehel A.[1], Vu Q.[2], Theodorou P. [1], Paxton R.J.[1]
[1] Institute for Biology, Martin Luther University Halle-Wittenberg, Halle, Germany; [2] Cuu Long Delta Rice Research Institute, Cantho City, Vietnam

Deformed wing virus (DWV) is an emerging infectious disease of the honey bee (*Apis mellifera*) which is efficiently transmitted between honey bees by *Varroa destructor* ectoparasitic mites. Both virus and mite are nowadays distributed worldwide except Australia and are likely a major cause of elevated losses of honey bee colonies over the past decades. DWV comprises two widespread genotypes, the originally described genotype A and the relatively recently (2004) described genotype B, otherwise known as *Varroa destructor* virus-1. In adult honey bees, DWV-B is more virulent than DWV-A. However, their comparative effects on earlier host developmental stages are unknown. Here we experimentally inoculated healthy honey bee pupae and tested for the relative impact of DWV-A versus DWV-B on mortality and wing deformities in eclosing adults. DWV-A and DWV-B caused similar, and only slightly elevated, pupal mortality (mean 20% greater mortality than control). Both genotypes caused similarly high wings deformities in eclosing adults (mean 62% greater wing deformities than control). Viral titre was high in all experimentally inoculated eclosing adults and was independent of wing deformities, suggesting that the phenotype 'deformed wings' is not directly related to viral titre or viral genotype. These viral traits favour the emergence of DWV by permitting the reproduction of its vector, *V. destructor*, in infected pupae, thereby facilitating the spread of DWV in honey bees infested by the mite.

112 Using an in vitro feeding system to investigate *Varroa*-vectored DWV variant transmission

Bradford E.L., Campbell E.M., Bowman A.S.
University of Aberdeen, School of Biological Sciences, Aberdeen, Scotland, UK

In recent years the health status of the European Honeybee (*Apis mellifera*) has been under threat. These health threats can be linked to the spread of the invasive parasitic mite, *Varroa destructor*, and increased damage associated with deformed wing virus (DWV) infection. In the absence of mites, infected bees are largely asymptomatic, but when vectored by mites, DWV can become a devastating overt infection. However, the underlying mechanisms of this *Varroa*-vectored DWV transmission are under investigated. When we consider DWV infection, there are two main variants: DWV-A and DWV-B. DWV-A is considered the classical variant, while DWV-B has been more associated with *Varroa*. Most transmission studies in this area used honeybees already infected with DWV. However, the high natural prevalence of DWV within honeybees can mask underlying DWV variant transmission from the mites. Using a newly developed in vitro feeding system for *Varroa*, we investigated the kinetics of the transmission of DWV and its variants from the mite and the DWV remaining within the mite. DWV levels within mites decreased enormously (≈ 99%) within a few days of in vitro feeding, when mite DWV levels are not being replenished by viral particles from the dietary (honeybee) source. Thus, replication rates of DWV within the mite cannot sustain the mite's DWV content when it is spitting DWV. As expected, the predominant of the two DWV variants (either DWV-A or DWV-B) within the mite was the major variant type transmitted during the initial feeding, but the amount of this variant passed into the feeding system decreased in subsequent days. In contrast, the less abundant variant within the mite increased in abundance both being transmitted and within the mite's body as the trial progressed. This observation held true whether mites were predominantly DWV-A or DWV-B prior to feeding. We found evidence of both variants replicating within *Varroa* during the entire period of the in vitro feeding experiment, and some evidence of increased replication of the minor variant over time. This information gives new insights into the underlying mechanisms of Varroa-vectored DWV transmission.

113 Europe-wide diversity of Deformed wing virus variants

Sircoulomb F.[1], Schurr F.[1], Blanchard Y.[2], Lucas P.[2], Dubois E.[1], Thiery R.[1], Smartbees consortium partners
[1] ANSES, Sophia Antipolis Laboratory, Honey Bee Pathology Unit, Sophia Antipolis, France; [2] ANSES, Viral Genetics and Biosecurity Unit (GVB), Zoopole, Ploufragan, France

Varroa destructor infestations (varroosis) are reported in many countries over the world. At the latest stage of infestation, bees can display shorten life span, shrunken wings, shorten abdomen, weakening and immunosuppression. These clinical signs and the subsequent overwintering colony losses are also associated to the transmission by *Varroa* of honeybee viruses, notably viruses belonging to the Deformed wing virus (DWV) cloud. Both stressors are often accused to represent one of the main causes of worldwide honey bee colony losses.
Varroa can vector DWV, Varroa destructor virus-1 (VDV1) and also DWV-VDV1 recombinant variants to the bees. It has been reported an increased viral load, virulence and reduced population diversity of the DWV in the parasitized bees leading to an overt infection. Additionally, it has been shown that DWV-VDV1 recombinants and VDV1 strains are more virulent than DWV strains and more importantly that VDV1 is closely linked to overwinter honeybee worker loss. However, little is known about the virus population structure and the virulence of strains isolated in Europe.
In the frame of the EU-funded project "Smartbees", foragers and/or bees with deformed wings were collected from 122 colonies spread across 14 European countries. Quantification of DWV and VDV1 loads was performed by selective RT-qPCRs at the colony level using a pool of bees and at individual level in bee heads. We detected VDV1 in bees from many European countries at high load. We also identified bees infected by both DWV and VDV1 in variable proportions. A representative set of DWV isolates was full-length sequenced in order to study phylogenetic relationship and genetic variation. The phylogeography of the studied DWV isolates will be discussed together with other metadata including varroa infestation level and honeybee subspecies.

114 Healthy honey bees - analysis of the virus population to assess rational *Varroa* control

Woodford L.[1], Evans D.J.[1], Bowman A.[2], Highet F.[3]
[1] BSRC (Biomedical Sciences Research Complex), University of St Andrews, UK; [2] Zoology, University of Aberdeen, UK; [3] SASA (Science and Advice for Scottish Agriculture), Edinburgh, UK

Varroa destructor is an ectoparasitic mite which is associated with significant losses of honey bee colonies globally. The mite acts as a vector for a range of pathogenic viruses, most important of which is Deformed Wing Virus (DWV). Overwintering colony losses, accounting for losses of ~25% of all colonies, are associated with high levels of Varroa-DWV infestation. Effective miticide treatments are available to control *Varroa* and so improve colony health. However, experience shows that treatment is rarely coordinated or used rationally, meaning controls are not implemented to maximise their efficacy and mite infestations are able to persist. This study uses coordinated treatment of *Varroa* in a geographically isolated environment (the Isle of Arran, Scotland). The goal is to determine whether rational, coordinated treatment is beneficial, using known characteristics of the DWV virus population as an indicator of colony health. It is reported that a high level of a near-clonal virus population is associated with *Varroa* infestation and colony losses, whereas *Varroa*-free healthy colonies carry only low levels of a diverse population of DWV. The study area contains ~50 colonies and 25 beekeepers. Sampling and virus analysis – strain diversity and viral loads – were conducted before and after treatment. Changes in virus diversity were quantified by genetic methods, including NGS analysis to determine population diversity. The first year of the three-year study is now complete and sampling has begun post-treatment in the second year. In parallel control studies, we are exchanging colonies between apiaries containing very high and low infestations of *Varroa* to ascertain the temporal changes in the virus population upon acquisition of the mite, or rational application of miticides. These control studies will inform our development of rationale *Varroa* control strategies for beekeepers in temperate regions.

115 Virome analysis on Belgian honeybees reveals multiple undescribed viruses including a diverse bacteriophage population

Deboutte W.[1], Beller L.[1], Yinda K.C.[1], Conceição-Neto N.[1] , Maes P.[1], de Graaf D.C.[2], Matthijnssens J.[1]
[1] Laboratory of Clinical and Epidemiological Virology, Rega Institute, KULeuven, Leuven, Belgium; [2] Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium

Advances in sequencing technologies and viral particle enrichment methodologies have resulted in the discovery of numerous, previously undescribed viruses. We applied high-throughput sequencing on Belgian *Apis mellifera* (honeybee) samples, treated according to the netoVIR protocol. In total 100 pools were sequenced, representing samples from 300 colonies sampled in the autumn of 2012 and 2013 in the scope of the EpiloBEE study. After quality control and de novo assembly, viral sequences were identified by BLAST-based approaches, domain searches and pHMMs. We were able to retrieve almost every known honeybee virus to date, and furthermore numerous novel divergent eukaryotic RNA viruses were discovered, including members of the Picornavirales, Mononegavirales and Orthomyxoviridae. We also describe divergent DNA viruses, including members of the Parvoviridae. Next, we used VIRsorter to identify prokaryotic viruses (bacteriophages) with a DNA genome, and pHMMs based on the RNA-dependend-RNA-polymerase to identify RNA bacteriophages. This resulted in the identification of more than 500 previously undescribed phage contigs. Using vContact2, an algorithm to classify these bacteriophages on the genera level, we could classify roughly 60% of the genomic contigs. When looking at the protein level, more than half of the ORFs did not show any amino acid similarity to proteins in public databases. Our analysis shows that viruses associated with honeybees are understudied, and despite having a relatively simple bacterial gut microbiota, the diversity of bacteriophages present in and on honeybees is a factor that has been neglected in most studies and can potentially have an impact on individual and/or colony health.

116 Apis Rhabdovirus-1, a negative-sense RNA virus present in populations of pollinators , replicates in *Apis mellifera* and *Varroa destructor*

Levin S.[1,2], Galbraith D.[3], Sela N.[4], Erez T.[1], Grozinger C.M.[3], Chejanovsky N.[1]
[1] Entomology Department, Institute of Plant Protection, Agricultural Research Organization, Israel; [2] Faculty of Agricultural, Food and the Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel; [3] Department of Entomology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA; [4] Department of Plant Pathology and Weed Research, Institute of Plant Protection, Agricultural Research Organization, Israel

Most of the viruses shared across bee species are positive-sense single stranded RNA viruses. However emerging data from metagenomics studies reveals that negative-sense RNA viruses are present and may be shared among pollinators. We found the negative-sense RNA enveloped virus, Apis rhabdovirus-1\Bee rhabdovirus-1 (ARV-1\BRV-1) in two bee species (*Apis mellifera* and *Bombus impatiens*) from bee populations in Israel and the United States as well as in *Varroa destructor* mites. Via quantitative Real-time PCR we discovered that in individual honey bees and mites ARV-1\BRV-1 can reach high titers (107-108 viral genomic copies). Furthermore, screening of honey bee colonies across Israel showed that the prevalence of the virus was about 20 %. Determination of the presence of the complementary sense RNA-strand indicated that ARV-1\BRV-1 replicates in *A. mellifera* and *V. destructor*. Our results suggest that *Varroa* mites could act as an ARV-1 vector; however, this may not be an absolute requirement for transmission among co-foraging bee species since ARV-1\BRV-1 we found it in *B. impatiens*.

117 A natural product inhibited the replication and expression of Israeli acute paralysis virus

Yang S.[1,2], Xu X.[1,2], Zhao H.[1,2,3], Deng S.[1,2], Chu Y.[1,2], Yang D.[1,2,4], Wang X.[1,2], Zhao D.[1,2], Diao Q.[1,2], Hou C.[1,2]
[1] Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; [2] Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Beijing, P.R. China; [3] Guangdong Institute of Applied Biological Resources, Guangzhou, P.R. China; [4] Graduate School of Chinese Academy of Agricultural Sciences, Beijing, P.R. China

Honeybee is fundamental to supply the pollination service for increasing the agricultural production and biodiversity. Recently in America and European countries, however, honeybee colony went through a large number of losses that has been linked with a RNA virus, Israeli acute paralysis virus (IAPV). Current knowledge about honey bee virus is limited, especially on virulence and pathogenicity of IAPV due to the lack of honey bee virus cell. Thus, it is crucial to construct a reverse genetic system to understand clearly the infection and develop effective drug to control IAPV. For this purpose, we constructed an infectious cDNA of full-length genomic clone of IAPV and rescued it from infected honey bee, and displayed identical phenotypes with wild virus. To further study the effect of natural product (named Q here) on inhibition of IAPV replication, we injected the healthy adult bees with constructed infectious IAPV and investigated the effect of Q application on their survival. Our results indicated that we not only constructed infectious clone of IAPV with virulence but also found one agent based on natural product to control the IAPV infection. Thus, we provided a power tool to study the molecular mechanisms involved in viral genome replication and virus pathogenesis, and found a potent antiviral agent that can be used widely in field. These results pave the way for further study the infection mechanism of honey bee virus as well as for antiviral treatment of bee viruses infected hives in practice. To our knowledge, our study provides the first infectious clone and antiviral agent based on the natural product and established a general model platform for studying the genetic characterization and gene functions of honey bee viruses.

118 Constructing infectious Sacbrood virus clone with green fluorescence protein expression

Mehmood S., Jin T., Huang H., Huang W-F.
College of Bee Science, Fujian Agriculture and Forestry University, Fuzhou, P.R. China

Sacbrood virus (SBV) is a common iflavirus causing larva death. Iflaviruses has only one open reading frame that translates into a polyprotein. How the polyprotein cleaves into functional proteins is not fully revealed, which leads to difficulties in creating mutations and exogenous gene introductions to the infectious clone. We successfully use bi-cistronic design to prevent the troublesomeness. We added intergenic internal ribosome entry site (IGR-IRES) of Black queen cell virus to the end of SBV genome, the end of 3′ nontranslated region. We add a commonly used enhanced green fluorescence protein gene and poly(A) after the IRES, resulting the genome structure: complete SBV-(BQCV IGR-IRES)-EGFP-poly(A). This design imitates the genome structure of dicistroviruses, which is phylogenetically close to iflaviruses. This clone recreated typical sacbrood symptoms with EGFP fluorescence in oral inoculation trials. Our bi-cistronic design provided a straightforward solution to add exogenous genes into SBV infectious clones. Clones of other bee-associated iflaviruses may be created using the same method.

119 Relevance of qualitative and quantitative changes in the virome to the survival of a Swedish *Varroa*-resistant honeybee population

Thaduri S.[1], Locke B.[1], Granberg F.[2], de Miranda J.R.[1]
[1] *Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2] *Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden*

The parasitic mite *Varroa destructor* in combination with viruses it transmits is the main cause for European honeybee, *Apis mellifera*, colony losses worldwide. However, there are several feral honeybee populations worldwide that manage to survive long-term without *Varroa* control. The best studied of these is on the Island of Gotland, Sweden which have survived without mite control treatment for more than 15 years. Additionally, recent studies with real-time PCR assays have shown that the Gotland mite resistant (MR) honeybees appear to be tolerant to honeybee viruses such as Black queen cell virus (BQCV) and Sacbrood virus (SBV) compared to mite susceptible (MS) honey bees. We have used next-generation sequencing (NGS) technologies to find more detailed metagenomics differences, especially the virome, and further confirm earlier findings of a strong reduction in virus titres in MR bees, both in absolute terms and relative to MS bees, as the season progresses from summer to autumn. The NGS analyses also discovered a distinct Lake Sinai virus (LSV) and *Apis rhabdovirus* (ARV) in Swedish honey bees. Further follow-up studies with real time PCR revealed also lower ARV and LSV titres in MR bees compared to MS bees, the same trend that was observed for BQCV and SBV. Phylogenetic analyses of the virus sequences in the NGS show a clear separation of the virus sequences in the two populations, but no evidence of progressive change during the season, in either population. These qualitative, genetic differences in virus sequence between the MR and MS population could be either part of the survival adaptation of the MR population, or due to pre-existing differences between the MR and MS populations, which are genetically isolated. Our results suggest that the Swedish mite resistant bees have a general tolerance to all viruses, although the mechanism behind this tolerance is still unclear, and that there may have been genetic co-adaptation by the viruses in the MR population.

120 European-wide analysis of *Paenibacillus larvae* genetic diversity: EuroPLarva

Sircoulomb F., Gaiani N., Paris L., Ribière-Chabert M., Rivière M.P.
ANSES, Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, Sophia Antipolis, France

The Gram-positive bacterium *Paenibacillus larvae* is the etiological agent of the most serious brood disease of honey bees, American foulbrood (AFB), causing considerable losses of honeybee colonies worldwide. Next generation sequencing (NGS) has greatly changed our approach regarding management of infectious diseases by shedding light on their genetic diversity, pathogenesis, evolution and detection. We used Illumina-based NGS and bio-informatic tools to analyse the genome of 64 *P. larvae* strains coming from 22 European countries. Comparison between the strains was performed at whole-genome, gene and genomic fragment level. We were able to identify conserved genomic fragments that could potentially improve the specificity of molecular identification. Additionally, the relationship between the strains revealed population genetic evolution and potential virulence factors. Finally, cgMLST typing further refine the typing of *P. larvae*. Our exploratory analysis of *P. larvae*'s genomes may improve its detection and contribute to better understand the pathological processes involved in AFB.

121 Health status and factors identified for winter losses of honey bee colonies by the Austrian surveillance study 2015/2016

Morawetz L., Köglberger H., Derakhshifar I., Mayr J., Moosbeckhofer R., Crailsheim K.
AGES GmbH, Vienna, Austria; [2] University of Graz, Graz, Austria

Austrian beekeepers experienced regularly high winter losses in their honeybee colonies in the recent years – a phenomenon also observed in other European regions. A surveillance study was conducted between summer 2015 and spring 2016 to describe the main factors for winter losses in Austria (part of the project „Future of honey bees"). The Austrian surveillance study took over the main concept of the EPILOBEE study, although Austria was not part of the study. During the Austrian study about 190 apiaries were visited three times within a year by trained bee inspectors. The health status from about 1500 colonies was examined, clinical signs of diseases were registered and bee samples were taken. The latter were used to determine varroa infestation rates by the detergent washing method. Of all noted clinical diseases, varroosis was most frequent (summer/autumn 5% of all colonies, spring 2%). Signs of chalkbrood were also found frequently in summer (4%), but less frequent in autumn and spring (2% and below). Signs of sacbrood and American foulbrood were seldom (all visits: <2% and <1%). No clinical signs of European foulbrood were detected. Univariate tests identified varroa infection rate as strong factor for winter loss ($P<0.001$). A high varroa infestation rate was correlated with DWV infection and clinical signs of varroosis. However, varroa infestation rate was a better predictor for winter loss than the other two factors. Thus, we recommend using varroa infestation rate for evaluating winter loss probability of bee colonies.

122 Should Varroosis include honeybee viruses as causative agents?

Schäfer M.O.[1,2]
[1] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald - Island Riems, Germany; [2] OIE Reference Laboratory for American foulbrood, Varroosis and infestation

The recent colony losses among honeybees (*Apis mellifera*) have worried not only beekeepers but also the public. Even simple causal relationships remain elusive and the picture has emerged that these colony losses are of multifactorial origin, it became more and more clear that infestation with *Varroa destructor* is playing a key role. Furthermore, it became clear that the parasite is triggering these losses in association with certain honeybee viruses, for which *Varroa destructor* is acting as mechanical and biological vector. Among the viruses vectored by *Varroa destructor*, the viruses of the AKI-complex or -clade (Acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus) and the DWV-clade (Deformed wing virus and *Varroa destructor* virus-1) have shown augmented prevalence and virulence worldwide. If one regards the clinical signs of these viruses, it becomes obvious that some of the clinical sings are already in use defining Varroosis. Therefore, these virus-clades should be included into the definition of Varroosis and the diagnostic techniques for identification of these viruses should be included into the manuals for diagnosis of Varroosis.

123 The yellow-legged hornet: its impact on European western honeybees and control methods

Pointeau S.[1,2], Rome Q.[3], Requier F.[4], Fournier A.[5], Decante D.[6], Vallon J.[1,2], Henry M.[2,7], Decourtye A.[1,2]
[1] Institut de l'abeille (ITSAP), Avignon, France; [2] UMT PrADE, Site Agroparc, Avignon, France; [3] UMS PatriNat – AFB, CNRS, MNHN, Muséum National d'Histoire Naturelle (MNHN), Paris, France; [4] Instituto de Investigaciones en Recursos Naturales, Agroecología y Desarrollo Rural (IRNAD), Sede Andina, Universidad Nacional de Río Negro (UNRN) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Carlos de Bariloche, Río Negro, Argentina; [5] Ecologie, Systématique et Evolution, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Paris, Orsay, France; [6] Association pour le Développement de l'Apiculture Provençale (ADAPI), Avignon, France; [7] INRA, UR 406 Abeilles et Environnement, Site Agroparc, Avignon, France

Since the accidental introduction of the Yellow Legged Hornet (*Vespa velutina*) in France before 2004, public authorities, scientists, and beekeepers have been looking for efficient control methods of this new predator of European western honeybees. Over the last 14 years, *V. velutina* has invaded almost all the French territory as well as the neighbouring countries (Spain, Portugal, Italy, Germany, Belgium, UK, and the Netherlands), which increases concern about this invasive predator. Since 2012, measures were taken by the French ministries of Agriculture and Ecology to list *V. velutina* among sanitary risk of second category for honeybees and invasive alien species. While beekeepers suspect *V. velutina* to increase substantially the rate of winter colony losses, little is known about the real impact of the hornet predation on honeybee colony dynamics and survival. To fill this gap, scientific works are currently carried out to assess both the impact of *V. velutina* on honeybees and potential damages to beekeeping. We propose an overview of advances in these works, with a special emphasis on control methods tested such as the spring queen trapping efficiency and the development of nest control through environmentally safe biocide.

124 Insights into honey bee pathogen and parasite dynamics and interactions using high-throughput analysis

D'Alvise P., Hasselmann M.
Institute for Husbandry Animal Science, University of Hohenheim, Stuttgart, Germany

Honey bee colony losses, if not caused by starvation or queen failure, are usually mediated by a number of viral, bacterial and eukaryotic pathogens. Colonies normally suffer from multiple infections before their final decline, yet little is known about the dynamics and interactions of inapparent infections.
We hypothesize that many infections are multifactorial, synergistic processes, and that co-occurrence of some pathogens may be predictive for colony decline. Testing this hypothesis requires quantitative measurements of all relevant pathogens over extended time periods in many colonies. Some of these will eventually develop diseases, and pathogen dynamics before the outbreaks can be analyzed for synergistic interactions. Quantitative analysis of all relevant pathogenic organisms in honey bee samples was rarely applied, but is necessary to understand pathogen dynamics.
We deploy high-throughput qPCR analysis to quantify honey bee pathogens, intestinal bacteria and the expression of selected immune and control genes in parallel in a large number of samples. The analysis is conducted on single bees, which allows us to study potential interactions of the pathogen and parasite species within the same host.
As a general result, bees from healthy colonies show low titers of pathogens, whereas diseased colonies showed increased titers of viral and bacterial pathogens and intestinal parasites. Within our current set of data from a long-term observation of six apiaries we found the intestinal parasite Nosema ceranae to be correlated with Black Queen Cell Virus and constituents of atypical intestinal microbiota. This observation may be a hint to synergistic parasite-pathogen interactions that may contribute to emergence of diseases.

125 Small hive beetle invasion risk under current and future climate scenarios: a modelling approach to foster mitigation efforts

Cornelissen B.[1], Schweiger O.[2], Neumann P.[3]
[1] Wageningen University & Research, Wageningen, Netherlands; [2] Department Community Ecology, Hemholtz Centre for Environmental Research, Germany; [3] Institute of Bee Health, University of Bern, Bern, Switzerland

Climate change and biological invasions are two major global environmental challenges and may interact, e.g. via altered impact and distribution of invasive alien species. The Small hive beetle (SHB, *Aethina tumida*, Murray) has spread from its native range to become established on all other habitable continents. Establishment in the context of biological invasion, requires SHB to survive and reproduce and ultimately complete the life cycle. Since SHBs pupate in soil, pupation performance is governed foremost by two abiotic factors, soil temperature and moisture, which will be affected by climate change.
In this study, we investigated the potential distribution and impact of SHB under current and future climate scenarios on a global scale. We did so by modelling pupal performance as a defining factor explaining the invasion risk of SHB.
We constructed a model explaining pupal performance (survival and development time) based on soil temperature and soil moisture data from published studies. We used presence data on SHB distribution to validate the model. We then linked pupal performance to global soil data in order to predict the likelihood of SHB establishment as a determinant for invasion risk under current and future climatic conditions. We were able to classify areas (resolution: 10 arcmin) as unsuitable, marginal and optimal for SHB pupation performance. The results show that many areas yet uninvaded are suitable for SHB to become established. Future climate change scenarios project a further expansion mainly in the northern hemisphere. This risk analysis will help areas to prepare adequately and may enable to mitigate the impact of global SHB invasions.

126 Survey of wild bee communities threatened by *Vespa velutina*

Carisio L., Lioy S., Porporato M., Manino A.
University of Turin, Department of Agriculture, Forest and Food Sciences, Turin, Italy

The yellow-legged hornet (*Vespa velutina* Lepeletier 1836) preys on of honey bees *(Apis mellifera* L.) and of other insects such as wild bees. It was accidentally introduced into France in 2004 and is rapidly colonizing other European countries. In Italy, the species is spreading throughout the northwestern part of the country and is causing losses to beekeeping industry and potentially to pollination ecosystem service. In the last decade, a global honeybees and wild pollinators decline has been reported and yellow-legged hornet could be a further deterioration factor. Since 2016 we are studying, under the EU funded LIFE STOPVESPA project, the status and trends of wild bee communities of Liguria region in order to evaluate any *V. velutina* impact on pollinators. Six areas with different yellow-legged hornet density were surveyed using a pan-trap sampling method. Richness, abundancy and species evenness (Pilou index) of sampled wild bee communities were recorded. Patterns of *V. velutina* colony growth and predation intensity through the season were also estimated and compared with wild bee flight periods. The effect of *V. velutina* presence was tested as correlate factor on wild bee abundancies using a GLMm model. Twenty-three survey sessions led to collect 643 specimens in 2016 and 1035 in 2017. More than 150 wild bee species were identified. We found 25 species not previously reported in Liguria region and one species (*Andrena asperrima* PÉREZ 1895) new for Italy. We detected an extremely variable abundancy, especially in the early summer period. The surveyed communities have the same evenness except for one site probably affected by intensive agricultural surroundings. Species richness is significatively different among sites. GLMm model does not show any effect on wild bee abundancy due to a short time yellow-legged hornet invasion. Significant *V. velutina* predation intensity appears to overlap with late summer flying wild bees in particular with species of the genera *Colletes*, *Hylaeus*, *Ceratina*. Further survey are necessary to highlight whether any *V. velutina* predation effect will occur after a longer time since the invasion.

127 Patterns of *Vespa velutina* invasion in western Iberia and Italy as revealed by molecular markers

Quaresma A.[1], Henriques D.[1], Godinho J.[2], Maside X.[3], Bortolotti L.[4], Pinto M.A.[1]
[1] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal; [2] National Institute of Agrarian and Veterinary Research (INIAV), Oeiras, Portugal; [3] Grupo de Medicina Xenómica, Universidade de Santiago de Compostela, Santiago de Compostela, Spain; [4] Honey bee and Silkworm Research Unit (CRA-API), Bologna, Italy

The Yellow-legged or Asian hornet (*Vespa velutina nigrithorax*) was naturally distributed in Southeast Asia. However, in 2004, it was accidently introduced in France from China and in the last decade it spread rapidly through the French territory and to other European countries. In the Iberian Peninsula it was reported for the first time in Spain, in 2010, and in Portugal, in 2011. Using a population genetics framework, the goal of this study was to test the genetic patterns of colonization of this invasive honey bee predator in the Atlantic side of Iberia and in Italy. A total of 246 individuals, each representing a single colony, were collected across the invaded area in Portugal (190), Spain (45) and Italy (11). Additionally, a dataset containing samples from France, Vietnam, South Korea, Indonesia and two provinces of China provided by Arca et al. (2015) was used as a reference for testing hypothesis about origin of the invasion. The genetic variability was assessed using 16 microsatellite loci and the mitochondrial cytochrome C oxidase. Population structure was inferred using the Bayesian approach STRUCTURE and diversity was estimated using GenAlex 6.5. Our results show that genetic diversity is low in Portugal, as expected from a founder effect originating from the French population. The Spanish population shows a higher genetic diversity and our data suggest that this is due to independent invasions originating from two range expansions: one from France and another from Portugal. The molecular data obtained for the Italian sample show diversity levels similar to those of Spain and supports introduction by range expansion from France. The mtDNA analysis revealed the presence of a single haplotype in Iberia and Italy, which has been also reported for France and UK. These results are in accordance with other European studies, further supporting an entrance of a small number of propagules or even of a single multi-mated queen in Europe.

128 Reproductive success in European orchard bee *Osmia cornuta* (Hymenoptera: Megachilidae) influenced by the number of males

Stanisavljević L.[1], Rašić S.[2], Stanisavljević J.[3]
[1] Department of Invertebrate Zoology and Entomology, Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, Serbia; [2] Faculty of Ecological Agriculture, Educons University, Sremska Kamenica, Serbi; [3] Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Belgrade, Serbia

The main objective of this study was to analyze the effect of the number of males mated with females on their fecundity and nesting potential. In the spring of 2017, the three experimental groups of bees were designated by the sex ratio (female/male) as 1:1, 1:2 and 1:3. Each group of bees, in the controlled laboratory conditions, consisted from 30 newly emerged females were released in the small cage (40x50x60 cm) with the designed numbers of males, provided with artificial carbohydrate food source of sucrose/water (50:50) solution. Males were emerged 2-3 days before females and immediately transferred to the cages. The first group of females spent 6h with males, and the second group 12h at the light regime 1:1, t=20±2 °C, and RH=65-70%. The body size and weight of both sexes are consistent with the average of our reared "population". After completion of mating, the females were transferred into a part of the field covered with a large cage (4x4x2 m) with a mixture of flowering plants (mostly rapeseed) and suitable natural nesting material.
During the mating and nesting activity were recorded behavior, longevity, and provisioning rate. The results of offspring production show statistically significant differences among experimental groups in both mating times. The group with equal sex ratio had a smaller number of progenies than others four: 1) mating time 6h: M=12.67±3.04, compared with M=17.93±3.33 and M=18.33±3.84, respectively; 2) mating time 12h: M=13.37±3.85 compared with M=20.30±3.39 and M=20.07±3.33, respectively. Sex ratio among offspring varies from 1:1.89 in the group with three males per female to 1:3.51 in the group with one male per female.
The low sex ratio in parent population is positively correlated with higher production of male progeny and vice versa. The main restriction factor for population abundance increasing is correlated with the available number of males.

Practically, these findings have a high importance for augmentation of *O. cornuta* populations and use as an orchard pollinator.
The same experiment was established in the springtime of 2018, and we are expecting first results in the late summer when adult bees becoming mature in cocoons.

129 Differential circular RNAs expression in ovary during oviposition in honey bees

Chen X., Shi W., Chen C.
Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, P.R.China

Circular RNAs (circRNAs) are non-coding RNAs newly identified and play important roles in RNA regulation. The mechanism and function of circRNAs have been reported in some species. However, little is known regarding circRNAs in honey bees. In this study, we analyzed circRNAs through bioinformatics, and predicted 12,211 circRNAs in the ovary of honey bee queens. 1340, 175 and 100 circRNAs were differentially expressed in comparisons of egg-laying queens vs virgin queens, egg-laying inhibited queens vs egg-laying queens and egg-laying recovery queens vs egg-laying inhibited queens. Further, functional annotation of differentially expressed circRNAs revealed several pathways that are closely related to ovary activation and oviposition, including insulin secretion and calcium signaling pathways. Moreover, the potential interactions among circRNAs, miRNAs, lncRNAs and mRNAs were investigated. Ame_circ_0005197 and ame_circ_0016640 were observed to sponge several reproductive related miRNAs. These findings demonstrate that circRNAs have potential effects in ovary activation and oviposition of honey bees.

130 Influence of DMSO with dextran, PVP or PEG in freezing extender on queens

Nur Z.[1], Çakmak S.S.[2], Çakmak I.[3], Önder N.T.[1], Gökçe E.[1], Üstüner B.[1], Alçay S.[1], Toker B.[1], Şen H.[3], Soylu M.K.[1]
[1] Uludag University, Faculty of Veterinary Sciences, Department of Artificial Insemination, Bursa, Turkey; [2] Ankara University, Faculty of Sciences, Department of Biology, Ankara, Turkey; [3] Uludag University, Beekeeping Development-Application and Research Center, Bursa, Turkey

The objective of this study was to elucidate the toxicity of widely used penetrating cryoprotective agents (DMSO) to frozen drone semen. DMSO was replaced totally or partially with the dextran, polyvinylpyrrolidone (PVP), and poyethylen gylchole (PEG); some of the non-penetrating cryoprotectant. The freezing extender with the %12, %8, %4 and %0 DMSO were supplemented with the %0, %4, %8 and %12 of dextran, PVP or PEG; respectively. For all treatment groups the final cryoprotectant concentration was %12 except control group was %0. Extended and straw (0.25ml) filled semen were frozen with LN vapors for the 7-10min and then were plunged in LN until thawing. Fresh and post-thaw semen motility and plasma membrane functional integrity were evaluated under phase-contrast microscopy (400×). Group with good motility and plasma membrane functional integrity (Dextran4, PVP4, DMSO12) and fresh semen were used for queen insemination (n=76) instrumentally. Two queen bees (2,6%) were died due to manipulation. The 36 (62%) of 58 live queens were observed to survive after a week, 23 (63.8%) live queens were observed to survive after three months. At the end of three months, 91.3% of the live queens continued to produce worker bees and 8,6% drones. At the end 10 month after overwintering there are two queens from PVP4 and tree queens from Dextran4 were survived and one queens from PVP4 and two queens from Dextran4 groups were continued to produce worker bees. In terms of rate of survival for tree month and worker bee, there were no differences among insemination groups (P>0.05). There were only 2 (25%) queens that were inseminated with 12DMSO produced drones.
This work was supported by TUBİTAK (Project Number: TOVAG215O586)

131 Queen temperature stress decreases sperm viability, queen performance, and colony productivity

Guarna M.M.[1], Pettis J.S.[2,3], Pernal S.F.[1]
[1] Agriculture and Agri-food Canada Bee Research Laboratory, Beaverlodge, AB, Canada; [2] USDA-ARS Bee Research Laboratory, Beltsville, MD, USA; [3] Current affiliation: Pettis and Associates

The health and performance of honey bee queens is an important factor determining colony productivity and survival. The aim of this project was to evaluate whether exposing queens to high and low temperatures affected the viability of queen's sperm, queen performance, and colony productivity
Objectives:
a) to monitor temperature in honey bee queen shipments, b) to evaluate the effect of temperature treatment on queen's sperm viability, queen performance, colony productivity and colony survival.
Methods: We monitored temperature of queen shipments using data-loggers. We then exposed queens to the observed temperatures and evaluated the viability of the sperm by fluorescent microscopy. Finally, in a 60 colonies field experiment, we compared the performance of temperature-treated queens and controls and evaluated colony productivity and wintering survival of colonies headed by temperature-treated queens and controls.
Results and conclusions: Queens can be exposed to both high and low temperatures during shipment which reduces sperm viability. These temperature treated queens, however, were otherwise indistinguishable physically or behaviorally from non-treated queens.
In a field study of 60 colonies, we observed a dramatic decrease in queen performance and colony productivity in colonies headed by temperature-treated queens. These colonies had poor brood patterns, only half of the adult population, and only one-third of the honey production compared with control colonies. In addition, while no overwintering losses were observed in colonies headed by control queens, 61% of the colonies headed by temperature-treated queens perished over the winter.
These striking results are of importance to the beekeeping industry as they can guide future efforts to improve shipping conditions as well as guide management decisions to improve queen performance and colony productivity.

132 Observations of the mating behaviour of *Apis melifera macedonica* and *Apis melifera cecropia* under natural conditions and under conditions of a control mating system (the Train of Virgin Queens)

Hatjina F., Charistos L.
Hellenic Agricultural Organization 'DEMETER', Division of Apiculture, Nea Moudania, Greece

An innovative method was applied to ensure controlled and at the same time natural fertilization of queen bees, as an alternative method to Instrumental Insemination. The Train of Virgin Queens (TVQ), also called the Joe Horner's System, is a method that shifts the natural mating of the queens hours later in the afternoon when there are no undesirable flying drones (The relevant video for the TVQ can be found here: https://www.youtube.com/watch?v=V8jXQeScgVg). The queen's nuptial flights were observed in terms of starting time, returning time and frequency of flights occurred every day and compared between the two different conditions, natural condition and TVQ. The ambient temperature were also recorded. Measurements took place in 2016 for the *macedonian* and in 2017 for the *cecropian* queens. The *macedonian* queens in May 2016 started the nuptial flights around 15.00 and returned after 16.00. In June, there was an average shift of at least 1 hour later on departure and return, and flight time was also prolonged by 15 minutes. Queens at the TVQ were released at 17.30 but always started their flight at least 30 minutes after transferring the nucleus outside, possibly to have a restored relative temperature and homeostasis to the hive forst. Thus, for May 2016, it was observed that most queens departed around 18.15 and returned around 19.15 (flight time of about 60 minutes), while in June they were released 30 min later, the departure occurred around 18.30, and eventually the return was observed between 19.00 and 20.00. A little different was the behavior of *cecropian* queens in 2017, when in June they normally started their flight after 16.30 in the afternoon and in July after 14.30. The above work is the first record of the coupling behavior of the Greek queens. The results will be very useful for limiting the daytime interval during which controlled coupling be made, while the system is evaluated for its usefulness in an area with a very high honeybee colony density.

133 Preservation of domesticated honey bee (Hymenoptera: Apidae) drone semen

Paillard M.[1,2], Rousseau A.[1], Giovenazzo P.[1,3,4], Bailey J.L.[1]
[1] Centre de recherche en sciences animales de Deschambault, Deschambault, Canada; [2] Departement des Sciences Animales, Centre de Recherche en Reproduction, Developpement et Sante Intergenerationnelle, Universite Laval, Pavillon Paul-Comtois, Quebec, Canada; [3] Departement de biologie, Universite Laval, Pavillon Alexandre-Vachon, Quebec, Canada

Preservation of honey bee (*Apis mellifera L.*, Hymenoptera: Apidae) sperm, coupled with instrumental insemination, is an effective strategy to protect the species and their genetic diversity. Our overall objective is to develop a method of drone semen preservation; therefore, two experiments were conducted. Hypothesis 1 was that cryopreservation (-196C) of drone semen is more effective for long-term storage than at 16C. Our results show that after 1 yr of storage, frozen sperm viability was higher than at 16C, showing that cryopreservation is necessary to conserve semen. However, the cryoprotectant used for drone sperm freezing, dimethyl sulfoxide (DMSO), can harm the queen and reduce fertility after instrumental insemination. Hypothesis 2 was that centrifugation of cryopreserved semen to reduce DMSO prior to insemination optimize sperm quality. Our results indicate that centrifuging cryopreserved sperm to remove cryoprotectant does not affect queen survival, spermathecal sperm count, or sperm viability. Although these data do not indicate that centrifugation of frozen-thawed sperm improves queen health and fertility after instrumental insemination, we demonstrate that cryopreservation is achievable, and it is better for long-term sperm storage than above-freezing temperatures for duration of close to a year.

134 Experimental evolution of parasite virulence in a eusocial insect

Lattorff H.M.G.[1,2,3]
[1] International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya; [2] Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany; [3] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany

Parasites are ubiquitous and affect almost all groups of animals. The effect on their host can be quite different ranging from benign to extremely harmful. One of the major factors affecting the virulence is the mode of transmission. Vertical transmission might result in reduced virulence, as this system ensures transmission, while under horizontal transmission higher virulence is expected to evolve. In social insects there is an additional level of social organization, the level of the colony. It is not well understood, whether the transmission between colony members is horizontal (members of the same generation) or vertical (transmission ensurance pathway) and it is not clear how such transmission events might affect the evolution of virulence. I conducted a serial passage experiment using primitively eusocial bumblebees and their intestinal parasite *Crithidia bombi*, a trypanosome. Six subsequent passages of *C. bombi* were conducted using individuals of the same colony and compared to parasite strains that were passaged through changing individuals of two different colonies. Each set up was replicated six times. Finally, the evolved strains were tested in two different genetic backgrounds closely related to the genotypes used for passages. The growth rate of *C. bombi* was determined for a four day period. The strains evolved within homogeneous hosts evolved a low virulence level, while changing the genetic background during passages resulted in higher virulence. These results indicate that transmission between closely related members of a social insect colony is very similar to vertical transmission, potentially due to the ensurance of transmission. Heterogeneous host background results in an increase in virulence. This has serious implications for the understanding of disease transmission and evolution of virulence of pathogens in pollinator insects.

135 Evolution of new gene functions by gene duplications - the case of major royal jelly proteins in the honey bee

Buttstedt A.[1,2], Mureșan C.I.[1,3], Helbing S.[1], Moritz R.F.A.[1]
[1] Martin-Luther-Universität Halle-Wittenberg, Zoologie - Molekulare Ökologie, Halle, Germany; [2] Technische Universität Dresden, B CUBE - Center for Molecular Bioengineering, Dresden, Germany; [3] Universitatea de Ştiințe Agricole şi Medicină Veterinară, Departamentul de Apicultură şi Sericicultură şi Biotehnologii, Cluj-Napoca, Romania

Gene duplications are terribly valuable for evolution as one can just fiddle around with the copy and change it hopefully into something useful enhancing the fitness of the carrier, whereas the function of the original remains untouched. However, in practice, it is not as simple as that and although gene duplications offer unprecedented opportunities for new functions, a duplicated gene is much more likely to become non-functional simply because deleterious mutations occur more often than advantageous ones. In addition, even if advantageous mutations occur, the probability that the respective allele becomes fixed within a population is rather low and advantageous alleles are frequently lost by chance. Thus, manifold gene duplications, driven mainly by unequal crossing over, resulting in multigene families provide considerably better conditions for the evolution of new gene functions than single gene duplications.
One of these multigene families that is perfectly suitable for the analysis of gene evolution is the major royal jelly protein (mrjp) gene family which evolved exclusively in the insect order Hymenoptera and comprises a total of ten copies within the genus *Apis*. We show here by combining research on DNA, RNA and protein level that *Apis*-mrjps experienced and are still experiencing manifold evolutionary pathways from pseudogenization of different mrjps in different *Apis* species (e.g. mrjp7 and 10 in *A. dorsata* and *A. mellifera*, respectively) to neofunctionalization (e.g. mrjp1). The neofunctionalization of MRJP1 simply ensures the survival of *Apis* bees, as the protein in complex with Apisimin defines the physicochemical properties of royal jelly, the food of honey bee queen larvae. A pH-dependent fibril network build by MRJP1 and Apisimin maintains the high royal jelly viscosity that is needed to raise queen larvae in their horizontally oriented queen cells.

136 Overview of the southwest Indian ocean honeybee: combining morphometry and genetics to investigate the original diversity of *Apis mellifera* spp. in Madagascar and surrounding archipelagos

Galataud J.[1], Delatte H.[2], Bernet C.[1], Techer M.[1,2,3], Reynaud B.[2], Clémencet J.[1]
[1] UMR PVBMT, University of La Réunion, Saint Denis, France; [2] CIRAD, UMR PVBMT, University of La Réunion, Saint Pierre, France; [3] Current Address: Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Recent studies characterizing the diversity and ancestry of the honeybee in the South-West Indian Ocean islands (SWIO), the endemic area of *Apis mellifera unicolor* ssp. (Latreille, 1804), established that the native range of *A. m. unicolor* is not restricted to Madagascar but extends to the surrounding archipelagos and involves a newly described African sub-lineage. Yet, these insular populations are highly genetically structured, due to old geographical isolation but also to modern introductions of European subspecies, especially in the Mascarenes (Reunion, Mauritius and Rodrigues island). The present study aims to outline the evolutionary processes that might have led to intraspecific diversification of the honeybee in the area, by coupling genetic results with morphometric approaches. For this, the forewing shape and the proboscis length of previously genetically characterized honeybees from 11 islands and 3 archipelagos (Seychelles, Comoros, Mascarene islands and Madagascar) were analyzed using classical and geometric morphometric methods (N ≈ 1000 specimens); European and African continental samples were also implemented as outgroups. Both traits present high geographic variability and proved to be highly relevant for the discrimination of lineages, subspecies, hybrids and ecotypes of *Apis mellifera* sp.. Thus, they were of particular interest within the SWIO area, a hotspot of diversity displaying many endemic flowering plants with which the honeybee interacts, and which also faces with recent imports of European lineages and subspecies. The observed patterns of diversification were highly congruent both at the inter- and intra-archipelago scales and reflected the finest genetic divergences detected via neutral genetic markers, including the ongoing hybridization process in Mauritius Island where European subspecies were recently imported. All together, these results shed light on the original diversity of the honeybee in the SWIO and the need to preserve it.

137 Genetic basis for the evolution of non-reproduction of *Varroa destructor* in populations of *Apis mellifera*

Conlon B.H., Moritz R.F.A., Routtu J.
Molecular Ecology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

The brood-parasitic mite *Varroa destructor* devastates colonies of the honeybee *Apis mellifera* around the globe. Managed colonies of *A. mellifera* usually require treatment with acaricides in order to survive more than three years post-infestation. However, natural selection can drive the evolution of resistance traits when populations are left untreated with acaricides. One such resistance trait, host-induced inhibition of *Varroa* reproduction, has evolved independently in several populations of *A. mellifera* which are able to survive more than three years of infestation without acaricide treatment. While these resistant populations are well studied, the genetic basis for this resistance trait is less well understood.
To this end, we studied Varroa-tolerant *A. mellifera* populations where the non-reproductive trait is not fixed. Using the presence of resistant and susceptible phenotypes in the same colony, we analysed whole-genome sequence data from drones and identified genomic regions associated with resistance. Due to the lack of within-locus dominance in the haploid genome of drones, we were able to accurately identify genome regions linked to *Varroa*-resistance in independently-evolved honey bee populations.

138 Floral landscape enrichment and semi-natural habitats improve honeybee health, as evidenced by a 'Landscape physiology' approach

Alaux C.[1,2], Allier F.[2,3], Decourtye A.[2,3,4], Odoux J-F.[5], Le Conte Y.[1,2], Henry M.[1,2]
[1] INRA, UR406 Abeilles et Environnement, Avignon, France; [2] UMT PrADE, Avignon, France; [3] ITSAP-Institut de l'Abeille, Avignon, France; [4] ACTA, Avignon, France ; [5] INRA, UE1255 Entomologie, Surgères, France

Anthropogenic-driven effects on landscape (habitat loss, fragmentation and degradation) expose most of insect pollinators to new and enduring environmental challenges. Therefore, there is an urgent need to understand how landscape alteration affects populations of pollinators, but also to promote landscape restoration, notably via agri-environment schemes. However, the assessment of landscape disturbance is often limited because the health state of populations is rarely considered. Furthermore, whether habitat-restoration techniques actually improve the health of targeted pollinator populations remains obscure. This gap could be filled thanks to a comprehensive understanding of how gradients of landscape quality influence pollinator physiology.
We therefore used this novel approach for honeybees (*Apis mellifera*) to test whether landscape patterns can shape bee health. We focused on the pre-wintering period since abnormally high winter colony losses have often been observed. We exposed colonies to different landscapes, enriched or not in melliferous catch crops and surrounded by semi-natural habitats, and then assessed the link between the landscape quality (catch crop and semi-natural habitats) and bee physiology (fat body mass and level of vitellogenin – marker of bee longevity).
We found that bee physiology was significantly improved by the presence of flowering catch crops, which were associated with a significant increase in pollen diet diversity. The influence of semi-natural habitats on bee health was even stronger. Vitellogenin level was in turn significantly linked to higher overwintering survival. Therefore, our experimental study, combining landscape ecology and bee physiology, offers an exciting proof-of-concept for better understanding the influence of the environment on pollinator health and setting the stage for more effective pollinator conservation.

139 Influence of major stresses on wild bees communities in an urban environment

Weekers T.[1], Leclercq N.[1], Hainaut H.[1], Caruso G.[2], Molenberg J-M.[1], Vereecken N.J.[1]
[1] Université Libre de Bruxelles, Agroecology Department, Brussels, Belgium; [2] Université du Luxembourg, Institute of Geography and Spatial Planning, Esch-sur-Alzette, Grand Duché du Luxembourg

In this presentation we aim to gain a deeper understanding on the interplay of major environmental stressors (in this case urbanization and pesticide exposure), their effect on wild bees' diversity and their interactions with flowering plants. Urbanization is known to be an important process and cause of habitat loss and reduction in pollinator species. We tested the hypothesis that increased urbanization levels have a negative effect on both the diversity of wild bees (through a decrease in species richness, functional and phylogenetic diversity), and also on their interactions with flowering plants (decreased number of interactions, increased generalization of the remaining pollinators due to the loss of more specialized pollinators species). Moreover, an increase in urbanization levels is expected to be statistically correlated with a decrease in areas and connectivity of managed green urban spaces (gardens, cemeteries, etc.), with a potential decrease in pesticide exposure.
Pesticide exposure is likely to be more prevalent at the countryside, but private gardens might also represent a threat to wild bees in an urban context. We assessed the extent to which mason bees (*Osmia cornuta* and *Osmia bicornis*) were exposed to pesticides by performing multi-residue analyses on the pollen contents of their brood cells in various areas of the Brussels Capital Region. We focused on pollen, as its chemical composition is known to impact bee health. Agrochemicals (including neonicotinoids, glyphosate, and other multi-residues analysis) were quantified from pollen breads (i.e. mixed of pollen and nectar collected by females to feed the larvae), using validated methods with high sensitivity (low limit of detection [LOD] and limit of quantification [LOW]) covering a large number of molecules. We will also discuss the co-occurrence of active substances and their impact on wild bee community structure and plant-pollinator networks.
These plant-pollinator networks were characterized for various sites subject to pesticide analyses, in distinct habitat types and along an urbanization gradient. Our results have important implications for urban green spaces management in a context of high real estate pressure and urban dwellers aspiring to more connections to nature in its various components.

140 The application of non-intrusive electronic bee hive monitoring to field studies

Evans H.A., Evans S.K.
Arnia Ltd, Jesmond, Newcastle upon Tyne, UK

Most honey bee studies tend to involve frequent physical manipulations however honey bees do not benefit from being continuously disturbed by persistent examinations; indeed there is a risk that these assessment methods bias the behavioural phenomena being studied. Non intrusive electronic bee hive monitoring greatly reduces this problem while offering simultaneous data collection from any number of colonies at any number of sites without the traditional need for associated human resource. The automation of data collection also removes many of the errors related to manual record logging. Electronic hive monitoring therefore enables better data management and truly scalable studies involving hundreds or thousands of colonies across various geographical areas over extended periods, thus facilitating the pooling of diverse sets of data and resources.
Hive monitors can reliably, frequently, consistently and objectively measure parameters such as hive homeostasis (brood temperature/humidity), bee activity (flight and fanning acoustics) and productivity (hive weight). In addition, a bee counter placed at the entrance of a hive can be used to monitor bee traffic; bees entering and leaving the hive. These counts provide valuable information about forager strength, nectar availability, in-field death rates, changes in forager behaviour due to disease and how the local environment influences bee activity. Furthermore, it is demonstrated that accumulating data is an invaluable resource for retro evaluating cause and effect relationships, as the system can provide "black box" data offering an audit log of the events leading up to colony failure. Metrological data such as apiary

air temperature, rainfall, humidity, sunshine and cloud cover are shown to add perspective to other sensor readings. Data is presented from studies on *Varroa* and in-hive treatments, a large scale European study on the effects pesticides have on colony performance and the effects of a fungicide treatment during almond pollination in California.

141 Changes in bumble bee species diversity and abundance over 50 years on red clover fields in Estonia

Karise R.[1], Mänd M.[1], Viik E.[2]
[1] Estonian University of Life Sciences, Tartu, Estonia; [2] Agricultural Research Centre, Saku, Harjumaa, Estonia

Intensive agriculture has caused severed declines in abundance and species diversity of natural pollinators. This phenomenon is positively correlated with the intensity of agriculture or human impact generally. European agricultural practice is classified to intensive ones. At the same time, Europe consists of countries with variable agricultural intensity and the northern-most countries are considered as with high natural diversity. From the territory of Estonia only 25% is agricultural land and about 50% is forested area, but at the same time landscape heterogeneity is very low.
The intensive agricultural practice in Estonia has bloomed since the eighties of past century. Despite some recession in mid-nineties, the intensity has been growing. The major change occurred in availability of leguminous hay crops (red clover, white clover, alfalfa), which were replaced firstly by cereals and later by oilseed crops. Similar changes in Great Britain have proved to lead to the decline of at least some wild bee species.
After Estonia joined The European Union, the demand to harness environmentally friendly agriculture came into effect here too. This was made through subsidizing environmentally friendly or organic agriculture. Since 2006 annual biodiversity monitoring program has been carried through to analyse the effect of production type on bumble bee abundance and species diversity. Among most important demands in environmentally friendly production was to include leguminous crops into crop rotation plan.
The aim of our study was to study, whether and what kind of are the changes in bumble bee abundance and species diversity in Estonia. We compare the monitoring data collected from red clover fields of 1955-1967 with data from 2007-2017. We have documented three new species for Estonia, but also proportional declines in some species abundances. The declines have occurred especially in long-tongued species, which are most sensitive to changes in plant communities.

142 European beech forests as a home for feral honey bee colonies

Rutschmann B.[1,2], Kohl P.L.[1]
[1] Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Würzburg, Germany; [2] HOBOS, University of Würzburg, Würzburg, Germany

It is a common belief that feral honey bee colonies (*Apis mellifera* L.) were eradicated in Europe through the loss of habitats, domestication by man and spread of pathogens and parasites. Interestingly, no scientific data are available, neither about the past nor the present status of naturally nesting honeybee colonies. We expected near-natural beech (*Fagus sylvatica* L.) forests to provide enough suitable nest sites to be a home for feral honey bee colonies in Europe. Here, we made a first assessment of their occurrence and density in two German woodland areas based on two methods, the tracing of nest sites based on forager flight routes (beelining technique), and the direct inspection of potential cavity trees. Further, we established experimental swarms at forest edges and decoded dances for nest sites performed by scout bees in order to study how far swarms from beekeepermanaged hives would potentially move into a forest. We found that feral honey bee colonies regularly inhabit tree cavities in near-natural beech forests at densities of at least 0.11–0.14 colonies/km2. Colonies were not confined to the forest edges; they were also living deep inside the forests. We estimated a median distance of 2,600 m from the bee trees to the next apiaries, while scout bees in experimental swarms communicated nest sites in close distances (median: 470 m). We extrapolate that there are several thousand

feral honey bee colonies in German woodlands. These have to be taken in account when assessing the role of forest areas in providing pollination services to the surrounding land, and their occurrence has implications for the species' perception among researchers, beekeepers and conservationists. This study provides a starting point for investigating the life-histories and the ecological interactions of honey bees in temperate European forest environments.

143 Potential role of wetlands for honey bees diversity, population density and conservation in Sudan

El-Niweiri M.A.A.[1,2,3], Lattorff M.G.H.[1,4,5]
[1]Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg, Halle/Saale, Germany; [2] Department of Biology, King Khalid University, Abha, Asir Region, Saudi Arabia; [3]Bee Research Department, National Centre for Research, Mohammad Najeeb Street, Khartoum, Sudan; [4] International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya; [5] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Information about the role of wetlands in honeybee biodiversity and population density is very rare and therefore the aim of this paper is to test the potential role of wetlands in the enhancement of honeybee diversity and population density in comparison to dry lands. Wetlands play a vital ecological role in Sudan by providing habitat for numerous species of plants and animals, but little information is known how they support beneficial insects, e.g. bees. Using microsatellite DNA markers we assessed the population structure of honeybees in both wetlands and dry lands. The wetlands showed significantly higher population densities and allelic richness for honeybees than the dry lands ($p < 0.05$), suggesting that wetlands provide good habitat for the enhancement of honeybee diversity and density and thus, are better conservation areas for honeybee species than dry lands.

144 Do floral traits play a role in animal plant interaction? A study case with orchid bees

Boff S.[1], da Luz C.F.P.[2], Raizer J.[3], Lima L.C.P.[4]
[1] University of Milan, Milan, Italy; [2] Institute of Botany, São Paulo, Brazil; [3] Federal University of Grande Dourados, Dourados, Brazil; [4] Federal University of Latin American Integration, Foz do Iguaçu, Brazil

Orchid bees (Apidae: Euglossini) comprise a fauna of important pollinator specially in the Neotropical region where it is originally distributed. They may contribute to 25% of native bees in some regions. However, most studies report individual males which are attracted to synthetic scents, not to bees visiting flowers, restricting information on bee-flower interaction. Thus, information on plant community-orchid bee interactions are still limited. In order to fill this gap, here we compiled information on plant-bee interaction by analyzing data of larval provision (pollen grains) from brood cells after adult emergence of seven different orchid bees' species. After analysis of pollen grains, we present plant communities (at family and genus level) visited by these bees. We also reconstruct flower morphology based on floral traits (flower type, main resource, pollination unit and symmetry) of 65 different genus and investigate through principal coordinate analysis (PCoA) approach the presence of model flower which orchid bee interacts most. Our finding indicates that orchid bee female visits flowers of several plant families but specialize in foraging for resources at flowers with specific floral traits. Our results reveal a novel paradigm to infer the floral traits which orchid bees seems to be attracted.

145 Are non-native plants a valuable resource for wild bees?

Seitz N.[1], vanEngelsdorp D.[2], Leonhardt S.D.[1]
[1] Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany; [2] Department of Entomology, University of Maryland, College Park, USA

Providing plants which provide optimal food resources for bees is a crucial element of bee conservation. Many lists of pollinator friendly plants have been compiled in recent years. However, most recommendations are lacking scientific basis. Additionally, the effect of non-native pollinator friendly plants on local wild bee communities is unclear. We need to examine visitation patterns on pollinator friendly plants in order to tailor plantings efficiently to the needs of wild bees. In a field experiment, we assessed native and non-native pollinator friendly seed mixes regarding their attractiveness to bees. The plant mixes included each 20 species of wildflowers as well as two grasses and were planted in plots at three different sites on farmland in Maryland, USA. For two years we recorded bee visitation to the specific flowers of each mix across all sites. Observations were done every two weeks throughout the flowering period.
Overall, non-native plants were readily accepted by the wild bee community. Most observed bee species visited both the native and the non-native plant plots with some species occurring uniquely in one or the other. The structures of the bee plant visitation networks were similar with medium network specialization. However, non-native plants established faster in the first study year and had an earlier onset of flowering in both years.
The use of non-native plants for bee conservation certainly has to be handled with caution. Nonetheless, our results show that they have the potential to provide suitable food resources for a broad range of bee species.

146 Landscape related plant and resource diversity increases foraging and colony fitness in a tropical social bee

Kaluza B.[1,2], Wallace H.[2], Heard T.[3], Leonhardt S.[1]
[1] Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany; [2] Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore QLD, Australia; [3] CSIRO Ecosystem Sciences, Brisbane QLD, Australia

Bee pollinators are threatened by anthropogenic activities, and habitat loss and –conversion are key causes for currently observed widespread declines in wild bees. Moreover, decreasing abundance and diversity of foraging plants directly limits bee foraging for plant resources (pollen, nectar and resin). Yet how plant richness and plant resource abundance influence foraging patterns and ultimately bee colony performance is still little understood.
In a long-term experiment, we placed hives of an Australian eusocial stingless bee, *Tetragonula carbonaria*, in their natural habitat (subtropical forests) and two landscapes differently altered by humans (suburban gardens and macadamia plantations). To better understand how plant resource availability and diversity in interaction with nutrient quality affect bees, we monitored foraging patterns and colony growth across seasons over three years.
We found that bees collected higher diversity and greater quantities of resources and reproduced most in gardens compared to forests and plantations. Fitness of individual workers was highly conserved across colonies and landscapes, but overall colony fitness declined with decreasing landscape resource availability and diversity. Nutritional quality of honey and resin storages was similar across landscapes, but was influenced by plant species composition. Pollen protein content however was high in plantations and gardens, not in forests. Our results thus demonstrate that high resource abundance, diversity and quality are not necessarily associated with large proportions of natural habitats within the bees' foraging range, but depend on overall plant species richness and composition and thus year-long resource availability, which ultimately drives reproduction rates in social bees.

147 Response of wild bee diversity and functional traits to vineyard management and landscape diversity across Europe

Kratschmer S.[1], Pachinger B.[1], Paredes D.[2], Macavei L.[3], Guzmán G.[4], Guernion M.[5], Nicolai A.[5], Fertil A.[5], Popescu D.[6], Bunea C.[6], Zaller J.[7], Winter S.[1,8]

[1] Institute of Integrative Nature Conservation Research, University of Natural Resources and Life Science (BOKU), Vienna, Austria; [2] Environmental Protection Department, Estación Experimental de Zaidín CSIC, Granada, Spain; [3] University of Modena and Reggio Emilia UNIMORE, Italy; [4] Institute for Sustainable Agriculture CSIC Córdoba, Spain; [5] University Rennes 1 UMR 6553, EcoBio Biological Station, Paimpont, France; [6] University of Agriculture Science and Veterinary Medicine, Cluj Napoca, Romania; [7] Institute for Zoology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria; [8] Division of Plant Protection, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Vineyards may inhabit many plant and animal species, especially when the inter-row space is vegetated with spontaneous vegetation or cover crops. Frequent soil tillage, has resulted in the loss of habitat quality, biodiversity and biodiversity-based ecosystem services. Wild bees are important pollinators of crops and wild plants and depend on floral resources and suitable nesting sites. We hypothesize that less tillage frequency, high flower coverage and landscape diversity affect wild bees in vineyards positively, but functional traits may show a different trend.

We sampled wild bees semi-quantitatively in 63 vineyards under different tillage regimes across Europe (AT, ES, FR, RO). In each vineyard a 200m² transect was sampled five times and floral resources were estimated by flower coverage. Tillage intensity was assessed by vegetation coverage (%) twice a year per vineyard. Landscape structure was mapped within a 750 m radius around each vineyard and the Shannon Landscape Diversity Index was calculated. The response of wild bees to averaged predictors was analyzed by considering species richness, abundance and characteristic traits (i.e. sociality, body size) using generalized linear models.

Wild bee species richness ranged between 22 and 64 spp. across Europe and was positively affected by extensive tillage and high flower coverage. Wild bee abundance increased significantly with high flower coverage combined with extensive tillage. High landscape diversity counteracted the negative effect of low flower coverage, while higher flower coverage increased eusocial bee abundance in simplified landscapes. Body size neither responded to inter-row tillage nor to landscape diversity.

To enhance wild bee diversity and abundance, the most significant measures in vineyards are to increase floral resources and to reduce soil tillage frequency. High landscape diversity can complete these activities for resilient pollination services in wine growing regions.

148 *Varroa destructor* feeds primarily on honey bee fat body tissue not hemolymph

Ramsey S.D.[1], Ochoa R.[2], Bauchan G.[3], Gulbronson C.[3], Mowery J.[3], Cohen A.[4], Lim D.[1], Joklik J.[1], Cicero J.M.[5], Ellis J.D.[5], Hawthorne D.[1], vanEngelsdorp D.[1]

[1] Department of Entomology, University of Maryland College Park, Maryland, USA; [2] Agricultural Research Service, Systematic Entomology Laboratory, United States Department of Agriculture, Maryland, USA ; [3] Agricultural Research Service, Soybean Genomics & Improvement Laboratory, Electron and Confocal Microscopy Unit, United States Department of Agriculture, Maryland, USA; [4] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina, USA; [5] Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

The parasitic mite *Varroa destructor* is the most significant single driver of the global honey bee health decline. A better understanding of the association of this parasite and its host is critical to developing sustainable management practices. Our work shows that this parasite is not consuming hemolymph as has been the accepted view, but damages host bees by consuming fat body, a tissue roughly analogous to the human liver. We conducted three studies with the objective of locating the feeding site in adult bees, verifying what host tissue(s) is being consumed, and determining what host tissue(s) is integral to survivorship and reproduction in *Varroa*. We imaged feeding wounds in adult honey bees for the first time showing that Varroa create a wound on the underside of the abdomen where fat body is the immediate underlying tissue. Fat body at the wound site showed evidence of external digestion. Hemolymph and fat body in honey bees

were then marked with fluorescent biostains. Fluorescence associated with the fat body was consistently detected in the gut of mites fed on these bees while comparatively little fluorescence was detected from the hemolymph biostain. Mites were then fed a diet composed of one or both tissues. Mites fed fat body tissue survived longer and produced more eggs than those fed hemolymph. Mites fed hemolymph showed fitness metrics no different than the starved control group. Collectively, these findings strongly suggest that Varroa are exploiting the fat body as their primary source of sustenance; a tissue integral to proper immune function, pesticide detoxification, overwinter survival and several other essential processes in healthy adult and immature bees. These findings fundamentally alter our understanding of the etiology of varroosis and underscore a need to revisit our understanding of this parasite and its impacts, both direct and indirect, on honey bee health.

149 *Varroa* mite saliva contains bioactive factors that aid mite feeding and manipulate the honeybee immune response

Campbell E.M., Bowman A.S.
Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, UK

Varroa destructor is an ectoparasite mite of the honeybee (*Apis mellifera*) that feeds on haemolymph of developing brood and transmits numerous pathogenic viruses including Deformed wing virus (DWV). The adult female mite feeds on developing bee larvae after cell capping and creates a communal wound site from which all of her subsequent offspring feed. It is hypothesised that bioactive factors in adult *Varroa* saliva supress the developing bee larvae immune response and prevent melanisation of the wound.
Saliva was collected by stimulation of adult female mites and subjected to nano-MS/MS proteomic approaches. Samples of saliva from 125 varroa and 274 salivary glands contained enough protein material to distinguish peptides of intensity and diversity to generate proteomes.
In total, 547 and 7044 peptides were detected in each sample from saliva and SG samples respectively. When whole varroa transcriptome database was searched with generated peptides this corresponded to 138 proteins from the saliva sample and 1302 proteins from the salivary gland. Each proteome contained a cohort of putative secreted bioactive factors present in both saliva and samples - many of which are homologous to factors found in haematophagous and insectivorous arthropods. In addition, peptides in saliva mapped to honey bee pathogens, including DWV, Bee Macula virus and Lake Sinai Virus. The saliva proteome, in particular, was dominated by DWV peptides.
Putative secreted bioactive factors were subsequently knocked down in *Varroa* by RNAi gene silencing. A dramatic impact on feeding physiology and host immune response was observed with host honey bee pupa becoming discoloured and failing to complete eclosion due to immune disruption.
In addition to the selected putative effector proteins above, *Varroa* saliva and salivary glands contained a number of other hits homologous with host immune evasion or modulation in the saliva of other acari and insects.
These approaches will allow an in depth analysis of the transmission of DWV and the role of salivary factors in the pathogen – *Varroa* – Honey bee axis.

150 Hormonal induction of *in vitro* egg production in the honeybee mite, *Varroa destructor*

Christie C.R.[1], Budge G.E.[2], Campbell E.M.[1], Bowman A.S.[1]
[1] Institute of Biological and Environmental Sciences, University of Aberdeen, UK; [2] Institute for Agri-Food Research and Innovation, Newcastle University, UK

The ectoparasitic mite, *Varroa destructor*, is viewed as one of the major causes of honeybee losses globally. Research in the biology and control of *Varroa* is currently hampered by a lack of standardised mites and their year-round availability. Through development of an artificial feeding system, adult female *Varroa* were successfully maintained off-host for 12 days and used for in vitro viral pathogen transmission and varroacide testing. Despite this, no eggs were laid from the

1000's of *Varroa* studied. To understand what stimuli were missing from our feeding system to permit oviposition, the expression of a suite of biomarker genes of egg production were measured. To quantify the first stage of egg production, vitellogenesis (egg plumping), EcR1, USP, Vg2, and LDLR were selected. For the later stage, choriogenesis (egg shell formation), FTZ-F1 was measured. In order to elicit egg laying in the feeding system, two hormonal stimuli were tested that we hypothesized would induce oviposition in vitro. One hormone (H#1) was topically applied to the *Varroa*, while the other hormone (H#2) was microinjected into the honeybee pupa. . Both hormones were successful in greatly improving oviposition rates in *Varroa* when feeding on pupae in vitro. When applied to the artificial feeding system, H#1 induced oviposition but H#2 did not. Through use of a chemosensory control setup, it was found that H#2 elicits laying in *Varroa* on the artificial feeding system without physical contact with the treated honeybee. In two independent trials, our *Varroa* artificial feeding system achieved 47% oviposition and development of all *Varroa* lifestages from protonymph to sexually mature female. With further refinement, we hope to have developed a continuous artificial rearing system for *Varroa*.

151 Transcriptome profiling of the parasite *Varroa destructor* provides new biological insights into the mite adult life cycle

Mondet F.[1], Rau A.[2], Klopp C.[3], Rohmer M.[4], Severac D.[4], Le Conte Y.[1], Alaux C.[1]
[1] INRA, UR 406 Abeilles et Environnement, Avignon, France; [2] INRA, UMR 1313 GABI Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France; [3] INRA, UR 875 MIAT Mathématiques et Informatique Appliquées de Toulouse, Castanet-Tolosan, France; [4] Institut de Génomique Fonctionnelle, UMR 5203 CNRS, U661 INSERM, Universités Montpellier 1 & 2, Montpellier, France

The parasite *Varroa destructor* represents the greatest threat to honey bee colonies. Although its impact on bees has been extensively studied, less is known about its biology and the functional processes governing its adult life cycle and adaptation to its host.
We therefore developed a full life cycle transcriptomic catalogue in adult *Varroa* and included pairwise comparisons with males, artificially-reared and non-reproducing females.
Our study provides the first full life-cycle transcriptomic catalogue in adult *Varroa* and reveals key genes and biological processes that are involved in the physiological, behavioural and functional changes occurring through the different stages. Our results suggest that phoretic individuals can be reared outside host colonies without impacting their physiology and that mechanisms underlying reproductive failure occur before oogenesis.
These new findings reveal the remarkable adaptation of *Varroa* to its host biology and notably to the switch from living on adults to reproducing in sealed brood cells. This work also argues for the value of transcriptomic data to derive new insight into the parasite biology (e.g. biological inferences gained from gene ontology and functional enrichment analysis) and potentially identify new targets for *Varroa* control. Finally, this publicly available transcriptomic dataset is intended to provide a resource for the research community to extend the exploration of *Varroa* biology.

152 Early *Varroa* infection transcriptomics in the Asian honey bee

Routtu J.[1], Conlon B.H.[1], Devaraju S.[2], Brockmann A.[2], Moritz R.F.A.[1]
[1] Martin-Luther-University, Halle-Wittenberg, Germany; [2] NCBS, Bangalore, India

Varroa destructor reproduces in both worker and drone brood in the European honey bee *Apis mellifera* causing great damage to an infected colony. However, in the original host the Asian honey bee *A. cerana*, *V. destructor* reproduces exclusively in the drone brood. While also infecting the worker brood, no reproduction is observed in the worker brood. This is one of the main causes of stable coexistence of the host and the parasite in this system. We studied the early onset of infection in the freshly capped brood of both workers and drones in the Asian honey bee by using transcriptomics approach. Our preliminary results suggest that the infected worker larvae change their gene expression drastically, but no such effect is observed in the infected drone larvae.

153 Lithium salts as varroacide - efficacy in the treatment of artificial swarms and side effects on bees and brood

Ziegelmann B.[1], Makosch M.[1], Hannus S.[2], Rosenkranz P.[1]
[1] University of Hohenheim, Apicultural State Institute, Stuttgart, Germany; [2] siTOOLs Biotech GmbH, Munich, Germany

After the discovery of the varroacidal activity of lithium salts, we examined more precisely the efficacy of lithium chloride and lithium citrate in artificial swarms as well as side effects on bees and larvae. Effects on the longevity of worker bees were examined under laboratory conditions in cages. The bees received lithium chloride diets of 2 mM, 10 mM and 25 mM either during the first 24 hours after hatching or continuously until death. While the short term feeding did not affect the longevity at all, a chronic exposure over several weeks significantly reduced the life span of worker bees. In order to analyze the effect of lithium on the larval development, worker larvae were reared in vitro according to Aupinel et al. (2005) and fed with lithium chloride and lithium citrate at concentrations of 1 mM to 2 mM. It could be clearly shown that worker larvae are more sensitive to lithium than adult bees and that a chronic exposure of certain concentrations may lead to maldevelopment and death of the larvae. However, first applications in free flying colonies with brood indicate a substantial lower susceptibility of the larvae under in vivo conditions. In an initial field test, artificial swarms with approximately 20,000 bees and one queen were established and fed for 3 days with either sugar solution, 10 mM lithium citrate, or lithium chloride at concentrations of 10 mM, 25 mM and 50 mM, respectively. After installing and feeding the swarms with sugar syrup, a final control treatment with Bayvarol was started before capping of the brood cells. All lithium treatments induced strong mite fall and, depending on the treatment, high efficacies with low variations could be achieved. Mean efficacies ranged from 85 % (10 mM lithium chloride) to 97 % (50 mM lithium chloride). Our results confirm the extraordinary high efficacy of lithium salts and the considerable potential of treating broodless colonies with low side effects on adult bees. However, for the treatment of colonies with brood, application methods which protect the more sensitive larvae from the exposure to toxic concentrations of lithium compounds must be developed.

154 *Using RNAi to control Varroa destructor – a novel biopesticidal tool for effective control of the parasitic mite of honey bees*

Gleit Kielmanowicz M., Verhaert J., Inberg A., Masucci J.D., Avni D., Cooper S., Durnel N., Back S.
Monsanto Company, Chesterfield, USA

It is now generally accepted that several factors contribute to the worldwide decline of honey bee health. The *Varroa* mite (*Varroa destructor*) is of particular significance. In many studies it was shown that high Varroa mite levels lead to colony decline. The mite infestation of the adult bees and bee brood, combined with the ability of Varroa to vector pathogenic bee viruses, eventually results in an unsustainable colony stress. If left untreated, the rising levels of the pest typically results in colony death. Synthetic miticides are struggling to provide a sustainable solution, mainly due to the narrow margin of safety to honey bees and rapidly developing resistance.
BioDirect™ technology leverages the high specificity of externally applied double-stranded RNA to design an effective biological *Varroa* control product. Following the initial encouraging laboratory results of using dsRNA to target *Varroa*, we've conducted field studies to evaluate the effectiveness of this treatment approach on mite population and bee hive survival. We demonstrated a significant mite population suppression in the treated colonies compared to the non-treated. Moreover, the hive survival improvement of the dsRNA treatment was comparable to the commercial Varroa control product used in the study. These results provide an encouraging evidence of dsRNA field efficacy against *Varroa*.
Our next phase of field testing is focused on evaluating the product performance in the context of various geographies, seasons, and Varroa control practices.

155 Kairomones in the hive involves in *Varroa destructor* mite behavior

Pojar-Fenesan M., Balea A., Ciotlaus I.
Babes-Bolyai University, "Raluca Ripan" Institute for Research in Chemistry, Cluj-Napoca, Romania

Varroa destructor mite (Arthropoda, Arachnida, Mesostigmata, Varroidae), is one of the major parasites that affect bee colonies. In the insect world, the living organisms communicate through chemical language using so-called semio-chemicals. In order to study possible mediators, kairomons, interspecific chemical communicators Varroa destructor – bees, in the experimental researches we collected and analysed the volatile from apiaries installed in Transylvania Cluj area.
In the hive, for reproduction, acarians "prefers" cells with drone brood to those with worker bee brood. Seems to drone brood have something different than worker bee brood.
For study the kairomones involves in Varroa mites attack, VOCs emited by the 4-10 days old living drone brood larvae was collected with SPME techniques and analysed by GC-MS Agilent 7890 & 5975 MS. The ethyl acetate extract of drone brood was analysed in GC-MS too.
Some of the chemicals identified in VOCs and in drone extracts was tested as baits into a techniques to capture acarians and to take out of the hive. These techniques don't use pesticides, neither predators nor bacteria but a "third way". The results in one year field tests shows viability of the method, this kind of control strategies for the mite avoiding side effects of conventional or biological control.

156 Reproduction of the mite *Varroa destructor* in original and new honey bee hosts

Lin Z.[1,2], Qin Y.[1], Page P.[2,3,4], Wang S.[1], Li L.[1], Wen Z.[1], Hu F.[1], Neumann P.[3], Zheng H.[1], Dietemann V.[2]
[1] College of Animal Sciences, Zhejiang University, Hangzhou, P.R. China; [2] Swiss Bee Research Center, Agroscope, Bern, Switzerland; [3] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [4] Plant Bioactive Compound Laboratory, Faculty of Agriculture, Chiang Mai University, Thailand

The mite *Varroa destructor* has shifted from its original host, the Eastern honey bee, *Apis cerana*, to the Western honey bee, *Apis mellifera*, with devastating effects on global apiculture. A key mechanism enabling the survival of mite-infested *A. cerana* colonies is the seeming absence of mite reproduction in worker brood. This could be due to a lack of host attractiveness, to a failure of the parasite to initiate reproduction and/or to a mechanism we previously coined social apoptosis, the altruistic sacrifice of infested brood through arrested development. Here, we tested 1) whether *A. cerana* worker brood is attractive to the parasite by using natural infestation assays, 2) whether social apoptosis prevents successful mite reproduction, with experimental infestations in the absence of adult workers, and 3) whether hygienic brood removal is correlated to the level of brood damage by comparing experimental infestations in the absence and presence of workers. Our data show that the absence of reproduction in *A. cerana* worker brood is neither due to a lack of host attractiveness nor to a failure of the parasite to initiate reproduction. However, our results confirm that successful mite reproduction was severely hindered by social apoptosis. Moreover, hygienic behavior occurred at the stage when brood development was arrested, suggesting that adult workers recognise and remove infested damaged worker brood with their parasites. As shown previously, a normal development of infested *A. cerana* worker brood with successful mite reproduction was highly exceptional. These results provide an explanation to the question why *V. destructor* mites infesting *A. mellifera* are not spilling back into sympatric *A. cerana* colonies and also offer new insights into the coevolution between honey bee hosts and their parasites in this system.

157

Tracking genomic footprints of successful host switches in honey bee *Varroa* mites

Techer M.[1], Roberts J.M.K [2,3], Mikheyev A.S.[1]
[1] Okinawa Institute of Sciences and Technology, Ecology and Evolution Mikheyev Unit, Okinawa-ken, Japan; [2] CSIRO, Canberra, ACT, Australia; [3] ADFCA, Research and Development Division, Al Ain, UAE

Varroa mites are ectoparasites infesting honeybee colonies and originally found only in Asia. The Western honeybee *Apis mellifera*, has been transported in almost every continent with beekeeping and ultimately met her sister species the Asian honeybee *Apis cerana*. This new, sudden contact gave the opportunity for parasite/pathogens spill over. *Varroa destructor* jumped at least twice from its original host *A. cerana* to *A. mellifera* and subsequently spread worldwide. *V. jacobsoni* has also emerged as a new threat by jumping twice in Papua New Guinea. Recently, another distinct *Varroa* sp. host switch was reported in Philippines. In light of these reports, additional host switch could occur but the genetic mechanisms behind the quick adaptation and success of *Varroa* are poorly understood. More broadly, the repeated host switches provide an opportunity to examine to what extent parallel evolutionary event occur using similar vs. different mechanisms. Using NGS technology, we aim to i) study the population structure between native and invasive *Varroa*, ii) identify and compare signatures of selection after each host switch, iii) detect whether gene flow has occurred or is still occurring among host populations. Whole genome sequencing of 24 V. destructor and 20 V. jacobsoni mites collected in their native area on *A. cerana* and *A. mellifera* show a population divergence with host specificity in *V. destructor*, while the pattern in *V. jacobsoni* suggests ongoing differentiation. In both species, host switches were associated with massive genetic differentiation across hosts, though the actual regions involved were different. Our results reveal cryptic diversity in the *Varroa* species complex with a new subgroup on *A. cerana*. To assess the contribution of native populations after jumps, we are estimating population size and migration rates under IM model for four host-parasite populations. Disentangling genetic and demographic factors for *Varroa* success could help to predict future invasion.

Abstracts

Posters

P001 Deformed wing virus variant strains in the Indigenous and exotic honey bee in Saudi Arabia

Hroobi A.[1,2], Alattal Y.[3], Alghamdi A.[3], Campbell E.M.[1], Bowman A.S.[1]
[1] University of Aberdeen, Institute of Biological & Environmental Sciences, Aberdeen, UK; [2] Albaha University, Biology Department, Albaha, Saudi Arabia; [3] King Saud University, Department of Plant Protection, Riyadh, Saudi Arabia

Deformed wing virus (DWV) is a RNA viral pathogen transmitted by *Varroa destructor* to honey bees, and is one of the leading causes of honey bee colony loss worldwide. Saudi Arabia imports approximately 100000 hives annually. The presence of honey bee viruses have never been investigated in the exotic imported honey bee Apis mellifera ligustica or in the indigenous honey bee *Apis mellifera jementica*. The objectives of this study were to compare the titre of DWV variant strains under different climatic conditions, geographical locations and proximity between hives of different honey bee subspecies and determined whether the virus transmitted between exotic bee and indigenous bee in Saudi Arabia. A real-time reverse transcription-polymerase chain reaction (qRT-PCR) assay was used to enable the specific detection and absolute quantification of DWV variants (DWV-A and –B). For some samples, a portion of the variable leader protein gene was sequenced and compared to the genetic databases. The results show that indigenous bees, *A. jementica*, have a much lower incidence of DWV compared to the imported bee, *A. ligustica* but that this titre was increased when A. *ligustica* colonies were kept in proximity to A. jementica colonies. DWV-B had the highest prevalence across all localities compared with DWV-A. Phylogenetic analysis showed highly similar viruses in *A. ligustica* and *A. jemetica* from adjacent hives indicating transmission between exotic and indigenous bees. The finding of the study has important implications for enhancing our understanding of the distribution and prevalence of DWV variant strains in Saudi Arabia and viral titres and provides baseline data for future analyses to increase the awareness of long-term impacts of DWV on bee populations in the country.

P002 The German bee monitoring (DeBiMo): report 2016/ 2017

Schroeder A.[1], von der Ohe W.[2], Genersch E.[3], Meixner M.[4], Otten Ch.[5], Berg S.[6], Schaefer M.[7], Martens D.[8]
[1] University of Hohenheim, Apicultural State Institute, Stuttgart, Germany; [2] Niedersaechsisches LAVES, Institut für Bienenkunde, Celle, Germany; [3] Laenderinstitut für Bienenkunde e.V., Hohen Neuendorf, Germany; [4] Landesbetrieb Landwirtschaft Hessen, Bieneninstitut, Kirchhain, Germany; [5] DLR Westerwald-Osteifel, Fachzentrum Bienen und Imkerei, Mayen, Germany; [6] Bayerische LWG, Institut für Bienenkunde und Imkerei, Veitshoechheim, Germany; [7] Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany; [8] Landwirtschaftliche Untersuchungs- und Forschungsanstalt LUFA, Speyer, Germany

This long-term project focuses on the monitoring of winter losses of honey bee colonies and the impact of bee diseases, pesticide residues and beekeeping management on these losses. For the winter 2016/ 2017, we will present and discuss prevalence of pathogens (i.e. *Varroa destructor*, *Nosema* spp., *Paenibacillus larvae* and four honey bee viruses), residues of 439 different pesticides and winter mortality based on data from 1,081 bee colonies and 109 apiaries and compare the data with results from previous years.

14.6% of the monitored colonies (N=1,081) and 16.4% of all wintered colonies of the involved apiaries (N=5,671) died during winter 2016/2017. The prevalence of acute bee paralysis virus (ABPV) was 10.4%, of sacbrood virus (SBV) 2.3%, of deformed wing virus (DWV) 41.1 % and of chronic bee paralysis virus (CBPV) 0.9% (N=565). Our data again demonstrate that the infestation level with *Varroa destructor* and the infection with deformed wing virus in autumn were significantly correlated with the winter losses of the monitored honey bee colonies (chi-squared test; $p<0.0001$) but not the infections with *Nosema* spp..
We identified 85 different pesticides in bee bread (N=152), most of them in traces. Only 5 samples (3.3%) were free of measureable residues. The most frequent pesticides originate from applications in flowering oil seed rape. We found up to 27 different substances in one sample with a mean of 7 different substances per sample. However, no differences in overwintering were observed between apiaries with high or low number of pesticides.

P003 Prevalence of protozoan parasites in *Vespa velutina* and in native European Hymenoptera (Vespoidea, Apoidea) from Galiza (NW-Iberian Peninsula)

Gabín-García L.B., Bartolomé C., Maside X.
Universidade de Santiago de Compostela, Grupo de Medicina Xenómica, CiMUS, Santiago de Compostela, Galiza, Spain

The Asian hornet *Vespa velutina* was first observed in France around 2004, from where it has rapidly spread across all neighbouring countries. It feeds on hydrocarbon-rich nectar and ripe fruit, and is a voracious predator of flying insects, particularly honeybees. These habits make it a double threat for the safety of European autochtonous hymenoptera: directly, by attacking and destroying nests and indirectly, by contributing to the spread of parasites. We focused on the latter and studied the prevalence of protozoan parasites in a collection of 173 isolates (>1000 specimens) representative of the populations of *V. velutina* and Hymenoptera native to the NW-Iberian peninsula (genera *Vespa*, *Vespula*, *Polistes*, *Bombus*). By means of molecular typing tools, we detected the presence of the most common parasites: e.g. *Nosema apis*, N. *cerana* (Nosematidae), *Lotmaria passim*, *Chritidia mellificae* and *C. bombi* (Trypanosomatidae) and *Apicystis bombi* (Apicomplexa), but also some putatively new taxons within this groups that deserve further characterization. Trypanosomatidae were the most prevalent parasites, with up to 50% presence in *Bombus* spp. *C. bombi* (17.7%), *A. bombi* (16,5%) and *N. thompsoni* (7.6%) were the most common parasites in *V. velutina*. All parasites identified the sample were detected in at least one of the *V. velutina* isolates, which is consistent with a putative role of this species in the spread of pathogens.

P004 Impact of nutritional stress on colony strength and pathogen infection

Branchiccela B.[1], Invernizzi C.[2], Mendoza Y.[3], Santos E.[2], Zunino P.[1], Antúnez K.[1]
[1] Instituto de Investigaciones Biológicas Clemente Estable, Microbiology Department, Montevideo, Uruguay; [2] Facultad de Ciencias, Universidad de la República, Ethology Division, Montevideo, Uruguay; [3] Instituto Nacional de Investigación Agropecuaria, Apiculture Section, Colonia, Uruguay

One of the negative impacts of agricultural intensification is the increase in the extent of monocultures. The availability of one pollen type may not be enough to accomplish the nutritional requirements that bee needs. Consequently, bees suffer nutritional stress, which has been proposed as one of the main factors associated with colony losses. In Uruguay, beekeepers relocate their colonies to *Eupcalyptus grandis* plantations in autumn, in order to increase honey production. Pollen from this trees is nutritionally poor because it has low lipids level and lacks a suitable proportion of essential aminoacids. In this environment, colonies get indefectibly infected with *Nosema* spp. This is a natural scenario suitable to study the interaction between nutritional stress and bee health. Our aim was to analyze the effect of nutritional stress on colony strength and pathogen infection. Sixty colonies were relocated to an *E. grandis* plantation in autumn (2015) and divided into two groups: 30 colonies did not receive any supplementation (stressed colonies) while 30 colonies were supplemented with 500 g of polyfloral pollen once every 15 days (supplemented colonies). Adult and brood population and infection level of *Nosema* spp. and viruses was analyzed before the experiment starts, once every 15 days for two months, and in spring (to evaluate long-term effects). During *E. grandis* blooming, non-supplemented colonies showed lower adult and brood population and higher *Nosema* spp. infection level in comparison with supplemented colonies. Interestingly, supplemented colonies showed higher infection level of ABPV, DWV and SBV, but not BQCV. In spring, non-supplement colonies remained with lower adult and brood population, compared to supplemented colonies, but both groups showed similar pathogens levels. No differences in colony losses between both groups were observed. In conclusion, nutritional stress had a direct impact on colony depopulation and *Nosema* spp. infection level.

P005 Does feeding pollen substitutes impact honey bee (*Apis mellifera*) colony strength parameters and Nosema infections?

Mortensen A.N.[1,2], Jack C.J.[1], Bustamante T.A.[1], Schmehl D.R.[1,3], Ellis J.D.[1]
[1] University of Florida, Entomology & Nematology Department, Gainesville, FL, USA; [2] Plant & Food Research, Hamilton, New Zealand; [3] Bayer Crop Science, Pollinator Safety, Research Triangle Park, NC, USA

U.S. beekeepers routinely feed their colonies commercial pollen and nectar substitutes in an effort to encourage growth and reduce colony losses. However, how supplemental protein feeding affects colony strength parameters is poorly understood. Therefore, we conducted a field study to examine if feeding protein substitutes affects colony strength (number of adult bees and capped brood cells) and *Nosema* spp. (number of spores) in commercially managed honey bee colonies in Florida, USA. Each of 124 colonies was randomly assigned to one of six treatments with at least 11 colonies composing each group. The six treatments were: (1) no pollen supplement, (2) wildflower pollen supplement, (3) MegaBee™, (4) Brood Builder™, (5) Bee-Pro® and (6) Ultra Bee™. Colony strength and *Nosema* were assessed prior to-, four weeks post- and eight weeks post-treatment. There was an overall decrease in *Nosema* intensity (avg. spores/bee) across all treatments over time. However, there were no statistically detectable differences in colony strength or *Nosema* between any of the pollen feeding treatments and the negative control treatment. Thus far, multiple investigations regarding supplemental protein feeding have failed to illuminate a clear consensus on the impact that this practice has on honey bee colony strength or productivity. Though challenging, more field level studies are needed to determine the impact of protein diet supplements on colony strength and health.

P006 Toward a novel diagnostic tool for the selection of varroa-sensitive hygiene honey bee colonies

Riva C., Beslay D., Le Conte Y., Mondet F.
UR406 Abeilles et Environnement, INRA, Avignon, France

The mite *Varroa destructor* is known to be the most damaging parasite for the domestic honey bee. The use of yearly treatments for the control of varroosis is the most common answer to prevent collapses of honey bee colonies. However, the number of effective acaricides is small and the mite tends to become resistant to these few actives molecules. Moreover, veterinary products tends to accumulated in beehive products and to show adverse effects on honey bee health. In this context, selection of varroa tolerant honey bees would be a more sustainable solution for apiculture.
Several cases of feral or unmanaged colonies surviving varroa infestation have been reported. Among traits observed in surviving bee populations, one involves the uncapping of infested cells and the removal of brood parasited by varroa. This behavior, currently named Varroa-sensitive hygiene (VSH), interrupts mite reproduction in the targeted cells and slow down the mite population growth in the colony. Hygienic behavior is one type of social immunity in which individual honey bees detect chemical stimuli from diseased larvae. Recently, 14 natural molecules have been identified as VSH triggers. These compounds are classified either as brood ester pheromones, or as specific to Varroa-parasited brood cells. The candidate molecules are currently tested in honey bee colonies to select the most effective cocktail, using a classical chemical ecology behavioral assay. In parallel, efforts are made to develop the assay into a practical tool for beekeepers. This step involves working on the delivery method through pharmaceutical techniques. These results are opening the way to a diagnostic tool currently developed, that could be use by bee keepers for the selection of VSH colonies.

P007 Virus prevalence across sympatric field samples of *Apis mellifera* and *Bombus* species

Panziera D., Theodorou T., Paxton R.J.
Martin Luther University Halle-Wittenberg, General Zoology, Halle, Germany

Managed honey bees have suffered significantly higher mortality in northern temperate regions of the world over the past three decades whilst evidence suggests that many populations of wild bee species have declined. While a number of factors are thought to be involved in these population declines, the increase in prevalence of viral pathogens is one of the most likely causes. Here we investigated patterns of cross-species sharing of three RNA viral targets among managed honey bees (*Apis mellifera*) and wild bee species (*Bombus* spp.) at eight independent German locations. In each location, flower richness as well as the abundance of flowers and honey bees were measured. In total, 320 individuals of *A. mellifera*, *Bombus lapidarius*, *Bombus terrestris* and *Bombus pascuorum* were sampled. All species were sampled and all were collected within a 5 day span of time at the same location. RNA was extracted from all and screened by qPCR for Black queen cell virus (BQCV), Deformed wings virus genotype A (DWV-A) and Deformed wings virus genotype B (DWV-B; aka Varroa destructor virus-1). Virus prevalence was generally higher in honey bees (mean prevalence 55%) than in bumble bee species (mean prevalence 35% across host species). There were also differences in prevalence between *Bombus* species, with *B. lapidarius* and *B. terrestris* showing higher prevalence (mean prevalence 40%) compared to *B. pascuorum* (mean prevalence 24%). Our results show that BQCV and DWV-B are quite common across sites while DWV-A is very rare. Additionally, a weak but positive relationship between virus prevalence in honey bees and Bombus species is suggested, a result which, when combined with the higher viral prevalence in honey bees, supports the hypothesis of viral spillover from honey bees to wild bees.

P008 Transmission via hive products: globalization of the honey bee virosphere?

Schittny D.C., Yañez O., Neumann P.
University of Bern, Institute of Bee Health, Bern, Switzerland

Global trade of honey bee, *Apis mellifera*, hive products has repeatedly raised concern about pathogen transmission amongst stakeholders. Viruses are key pathogens and regularly found in hive products. However, the efficacy of hive products for virus transmission is poorly understood. Here, we investigate hive products for their deformed wing virus A (DWV-A) transmission capacity in a fully-crossed hoarding cage experiment and screened commercial products for virus levels. *A. mellifera* workers were provided with honey, pollen and wax either contaminated with high (~1*108 copies/g), medium (~5*106 copies/g), low (~1*105 copies/g) or no (control) DWV-A copies. For 10 days, bee mortality was monitored. Then, virus copies were quantified in bee heads and in 38 commercial hive products using RT-qPCR. For honey and pollen, a positive correlation between DWV-A concentration and bee mortality was observed. High concentrations always resulted in infections, medium ones in 47% and low ones still in 20% of cases. No significant differences were observed between the tested products. In the commercial honey 7.7*102 - 1.8*105 (mean = 1.3*104) DWV-A copies per gram were found, in commercial pollen between 1.4*103 and 1.3*104 (mean = 3.6*103) DWV-A copies per gram. The results clearly show that DWV-A transmission via hive products is feasible.
The risk of introducing novel viruses and/or new strains of already established ones via global trade should be considered in regulations. It appears prudent to include virus analyses for health certificates in the import/export regulations of honey bee products.

P009 Validation of three Real-Time PCR assays for the rapid differentiation of *Aethina tumida* from other *Nitidulidae* (Coleoptera) species

Cirasella L.[1], Bergamini F.[1], Bassix S.[1], Antognetti V.[2], Formato G.[2], Cersini A.[2], Carra E.[1]
[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione di Modena, Modena, Italy; [2] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy

The small hive beetle (SBH), *Aethina tumida* (Coleoptera; Nitidulidae), a parasite of European honeybee colonies (Apis mellifera), has shown a significant range expansion from the native Southern Africa to other Continents. In 2014, it was detected in Southern Italy (Calabria and Sicilia), and its presence is so far confined in Calabria region.
Some other *Nitidulidae* species could be associated with the honeybees colonies, but they could be confused with the SHB. Among these, *Cychramus luteus*, *Brachypeplus glaber* and *Meligethes aneus* could potentially be present in the territory under SHB surveillance, interfering with the analysis of identification of SBH.
To assist the surveillance and control activities of SHB, three distinct TaqMan Real-Time PCR assays were developed and validated to ease the differentiation of SHB from these mentioned *Nitidulidae* species, above all when parts of insects are found in hive debris and in brood honeycombs.
Three new sets of specific primers and MGB probes, targeting the COI sequences, were designed in silico. Distinct recombinant plasmids, containing one copy of the target sequences, were used as standard reference DNA. The diagnostic specificity of the three real-time PCR was evaluated by testing DNA extracted from SHB larvae or adults, giving no positive results. The analytical sensitivity was determined by the limit of detection, generated by serial 10-fold dilutions of each standard DNA with a known copy number, either in water and in negative debris and brood honeycombs extracts, resulting to be 50 copies/ µl in all matrices. For each PCR, the intra and inter-assay variability were determined using negative honeycombs samples artificially contaminated with different load of the standard DNA. All the replicates tested resulted positive.
The Real-Time PCR assays we developed, could represent a reliable support to get a quick differential identification of SHB from Cychramus luteus, Meligethes aeneus and Brachypeplus glaber.

P010 Identification of honeybee colonies infected by *Paenibacillus larvae* through the powdered sugar examination

Bassi S.[1], Carpana E.[2], Bergamini F.[1], Loglio G.[3], Palminteri S.[4], Galletti G.[5], Carra E.[1]
[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione di Modena, Modena, Italy; [2] CREA – API, Consiglio per la Ricerca in Agricoltura e l'Analisi dell' Economia Agraria, Unità di Ricerca di Apicoltura e Bachicoltura, Bologna, Italy; [3] Agenzia di Tutela della Salute di Bergamo, Dipartimento di Prevenzione Veterinario, Trescore Balneario (BG), Italy; [4] Azienda USL di Bologna, Dipartimento di Sanità Pubblica, Bologna, Italy; [5] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sorveglianza Epidemiologica Emilia-Romagna, Bologna, Italy

We studied the possibility to identify *Paenibacillus larvae* infected honeybee colonies and determine their level of infection by using the powdered sugar examination.
105 colonies belonging to 10 apiaries were examined: Group A - 15 colonies with AFB symptoms; Group B - 45 colonies without symptoms of disease but belonging to apiaries with AFB diseased colonies; Group C - 45 colonies of apiaries where no cases of AFB were reported in the last two years.
Fifty grams of powdered sugar was dusted on the top bars of each brood combs and collected after 20 min on sheets of papers placed on the bottom of the hives.
The sugar samples were examined by cultural method and with a new molecular method: a 16S rRNA gene based quantitative TaqMan real-time PCR.
The results obtained by culture method are the following:
Group A: all samples were positive with a spore load between 1x10^4 and 2,8x10^7 CFU/g.
Group B: 10 samples were negative; 34 positives with a spore load between 2x10^1 and 6,2x10^3 CFU/g and 1 positive with a load of 1,7x10^4 CFU/g.
Group C: 37 samples were negative and 8 positive with a spore load between 2x10^1and 1,4x10^2 CFU/g.

Concordance between culture method and qPCR was observed in 82/105 (78%) samples: 52 resulted positive and 30 negative by both methods.
Discordance was observed in 23/105 (22%) samples: 6 samples resulted positive by culture method but negative by qPCR, and 17 samples resulted negative by culture method but positive by qPCR. In 46 samples out of 52 positive by both methods the qPCR found a greater number of spores than culture method. The real-time qPCR detected more positive samples and higher infection level than culture method. The results obtained by both methods were closely concordant with the clinical-epidemiological situation of the hives, and confirmed that the powdered sugar can be a very useful indicator of P. larvae infection in honeybees colonies.

P011 Artificial brood interruption associated to the winter *Varroa* treatment

Gianessi E.[1], Garrido C.[2], Nanetti A.[1]
[1] CREA-AA Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy; [2] BeeSafe, Leverkusen, Germany

In many regions, varroa management bases on an annual two-treatment concept. Of the two - one in winter and one in summer – the winter treatment is pivotal in that the broodless condition allows to reach high efficacy in the short term. However warm winters are becoming increasingly frequent in temperate areas, which promotes continuous brood rearing all over the year. Winter mature brood offers to *Varroa* mites a) uninterrupted reproductive opportunities and b) protection against the administered acaricides, possibly resulting into excessive spring infestations and colony losses.
This investigation aimed at preliminary testing the possibility to force a colony into broodless condition (and thus the mites into the phoretic stage) by confining the queen in a large queen excluder cage (MeGa, *Menna apicoltura*), as an adaptation to the abovementioned consequences of the climate change.
In November 2016, an apiary of 60 colonies in the area of Bologna, Italy, was split into 4 homogeneous random groups. The queens of three groups were caged in the centre of their winter cluster and released after 23, 76 or 93 days. The fourth group served as a non-manipulated control. The day 23 all the colonies were treated by trickling with Api-Bioxal (oxalic acid).
No significant difference was detected in queen survival, spring (March 2017) adult population and spring brood area, which indicates good tolerability of winter caging for the colonies.
Results gave indications of negative correlation between caging period and overwintering success, but clear statistical significance was not reached. If confirmed by further trials, this may open to a possible use of winter caging also in the regulation of spring colony build up.
Study conducted with the financial contribution of the Emilia-Romagna Region (Reg. (UE) 1308/2013).

P012 Nosema ceranae found in the abdomens of small hive beetle (*Aethina tumida*) imagoes

Cilia G.[1], Cardaio I.[1], Jarmela dos Santos P.E.[1], Ellis J.D.[2], Nanetti A.[1]
[1] CREA-AA Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy; [2] Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

The possible presence of the microsporidium *Nosema ceranae* in *Aethina tumida* (small hive beetle = SHB) imagoes was investigated in forty beetle specimens sampled in Gainesville (Florida) during summer 2017 from a colony without evident sings of nosemosis. Two subsamples were created: one of 30 SHBs for pool analysis and one of 10 SHBs for quantifications at individual level.
The SHBs were washed with ethanol, the abdomens crushed for DNA extraction and a qPCR (based on 16S rRNA gene) was then performed.

N. ceranae was detected in 7 out of 10 individually analysed specimens. If all the collected samples are considered, an average of 564 N. ceranae copies per individual SHB were found.
This is the first study reporting *N. ceranae* associated to SHB imagoes. However further research is needed to elucidate the SHB contamination route and its possible role in the transmission of *N. ceranae* infections to honey bees and to other pollinators.
Study conducted within the AETHINET project, financed by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF).

P013 Histological features of the larvae of *Apis mellifera* on day 5 fed with toxic nectar in vitro

Pedrana G.[1], Viotti H.[1], Presentado M.L.[1], Arredondo D.[2], Antúnes K.[2], Juri P.[3], Nogueira E.[3], Invernizzi C.[4]
[1] University of the Republic, Department of Morphology and Development, Histology and Embryology, Montevideo, Uruguay; [2] Instituto de Investigaciones Biológicas Clemente Estable, Microbiology Department, Montevideo, Uruguay; [3] University of the Republic, Department of farm animals, Apiculture Area, Montevideo, Uruguay; [4] University of the Republic, Department of Animal Biology, Ethology Section, Montevideo, Uruguay

In previous studies we determined that honey bee colonies were affected with "River disease" syndrome in early summer. This syndrome was produced by a toxic fluid from nectar giving to the larvae, collected honeydew of a planthopper Flatidae in leaves of *Sebastaiana schottiana* tree. The massive death of young larvae is the main sign of these syndrome. We seek to understand how the intoxication affect the larvae morphology. Therefore, we develop an in vitro model that include the toxic nectar from affected colonies. Larvae reared in vitro were fed with healthy nectar (n = 5) and toxic nectar with RD (n = 5). At day 5 all larvae were fixed (formaldehyde, acetic acid, alcohol) and paraffine embedded to obtain 5 um sections stained with hematoxylin-eosin. Histological images at 400 magnification were analysed with ImageJ software. Volume occupied by fat body, digestive system and nervous systems was analysed. The results showed a decrease in fat body cells in larvae fed with toxic nectar compared with healthy nectar. Moreover, at day 5 larva in healthy group showed the development of the nervous systems and increasing volume of fat body with lipid droplets in cytoplasm of trophocytes and evident euchromatic nucleus. However, larva fed with toxic nectar showed trophocytes with eosinophilic cytoplasm and less differentiation patterns. Eonocytes in larvae fed with toxic nectar showed heterochromatic nucleus and an increase un nucleus volume compared with the cytoplasm. In contrast, healthy larvae showed trophocytes with euchromatic nucleus and presence of nucleolus. Also, the epithelium of Malpighi ducts showed cells with apoptotic nucleus in larvae fed with toxic nectar. In conclusion, in vitro feeding with toxic nectar showed alterations of essential cells for metamorphosis in larvae of *Apis mellifera* suggesting that there is less possibility to achieve the next step of development.

P014 Deformed wing virus variants in *Varroa destructor* susceptible and tolerant honey bee colonies

Castelli L.[1], Branchiccela B.[1], Mendoza Y.[2], Invernizzi C.[3], Antúnez K.[1]
[1] Microbiology Department, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; [2] Apiculture Section, Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay; [3] Ethology Section, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

Varroa destructor is the major threat that affects honey bee colonies worldwide. In Uruguay, two contrasting scenarios are found, bee populations located in the South-West of the country are susceptible (S), collapsing if they are not chemically treated, while bee populations located in the East are tolerant (T), being able to survive without treatment. In a previous study, we compare the host-parasite interaction in those populations. One experimental apiary was located in each region, with S or T colonies (N=23 and 21, respectively). No miticide treatment was applied. In autumn, S colonies showed higher *V. destructor* parasitation level (K-haplotype), lower grooming, and lower hygienic behaviour than T colonies.

Besides that, S and T colonies were infected by similar levels of ABPV, BQCV and SBV. However, S colonies showed higher DWV infection level compared to T colonies. In winter, all S colonies died while only 9% of T colonies died. In order to deepen in the study of DWV-varroa interaction, the aim of this study was evaluate the DWV variants circulating in bees and mites from S and T colonies. Two S and two T colonies from our previous study were randomly selected. Twenty nurse bees and ten varroa mites per colony, collected in autumn were used. Total RNA was extracted, retrotranscribed, and a fragment of the replicase polyprotein gene was amplified by qPCR using High Resolution Melting. Amplicons were cloned and 8-10 clones per sample were sequenced. Simultaneously, individual clones were analyzed by HRM. Bees and mites collected from all colonies were infected by a predominant DWV variant, identified as variant A. Extremely low genetic variation was detected. Those results indicate that the difference observed in S and T colonies is not associated to the DWV variant. According to Martin et al. (2012), the low diversity and predominance of a single DWV variant is consistent with the long establishment of *V. destructor* in Uruguay.

P015 Detection of viruses in virgin and mated queens of the honey bee *Apis mellifera L.*

Blažytė-Čereškienė L., Tamašauskienė D., Būda V.
Nature Research Centre, Laboratory of Chemical and Behavioural Ecology, Vilnius, Lithuania

The presence of six honey bee viruses including acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), black queen cell virus (BQCV), deformed wing virus (DWV), Kashmir bee virus (KBV), and sacbrood virus (SBV) were examined in individual honey bee queens using molecular methods. Three groups of queen samples were analyzed: 1) virgin 2-day-old queens; 2) mated and egg lying 30-day-old queens from nucleus colonies, and 3) mated and egg lying 2-year-old queens from normal colonies.
We did not detect any of tested viruses in the virgin queens, whereas three viruses – DWV, SBV, and BQCV - were detected in the mated queens. The most prevailed virus was BQCV in mated and egg lying queens. It was detected in 75,3 % of 30-day-old queens and in 46 % of 2-year-old queens. DWV was detected in 3-4 % of the mated 30-day-old queens. SBV was detected in co-infection with BQCV in 3,3 % of the mated 30-day-old queens. ABPV, KBV, and IAPV were not detected in honey bee queens.

P016 Inhibition of growth of *Paenibacillus larvae* by bacteriocin from *Brevibacillus laterosporus*

Miljković M.[1], Kabić J.[2], Fira Đ.[2], Kojić M.[1], Stanisavljević L.[2]
[1] University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia; [2] University of Belgrade, Faculty of Biology, Belgrade, Serbia

Paenibacillus larvae strain pc19726256 have been isolated from American Foulbrood infected/diseased colonies of honey bees in southwest Serbia. *P. larvae* was grown in brain heart infusion (BHI) medium (37 g of BHI), supplemented with 3 g of yeast extract, 2% (wt/vol) glucose, 0.25% (wt/vol) potassium aspartate, 1 mM CaCl2, 1 mM MgCl2, 1 mg of L-tryptophan, L-phenylalanine and thiamine hydrochloride per liter at 30°C with shacking (150 rpm). Solid medium was prepared by adding 1,5% of agar and 5% (vol/vol) sterile defibrinated horse blood.
For testing of sensitivity *P. larvae* to bacteriocins (nisin A, lactolisterin BU, laterosporulin SP7 and laterosporulin SP11) agar-well diffusion assay was used.
Results: It was shown that already germinated strain *P. larvae* was sensitive to nisin A, laterosporulin SP7 and laterosporulin SP11, but not to lactolisterin BU. The strongest zone of inhibition (40 mm) was obtained by laterosporulin SP11.
Laterosporulin SP7 and laterosporulin SP11 were isolated by ammonium sulfate precipitation from the supernatant of culture of Brevibacillus laterosporus BGSP7 and BGSP11, respectively, grown overnight in LB medium at 37°C with shaking (180 rpm).
The next steps are to assess whether the laterosporulin SP11 could prevent *P. larvae* germination and determine whether this bacteriocin would be effective in laboratory-reared honey bee larvae.

P017 Nosemosis control in European honey bees *Apis mellifera* by silencing the gene encoding *Nosema ceranae polar* tube protein 3

Rodríguez-García C.[1,3], Evans J.D.[1], Branchiccela B.[2], Heerman M.C.[1], Banmekea O.[1], Hamilton M.[1], Higes M.[3], Martín-Hernández R.[3,4], Chen Y.P.[1]
[1] USDA-ARS Bee Research Laboratory, BARC-East, Beltsville, MD, USA; [2] Instituto de Investigaciones Biológicas Clemente Estable, Department of Microbiology, Montevideo, Uruguay; [3] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental, IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [4] Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FEDER), Fundación Parque Científico y Tecnológico de Albacete, Spain

RNA interference (RNAi) is a post-transcriptional gene silencing mechanism triggered by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. RNAi is conserved in a wide range of eukaryotic organisms. This mechanism has provided unique opportunities in combating honey bee diseases caused by various parasites and pathogens. Nosema ceranae is a microsporidian parasite of European honey bees, Apis mellifera and has been associated with honey bee colony losses in some regions of the world. Here we explored the possibility of silencing the expression of a *N. ceranae* putative virulence gene encoding polar tube protein 3 (PTP3) which is involved in the host cell invasion as a therapeutic strategy for controlling *Nosema* disease in honey bees. Our studies showed that the oral ingestion of a dsRNA corresponding to the sequences of *N. ceranae* ptp3 could effectively suppress the expression of the ptp3 gene in *N. ceranae* infected bees and reduce *Nosema* load. In addition to the knockdown of ptp3 gene expression, ingestion of ptp3-dsRNA also led to improved innate immunity in bees infected with *N. ceranae* and an improvement in physiological performance and lifespan compared to untreated control bees. These results strongly suggest that RNAi-based therapeutics hold real promise for the effective treatment of honey bee diseases in the future and warrant further investigation.

P018 Main pathogens in bumblebee species from Spain

Benito M.[1], De la Rúa P.[2], Ornosa C.[3], Bartolomé C. , Maside X.[4], Martín-Hernández R.[1,5], Higes M.[1]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Marchamalo, Guadalajara, Spain; [2] Departamento de Zoología y Antropología Física, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain; [3] Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain; [4] Xenómica Comparada de Parásitos Humanos, CIMUS, Medicina Xenómica, Universidade de Santiago de Compostela, CIMUS, Medicina Xenómica, Santiago de Compostela, Spain; [5] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain

Since the middle of the last century, the populations of bumblebees have decreased in different parts of the world. This decline of pollinating insects is due to multiple causes, but pathogens may play an essential role. In this work, we present the results of the detection of different pathogens for bumblebees to determine the presence in Spanish bumblebee populations.
Bumblebee sampling was carried out in 2013, 2014, 2015 in some Spanish national parks, as Pyrenees, Sierra de Guadarrama and Sierra Nevada, as well as in other complementary localities. A total of 1,016 bumblebees were collected and preserved in alcohol until analysis. All the samples were analyzed individually for *Nosema* Spp. presence and 736 of them were also analyzed to detect trypanosomatids. Additionally, in 2015, 100 individual bumblebees from the same territories were collected in RNAlater Ò and analyzed for virus detection. All pathogens were analyce using PCR or RT-PCR techniques previously described
Only 2.7% of bumblebees were positive for the genus *Nosema*, (27 out of 1.016) and three species belonging to this genus were found: *Nosema bombi* (1.97%), *Nosema ceranae* (0.20%) and *Nosema thomsoni* (0.39%). Besides, 13.1% of the samples were positive to trypanosomatids (97 out of 736) although in this case it was not possible to identify

the species, because primers used were just Trypanosomatida order specific. Regarding viruses, a 25% of bumblebee analyzed for them were positive to Deformed Wings Virus, 65% to Black Queen Cell Virus, and 2% to the ABPV- KBV- IAPV complex.
The implication of these pathogens in the decline of bumblebee populations is still to be determinated.

P019 Comparative analysis of PCRprotocols to detect bee trypanosomatids

Buendía-Abad M.[1], Martín-Hernández R.[1, 2], Benito M.[3], Bartolomé C.[4], Maside X.[4], Higes M.[1]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Marchamalo, Guadalajara, Spain; [2] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain; [3] Departamento de Biodiversidad, Ecología y Evolución; Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain; [4] Grupo de Medicina Xenómica, CIMUS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Trypanosomatids are flagellated kinetoplastids that colonize the intestinal tract of insects (among other hosts), and have been known to infect *Apis mellifera* since the last century. These parasites are receiving increasing attention due to their high prevalence and potential relationship with colony losses, although their role in bee mortality is still to be determined. Up to now, two trypanosomatid species have been described in honey bees, *Crithidia mellificae* and *Lotmaria passim*; their correct identification relies on the recent development of a few species-specific PCR protocols.
In order to identify the most reliable method available for the detection of these pathogens in *A. mellifera*, we compared the sensitivity, specificity and reproducibility of a new method developed by our group with other species-specific protocols described in the literature. The assays were performed using dilutions of DNA extracted from ATCC strains (PRA-403 and 30254, for *L. passim* and *C. mellificae*, respectively).
The results suggest that, although all the protocols tested were highly specific, the one developed by our group was more sensitive and reproducible than others, allowing the detection of lower trypanosomatid infection levels in field samples.
This study has been funded by the project ERTA2014-00003.

P020 Bee pathogens colonizing bee gums

Sokół R., Michalczyk M.
Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medine, University of Warmia and Mazury, Olsztyn, Poland

Objectives: Various pathogens were identified in honey bee (*Apis mellifera*) colonies in apiaries and in colonies of wild honey bees. Wild bee colonies inhabiting trees (*Pinus sylvestris* L.) in north-eastern Poland were analyzed. The presence of pathogens was evaluated in five (1-5) bee gums that had been naturally colonized by bees for 3 years.
Material and Methods: Sixty live worker bees (group A) and 30-60 dead worker bees (group B) were sampled from each bee gum in March and September of 2016 and 2017. Live bees were collected over the outlet from the gum tree beehive, and dead bees were collected at the base of the gum tree. Bee samples were analyzed by PCR with the use of OIE-certified kits and specific primers for two *Nosema* species and the following viruses: acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV) and the American foulbrood (AFB).
Results: In March 2016, pathogens were not detected in group A bees, whereas in group B bees, *N. ceranae* was found in bee gums 1, 4 and 5, and DWV was identified in bee gums 2, 3 and 5. In September 2016, pathogens were not detected in group A bees, whereas in group B bees, *N. ceranae* was found in bee gums 1, 2, 3 and 5, and DWV was identified in bee gums 2 and 5. In March and September 2017, pathogens were not detected in group A bees. In group B bees, *N.*

ceranae was determined in bee gums 1 and 4, and DWV was identified in bee gum 5 in March 2017, and *N. ceranae* was determined in bee gum 4 and DWV was identified in bee gums 1 and 3 in September 2017.The both groups of bees were free of AFB.
Conclusions: Monitoring studies of bee gums not only expand our knowledge of bee pathogens, but also contribute practical information about the progression of life-threatening diseases in bee colonies. The results can be used to develop effective procedures and treatments for preventing the uncontrolled development and spread of bee diseases.

P021 A survey of the different Varroa control methods used in Scotland and their efficacy

Tice G.[1], Highet F.[2], Barnett M.W.[3]
[1] Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland; [2] Science and Advice for Scottish Agriculture (SASA), Roddinglaw, Scotland; [3] The Roslin Institute, The University of Edinburgh, Scotland

The hematophagous mite, *Varroa destructor*, is considered to be the worst threat facing beekeeping in the world today and has considerable negative impact on bee health. If levels of the mite are left unchecked, the result is usually the demise of the colony due to the mite being a potent vector or activator of a number of bee viruses in both the pupal and adult stages. With the assistance of the Scottish Beekeepers Association and Science and Advice for Scottish Agriculture (SASA), we have conducted a survey of the different types of *Varroa* control methods used by beekeepers in Scotland. *Varroa* reached Scotland in the late 1990s after first being detected in south west England in 1992, although there are still some areas in the Hebrides that remain varroa-free eg the Isle of Colonsay and the Isle of Mull. To gain some insight into the efficacy of the different treatments being used, we will analyse a subset of the apiaries by posting out inserts suitable for collecting *Varroa* mites dropping through open mesh floors for a specified time period. Inserts will be returned to our laboratory where *Varroa* mites will be counted by a team of citizen scientists who previously performed a survey of honey bee diseases in Scotland in 2013 and 2014. Estimated number of varroa per colony will be correlated with *Varroa* treatment(s) used, apiary density in the area and geographical information.

P022 Study of some pathogens in bumblebees and honeybees at four different locations in Slovenia

Pislak Ocepek M.[1], Toplak I.[2], Zajc U.[2] Jenčič V.[1], Žvokelj L.[1], Vreček Š.J.[1], Bevk D.[3]
[1] Institute for pathology, wildlife, fish and honeybees diseases, [2] Institute for microbiology and parasitology, Veterinary Faculty University of Ljubljana, Slovenia; [3] National institute of biology, Ljubljana, Slovenia

Wild pollinators as well as honeybees, have an invaluable role in plants pollination. Several studies have been done in relation to bee diseases, but less is known about pathogens in bumblebees and the possible transmission of pathogens between them. Therefore, in Slovenia a study of some pathogens in honeybees and bumblebees and their comparison was carried out.
Fifty individuals of bumblebees of different species and honeybee workers were sampled at the same time in August 2017 at four different locations in Slovenia. In the laboratory, a suspension was prepared from each bumblebee while the honeybee samples from each location were pooled. The suspension was divided for DNA and RNA isolation. Isolated RNA was used in the RT-PCR method to diagnose honeybee viruses: deformed wing virus (DWV), chronic bee paralysis virus (CBPV), acute bee paralysis virus (ABPV), black queen cell virus (BQCV) and sacbrood virus (SBV). Isolated DNA was used in the PCR method to diagnose *Nosema bombi*, *Nosema ceranae*, *Nosema apis*, *Apicystis bombi*, *Crithidia bombi* and *Crithidia mellificae*.
All samples were negative for CBPV. 14% of bumblebees and honeybees from three out of four locations were positive for ABPV, 32% of bumblebees and honeybees from all locations were positive for BQCV, 16% of bumblebees and honeybees from one location were positive for DWV and 18% of bumblebees and honeybees from two locations were positive for SBV. For *Nosema ceranae*, 4% of bumblebees and honeybees from three locations were positive. There were

no positive samples for *Nosema apis*. 6% of bumblebees were positive for *Nosema bombi* while honeybees from all locations were negative. 8% of bumblebees and honeybees from three locations were positive for *Crithidia bombi*, 10% of bumblebees and honeybees from all four locations were positive for *Crithidia mellificae* and 6 % of bumblebees and honeybees from one location were positive for *Apicystis bombi*.
From the results, the link between the infection of bumblebees and honeybees with some pathogens could be concluded. The possible transmission of several pathogens is even more evident if we look at the results for individual locations. We will continue this research with more samples and locations included.

P023 BeeTyping™, an approach for monitoring bee health inspired from clinical microbiology biotyping

Bulet P.[1,2], Arafah K.[2], Voisin S.[2], Bocquet M.[3], Le Conte Y.[4], Alaux C.[4], Dalmon A.[4], Gauthier L.[5], Simuth J.[6], Bilikova K.[6]
[1] University Grenoble Alpes, La Tronche, France; [2] Plateforme BioPark d'Archamps, Archamps, France; [3] Michel Bocquet, Annecy, France; [4] INRA UR 406, Avignon, France; [5] Institut du Liebefeld, Berne, Suisse; [6] Institute of Forest Ecology, Slovak Academy of Sciences, Bratislava, Slovakia

Honeybee population decline is being attributed to stressors such as parasites (viruses, *Varroa* mite), pesticides, nutritional and climatic changes. Most researches focused on identifying the stressors' presence instead of their impact on honeybee colonies. BeeTyping™ aims at profiling the infected bees' peptidome, in order to deliver practical applications for bee health management. Hemolymph samples were collected from individuals from monitored colonies with a diagnosed infection, and from individuals experimentally infected with a pathogen. Virus presence was confirmed by quantitative PCR. Peptide and protein content was analyzed and compared by MALDI-MS, directly or after reduction-alkylation of the hemolymph. Top-down analysis by LC-MS/MS was conducted to confirm peptide and protein identities. Hemolymph analyses can track key peptides and proteins of the bee systemic immune response (i.e. apidaecin, abaecin, hymenoptaecin…), and resulted in different molecular fingerprints in function of the bees' infectious conditions. These differences were confirmed by statistical comparison of MS profiles by principal component analysis. Virus-infected bee, with or without *Varroa destructor* co-infection, ended up in a cluster of their own inside the overall *Varroa* cluster. These first results strongly support the robustness of our monitoring approach in the case of co-infections, its potential as a plausible strategy to monitor honeybees' health, and a mean for a better understanding of the molecular immune response of this social insect, in both experimental and natural infections. Other infection models are under investigation, notably for microsporidia (*Nosema*) and entomobacteria.

POSTERS

P024 Sanitary impacts and virus diversity in *Apis mellifera unicolor* population on Reunion Island, one year after *Varroa destructor* first detection

Esnault O.[1,3], Xavier B.[1], Thibodeau L.[2], Garcia P.[2], Lebreton G.[3], Chauzat M.P.[4,5], Delatte H.[3]
[1] GDS Réunion, La Plaine des Cafres, Réunion; [2] DAAF Service SALIM, Saint-Pierre, Réunion; [3] CIRAD UMR PVBMT, Saint-Pierre, Réunion; [4] ANSES, Laboratoire national de référence sur les maladies des abeilles, Laboratoire de référence de l'Union européenne pour la santé de l'abeille, Sophia Antipolis, France; [5] ANSES, Unité de coordination et d'appui à la surveillance, Maisons-Alfort, France

Varroa destructor is the major sanitary threat for honeybee colonies in several parts of the world. Reunion Island, is a small island of 2,511 km² located in the South West Indian Ocean. Beekeeping is a major social and economic activity on the island with XX apiary declared. A first sanitary survey conducted in 2013 over the whole island demonstrated that the population of *Apis mellifera unicolor* were *V. destructor* free, and no colony death being reported. Nonetheless, several pathogens had been detected: *Melissococcus plutonius*, BQCV, CBPV, DWV, *Acarapis* sp. and *Nosema ceranae*. Regarding to this relatively good sanitary situation without *V. destructor*, American foulbrood or *Aethina tumida* pathogens, and in order to try to keep it the same, a network of sentinel hives had been set up in 2015.
Nevertheless, the 4th of May 2017, the first *V. destructor* mites were early detected (without varroosis) in the North of

the island within sentinel hives. Several sanitary measures were applied by veterinary services to try to contain the mite spread. A first epidemiological survey was conducted between May and June 2017 to evaluate the spread of the mite on the island. During this survey 270 colonies were sampled. A second epidemiological survey was conducted between March and May 2018 to assess colony health and mortality rates, post invasion of the mite. The hazard perception caused by the mite introduction was also recorded.
All those results will be discussed regarding to i) the tropical sanitary context ii) the indigenous status of the honeybee from la Réunion, iii) the impact on beekeeping management on La Réunion, iv) similar invasion situations observed on island ecosystems.

P025 The *Tropilaelaps* mites threat: an examination of the injuries inflicted on Apis mellifera host

Phokasem P.[1,2], de Guzman L.I.[3], Khongphinitbunjong K.[4], Frake A.M.[3], Chantawannakul P.[1,5,6]
[1] Bee Protection Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand; [2] Graduate School, Chiang Mai University, Chiang Mai, Thailand; [3] USDA-ARS, Honey Bee Breeding, Genetics and Physiology Laboratory, Baton Rouge, Louisiana, USA; [4] School of Science, Mae Fah Luang University, Thailand; [5] Environmental Science Research Center, Faculty of Science, Chiang Mai University, Thailand; [6] International College of Digital Innovation, Chiang Mai University, Thailand

Tropilaelaps spp. are the most serious parasites of *Apis mellifera* in Asia. Like *Varroa* mites, *Tropilaelaps* mites puncture through the integuments of bee hosts to feed on hemolymph. In this study, we examined the types of injury inflicted by *Tropilaelaps mercedesae* on different stages of *A. mellifera* in Northern Thailand. The injuries inflicted on infested bee and uninfested bee, *Apis mellifera*, were investigated after visualisation by vital staining with trypan blue. The average injuries were found high in prepupae (PP) in both group. We report here for the first time that these mites feed on unsealed larval stages preferably fourth larval instar (L4). *Tropilaelaps* mites cause multiple wounds especially on larval stages. In addition to wing deformation, injuries were observed on different parts of the bees' body including the mouthparts, legs, abdomen, thorax and antennae. It is interesting to note that the antennae of deformities wing bees were found injuries. Moreover, we also found *Tropilaelaps* mites are vectors of deformed wing virus.

P026 Field tests of the new varroacide VarroMed® in honey bee colonies with brood in late summer and autumn

Rosenkranz P.[1], Buck R.[2], Kustermann T.[1], Frey E.[1]
[1] University of Hohenheim, Apicultural State Institute, Stuttgart, Germany; [2] Goldstück Beekeeping Business, Vogt, Germany

VarroMed®, a recently registered new varroacide is available for European beekeeper since autumn 2017. Main components are oxalic and formic acid and it is recommended for multiple trickling of honey bee colonies with brood in spring and autumn. We started a first field study with 56 colonies in mid-August 2017: Half of the colonies were treated with VarroMed® in 6-day intervals, the other half was treated two times with formic acid. The *Varroa* mite fall was recorded continuously in sticky bottom boards throughout the whole test period. In all colonies, oxalic acid winter treatments under brood free condition were performed. In a second study, VarroMed® treatments only started in mid-September with a total of 37 colonies using the same protocol but without a formic acid treated group and the final winter treatment performed with Perizin®.
Each application of VarroMed® elicited a significant increase in mite fall; however, depending on the infestation level and the start of the treatments the efficacy was highly variable. In the study with the early start of the treatment the average efficacy of 5 VarroMed® applications was below 50% when compared to the later winter treatments, similar to the likewise low efficacy of the formic acid treatments at the same apiary. From the colonies with a later start of the treatment the average efficacy of 3 – 5 VarroMed® applications was calculated with satisfactory 94%. According to the season, these

colonies were weaker and had lower brood amounts which might have favored the efficacy of the VarroMed® treatments. The crucial period for a *Varroa* treatment concept in Middle and Northern Europe is July/ August, when the infestation rates of the colonies should be rapidly reduced in order to produce healthy winter bees. Based on our results VarroMed® cannot be recommended as an exclusive treatment for this period but it could be an option for a later on treatment in September in combination with a previous formic acid application.

P027 Biofilm formation of an Iranian isolate *Paenibacillius larvae*, the etiological agent of American FoulBrood Disease in honey bees larva

Betesho Babrud R.[1,2], Kermanshahi R.K.[1], Motamedi Sede F.[2], Zahra Mousavinejad S.[5]
[1] Department of Microbiology, [3] Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Vanak, Tehran, Iran; [2] Research School of Radiation Application, Nuclear Science and Technology Research Institute, Iran

American FoulBrood(AFB) caused by *Paenibacilus larvae* is the most lethal disease in honey bee (*Apia mellifera*) larvae. The aim of the present work was to investigate the biofilm formation in Iranian isolate of *P.larvae* . The strain was isolated from a suspected comb with the sign of AFB from Isfahan (one of the central provinces) in summer 2016 and identified on the basis of colony morphology on MYPGP agar and CSA, biochemical characteristics (gram staining, catalase reaction, casein and gelatine hydrolysis) and partial 16srRNA gene sequencing using universal primers for PCR. The isolate was studied for the ability to form invitro biofilm formation by the slide, microplate technique assay, and scanning electron microscopy. *P.larvae* was identified on the basis of colony morphology on CSA and MYPGP agar media and in gram staining, from liquid culture seen as long gram-positive bacilli appeared single or arranged in short or long chains. Colonies with catalase negative, gelatinase, and Methyl Red positive, unable to use starch and unable to grow in Nutrient broth were selected for molecular identification. The alignment results of amplified base pairs in 16srRNA with database revealed 99-100% identity with *P.larvae* subspecies larvae. According to the phylogenetic tree *P.larvae10* and other isolates formed a distinct cluster. Biofilm formation ability of *P.larvae10* in MYPGP broth was investigated by the slide, microplate method and SEM indicated that the isolate was able to form biofilm in vitro. The quantified biofilms of 18 and 24 hours were weak and intermediate respectively.In conclusion; biofilm formation which is one of the virulence factors of bacteria was observed for the first time in an Iranian isolate of *P.larvae*.

P028 Chronic stress in honey bee colonies induced by acaricide residues and pathogens. A case study

Higes M.[1], Almudena Urbieta A.[1], González-Porto A.V.[1], Bernal J.L.[2], Bernal J.[2], Meana A.[3], Alonso-Prados E.[4], Martín-Hernández R.[1,5]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental, IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [2] I.U.CINQUIMA, Analytical Chemistry Group. University of Valladolid, Valladolid, Spain; [3] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain; [4] Unidad de productos Fitosanitarios. Dirección Técnica de Evaluación de Variedades y Productos Fitosanitarios (DTEVPF), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain; [5] Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYTFEDER), Fundación Parque Científico y Tecnológico de Castilla – La Mancha, Spain

Bees are key elements in economic and environmental ecosystems, hence the alarm generated by studies identifying factors that compromise bee survival, such as pathogens and pollutants. To gain insight into how such elements affect the welfare of bee colonies, we have carried out a comprehensive screening of colonies at a professional Spanish beekeeper with important problems on colony health status and high colony losses. Several factors that potentially favoured colony collapse were identified, including: *Nosema ceranae* infection, alone or in combination with other factors (e.g. BQCV, DWV infection); and the accumulation of acaricides commonly used to control *Varroa destructor* (coumaphos and tau-fluvalinate) in beebread. These data highlight the importance of evaluating these factors in future monitoring

programmes, as well as the need to adopt appropriate preventative measures in national and international welfare programmes aimed at securing bee fitness and health.

P029 Trypanosimatids: a new threat for honey bee colonies?

Higes M.[1], Buendía M.[1], Benito M.[1], García-Palencia P.[2], Barrios L.[3], Jiménez-Antón M.D.[4], Olías-Molero A.I.[4], Martín-Hernández R.[1,6], Alunda J.M.[4], Gómez-Moracho T.[1,5]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA), IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [2] Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Spain; [3] Consejo Superior Investigaciones Científicas, SGAI-CSIC. Statics Department, Madrid, Spain; [4] Department of Animal Health, Group ICPVet, Faculty of Veterinary Medicine, University Complutense, Madrid, Spain; [5] Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI), University Paul Sabatier, CNRS, UPS, France; [6] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain

Trypanosomatids are flagellated Protista parasites with a wide range of hosts. Recently, trypanosomatids of insects, especially those parasitizing the Western honey bee *Apis mellifera*, have received much attention given their increasing prevalence in honey bee colonies in last years and their possible implication on colony losses. However, either their pathogenicity in honey bees or their possible role on honey bee losses are still unresolved
Here, we investigate the infectivity and pathogenicity of *Lotmaria passim* (ATCC PRA403) and *Crithidia mellificae* (ATCC 30254) in the honey bee *A. mellifera* by an experimental infection in caged bees with infective promastigotes cells obtained in the laboratory from specific culture media. Honey bees individually infected with promastigotes of *L. passim* of 96 hours of growth died significantly faster than the other infected groups (promastigotes of *C. mellificae* of 144 and 96 hours of growth and promastigotes of *L. passim* of 144 hours of growth) and uninfected control bees. Scanning electron microscopy revealed that only bees infected with promastigotes of *L. passim* of 96 hours of growth, exhibited spherical-shaped cells morphotypes adhered to the surface of their hindgut epithelium. Our results suggest that *L. passim* could bee a pathogen of honey bees, diminishing the lifespan of the infected bees in laboratory experiments. We discuss the role of *L. passim* at the individual level and its possible role in colony survival.

P030 Tissue tropism of *Nosema apis* and *Nosema ceranae* in experimentally infected worker honey bees (*Apis mellifera*)

Higes M.[1], García-Palencia P.[2], Urbieta A.[1], Nanetti A.[3], Martín-Hernández R.[1,4]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA), IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [2] Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Spain; [3] CREA-AA, Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy; [4] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain

The microsporidia *Nosema apis* and *Nosema ceranae* are major honey bee pathogens with different characteristics in terms of symptoms, evolution and transmission. Although the ventricular epithelium is generally considered the target tissue of both species, indirect observations have led to speculate that *N. ceranae* may also target other structures, which might explain at least some of its peculiarities. To investigate the tropism of Nosema spp. for honey bee tissues, we performed controlled laboratory infections with fresh spores of either species, that were orally administered at two doses. Organs from the digestive (esophagus, ventriculus, ileum, rectum), excretory (Malpighian tubules), circulatory (aorta, heart), respiratory (thoracic tracheas), exocrine (hypopharyngeal, mandibular and labial, cephalic, thoracic salivary glands) and sensory/nervous (brain, eyes and associated nerve structures, thoracic nerve ganglia) systems, as well

as the fat body were dissected from the infected bees 7 and 10 days post infection and examined by light and electron microscopy. Both *Nosema* species were found to infect epithelial and basal regenerative cells in the ventriculus, whereas ileum and rectum contained spores of the microsporidia but failed to show overt signs of infection. No stages of the parasites or tissue damage were detected in the other organs that were examined, confirming high tropism of both species for ventricular epithelium. Our direct histopathological observations do not support the hypothesis that the two *Nosema* species exhibit tropism for honey bee organs other than the ventriculus.

P031 Efficacy of Polyvar Yellow® for controlling *Varroa destructor* in Spanish honey bee colonies

Higes M.[1], Martín-Hernández R.[1,5], Meana A.[2], Hernández-Rodríguez C.S.[3], Krieger K.[4], González-Cabrera J.[3]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA), IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [2] Veterinary Faculty, Universidad Complutense de Madrid, Madrid, Spain; [3] ERI BIOTECMED, Universitat de València, Valencia, Spain; [4] Bayer Animal Health, GmbH., Monheim, Leverkusen, Germany; [5] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain

Varroa destructor mite is a serious and devastating ectoparasite that affects honeybee colonies worldwide. It is believed that this parasite is one of the drivers in the colony loss phenomenon suffered by beekeeping around the world in recent decades. To prevent the mite causing the death of colonies, it is necessary to systematically apply acaricide treatments. However, the number of acaricides registered as veterinary medicaments for their treatment is limited and their efficacy is not enough to control de mite. Last year, the Spanish regulatory agency approved a new medicine based on the pyrethroid flumethrin to control of varroosis (PolyVar Yellow®-flumethrin 275 mg).
To determine the efficacy of this product in the early autumn, we conducted a clinical trial following the requirements of the "Guidelines on veterinary medicinal products controlling Varroa destructor parasitosis in bees of EMEA". Twenty homogeneous *Apis mellifera iberiensis* colonies were naturally infested with *V. destructor*. They showed normal behaviour and no signs of other infectious diseases at the beginning of the trial. No acaricidal treatment was allowed for at least 6 months prior the experiment (last treatment coumaphos). Ten colonies were treated randomly with PolyVar Yellow® and the other ten received Apivar® (amitraz, 500mg/strip), both treatments lasted 56 days according to the standard procedure. The mean acaricidal efficacy recorded for Apivar® was 97% (range 93.2-99.8%) and 89.3 % (range 80.3-99%) for PolyVar Yellow®. A high throughput genotyping assay based on TaqMan® was conducted to determine whether the cause of the lower efficacy in the treatment with PolyVar Yellow® was correlated with the presence of pyrethroid-resistant mites in the population in some colonies of treated group. The results showed that indeed, the frequency of resistant mites were higher in colonies showing lower efficacy.
Overall, our data evidenced that PolyVar Yellow® can be an effective alternative to manage the parasitism in colonies where there is no resistant mites or where their frequency is very low. The implementation of an IPM strategy based on a strict rotation of different acaricides with other management approaches should be the key for a long-term control of the mite.

P032 BEEHEAL: standardization of laboratory methods for sample processing, nucleic acids extraction and PCR for microsporidia and viruses analysis

Martín-Hernández R.[1,2], Benito M.[1], Chejanovsky N.[3], Le Conte Y.[4], Dalmon A.[4], Higes M.[1], Pinto M.A.[5], Reyes-Carreno M.[3], Soroker V.[3]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA), IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [2] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain; [3] Agricultural Research Organization (ARO), TheVolcani Center, Israel; [4] Abeilles et Environnement, INRA, Avignon, France; [5] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança,Bragança, Portugal

BEEHEAL is a project designed to determine the phenology and interaction of *Nosema ceranae* and viruses in four Mediterranean countries: Spain, France, Portugal and Israel, including some territories where *Varroa destructor* is not present (Azores and Ouessant islands). This will allow us to study and compare the interactions between pathogens in a wide range of hosts, beekeeping and climatic conditions.
The honey bee samples collected along the year in the different countries will be analysed for pathogens in three laboratories. This requires a standardisation of methods to compare the results in order to assess the effect of every variable in a reliable way. To that end, the participating laboratories have been working together to establish the sampling methodology, the conservation of the samples, the nucleic acids extraction and the PCR analysis.
We analysed the nucleic acid extraction using different buffers or commercial kits. The incubation of sample in TE buffer at 95°C for 20 minutes showed a good sensibility level and good value for N. ceranae DNA extraction. The integrity of RNA was also evaluated to guaranty that the same sample (either individual bees or composite samples) can be analysed for Microsporidia and viruses detection.
A joint protocol for sample processing, DNA and RNA extraction and PCR analysis has been developed and adjusted to the particular conditions and equipment of each laboratory. The standardisation of methods to be implemented by each participating laboratory will avoid the biases on conclusions based on the diverse methods applied.
This work has been developed under the BEEHEAL project. BEEHEAL is funded through the ARIMNet2 2016 Call by the following funding agencies: INIA (Spain), MOARD (Israel), ANR (France), and FCT (Portugal). ARIMNet2 (ERA-NET) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 618127.

P033 Infection levels of Nosema ceranae (fries et al., 1996) in honey bee colonies in Poland

Martín-Hernández R.[1,2], Bravo S.[3], Ornosa C.[3], Iminska U.[4], Gajda A.[4], Topolska G.[4], Higes M.[1]
[1] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA), IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [2] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain; [3] Facultad de Biológicas, Universidad Complutense de Madrid, Madrid, Spain; [4] Department of Pathology and Vaterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

The epidemiology of honey bee transmissible diseases is influenced by several factors related to the host and parasite but also by environmental and beekeeping conditions.
While the epidemiology of the infection by *Nosema ceranae* in *Apis mellifera* is well characterized in Spain (Mediterranean country) and some other warm regions, there are some colder regions of Europe where the studies are limited to some specific moments of the year and the data on infection level expressed by a percentage of infected bees in colonies in those regions are scarce or hardly available.
In this study, the infection of *N. ceranae* was studied in hybrids of *Apis mellifera carnica* and *Apis mellifera ligustica* in Poland where different pathological repercussions of the infection than in Spain are reported. Interior bee samples were collected from 5 colonies located at the apiary in the Warsaw University of Life Sciences from October 2015 until March 2017. The number of bees infected per colony was analysed by PCR to determine the percentage of infection by *N. ceranae* and *Nosema apis*.
The infection by *N. ceranae* was detected in every sampled month, although the lower levels were found in July (2016) when it was on average only 0.8% and the higher in March (2017) when about 83.3% of bees were infected. Conversely, *N. apis* was only detected in August 2016 in a colony. This pattern shows differences with those described in Mediterranean areas where the lowest level of Microsporidia infection is usually found during the spring.

P034 Does thiametoxan seed-treated oilseed rape have an impact on honey bee mortality?

Higes M.[1], García-Villarubia C.[2], González-Porto A.[3], López A.[2], Urbieta A.[1], Alonso-Prados E.[4], Bernal J.L., Bernal J.[5], del Nozal M.J.[5], Martín-Hernández R.[1]
[1] Laboratorio de Patología Apícola, [2] Departamento de Agricultura, [3] Laboratorio de productos de la colmena. Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [4] Unidad de productos Fitosanitarios. Dirección Técnica de Evaluación de Variedades y Productos Fitosanitarios (DTEVPF), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain; [5] I.U.CINQUIMA, Analytical Chemistry Group. University of Valladolid, Valladolid, Spain; [6] Fundación Parque Científico y Tecnológico de Albacete, Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT), Albacete, Spain

To study the influence of thiamethoxam in the colonies strength and *Nosema* parasitation an apiary formed by 5 honeybee colonies of *Apis mellifera iberiensis* was installed in front of an experimental plot planted with Oilseed rape (Ginfizz variety), treated with Cruiser 350 FS at an equivalent application rate of 18 g thiamethoxam/ha. 750 m apart a second apiary (5 honey bee colonies) was located in front of an experimental plot planted with untreated oil seed rape (OSR).
Colonies had a similar sanitary status with naturally mated sister queens of the same age, and comparable colony strength (10 brood chamber combs covered by bees). *Varroa destructor* was controlled with Apitraz© twice a year.
The exposition phase lasted from 8th April to 11 May 2015. At the start of the exposition, brood combs with beebread and honey were removed to stimulate foraging activity.
Corvicular pollen was collected by standard pollen traps activated 24 hour at three time points, at the beginning (8 April), in the middle (15 April) and at the end of the bloom (29 April). The presence of OSR in the corvicular pollen and honey was confirmed by palinological analysis
Analysis of neonicotinoid residues was conducted at the end of exposition phase in honey (LOQ=10 ng/g) and corvicular pollen (LOQ =2.1 ng/g)
Nosema ceranae parasitic load, presence of BQCV, DWV, LSV-complex and AKI-complex and colonies strength were conducted 15 days before and after bloom, in 3 different months before wintering and just after winterin. *N. ceranae* parasitation was controlled with fumagillin in September 2015, to avoid the winter collapse.
During the exposition phase, dead foragers were statistically different between apiaries (Kruskall Wallis, P-Value = 0,03). These results are confirmed by a significant higher % of OSR honey in the exposed apiary (p value, 0,009), despite the residues of thimethoxam were always below LOQ in all analysed matrices.
No statistical differences were observed between apiaries in relation to colony strength presence of viruses and *Nosema* parasitic load during and after the exposition phase and after wintering.
However, a significant increase of the parasitation of *N. ceranae* was observed after wintering (p-value= 0,0002) with respect the start of the study.
Funding: this work was supported by INIA-FEDER founds (National public research project RTA2013-00042).

P035 Antimicrobial activity of phytomolecules against *Paenibacillus larvae*

Szawarski N.[1,2], Giménez Martínez P.[1,3], Fuselli S.R.[1,4], Eguaras M.J.[1,2], Lamattina L.[2,5], Maggi M.D.[1,2]
[1] Centro de Investigación en Abejas Sociales (CIAS- IPROSAM), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina; [2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; [3] Agencia Nacional de Promoción Científica y Tecnológica, Buenos Aires, Argentina; [4] Comisión Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina; [5] Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), Mar del Plata, Argentina

Pollination is vital to the maintenance of both wild plant communities and agricultural productivity. However, pollinators like *Apis mellifera* are threatened by different biotic and abiotic stressors that affect the bee survival. Pathogens and

parasites are among the most important factors that cause mortality of bees. *Paenibacillus larvae*, the causative agent of American foulbrood, is one of the most important bacterial pathogens that affect bee health. The use of antibiotics, particularly tetracycline hydrochloride, is the most common method for prevention and treatment of infected colonies. The extended use of synthetic drugs is known to generate several problems, including the presence of chemical residues in the beehive products and occurrence of resistant strains. An ecological alternative is the use of vegetal molecules that can be found in floral rewards, such as polyphenols and phytohormones. In recent years the study of this type of molecules has been put in focus, showing that they can be extremely important and very beneficial in the immunity of bees. The aim of the present study was to evaluate the antimicrobial activity of abscisic acid (ABA), indole acetic acid (IAA), gibberellic acid (GA) and p-coumaric acid against *P. larvae* by the broth microdilution method. The p-coumaric acid showed antimicrobial activity against *P. larvae* genotype ERIC I, resulting the MIC equal to 650 μgmL-1 and MNIC to 500 μgmL-1. On the other hand, ABA, IAA and GA did not show antimicrobial activity in the range from 1000 to 15.6 ugmL-1. Of all the phytomomolecules analyzed in the present study, only p-coumaric acid showed antimicrobial activity. This molecule could be a possible potential as a natural alternative for the control of *P. larvae*, avoiding the problems generated by the use of synthetic antibiotics, such as the presence of residues in the beehives products and the appearance of resistant strains.

P036 Antimicrobial activity of phenolic extract of apple pomace against *Paenibacillus larvae* and its toxicity on *Apis mellifera*

Giménez Martínez P.[1,2], Ramírez-Ambrosi M.[3], Gallo B.[3], Berrueta L.A.[3], Alonso-Salces R.M.[1,4], Maggi M.D.[1,4], Fuselli S.R.[1,5]
[1] Centro de Investigación en Abejas Sociales (IPROSAM), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina; [2] Agencia Nacional de Promoción Científica y Tecnológica, Buenos Aires, Argentina; [3] Departamento de Química Analítica, Facultad de Ciencia y Tecnología, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Bilbao, España; [4] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; [5] Comisión Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina

Apis mellifera populations are threatened by different biotic and abiotic stressors that affect the bee survival. Pathogens and parasites are among the most important factors that cause mortality of bees. *Paenibacillus larvae*, the causative agent of American foulbrood, is one of the most important bacterial pathogens that affect bee health. The use of antibiotics, particularly tetracycline hydrochloride (OTC), is the most common method for prevention and treatment of infected colonies. The extended use of synthetic drugs is known to generate several problems, including the presence of chemical residues in the beehive products (honey, pollen and wax), which eventually may even affect consumer health. However, this application can increase the risk of occurrence of resistant strains. An ecological alternative is the use of vegetal extracts containing bioactive compounds, such as polyphenols. The aim of the present study was to evaluate the antimicrobial activity of phenolic extracts of apple pomace, coming from the cider industry, against *P. larvae* by the broth microdilution method. Besides, the toxicity of the extracts on *A. mellifera* was evaluated using the complete exposure method. All extracts contained from 1022 to 9364 mg/g of total phenolic contents, determined by HPLC-DAD, showed antimicrobial activity against *P. larvae* genotype ERIC I, ranging from 20 μgmL-1 to 150 μgmL-1.Toxicity assays of apple pomace extracts on adult bees exhibited a maximum mortality of 18% after 48h. Although some extracts analyzed in the present study showed higher MIC values than OTC, apple pomace extracts are still promising, since being natural products they would lack the problems that synthetic antibiotics have, including the presence of residues in the beehive products and the occurrence of resistant strains. Furthermore the reuse of agro-industrial residues such as apple pomace that are generated in great quantity causing environmental problems, is being paid attention by governments and environmental protection institutions.

P037 Honey bee feeding and its influence on the biochemical response to fluvalinate acaricide

Mitton G.A.[1], Mitton F.M.[2], Szawarski N.[1], De Piano F.[1], Eguaras M.[1], Maggi M.D.[1], Ruffinengo S.[1,3]
[1] Centro de Investigación en Abejas Sociales (CIAS), Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM - CONICET - CIC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina; [2] Laboratorio de Ecotoxicologia Ambiental, Instituto de Investigaciones Marianas y Costeras (IIMyC), Universidad Nacional de Mar del Plata, Mar del Plata, Argentina; [3] Grupo Apicultura, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Argentina

Varroa destructor is a world-wide pest of the honey bee *Apis mellifera*. The control of this parasitic mite is carried out through synthetic acaricides such as pyrethroids (flumethrin and fluvalinate). It has been shown that the nutritional quality of *A. mellifera* diet appears to play an essential role in honey bee health, particularly in relation to the pesticides effects. The aim of this work was to study the feeding influence in the stimulation of the detoxification mechanisms involved in the fluvalinate susceptibility. Nurse bees were fed during 5 days with: sugar syrup 2:1 (control), sugar syrup 2:1 + acid-p-coumaric (600 μM) (coumaric) or sugar syrup 2:1 + indole-3acetic acid (20 μM) (IAA). On the sixth day, a group of bees of each condition was topically exposed to fluvalinate DL50 (15 μg/bee), controls were made with bees that only received topical administration of the solvent (acetone). After 24 h, bees were removed and stored -80° for later determination of protein content, glutathione-S-transferase (GST), acetyl cholinesterase (AChE), Cytochrome oxidase (P450) and glutathione reductase (GR). AChE was measured in heads and GST, P450 and GR in abdomens. All abdomens presented higher protein content than the heads ($p<0.05$), for all conditions. Protein content was higher on abdomen of the bees exposed to the acaricide ($p<0.05$). Bees fed with coumaric or IAA showed a tendency to increase P450 activity in exposed and non-exposed conditions compared with the control group ($p>0.05$). A tendency to decrease AChE activity was observed on bees fed with coumaric or IAA, in exposed and non-exposed conditions compared with the control group ($p>0.05$). The feeding with coumaric affected significantly the GST and the GR activity ($p<0.05$) in exposed and non-exposed nurse bees. Exposed bees showed lower GST activity than non-exposed ones ($p<005$) under coumaric feeding, while for GR, exposed and non-exposed nurse bees presented higher activity than control bees. However, the GR activity was lower in exposed nurse bees ($p<0.05$), as well as GST activity. Our results underscore the importance of phytochemicals naturally present in nectar and pollen, and their function as regulators in detoxification processes.

P038 The Belgian national reference laboratory on bee diseases

Matthijs S., Roels S., De Regge N.
Infectious diseases in animals, Sciensano, Brussels, Belgium

The Belgian National Reference Laboratory (NRL) on Bee Diseases is responsible for the diagnosis of the five notifiable bee diseases and pests in Belgium: the bacterial diseases American foulbrood (caused by *Paenibacillus larvae*) and European foulbrood (caused by *Melissococcus plutonius*); the parasitic mites *Acarapis woodi* and *Tropilaelaps* sp. and the small hive beetle *Aethina tumida*. We use standard procedures for microscopy, bacteriological analyses and molecular methods to diagnose various diseases and parasites.
The NRL performs diagnoses for and gives advice to beekeepers, veterinarians, companies, the Federal Agency for the Safety of the Food Chain and cooperates with the European Union Reference Laboratory for Honeybee Health and the Belgian government. It was and is included in several official National and European surveillance programs.

P039 Detection of RNA viruses in bumblebees *Bombus atratus* in Uruguay

Salvarrey S.[1], Antùnez K.[2], Arredondo D.[2], Invernizzi C.[1]
[1] Ethology Division, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay; [2] Microbiology Department, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

Bumblebees belonging to the genus *Bombus* are excellent pollinators, contributing to the manteinance of natural ecosystems and the production of comercial crops. Like honey bees, bumblebee populations are threatened by different factors, including multiples pest and pathogens. *Bombus atratus* is an American species widely distributed in Uruguay. The aim of this study was to evaluate the presence of RNA viruses on workers and queens of *B. atratus* collected in the field and on workers obtained in artificially reared colonies. We analyzed the presence of Acute Bee Paralysis Virus (ABPV) , Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV) and Sacbrood Bee Virus (SBV) in 16 workers and 12 queens collected in the field, and 40 workers obtained in artificially reared colonies. Total RNA was extracted from each sample, retrotranscribed and subjected to quantitative PCR using specific primers for each virus. The four analyzed viruses were detected in bumble bees. Workers collected in the field were infected by ABPV (18.8%), BQCV (93.8%), DWV (37.5%) and SBV (31.2%); queens collected in the field were infected by ABPV (33.3%), BQCV (66.7%), DWV (16.7%) and SBV (50.0%), workers and artifitially reared were also infected by ABPV (12.5%), BQCV (80.0%), DWV (7.5%) and SBV (20.0%). BQCV was the most prevalent virus detected in bumble bees, independently of the cast and origen; while DWV, ABPV y SBV showed lower prevalences, specially in artifitially reared individuals. RNA viral prevalences in bumble bees are similar to that reported for *Apis mellifera* in Uruguay. The domestication of different aphids such as *A. mellifera* and some bumblebees species, has been reported as one of the causes that facilitates that pathogens, parasites and viruses find new hosts, a phenomenon known as spill-over. The obtained results indicate that ABPV, BQCV, DWV and SBV can be hosted by honeybee and bumblebees.

P040 Effect of different MRS-media on the growth of *Melissococcus plutonius*

Dostálková S.[1], Lamei S.[2], Danihlík J.[1], Stephan J.G.[2], Forsgren E.[2]
[1] Department of Biochemistry, Palacký University Olomouc, Olomouc, Czech Republic; [2] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Melissococcus plutonius, causing European foulbrood (EFB) in honeybee brood, is one of the major bacterial pathogens of honeybees. Melissococcus plutonius is a Gram-positive coccus which needs microaerophilic to anaerobic conditions and carbon dioxide to multiply. The nutritional environment needed for isolation and maintenance of bacterial cultures is often provided as a culture medium based on special needs for any particular bacteria. There are published protocols for selective culture media for *M. plutonius*, but this fastidious microorganism can be difficult to cultivate and there is variation in nutritional requirements of different field isolates. This can be problematic when the bacterium is used for research purposes and maintained under laboratory conditions.
During isolation and maintenance of bacteria from the genus *Lactobacillus* it was observed that the MRS medium (De Man, Rogosa and Sharpe agar) used for culturing Lactobacilli had a positive effect on the growth of *M. plutonius*. We present results from a study where we tested different MRS-media on the growth of *M. plutonius* and the data confirm that some of the tested MRS-media can improve the growth of this bacterium. Further investigations based on these preliminary results may help identify essential compounds which can be used as supplements in culture media for improved growth of *M. plutonius*.

P041 Diagnosis of the *Tropilaelaps* mites (Acari: Laelapidae) infestation: optimisation and standardisation of two identification methods based on morphological examination and PCR

Franco S., Cougoule N., Delcont A., Chauzat M-P., Duquesne V.
Honey Bee Pathology Unit (European Union Reference Laboratory for Honey Bee Health), Anses, Sophia Antipolis, France

Mites of the genus *Tropilaelaps* (Acari: Laelapidae) are ectoparasites of bees. They feed on bee brood, and cause malformations, mortality and gradual colony decline, in association with the viruses that they vectorise. Four species of the genus *Tropilaelaps* are described on bees: *T. clareae*, *T. mercedesae*, *T. koenigerum* and *T. thaii*. Originally, each species is hosted by a particular giant honey bee in Asia. However, following the introduction of the European honey bee Apis mellifera into regions infested with Tropilaelaps, two species, *T. clareae* and *T. mercedesae*, successfully adapted to parasitize this new host.
Currently present only in Asia, *Tropilaelaps* mite is a threat to other territories. Therefore, the infestation is regulated in the European Union and belongs to the OIE list of diseases. Considering these sanitary issues, rapid identification is crucial in case of suspicion in order to implement sanitary measures to avoid spreading.
Two methods have been developed by the European Union Reference Laboratory for Bee Health to ensure the quality of analytical results: morphological examination and molecular identification. Morphological examination is performed for primary diagnosis. Specific criteria have been selected to differentiate *Tropilaelaps* genus from other mites currently found in honey bee hives (such as *Varroa destructor* which is widespread). In a second stage, molecular identification is carried out for confirmation, particularly when specimens are damaged, and to identify the Tropilaelaps species. This method adapted from a previously published assay is based on PCR and sequencing. The complete method has been characterised and validation steps have been conducted. Reference materials have been produced to control the parameters of the PCR and of the complete method.
These two methods were published in the OIE Manual of diagnostic tests and vaccines in 2017 in order to ensure reliable diagnosis of Tropilaelaps at the international level.

P042 Prevalence of *Paenibacillus larvae* in central bohemian region: a case study during spring 2018

Jerabkova V.[1], Kamler M.[1], Ovcarik R.[2], Titera D.[1], Vinsova H.[1]
[1] Bee Research Institute Dol, Libcice nad Vltavou, Czech Republic; [2] Czech Beekeepers Union, Praha, Czech Republic

The analysis of the hive wax debris fallen on bottom board is the most common method for monitoring *Paenibacillus larvae* in the Czech Republic (CR). Detection of clinically significant amount of *P. larvae* is based on the cultivation technique from samples of mixed debris collected from colonies in apiary. Non-invasivity and quick sampling are the benefits of this method. Cultivation of *P. larvae* from hive wax debris samples is successfully used for more than 10 years for monitoring of American foulbrood (AFB) in the CR.
Bacterial pathogen *P. larvae* is obligatorily examined in wax debris samples at queen breeders and at migrating beekeepers in the CR every year. This obligatorily monitored group of beekeepers represents approximately 5 % of all beekeepers in the CR. Thus, colonies of 95 % beekeepers remain with unknown status of P. larvae presence being a potential risk of hidden AFB epicentres.
Some regional governments in the CR support the screening of this pathogen to prevent the outbrake of AFB. E. g. South Moravian Region is continuously monitoring AFB in hive wax debris for last 10 years after the outbrake of AFB epidemy in 2006. Thanks to that, the epidemy was successfully eradicated. Now we present data from a new project focused on occurrence of AFB in honeybee colonies in the Central Bohemian Region.
Around 2000 of mixed hive wax debris samples were analyzed for presence of P. larvae from January to April 2018. Nine samples positive for *P. larvae* were detected by the cultivation method. All honeybee colonies (n=69) at 9 apiaries with positive results were inspected for symptoms of AFB. Colonies with clinical manifestation of AFB (n=11) were found at 8 inspected apiaries. Thanks to wide screening of *P. larvae* in hive wax debris we were able to reveal hidden clinical cases of AFB.
Screening project is supported by Central Bohemian Region administration and researchers of BRI Dol are supported by the Ministry of Agriculture, grant No.QK1710228.

P043 Distribution of Deformed wing virus of honeybees (*Apis mellifera intermissa*) in the different regions of Algeria

Adjlane N.[1,3], Haddad N.[2], Baz A.[3]
[1] Département d'Agronmie, Université M'hamed Bougara, Boumerdes, Algeria; [2] Bee Research Department, National Center for Agricultural Research and Extension, Baqa, Jordan; [3] Laboratoire de Biologie et de physiologie animale, ENS, Kouba, Algeria

Deformed wing virus (DWV) can cause wing deformity and premature death in adult honeybees, although like many other bee viruses, DWV generally persists as a latent infection with no apparent symptoms. 82 honeybee samples from 15 regions located in the north and south of Algeria were analyzed for the presence of deformed wing virsu. detection of deformed wing virus (DWV) in *Apis mellifera* L. based on the reverse transcriptase polymerase chain reaction (RT-PCR) technology.
The prevalence of DWV throughout in Algeria was variable. The virus is present in practically all the apiaries analyzed. No differences were recorded between the two subspecies *Apis mellifera intermissa* and *sahariensis*. On the other hand, higher rates have been recorded in the north than in apiaries in the south. No differences in bee mortalities were reported between the two regions. The north-central region of Algeria is the most contaminated zone with 100% of the bees infected by the virus. This zone is the largest in number of beekeepers and hives in Algeria.
An epidemiological survey on DWV and other viruses in relation to *V. destructor* covering most of the country is necessary to show the spatial and temporal dynamics of the distribution of the virus.

P044 Honey bee colony losses and associated viruses in *Apis mellifera intermissa* in Algeria

Adjlane N.[1,3], Haddad N.[2], Baz A.[3]
[1] Département d'Agronmie, Université M'hamed Bougara, Boumerdes, Algeria; [2] Bee Research Department, National Center for Agricultural Research and Extension, Baqa, Jordan; [3] Laboratoire de Biologie et de physiologie animale, ENS, Kouba, Algeria

Worldwide elevated mortality of honey bee colonies is therefore a worrying problem for beekeeping and agriculture, most mortalities have been attributed to high loads of parasites and pathogens, such as high infestations by the ecto-parasitic mite *Varroa destructor* , together with associated viruses. Our aim is to examine the occurrence and prevalence of five bee viruses (ABPV, BQCV, CBPV, DWV, IAPV) in some apiaries located in northern Algeria. These apiaries have recorded more than 20% mortality. We discuss the prevalence and distribution of pathogens observed in the context of global colony losses.
Three colonies were randomly sampled per apiary. Samples of approximately 100 worker bees were collected from January to March 2017. Totally 50 samples, , were analysed for 5 different viruses. RNA was extracted and analyzed with qRT-PCR.
The 5 viruses are present in all the colonies, the virus DWV and CBPV form the two viruses which dominate with rates of 100% followed by the other 3 viruses (less than 80%). The rate of varroa infestation in adult bees for all the colonies studied exceeds 12%. The majority of the apiaries studied were treated using traditional and unregistered methods, which explains the high rate of infestation. The association of *Varroa* and viruses is probably one of the causes of the increase in mortality in the area studied.

P045 Neonicotinoids affect brain structural plasticity and olfactory memory in honey bees

Cabirol A.[1], Fayolle M.[1], Haase A.[1,2]
[1] Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy; [2] Department of Physics, University of Trento, Trento, Italy

The foraging efficiency of honey bee colonies has recently been related to the sophisticated cognitive skills of their individuals. Any damage to the brain and cognitive functions of foragers can therefore affect the whole colony equilibrium. In this context, it is critical to understand how neonicotinoid pesticides, which have been identified as partly responsible for the decline in pollinator populations, affect both brain structure and function. Here, we investigated whether imidacloprid, the most widely used neonicotinoid, could affect brain structural plasticity and thereby olfactory learning and memory in honey bees.
Following a chronic oral treatment with field-realistic doses of imidacloprid, the ability of bees to learn and memorise olfactory information was assessed using the well-established protocol of appetitive conditioning of the proboscis extension response. Because olfactory long-term memory has previously been associated with structural changes in specific brain centres, the mushroom bodies, we performed the same measures of structural plasticity either after the imidacloprid treatment or after the memory tests.
Our study focuses on potential variation in the number of synaptic boutons within the mushroom bodies after the imidacloprid treatment. This can have consequences on the ability of bees to memorise olfactory information in the long-term by affecting the changes in synaptic bouton number required for memory formation. Our study is the first to relate the impact of neonicotinoids on brain structural plasticity with cognitive performance.

P046 A new design for 10-day adult chronic toxicity tests: exposing honey bees (*Apis mellifera* L.) to active ingredients in pollen, sugar water, and wax matrices

Jack C.J.[1], Mendonça G.[1,2], Dai P.[1,3], Bloomquist J.R.[4], Ellis J.D.[1]
[1] Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; [2] Universidade Federal do Rio Grande do Norte – UFRN, Campus da Escola Agrícola de Jundiaí, Macaíba – RN, , Brasil; [3] Key Laboratory of Pollinating Insect Biology, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; [4] Emerging Pathogens Institute, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

Honey bees (*Apis mellifera* L.) are potentially exposed to pesticides via nectar, pollen and wax; however, pesticide exposure via pollen and/or wax often is not considered in Tier 1 tests for honey bee risk assessments. The purpose of this study was to develop a method whereby the impacts of pesticide residues in pollen and wax on honey bees can be determined in a 10-day adult chronic toxicity test. The addition of wax and pollen to such a study design requires modifying typical in vitro cages to accommodate the new matrices, determining the appropriate concentration of the toxic standard (dimethoate) needed in each matrix to kill 50% of the adult honey bees (LC50) in a positive control, and integrating the test substance into the matrix consistently and evenly. Five treatments (five concentrations of dimethoate), a solvent control (acetone) and a negative control (no solvent or test substance) were established for each of three matrices, including sugar water (the standard delivery matrix in a Tier 1 test), pollen, and wax. Each group was tested with three cages and ten bees/cage following EPA and OECD guidelines for Tier 1 tests. Log rank tests indicated that there were significant differences in survival among bees exposed to different concentrations of dimethoate in nectar, pollen and wax. Mortality was significantly different between the controls and dimethoate treatments at 0.1875 – 3 ug/g dimethoate in nectar, 48 – 192 ug/g dimethoate in pollen, and 30 – 120 ug/g dimethoate in wax. The LC50 values for nectar, pollen and wax were 0.41, 57.42, and 33.11 µg/g respectively. The addition of wax and pollen as matrices for test substance screening could increase the biological relevance of honey bee risk assessments where the test substance can be delivered in all the matrices in which it is encountered by honey bees in their hives.

P047 Synergistic effect of *Varroa destructor* and imidacloprid on immunity during honey bee development

Tesovnik T.[1], Zorc M.[1], Gregorc A.[2,3], Narat M.[1]
[1] Department of Animal Sciences, Chair for genetics, animal biotechnology and immunology, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia; [2] Agricultural Institute of Slovenia, Ljubljana, Slovenia; [3] Faculty of Agriculture and Life Sciences, University of Maribor, Maribor, Slovenia

The side effect of intensive pesticide use in agriculture settings has been a concern to a beekeeping industry. Accumulating evidence suggests that pesticides may have negative impact on honey bees. Additionally, honey bees exposed to different stressors, as parasites and pathogens they vector, further affect their health. Previous studies investigated gene expression in adult honey bees exposed to pesticide imidacloprid or *Varroa destructor* mites individually. The aim of this study was to investigate combined effects of two stressors on honey bees in environmentally realistic conditions, where varroa infested colonies are transported to crop fields treated with imidacloprid. We carried out laboratory experiment where honey bee larvae were treated with neonicotinoid per os and varroa were applied on their surface before pupation. Here we present an expression profile of immune-related genes during pupae development (white-eyed, brown-eyed pupae, and emerged honey bees). Influence of neonicotinoid imidacloprid exposure in larval stage showed that most of immune related genes were downregulated in white-eyed and brown-eyed pupae. However, two of immune related genes, lysozyme-2 and PPO, were still significantly upregulated. Gene expression pattern of varroa infested honey bees has changed during development, where the number of significantly elevated genes increased from white-eyed pupae to newly emerged honey bees. Simultaneous effect of both stressors, varroa and imidacloprid, was most noticeable in white-eyed pupae where we detected significantly changed expression of seven genes (four genes were downregulated, and other three genes were upregulated). We discuss the impact of imidacloprid and varroa parasitism that both appear in environmental conditions as an important external factors on bee immune system development.

P048 Survival and physiological impacts of pesticides combinations in the honeybee (*Apis mellifera*)

Almasri H., Pal E., Tavares D.A., Tchamitchian S., Sene D., Brunet J.L., Belzunces L.P.
INRA, Laboratoire de Toxicologie Environnementale, UR 406 Abeilles & Environnement, Avignon, France

During the last twenty years, the honeybee populations have been in constant decline. This phenomenon was attributed to multiple factors, including the pesticide use. Studies have shown that the nectar, pollen, honey, bee bread, and wax, contain pesticide residues. These pesticides, even at very low concentrations, may act alone or in combinations to lead to synergistic harmful effects. In this study, winter worker bees were chronically exposed for 16 days to a food containing pesticides. The pesticides were imidacloprid, a neonicotinoid insecticide, difenoconazole, a triazole fungicide, and glyphosate, an amino-phosphonate glycine herbicide. The pesticides were present at the concentrations of 0 (control), 0.01, 0.1, 1 and 10 μg/L, alone or in binary and ternary mixtures (imidacloprid + difenoconazole, imidacloprid + glyphosate, difenoconazole + glyphosate, imidacloprid + difenoconazole + glyposate). Mortality rate and food consumption were recorded daily and the potential impacts of these mixtures on antioxidant defenses and oxidative stress were assessed by measuring the activity of glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPox) and Glucose-6-phosphate dehydrogenase (G6PDH) at day 16 at the concentrations of 0.1 and 1 μg/L. Survival rates showed that the mixtures of pesticides have greater impacts than pesticides alone. A synergistic effect was observed with the ternary mixture at 0.1 μg/L and with the imidacloprid-glyphosate mixture at 0.01, 0.1 and 10 μg/L. An additive effect was observed at the 4 different concentrations of the imidaclopride-difenoconazole mixture and at 1 and 10 μg/L for the ternary mixture. Statistical analysis shows that the effects on bees may not be dose-dependent. The analysis of physiological biomarkers indicates that an oxidative stress took place, as shown by CAT activity. In the head, the CAT activity was decreased in bees exposed to imidacloprid and difenoconazole while this activity was reduced in the midgut with the imidacloprid-glyphosate mixture. These results suggest that imidacloprid and difenocozole alone decrease the CAT activity whereas their actions are suppressed in combination. With the increase of sprayings containing several pesticides and the multi-pesticide contamination of honeybee food, understanding the modes of action of pesticide mixtures will finally help protecting the honeybees.

P049 Impact of imidacloprid, difenoconazole and glyphosate alone or in mixtures on honey bee

Tavares D.A., Almasri H., Tchamitchian S., Sene D., Brunet J-L., Belzunces L.P.
INRA, Laboratoire de Toxicologie Environnementale, UR 406 Abeilles & Environnement, Avignon, France

Residues of pesticides present in the agricultural environment can lead to different exposure scenarios for honey bees. Investigations on the effects of pesticide mixtures in honey bees are still scarce. In this study, we evaluate the impact of imidacloprid, glyphosate and difenoconazole alone or in binary and ternary mixtures on the survival and physiology of Apis mellifera. Honey bees of different ages were collected from frames in the hive body and arranged in plastic cages (30 bees per cage and 7 cages per experimental group). The cages were placed in an incubator under controlled conditions (33 ± 2C, 40 ± 10% relative humidity, and darkness). The honey bees were chronically exposed for 20 days through sucrose solutions containing 0 (control), 0.1, 1 and 10 μg/L of the pesticides alone or in mixtures. Daily mortality and food consumption were recorded. All pesticides, alone or in mixtures, affected the survival of honey bees. Different results were obtained according to the concentration but, remarkably, at all concentrations, the greatest adverse impacts were caused in bees exposed to binary and ternary mixtures. The effects of pesticides on the physiology of honey bees were evaluated through the determination of the tissue activity of acetylcholinesterase (AChE) and glutathione-S-transferase (GST) at two sample dates (day 10 and day 20). Honey bees exposed to pesticides showed a modulation of AChE and GST activities according to concentration and time of exposure. For AChE, different scenarios were observed. However, the ternary mixture caused an increase in its activity at all concentrations and times of exposure. Pesticides, alone or in mixtures, decreased GST activity in all groups and times of exposure, independently of the biological compartment (head or midgut) considered. On day 20, the impact of binary and ternary mixtures on GST is clearly greater than those of isolated pesticides. This study provides information on the toxicity to bees of pesticides not only alone, but also in mixtures.

P050 Trinitrotoluene bioaccumulation in the honey bee hive

Filipi J.[1], Glackin J.M.E.[2], Gillanders R.N.[2], Turnbull G.A.[2], Dražić M.[3], Babić Z.[4], Muštra M.[5], Simić M.[4], Pavković N.[6], Kezić N.[6]
[1] Department of ecology, agronomy and aquaculture, University of Zadar, Croatia; [2] Organic Semiconductor Centre, School of Physics & Astronomy, University of St Andrews, Scotland, UK; [3] Croatian Agricultural Agency, Zagreb, Croatia; [4] Faculty of electrical engineering, University of Banja Luka, Bosnia and Herzegovina; [5] Faculty of transport and traffic sciences, University of Zagreb, Croatia; [6] Croatian centre for testing, development and training, Zagreb, Croatia

Honey bees are well-known for their capacity to collect particles from the surrounding environment on their body hair and transporting this material back to the hive. This subsequently leads to bioaccumulation in the hive, the level of which is ultimately determined by the number of honey bees and time. This phenomenon can be exploited for monitoring various compounds in the environment, for instance, pesticides or radionuclides. Trinitrotoluene (TNT) vapours are difficult to detect partly due to low vapour pressure and environmental factors. Under the Bee4Exp project, honey bees have been applied to detecting explosive material from landmines using both an active method of honey bee conditioning and a passive method of hive air and particulate sampling after free-flying and subsequent analysis. In the passive method the sensing is performed by monitoring the loss of light emission from a luminescent thin film sensor when it comes into contact with a nitroaromatic material that has bioaccumulated in the hive, such as TNT. Explosives were sampled by air pump from the hive interior for vapours, and particulates from polymer mats on the hive entrance. The results indicate that the passive method can be a promising tool for detecting trace explosive vapours in complex real-world environments, with the polymer mats showing higher TNT retention.

P051 Neonicotinoids decrease sucrose responsiveness of honey bees at first contact

Démares F.J., Pirk C.W.W. Nicolson S.W., Human H.
Social Research Insect Group, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

Neonicotinoid insecticides are known to have harmful effects on the behaviour and physiology of many insects. Through pollination services, honey bees are exposed to these insecticides in pollen and nectar. Impaired navigation and decreased foraging activity are some of the negative effects reported for neonicotinoids. We exposed caged foragers to sublethal acute doses of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) and tested them individually for sucrose responsiveness. We also tested the effect of a range of sucrose solutions laced with neonicotinoids on bees previously unexposed to neonicotinoids, to mimic the situation where foragers would first encounter poisoned nectars varying in sugar concentrations. Thus, bees were exposed to the insecticides either in the diet fed to caged foragers for 24 hours before testing or in the test solutions used to measure sucrose responsiveness, or both. We report a detrimental effect on honey bee responses to mid-to-high sucrose concentrations under all experimental conditions. Previously unexposed bees displayed unexpectedly low responses to the higher sucrose concentrations tested. This attenuation of sucrose response is further evidence that neonicotinoids are multisensory disruptors, with potent actions at first contact, against pollinators and other beneficial insects.

P052 Effects of neonicotinoid insecticide exposure on nest-founding bumblebee queens

Leza M.[1], Watrous K.M.[2], Bratu J.[2], Woodard S.H.[2]
[1] Laboratory of Zoology, Department of Biology, University of the Balearic Islands, Spain; [2] Department of Entomology, University of California, Riverside, Riverside, CA, USA

Bumblebees are among the world's most important groups of pollinating insects in natural and agricultural ecosystems. Each spring, queens emerge from overwintering and initiate new nests, and the success or failure of these efforts shapes the spatial patterning and abundance of pollination services by workers later in the season, as well as bumblebee population dynamics and persistence along greater timescales. Here we present the first laboratory experiment with the model bumblebee species Bombus impatiens that explores how early nesting success is impacted by the effects of temporary or more chronic exposure to sublethal levels of a neonicotinoid-type insecticide (imidacloprid at 5 ppb in nectar) one factor that have been previously implicated in bumblebee decline. We found that queens exhibited increased mortality and dramatically reduced activity levels when exposed to imidacloprid, as well as delayed nest initiation and lower brood numbers in the nest, but partially recovered from these effects when they only received early, temporary exposure. These findings speak to the sensitivity of queen bumblebees during the nest initiation phase of the colony cycle, with implications for how queens and their young nests are uniquely impacted by exposure to threats such as pesticide exposure.

P053 Monitoring of honeybee colonies exposed to pesticide contamination in apple orchards and vineyards by means of Melixa systems

Fontana P.[1], Zanotelli L.[1], Malagnini V.[1], Tonidandel L.[1], Benedetti M.[2], Angeli G.[1]
[1] Centro Trasferimento Tecnologico, Fondazione Edmund mach, San Michele all'Adige, Italy; [2] Melixa S.r.l., Trento, Italy

The study of pesticides effects on honeybees is a crucial topic in the current research on honeybee decline. Frequently the estimation of these effects is difficult in particular in the field. Since many crop protection products do not produce evident signs in the hives, their effects can only be verified by measuring the adult bees' population, the presence and

the persistence of brood, colony food stocks and honey production into honeybee colonies. This monitoring, as well as being very time consuming, requires precise environmental conditions to be done and often the controls between different stations occur at too long a time distance. For this reason, it was decided to adopt Melixa Systems as tools to evaluate if the data relative to the flight activity and the weight of the honeybee colonies can equally provide an effective image of the development of the colonies, in relation to the presence or absence of contaminants in the main matrices of the hive: wax, pollen and honey.
Melixa Systems are electronic monitoring devices applied to hives and they may offer an important support in this kind of experimentation, recording and transferring to the pc the following remoting information:

1. Flight activity in relation to weather conditions
2. Trend of the honeybee colony weight in relation to weather conditions

Two parallel field experiments were conducted, during 2018, to evaluate the effects on bees of the exposure to crop protection products in two different agricultural environments: apple orchards and vineyards. Small apiaries were made up in three different vineyards and in three orchards, all in Trentino (Northern Italy). In every apiary, three Dadant Blatt hives were equipped with Melixa System and simultaneously other hives were used to collect wax, honey and pollen loads.
The analysis of both residues on hives matrix and the data obtained by Melixa Systems, allowed the evaluation of the health state of the colonies and, in case of contaminations, the severity of the colony impairment.

P054 Effects of pesticides on walking behavior of *Apis mellifera*

Resnik V., Grünewald B.
Bee Research Institute, Oberursel, Germany

Different pesticides are known to influence honeybees´ physiology and behavior. Furthermore, there is growing concern that multiple pesticides display more severe effects than assumed when added up independently. Yet understudied are the effects of pesticides on walking behavior of honey bees, although this can be well assessed in laboratory environment.
Presenting preliminary results, we show that pesticides in sublethal concentrations affect honeybees walking behavior especially when combined. We orally administered insecticides and acaricides (thiacloprid, clothianidin, flumethrin, amitraz, coumaphos, tau-fluvalinate and lambda-cyhalothrin) and tracked walking distance, velocity and patterns for 60 sec 30 and 60min after the administration, using a round glass arena and video recordings. Compared to control groups, honeybees that ingested pesticides displayed a variety of behavioral changes, which will be presented in the poster. Amongst them are changes in walking distance and altered walking patterns. Other impacts of pesticide treatment such as diarrhea, tremor and hyper excitation were also observed.
So far, our results present a variety of impairments by single and mixed pesticide administration, with varying effect durations. With our results we hope to provide a better risk assessment of interacting effects and multi-factorial damage to individual honeybees and hives by pesticides in the future.

P055 Impacts of nutrition on the bumblebee's sensitivity to pesticides

Barraud A.[1], Nadarajah S.[2], Vanderplanck M.[1], Denis M.[1]
[1] Research Institute for Biosciences, Laboratory of Zoology, University of Mons, Mons, Belgium; [2] University of Pierre et Marie Curie, Jussieu, Paris VI, France

With ~2.000 species currently recorded in Europe, bees are a highly diversified and efficient group of pollinating insects. Their decline could therefore lead to a risk for ecosystems functioning and crop yields. The drivers of this decline have been well documented in Europe, specifying multiple factors such as pesticides, pathogens, poor nutrition or climate change. Moreover these factors can potentially interact synergistically. For exemple, how organisms deal with

a toxicant (toxicokinetics) and how a toxicant affects an organism (toxicodynamics) depend on the physiological background of that organism. Variation in diet may affect this physiological background and therefore health, development and survival, as well as sensitivity to pesticide exposure. Pesticides, in turn, may compromise food intake, digestion and metabolic pathways. We urgently need a clear understanding of how nutritional diversity, quality and quantity can affect the health, fitness and development of bees, and how appropriate nutrition can buffer the effects of agrochemical exposure.
The aim of this work was first to assess the impacts of agrochemical and nutritional stress on bumble bee development, and secondly to determine how sensibility to pesticides can be related to proteins content. We performed bioassays on 9 groups of 10 *Bombus terrestris* microcolonies (i.e. queenless colonies) in a fully crossed experience including three different doses of neonicotinoid imidacloprid (0, 2 and 20µg/L) and three pollen diet [willow pollen, diluted willow pollen (30% of cellulose) anddiluted willow pollen plus soy protein]. All colonies were fed *ad libitum* with sugar solution and pollen. We measured during 28 days the consumption of pollen and syrup; and after the 28 days the total brood mass, total dilution (ratio pollen/syrup consumption) and total efficiency (ratio pollen/brood mass).
All measured parameters were affected by the pesticide, especially the efficiency: -55% and -93% for 2 and 20µg/L, respectively. As expected, efficiency was also affected by pollen diet quality in the control groups (i.e. 3 groups without pesticide). However, the diet quality did not have any effect on brood mass and efficiency in the groups exposed to neonicotinoid. In other words, micro-colonies were affected in the same way by pesticides no matter what they were eating on. Regarding the behaviour of dilution, the optimal diet group (i.e. full willow pollen) increased the dilution when exposed to pesticide while the two other groups did not change their behaviour. Overall we showed that diet quality (dilution or protein level) did not affect sensitivity to pesticide exposure. It seems therefore that the negative effect of pesticide exposure cannot be compensated by a diet of high quality.

P056 Preliminary protocol to identify the decapped-recapped cells by worker bees, in order to estimate the hygienic behavior in connection with the capped brood

Siceanu A., Căuia E., Vișan G.O.
Institute for Beekeeping Research and Development, Bucharest, Romania

Some studies (J. Harris, R. Danka, J. Villa, 2013) suggests that some worker bees have the ability to identify some cells infested with varroa and to decap and recap them. This behavior seems to influence the reproduction success rate of varroa mite. In order to estimate this trait a protocol was developed and could be used for a correct and quick identification of the decapped-recapped cells. The method consists in the artificially decapping brood in order to notice the internal part of cappings, which is a good indicator of the decapping/recapping behavior performed by bees. The difference between a not deccaped cell and a decapped-reccaped cell is the presence/absence of the part of the cocoon which is in contact with the wax cap. Working technique consists in the artificial removal of cappings, of a certain surface of capped brood, by soldering with melted beeswax and snatching, using a decapping element. Thus, the decapping element become the evaluation element/reading element for the bee colony in combination with a photo of the decapped brood. The big advantage of this method is that we don't have to cut and preserve combs with brood in the freezer or to make evaluations in the field, but only to take these 2 elements (decapping element and photo) which can be stored for the evaluation in any conditions of room temperature. The protocol, which can be applied in the research and breeding field, will present the work methodology by specific stages, being well illustrated by photos.

P057 The pheromones semiochemical effect study in a new solution for honey bee colony development

Balea A., Ciotlaus I., Pojar-Fenesan M.
Babeş-Bolyai University, Raluca Ripan Institute for Research in Chemistry, Cluj-Napoca, Romania

The downward trend and effective decreased egg-laying queen is one of the causes leading to a decline of bee numbers worldwide, with a major effect of this seen in crop damages due to disfunctioning ecosystems. The aim of this study is find chemical formulations that lead to the development of the bee colony by increased egg-laying queen.
In this paper the results of several experiments on feeding bee colonies are presented. Experiments were conducted early spring when natural resources for bee food are missing. For fair and accurate results, each variant was tested in five hives. The first time, three feeding options were tested, two of which were simple bee food with protein and the third one was supplemented with essential fatty acids. All three variants were administered in the presence of pheromones. A forth food variant was used as a benchmark, this variant containing only bee food with protein, without any brood pheromones. The variants of the proposed chemical formulation of pheromones are configured according to the volatile emitted by the bee brood. The volatiles were collected through SPME and analysed by GC-MS. The nutritional effect for fatty acids incorporated in protein cake was tested in comparation to protein food without fatty acids.
The evaluation of the results was done by: assessing the amount of food consumed, respectively assessing the brood mass, observing and measuring the brood comb area. The best results were obtained with bee food supplemented with essential fatty acids and administered in the presence of brood pheromones.

P058 Bumblebee foraging preferences and strawberry pollination effectivity according to surrounding biotopes

Bonchuchnaia A., Karise R., Mänd M.
Plant Protection, Estonian University of Life Sciences, Tartu, Estonia

Pollinators decline is a worldwide problem with a major effect on crop production. It is known that agriculture through intensification negatively affects pollinators' density and diversity. Mass flowering crops with high floral resource abundance concentrated in time couldn't be enough supported by natural pollinators even in case of sufficient supply by the surrounding biotope. Then, extra pollinators are needed to relieve pollination deficiency. Therefore, it is necessary to estimate the supporting capacity of anthropogenic landscapes for the pollinator colony development and pollination potential on entomophilous crops.
The aim of the study is to evaluate the bumblebee foraging rate of strawberry pollen in different landscapes and the impact of surrounding biotopes on colony development. Commercial bumblebee hives (*Bombus terrestris* L.) were placed in natural, semi-natural and anthropogenic landscapes near the flowering strawberry fields, and were observed during a 3-week period in the year 2014 and 2017. Bumblebee corbicular pollen was collected and analysed for botanical origin. The results demonstrated that the average proportion of the strawberry pollen in collected samples significantly varied by years and landscapes. In addition, bumblebee-collected pollen showed food plant constancy with a domination of *Brassicaceae*, *Lamiaceae*, *Caragana*, *Rosaceae* and *Fabaceae* plant families in both years and landscapes. Estimation of hive biomass increase showed no differences between the biotopes.
We conclude that Estonian agricultural landscape is suitable for pollinator colony development. Using commercial bumblebees as extra pollinators, however, might be affected by the surrounding biotopes.

P059 Queen tracking inside the hive : a new methododology for the study of queen movement

Rüger C[1,2,3], Bompa J-F.[4], Nozet G.[4], Grateau S.[5], Ricard E.[4], Béguin M.[1,3], Guirao A.L[1,3], Basso B.[1,3]
[1] ITSAP-Institut de l'abeille, Avignon, France; [2]UR 406 Abeilles et Environnement, INRA, Avignon, France; [3]UMT PrADE, Avignon, France; [4]GenPhySE, Universite de Toulouse, INRA, INPT, INP-ENVT, Castanet Tolosan, France; [5]UE Abeilles, INRA, Le Magneraud, France

An electronic device system by RFID has been developed to track queen movement inside her colony.
This is the first system able to track queen movement surrounded by her workers without creating an artificial environment (glass hive for example) away from her living conditions. A 9 frame Dadant colony has been created, the queen has been tagged with a small RFID chip and each frame has been equipped with 4 antennas. Only one reader is needed for the entire colony. A specific algorithm was developed with the supervising PC interrogates the correct antenna to track the queen movement. At the end, the system informs, second by second, on which quarter of frame-between is the queen of the colony.
This prototype operated throughout the 2017 season, and colony were monitored monthly (health, quantity of open and sealed brood, reserves).
Such a system generates a lot of data (around 1 each second) to process. As a first step, the analysis compares the activity of the queen according to the hours of the day and according to the season. The comparison of the presence of the queen on a frame in relation to the egg-laying activity was also studied.
The possibility to follow the movements of queen inside the hive by means of RFID system is fully mastered. The technique we reported here will allow researchers to study topics related to the queens' health and genetic.

P060 Apolipophorin III haemolymph content and weight as putative biomarkers of precocious foraging

Cabbri R., Isani G., Galuppi R.
University of Bologna, Department of Veterinary Medical Sciences, Ozzano dell'Emilia, Italy

Due to the superorganismic nature of the hive, the balance between honey bee sub-populations is of great importance. The transition from house bee to forager is not fixed and can be modulated in response to many stimuli: malnutrition, wax deprivation, lack of pollen, forager loss and action of pathogens. This suggests the presence of a common host response to different stressors, possibly leading to a common pathway of colony collapse, as recently proposed by other authors. Thus, biomarkers of population unbalance could be useful tools for early detection and counteraction of hive depopulation. To simulate this condition, we conducted a trial with single cohort colonies made with approximately 2500 workers of same age and a fertile queen. With this experimental setup, we were able to sample: precocious foragers (n=15), proper aged nurses (n=21), proper aged foragers (n=15) and overaged nurses (n=19). Haemolymph was drawn from each bee after weighing it on a balance with 0.001 g sensitivity. Haemolymph proteins were separated via SDS-PAGE and most relevant bands were identified using MALDI-TOF technology. The abundance of apolipophorin, vitellogenin and apolipophorin III was calculated by densitometry comparing the bands with an internal standard (lactate dehydrogenase). Precocious foragers showed a significantly higher amount of apolipophorin III (M= 0.068 ug, SD= 0.063 ug) than normal aged foragers (M= 0.040 ug, SD=0.063 ug), t = -2.6007, p-value = 0.01469. Moreover, they showed a significantly higher body mass (Mdn=0.082 g, IQR= 0.007 g) than normal aged ones (Mdn=0.078 g, IQR=0.007 g), (t = -1.7688, p-value = 0.04012). Given the results obtained, apolipophorin III and weight may be promising biomarkers for the identification of alterations in foraging force. Further studies are needed to validate these candidates and to find putative markers also for the nurses.

P061 Hygienic behaviour in different lines of honey bee colonies

Tamašauskienė D.[1,2], Balžekas J. ,Blažytė-Čereškienė L.[2]
[1] *Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture, Akademija, Kėdainiai distr., Lithuania;* [2] *Institute of Ecology, Nature Research Centre, Vilnius, Lithuania*

It has been noticed that some honey bee colonies recognize diseased, damaged or dead bees in covered brood, and then remove them from the nest. These bees are termed as hygienic bees. They eliminate the source of infection and limit the spread of the disease in the colony, thereby protecting themselves from Varroa destructor mites and other infectious diseases such as European foulbrood, American foulbrood, chalkbrood and others. Hygienic behaviour is a heritable genetic trait. The honey bees with a high hygienic behaviour can be derived by proper selection.
Method: Hygienic behavior of *Apis mellifera carnica* honey bee colonies (N=271) using a freeze killed brood method was tested in 2014-2017. In the experiment five different lines (Cnor, Ctrc, Cct-19, Cslov, and Cvig) of *A. mellifera carnica* with one, two, and three year old bee queens were used. In this assay, a comb section of sealed brood containing approximately 100 cells on each side is cut from a frame and frozen for 24 hours at -18o C.
Results: The test revealed 58 bee colonies (21.4 %) that had a high and very high hygienic behavior (i.e. 81 % - 100 % of cells was cleaned), and 127 bee colonies (46.9 %) did not have hygienic behavior, i.e. they cleaned less than 51 % of brood cells. High hygienic behaviour was detected approximately in 27 % of colonies in lines Cnor and Ctrc, in 20 % of colonies in lines Cct-19 and Cvig, whereas in line Cslov only 15 % of colonies were detected as hygienic.
No significant differences were estimated between hygienic and non-hygienic honey bee colonies in wintering, strength and development rates.

P062 Use of quadcopter drones to estimate honey bee population densities and define honey bee drone congregation locations

Crewe R.M., Human H., Yusuf A.A.
University of Pretoria, Social Insects Research Group, Pretoria, South Africa

The use of quadcopter drones (unmanned aerial vehicles or UAV) as a tool to discover and define areas of honey bee drone presence will be described. The UAVs are used to to carry honey bee queen substance lures that are used to attract the drones. Since the location and position of the UAV can be accurately recorded, it is possible to define the area in which drones can be attracted. Subsequently, the UAV's camera is used to reconstruct a three dimensional topography of the areas where drones are found and to define the physical attributes of these areas.
In addition to using the UAV as described above, they are used to carry Williams drone traps in order to sample honey bee drones at the particular location being investigated. These samples are used to estimate colony densities in the catchment area of the drone congregation.
Data on the use of this tool at a number of locations will be presented in order to demonstrate its utility in studying mating behaviour and in estimating population sizes in wild populations of honey bees.

P063 The assessment of non-reproduction rate of *Varroa* (*Varroa destructor*) in a selection apiary in Romania – a comparative approach 2015-2016

Căuia E.[1], Siceanu A.[1], Buchler R.[1], Uzunov A.[2,3], Vișan G.O.[1], Căuia D.[1]
[1] *Institute for Beekeeping Research and Development, Bucharest, Romania;* [2] *Landesbetrieb Landwirtschaft Hessen, Bieneninstitut Kirchhain, Kirchhain, Germany;* [3] *Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Republic of Macedonia*

Recent international research attempts, concerning the natural resistance of honey bee colonies to *Varroa* (*Varroa destructor*), are focused on specific traits such as *Varroa* sensitive hygiene and Suppressing mite reproduction. Some international researchers demonstrated that the low reproduction rate of varroa can be influenced by an important genetic trait of bees which could be identified and used for selection. Thus, since 2014 a standardized assessment procedure for this trait was established in the frame of RNSBB network and Smartbees project. The first evaluations on Romanian bee (*A.m. carpatica*), regarding the mites' non-reproduction rate, using the established protocol, were done on samples collected in 2015-2016 and the results show a relatively high variability of the values. Additionally, on the same test population in 2016 we used a particular developed by our side, artificial brood decapping method in order to evaluate the impact of this technique on the mites' reproduction rate. The results of the experiments reveal that the mites' non-reproduction rate in the infested brood samples ranged between 18% and 38% in 2015 and between 18% and 58% in 2016, respectively. In artificially decapped brood the mites' non-reproduction rate increased in average by 9,79%, being artificially generated by the adult females escaping out of the cells before finishing their biological cycle. The findings of the evaluations in this experiment (2015-2016) will be analyzed and presented. The experiments were done in the frame of Smartbees project, grant 613960, funded by European Commission.

P064 The behaviour of adult honey bees, *Apis mellifera* L., that were reared artificially

Mortensen A.N.[1,2], Ellis J.D.[1]
[1] Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; [2] Pollination & Apiculture, Plant & Food Research, Hamilton, New Zealand

The extent to which the juvenile environment may alter the adult behaviour of honey bees, *Apis mellifera* L, is not well understood. Cooperative behaviours observed in honey bees are often regarded as simple instinctual responses to positive and negative feedback. However, learning and cognition also play a role in some honey bee behaviours. We compared the behaviour of adult honey bees reared in the laboratory (in vitro) or their parental colony (in vivo). Adults were collected at emergence from both rearing environments and held in hording cages with other individuals from the same colony and rearing condition. Laboratory assays were conducted to compare key social behaviours such as trophallaxis, queen response, and brood rearing between workers reared in both environments. Additionally, adults from both rearing environments were introduced into an observation hive and their behaviour monitored for 28 days. There were no statistically detectable differences in trophallactic food sharing or queen response between bees reared in vitro or in their parental colony. Furthermore, there was not a detectable effect of rearing environment on the age at which artificially reared bees conducted tasks in the observation hive compared to that of bees reared in their parental colony. However, in the nursing behaviour laboratory assay, there was a statistically detectable reduction in the number of cell visits and the duration of cell visits made by bees reared in vitro compared to that for bees reared in their parental colony. These data increase our understanding of the interaction between the developmental environment and honey bee behaviour. Furthermore, in vitro rearing of honey bees is a popular risk assessment tool, and our data offer additional metrics that can be used to assess sublethal effects of environmental stressors during honey bee development.

P065 Large scale monitoring of honey bee colony losses in Latin America

Requier F.[1], Antúnez K.[2], the "Colony Losses" working group of the Bee Research Latin American Society (SOLATINA)
[1] Instituto de Investigaciones en Recursos Naturales, Agroecología y Desarrollo Rural (IRNAD), Sede Andina, Universidad Nacional de Río Negro (UNRN) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Carlos de Bariloche, Río Negro, Argentina; [2] Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Montevideo, Uruguay

Current global changes affect biological process and ecosystems functioning, for which large-scale monitoring of bio-

diversity are needed to understand the cause and consequence of such disturbances, towards the prediction or the adaptation to future trends. Benefits in large-scale monitoring programs applied to bees allowed, for instance, to pinpoint the current pattern of decline in European wild bee populations and in managed honey bee population in the United States and across Europe. Although Latin America implies a large livestock in managed honey bee colonies and plays a major role in the global beekeeping activity, large-scale monitoring and estimates of bee colony losses are lacking. We developed an unified questionnaire of colony losses based on surveys that have proved to be effective in other regions, specifically, those developed by the Bee Informed Partnership, COLOSS and EPILOBEE. The questionnaire was adapted to LA climatic conditions, for example, by considering determinants of the season of honey bee low activity other than thermic winter, more representative of tropical and subtropical regions (e.g., dry or rainy season). Moreover, we included other types of beekeeping activities, like meliponiculture, an activity well established and developed in Latin America. More than one thousand of beekeepers participated to this volunteer-based survey of colony losses during the 2016-17 year. The spatial scale of this survey included the complete latitudinal gradient of the Latin America, i.e. from Mexico to Argentina, involving 13 countries (i.e. Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Ecuador, Mexico, Paraguay, Peru, Dominican Republic, Uruguay and Venezuela), and an important climate gradient. Moreover, both beekeeping and meliponiculture activities were represented among the respondents, i.e. using the exotic honey bees vs. the native stingless bees, respectively. In this talk we present the effects of the climate, beekeeping practices, and the type of bee colony used, i.e. native vs. exotic, on the spatial distribution of the colony losses over Latin America. This standardized survey provides also the first dataset to compare the rate of bee colony losses between Latin America countries.

P066 Genetic analysis of royal jelly production and behavioural traits in *Apis mellifera*

Phocas F.[1], Vidal M.[2], Le Bihan C.[3], Basso B.[2]
[1] GABI, INRA, Jouy-en-Josas, France; [2] ITSAP, Site Agroparc, Avignon, France; [3] GPGR, Agrapole, Lyon, France

The French association of royal jelly producers implemented a breeding scheme at a national scale to improve a honey bee population for traits of economic interest for royal jelly production. This contribution aims to estimate genetic parameters for those traits because selective breeding requires knowledge of heritability and genetic correlations between the relevant traits. Data from 1,006 colonies were collected from 2011 to 2017 in 37 apiaries. Queens of those colonies were produced by 97 inseminated dam queens. Quantity of royal jelly of the first two harvests in the production season were collected for the 1,006 colonies and the average performance was the production trait studied (PROD). Three functional traits were recorded for 642 to 752 of those colonies at the beginning of the harvest season: a sanitary score (SANI) and two behavioral scores : gentleness (GENT) and calmness (CALM). They were assessed by subjective scoring on a 4-mark scale, with the mark 4 being the most favorable one. Genetic parameters were estimated with a multiple trait animal model considering the performance of the colony as a trait of the queen. The bee genetic (male haploidy) and reproductive (polyandry) specificities were accounted for in the derivation of the relationship matrix. Estimates of genetic parameters were dependent on the numbers of mating drones (d) and drone-producing queens (q). In the range of likely values for d and q, heritability estimates for PROD and GENT were moderate (20-30%) and heritability estimates for CALM and SANI were lower (5-15%). Estimates of genetic correlations between traits were more sensitive to d and q values than heritability estimates. In any cases, GENT and CALM were genetically strongly correlated. While CALM was also favourably correlated to PROD, GENT was weakly associated with PROD. In addition, a tendency toward unfavorable genetic association between GENT and SANI was observed. These preliminary results have to be confirmed by a future analysis on a larger dataset. In conclusion, genetic improvement for bee gentleness and royal jelly production is possible by selective breeding but attention should be paid not to deteriorate the sanitary status of the colonies.

P067 Beehive ventilation: a research agenda

Linton F.
Colonymonitoring.com, Chevy Chase, Maryland, USA

A healthy and productive honey bee colony requires hive ventilation to control temperature, humidity, and products of respiration. Exterior conditions vary over the day, season, and year, and interior needs vary according to activities such as brood rearing, nectar processing, and winter clustering. Honey bees may expend significant effort to maintain optimal interior conditions over varying exterior conditions. Yet the losses owing to suboptimal ventilation are unknown, the effort required to maintain adequate ventilation is unknown, and the productivity to be gained by minimizing bees' effort to maintain optimal ventilation is also unknown.
A research program is needed to determine the effort a colony must make to provide an optimal hive environment as external conditions change and internal activities vary over time; how hive modifications might minimize that effort; the beneficial effects of these modifications; and how to automate the modifications to compensate for changes in external conditions and internal activities.
Research Hypotheses

1. A significant, yet highly variable, amount of honey bee labor is required to maintain optimal conditions within the hive for the activities of maintaining the winter cluster, raising brood, and processing nectar into honey, under various external conditions (weather, seasons, and daily fluctuations).
2. Beekeeper actions such as enlarging or reducing hive apertures, exposing or sheltering the hive from sun and wind, and adding or removing insulation will greatly reduce bee labor in each of these circumstances.
3. When bee labor required to maintain optimal conditions is minimized, the colony will increase its health and productivity.
4. Colony monitoring sensors, coupled with environmental controls (for ventilation, wind, and solar exposure) can be automated reliably and economically, and will minimize bee labor devoted to in-hive environmental optimization, thereby increasing colony health and productivity.

P068 Screening of volatile organic compounds with semiochemical role from melliferous plants

Ciotlăuş I., Pojar-Feneşan M., Balea A.
Babeş-Bolyai University, Raluca Ripan Institute for Research in Chemistry, Cluj-Napoca, Romania

This floral volatiles play an important role in the chemical comunication between plants and insects. The aim of the study was to characterize the volatile profile of melliferous plants from Transilvania area. It was pursued in special the identifying of components with semiochimical role. Some of the floral compounds have a semiochemical role, they are involved in interspecific communication from the hive.
In this paper are presented the melliferous potential of two Forest Species: *Robinia pseudoacacia* and *Tilia cordate* and two Agricultural Crops: *Brassica napus L* ssp. *Oleifera D.C.* and *Helianthus annus*. The analyses of volatiles present in the flower parts were performed by SPME/GC-MS technique. In the volatiles collected from *Acacia* flower there are eight specific compounds. Linalool - floral compound and major constituent of many scents is known to cause behavioral changes to the bee. It is an attractant for the Queen honeybee. β- ocimene E is a constituent of the pheromones of many pollinators. In the volatiles collected from Sun flower there are twenty-four specific compounds. The major component belonging to the sun flower volatiles is α-pinene. Many species of the pollinating insects utilize α-pinene in their chemical communication system. In the volatiles collected from Linden flower there are eleven specific compounds. D-limonene, is the major component from Linden flower volatiles. It is an attractant and constituent of the pheromones of many pollinators. For Rape flowers, there are eighteen specific compounds. The floral markers are: benzeneacetaldehyde and phenyl ethanol. They were identified a total of 72 compounds, from which some semiochemical compounds were already known, but also new compounds were discovered.
Demonstrating the semiochimic role of newly identified components requires biological tests. A solution may be the use of electrophysiological methods which have been so successfully used in pheromone chemistry.

P069 Extraction of sugar from vegetables and supplement carbohydrate sources to honeybee, *Apis mellifera*

Kim J-E., Koo H-Y., Kim D-I., Kim S.Y., Choi Y-C.
Entomology and Sericulture Research Center, Agricultural Research & Extension Services, Jeollanam-do, Jangseong-gun, South Korea

Honeybees (*Apis mellifera*) are well-known for their elaborated division of worker with each bee performing a series of social mission. Colony organization is based on age-dependent division of worker. Honeybees performed several mission: nursing, cleaning or sealing brood cells, producing honey, collect pollen and nectar. The nectar is carbohydrate source for making energy. Korea has a very short blooming season for producing honey due to climate condition. So, almost Korean beekeepers have been supplied sugar syrup to honey bee for their carbonate source. Unfortunately, recently, the price of sugar has risen so much that the production cost of beekeeper has risen. We are extracted several sugars from plants for using carbohydrate source to bee. 3 sugar sources were extracted from plants (watermelon, cabbage, and mandarin). Almost sample showed high level of insecticidal rate and low level feed intake rate. We were selected 2 type of cabbage sugar syrup that low level insecticidal rate and high level feed intake rate (No 6 cabbage(+fructose) was mixed with 10% fructose and No. 7 cabbage(+pollen) was made with 10 % pollen). Cabbage sugar solution has much more impurities than purified sugar. So, No.6 and No 7 sample can do up-regulation of antimicrobial genes (apideacin, defencin, abacin, and hymenopteacin). Our results suggest that up-regulation of antimicrobial genes might be involved in worker through carbohydrate impurities related immune pathways.

P070 Optivar: Promoting the development of selection and breeding for honey bee colonies that are able to naturally limit the burden of *Varroa* infestations

Tison L.[1], Maisonnasse A.[2], Kretzchmar A.[3], Leconte Y.[4], Mondet F.[5]
[1] INRA PACA, Unité Abeilles et Environnement, UMT PrADE, Avignon, France; [2] ADAPI, UMT PrADE, Avignon, France; [3] INRA PACA, BioSP - Biostatistiques et Processus Spatiaux, Avignon, France; [4] INRA PACA, Unité Abeilles et Environnement, UMT PrADE, Avignon, France; [5] INRA PACA, Unité Abeilles et Environnement, UMT PrADE, Avignon, France

Varroa resistance can be defined as the ability of honey bee colonies to survive the parasite for several years in the absence of any treatment against the mite. Long-term survival of untreated *Apis mellifera* populations has been reported in the US and Europe. The ability of honey bee colonies to survive varroa mite infestations has been associated with the development of the *Varroa* Sensitive Hygiene behavior (VSH). Resistant colonies are able to detect the presence of varroa through the cap of developing brood and to remove parasitised brood and the mites. Our study aims to validate a new method of evaluation of the resistance potential of honey bee colonies through estimation of the VSH expression level. To ensure the appropriate use of the method, a simple and clear protocol is needed, but it must be accompanied by recommendations on the environmental conditions in which the test may be used. Such efforts are particularly important to standardise testing in different locations, a feature that is essential to ensure the success of breeding efforts.

P071 Are effective microorganisms possible supplement for honey bee colonies?

Smodis Skerl M.I.[1], Bubnic J.[1], Presern J.[1], Moskric A.[1], Tlak Gajger I.[2]
[1] Agricultural Institute of Slovenia, Animal Science Department, Beekeeping, Ljubljana, Slovenia; [2] Veterinary Faculty, University of Zagreb, Department of Biology and Pathology of Bees and Fish, Zagreb, Croatia

Honey bees as essential pollinators have experienced increased mortality in recent years. One of the possible reasons could involve several disturbances of their microbiota composition. Probiotic microorganisms compete with pathogenic

microbes in the gastrointestinal tract that involves adhesion to the intestinal epithelium. The presence of probiotics can lead to a better availability and utilisation of nutrients in honeybees. The aim of this study was to assess some effects of the EM probiotic® supplement in the honey bee diet on bee longevity and food ingestion in hoarding cages, and winter survival in experimental colonies. In laboratory experiments, the addition of the 2.5, 5 and 10 % probiotic in sugar syrup (1:1, w:w) decreased worker longevity in comparison to the control group. Continuous feeding of experimental colonies with 5 % probiotic in sugar syrup from July to September 2017 showed no differences in winter survival. Possible use of effective microorganisms as a suppliment in bee diet is discussed.

P072 Number of queen cells and reproductive swarms in the dwarf honey bee, *Apis* florea

Phiancharoen M., Chomphuphan S., Khongkhuntian T.
King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand

The dwarf honey bee, *Apis florea* is distributed throughout Thailand where it is an important pollinator. It builds its single-comb nests around branches of small trees or shrubs. The combs without bees but with honey, pollen and brood are seasonally harvested and sold locally. We observed eight colonies of *A. florea* during flowering season, when there is ample supply of nectar and pollen. At Chom Bueng, Thailand (13º 59′ N, 99º 51′ E), this season stretches from April to August. We recorded the time when drone cells were constructed and their number as well as the time and number of queen cells and the number of reproductive swarms per colony. Drone cells occurred, when the colonies had in average of 7.479 + 0.54 worker brood cells in the time of middle of April until end of May. After the queen had laid drone eggs for a period of times, queen cells were built at the lower rim of the drone brood cells, none at worker cells.The average number of sealed queen cells was 7.25 ± 2.76 (min 3, max 10). Six colonies produced two swarms, one primary swarm with the mother queen and one reproductive swarm with a daughter queen while the other two colonies had three swarms, one primary and two reproductive swarms. At the end, all swarms left the empty comb.This differs from cavity nesting Apis species where the last queen inherits the cavity with combs with brood and food. The fate of the surplus queen cells and emerged queens are discussed. Altogether, the reproduction rate of 1.2 is smaller than calculated before from the number of queen cells.

P073 Evidence for short term evolutionary memory of genetic and environment interactions

Dall'Olio R.[1], Battaglia F.[2], Battaglia C.[2]
[1] BeeSources, Bologna, Italy; [2] Azienda Agricola Fortunato Battaglia, Palermo, Italy

Two different honey bee subspecies are endemic of Italy: *Apis mellifera ligustica* (Spinola, 1806) in the Italian peninsula and *Apis mellifera siciliana* (Montagano, 1911) in the island of Sicily. The importance of the genetic origin with respect to local adaptation have already been tested at European scale during the COLOSS GEI Experiment (Buchler et al., 2014); however, the importance of mating location for the F1 generation, a common event in practical beekeeping where locally adapted mother queens are reproduced in "exotic" locations, have not been tested thus far.
In our experiment we monitored 6 *A.m.siciliana*, 6 *A.m.ligustica* and 6 *A.m.sicilianaXA.m.ligustica* colonies for colony strength, behavioural observation, *Varroa* mite infestation and honey production during 2016 beekeeping season. All of the queens were mated locally in Sicily and among the A.m.ligustica colonies we selected 4 'F1' and 2 'F2' generations (while 'P' generation sexuals were mated in continental Italy).
Results showed comparable behaviour between *A.m.siciliana* and *A.m.sicilianaXA.m.ligustica*, while *A.m.ligustica* reported higher mortality and higher mite infestation levels with respect to other groups, evidencing that local adaptation cannot be achieved through local mating in only one or two generations.

P074 Finding Home: bumblebee homing in rural and urban environments

Herascu R.[1], Hall K.[1], Wright O.[1], Edmonds H.[1], English R.[2], Osborne J.[3], Hempel de Ibarra N.[1]
[1]Centre for Research in Animal Behaviour, University of Exeter, Exeter, UK; [2]South Devon Area of Outstanding Natural Beauty, South Hams District Council, Totnes, UK; [3]Environment and Sustainability Institute, University of Exeter, Penryn, UK

In order for a bumblebee colony to survive, its workers must successfully navigate to and from their nest. *Bombus terrestris* workers have been found to return to their nests when displaced from up to 9.8km; while estimates of their foraging range vary from several hundred metres to 2.2 km. We investigated the homing ability of *Bombus terrestris* workers in two rural and two urban sites in South West England. Bumblebee colonies were first introduced into the chosen environments and allowed to forage freely and explore the landscape for a period of five days. During this time, bumblebees were marked with Radio Frequency Identification (RFID) tags upon return to their nest. Tagged bumblebees were then released at distances of 300m, 1km and 2.5km from their nest in all four cardinal directions (rural sites: *N=229*; urban sites: *N=254*). Each bumblebee was released only once.
At all sites, the proportion of returning bumblebees significantly decreased as release distance increased. Whilst only two rural and two urban sites were tested, a significantly lower proportion of bumblebees returned to their nest in the urban environments when compared to the rural environments (300m: 90% rural vs 71% urban; 1km: 78% rural vs 54% urban; 2.5km: 34% rural vs 26% urban). Furthermore, a higher proportion of bumblebees stayed out overnight in the urban environments. Although the number of site replicates is low, this study gives some evidence that bumblebees found it more difficult to navigate back to their nest in urban environments compared to rural environments. It is hypothesised that this may relate to the resource availability around their nest and the distances that each bumblebee had flown prior to experimental displacement. It could also be the case that the structure of the urban environment may be more challenging to navigate.

P075 *Nosema* infected bees increase protein intake to fight parasites

Gómez-Moracho T.[1], Hebb P.[2], Pasquaretta C.[1], Lihoreau M.[1]
[1] Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier, Toulouse, France; [2] Laboratoire Evolution et Diversité Biologique, UMR 5174 Centre National de la Recherche Scientifique, Université Paul Sabatier, Toulouse, France

Insects regulate their nutrient intake to maximize fitness traits. Increasing evidence show that individuals infected with parasites can change their food preferences to acquire nutrients that help them combat the infection. However, whether and how such dietary self-medication is accomplished in social species, where a small number of individuals collect food for all their nestmates, is unresolved. Here we explored how bumblebees exposed to the gut parasite Nosema ceranae regulate their protein and carbohydrate intake. Bumblebees were either given a simultaneous choice between a low protein diet and a high protein diet (choice experiment) or restrained to just one of them (non-choice experiment) during two weeks. Nosema-exposed bumblebees showed higher protein intake overall and presented lower infection rate, but died faster than non-exposed bees. Proteins help to boost the immune system and therefore this choice could be beneficial to combat parasite infections. We will discuss the role of nutritional regulation to fight parasites at the individual level and its potential implication as a social immunity mechanism at the colony level, where different rates of bees may be infected.

P076 Melliferous and polliniferous ressources of *Apis mellifera unicolor* in a tropical rainforest of Reunion Island

Rasoloarijao T.M.[1,2,3], Clemencet J.[3], Lebreton G.[2], Ramavovololona P.[1], Ramamonjisoa Ralalaharisoa J.[1], Leperlier C.[2], Angebault G.[2], Riviere E.[2], Delatte H.[2]
[1] Université d'Antananarivo – Département de Biologie et Ecologie Végétales – Laboratoire de Palynologie Appliquée – Madagascar, Antananarivo, Madagascar; [2] CIRAD, UMR PVBMT, Saint Pierre, La Réunion, France; [3] Université de la Réunion, UMR PVBMT, La Réunion, France

The honeybee *Apis mellifera* L. is native from various regions of the globe such as the tropical islands of the Indian Ocean, known as a "hotspot" of biodiversity. The interaction between the native flora and the indigenous honeybee subspecies *A. m. unicolor* is crucial for the conservation and the management of this exceptional biodiversity. Honeybees collect pollen passively during flower visiting or nectar harvesting, but honeybees can also collect pollen actively with the aid of various structural and behavioral adaptations. The aim of this study was to investigate the interaction between this native pollinator and the native flora in a tropical rainforest of La Reunion. For doing so, we analyzed pollen pellets sampled from hives located in Mare Longue indigenous forest (eastern of Reunion Island) monthly during sixteen months. We also analyzed the honey produced, following the method of melissopalynology, during the same period. The phenology of plant species of the foraged zone was evaluated along 15 transects located up to 1 kilometer around the hives. A total of 73 different pollen types were identified in honey and/or in pollen pellets (55% from exotic flora, 45% from native flora) belonging to 22 plant families. In the pollen pellets, 13 pollen types on average were observed by date. In 13 of the 16 dates, indigenous taxa were dominant in quantity of pollen pellets collected by honeybees. For 11 of the 16 dates, the honey samples presented a dominance in quantity of indigenous pollens. The phenology study followed monthly 120 species present in the study area, among which they were 64 exotic and 56 indigenous species. Our results suggest that the honeybee *A. m. unicolor* could play a major role in the pollination of indigenous flora. More studies concerning plant-honeybee interaction would be valuable for habitat management.

P077 Experimental infection of bumblebees (*Bombus terrestris*) with Deformed wing virus and Black queen cell virus

Tehel A., Paxton R.J.
General Zoology, Martin-Luther-University Halle-Wittenberg, Germany

Viruses associated with honey bees and that spill over to infect other bee species are suggested as one out of many possible reasons for wild bee decline. Deformed wing virus (DWV) in particular seems to be highly virulentfor honey bees and one major key factor for high overwinter mortality. Already many studies shown viruses like DWV or Black queen cell virus (BQCV) at high prevalence in a variety of screened bumble bee species. However these descriptive data say nothing about the effect of these viruses on the host. Experimental infection data could be more useful, and would help push forward investigation of the role of viruses in wild bee decline.
In this study, infections with DWV genotype A (DWV-A), DWV genotype B (DWV-B) and BQCV were established experimentally in commercially reared bumblebee workers (*Bombus terrestris*) through feeding with inocula containing one of the viruses. After a starvation period, two day old worker bees were fed once individually with 1 × 10^9 genome equivalents of the respective inoculum. Inocula and bumble bee colonies were tested by qPCR for common honeybee-associated viruses to ensure lack of cross-contamination (DWV-A, DWV-B, BQCV and four other viruses: SBPV, SBV, ABPV, CBPV).
After inoculation, bees were kept in small groups in incubators at 30°C and were checked every day for mortality. To ensure the presence of virus in the experimentallyinfected bees, a few individuals were removed from the experiment after 20 to 25 days, freeze killed and tested for the fed viruses.
In this experiment adult bees orally infected with DWV-A, DWV-B or BQCV seem to show no significant difference in their mortality compared to a control group. But we cannot exclude that these result may underestimate the impact of these viruses on bumble bees because we only used a single infection event. In nature, bumble bees maybe exposed to viruses more regularly, which results in a potential accumulation of the virus. Furthermore an earlier time point of infection during life could make a big difference by increasingthe infection intensity.

P078 Determining the efficacy of oxalic acid sublimation as a control for the honey bee pest *Varroa destructor* in Florida, USA

Jack C., Ellis J.D.
Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

Oxalic acid (OA) is a natural compound that has been used to control the honey bee (*Apis mellifera*) pest *Varroa destructor*. We tested the efficacy of OA sublimation (1 g/application) combined with artificial brood interruption (queen caging in the hive for 24 d) as a *Varroa* control in the fall of 2016. Sixty experimental colonies were randomly assigned to one of six treatment groups with 10 colonies composing each group. The six treatments were: (1) OA applied once, brood interruption, (2) OA applied three times, brood interruption, (3) no OA, brood interruption, (4) OA applied once, no brood interruption, (5) OA applied three times, no brood interruption, and (6) no OA, no brood interruption (negative control). An additional 10 colonies served as positive controls and were treated with the miticide amitraz (Apivar). We observed high colony mortality wherever brood interruption was applied. Colonies receiving any application of OA had high Varroa levels, while those receiving the standard amitraz treatment were generally healthier and had better survival. As a follow-up study, we sublimated different doses of OA to determine the amount of OA needed to control Varroa effectively. Forty experimental colonies were assigned to one of four treatment groups, with ten colonies composing each group. The four treatments were: (1) 1 g OA, (2) 2 g OA, (3) 4 g OA, and (4) no OA. The OA was applied once per week for three weeks. We observed high Varroa mortality in the 4 g OA treatment, which was significantly different than that in the 1 g and control treatments ($p < 0.05$). There were no significant differences between the treatments in any of the measured colony strength parameters (adult bees, brood cells, honey cells or pollen cells) ($p > 0.05$). Collectively, our results suggest that the label rate for OA use in bee colonies in the U.S. should be higher to be effective against Varroa and that increasing the application rate to 2 or 4 g per appliation does not otherwise harm colony strength parameters.

P079 A new multiplex PCR protocol to detect mixed trypanosomatid infections in species of *Apis* and *Bombus*

Bartolomé C.[1], Buendía M.[2], Benito M.[2], De la Rúa P.[3], Ornosa C.[4], Martín-Hernández R.[2,5], Higes M.[2], Maside X. [1]
[1]Universidade de Santiago de Compostela, Grupo de Medicina Xenómica, CiMUS, Santiago de Compostela, Galiza, Spain; [2] Laboratorio de Patología Apícola. Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA), IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain; [3] University of Murcia, Department of Zoology and Physical Antropology, Murcia, Spain; [4] Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain; [5] Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYTFEDER), Fundación Parque Científico y Tecnológico de Castilla – La Mancha, Spain

The recent development of PCR-derived protocols for the detection of honey and bumblebee pathogens has drawn increasing attention towards trypanosomatids. But most of these methods do not allow for the identification of the infecting species, which would require further sequencing. Moreover, the resolution limits of direct sequencing mean that parasites at lower loads in mixed infections will likely go unnoticed. To overcome these drawbacks, we developed a multiplex PCR protocol to readily identify in a single reaction the main trypanosomatids present in these hymenopterans (*Lotmaria passim*, *Crithidia mellificae* and *Crithidia bombi*), which will facilitate the study of their epidemiology and transmission dynamics.
A battery of primers, designed to simultaneously amplify fragments of the RNA polymerase II large subunit (RPB1) of L. passim, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of C. mellificae and the DNA topoisomerase II (TOPII) of C. bombi, was tested for target specificity under single and mixed template conditions using DNA extracted from cell cultures (*L. passim* ATCC PRA403; *C. mellificae* ATCC 30254) and from a bumblebee specimen infected with *C. bombi* only (14_349). Once validated, the performance of the method was assessed using DNA extractions from seven *Apis mellifera* (Linnaeus, 1758) and five *Bombus terrestris* (Linnaeus, 1758) field samples infected with trypanosomatids whose identity had been previously determined by PCR-cloning and sequencing (P-C-S). The new method confirmed the results obtained by P-C-S: two of the honeybee samples were parasitized by *L. passim*, *C. mellificae* and *C. bombi* at the same time, whereas the other five were infected with *L. passim* only. The method confirmed the simultaneous presence of *L. passim* and *C. mellificae* in two *B. terrestris*, where these parasites had not previously been reported.

P080 Three years controlling the yellow-legged hornet (*Vespa velutina*), a new predator of honeybees in the Balearic Islands

Herrera C.[1], Marqués A.[1], Colomar V.[2], Leza M.[1]
[1] Laboratory of Zoology, Department of Biology, University of the Balearic Islands, Palma, Illes Balears, Spain; [2] Consortium for the Recovery of the Fauna of the Balearic Islands (COFIB), Santa Eugènia, Illes Balears, Spain

The yellow-legged hornet, *Vespa velutina nigrithorax* is an Invasive Alien Species introduced into Europe in 2004 and was detected for the first time in the north of Spain in 2010. Here we present the first detection of *V. velutina* in the westernmost Mediterranean islands and the methodology used to achieve the eradication of this predator. The first detection in Majorca (3667 km2. Balearic archipelago, situated 176 km off the mainland) was in 2015 when only one secondary nest was found in the northwest of the island. During 2016, nine nests were found and removed from August to November and in 2017 this number grew up until twenty nests from June to October. During 2015 and 2016 all nests were detected in evergreen tree species (pines, holm oaks, and common cypress) in the ''Serra de Tramuntana'', a mountain range located in the northwest of the island of Majorca However, in 2017 one of the nests was located 20 km like maximum distance from the first nest located in 2015, through the mountains to the foot of the mountain. In order to detect the nest, feeding points with protein attractant (raw fish) were set in the area in order to locate and follow adults approaching the traps. Flight routes of observed adult hornets from two or three feeding points were followed by drawing a triangulation on the map that allowed location of the nest by visual inspection. (2) Visual observation in the apiaries around the island and active monitoring in natural zones carried out by fifty-four environmental Agents throughout the island. (3) Public awareness and environmental education. It is the first report on an island of destroying nests as a means of controlling the spread, a scenario very different to mainland Europe.

P081 Black garden ants are alternative hosts of honey bee viruses

Schläppi D.[1], Yañez O.[1], Chejanovsky N.[1,2], Neumann P.[1]
[1] University of Bern and Agroscope, Vetsuisse Faculty, Institute of Bee Health, Switzerland; [2] Agricultural Research Organisation, the Volcani Center, Department of Entomology, Israel

Emerging infectious diseases are often the product of host shifts, where a pathogen jumps from its original host to a novel species. Separating mechanical from biological vectors is essential, especially for RNA viruses that exhibit high mutation rates and often cross species barriers. Adaptive changes of viruses enabling replication in novel hosts can ultimately result in a higher virulence to the new host and to the original host after spillback. Although it is in general known that ants can be biological vectors of honeybee viruses, the possible role of the common black garden ant, Lasius niger, as an alternative host of Acute bee paralysis virus (ABPV) and Deformed wing virus type B (DWV-B) is currently unknown. Laboratory *L. niger* colonies (26-741 workers) were fed with ABPV and DWV-B infected honey bee pupae (N=18) or with non-infected collembola (N=2). Adult queens and pooled worker samples were tested for virus infections by qPCR and minus-strand specific PCR as a proxy for virus replication. The data show that *L. niger* queens and workers can carry both ABPV and DWV. Minus-strand RNA was only detected for ABPV, including one field collected queen (control group). Since PCR detection of honey bee viruses does not necessarily imply that other species are actually serving as novel hosts, additional confirmation of viral infection symptoms is required. Therefore, new *L. niger* colonies (107-392 workers) were fed with ABPV infected honey bee pupae (N=8) or non infected pupae (N=8) and evaluated for ABPV infection symptoms. Symptoms were found both at colony (fewer emerging workers) and individual level (impaired locomotion and movement speed). These results demonstrate that L. niger is an alternative host of ABPV, possibly acting as a biological vector of ABPV and as mechanical one for DWV. Ants seem to be ubiquitous biological reservoirs at least for ABPV and possibly other honey bee viruses. The impact of virus spillback to honey bees remains to be investigated.

P082 Supplementation of *Apis mellifera* colonies with a beneficial microbes mixture based on *Lactobacillus kunkeei* strains

Arredondo D.[1], Añón G.[1], Zunino P.[1], Harriet J.[2], Campá J.[2], Antúnez K.[1]
[1] Department of Microbiology, Division Microbiological Sciences.Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; [2] Beekeping Section, DILAVE, Ministry of livestock, agriculture and fisheries, Montevideo, Uruguay

The administration of beneficial microbes as food additives is a widely used approach to improve the health of different hosts. In previous studies our research group obtained a mix of beneficial microbes (BM) composed by 4 strains of *Lactobacillus kunkeei*, isolated from the gut microbiota of bees. Previous results using larvae and adult bees infection models suggested that this product is effective in preventing the infection by *Paenibacillus larvae* and *Nosema ceranae*. The aim of this study was to evaluate the effect of the administration of these beneficial microbes mixture on commercial colonies, on the *Varroa destructor*, *N. ceranae* and virus infection, as well as their effect on colony strength.
Cells of the 4 *L. kunkeei* strains obtained from fresh cultures on MRS broth were harvested by centrifugation, re-suspended in sugar syrup (1:1) and mixed on equal concentrations (final concentration of 1x106ufc/ml).
Three groups of 15 professional colonies were used. All the colonies were treated with amitraz (4%) at the beginning of the experiment (march, autumn). Colonies from group one received the beneficial microbes mixture over the brood combs (50 ml) and also in feeders (200 ml) once a week during three weeks. Colonies from group two received sugar syrup in the same way of group one, and colonies from group three did not receive treatment.
One and three months after treatments, colony strength was estimated and infection level of pest and pathogens was evaluated.
The administration of the beneficial microbes mixture on colonies was safe, since colony strength (population of larvae and adults) was not affected. In July (winter) *V. destructor* levels were significantly lower in the BM-treated colonies compared with control colonies. Also, in the BM-treated colonies *N. ceranae* spore count decrease faster than in the other groups. However, However, the BM did not significantly affect the infection level of ABPV and DWV during the assay.
Those results are promising and encourage the development of new studies using this beneficial microbes mixture.

P083 Trapping *Vespa velutina* queens as a control method and its impact on honey bee colony strength

Romano A., Laurino D., Capello M., Manino A., Porporato M.
Department of Agricultural, Forest and Food Sciences, (DISAFA) - University of Turin, Grugliasco, Italy

The Asian Yellow legged Hornet, *Vespa velutina* Lepeletier 1836, was accidentally introduced in France in 2004 and rapidly colonized most of Western Europe. In Italy, the first hornet was trapped in Loano (Liguria) in 2012, while the first nests were found in the 2013 in Liguria and Piedmont Regions. Nowadays, the Asian hornet in Italy is present in Liguria and Piedmont Regions, with sporadic detections in Tuscany and Veneto Regions. Within the LIFE14/NAT/IT/001128 STOPVESPA Project, the trapping of *V. velutina* queens in spring as a control method is tested since 2017. The aim of the work is to correlate the spring catches of queens with the number of colonies founded by *V. velutina* in the same season and the predation pressure on honey bee colonies later in the year. To check that, three apiaries have been installed and 40 TrapTrap® traps were placed around each apiary within a radius of 700 m. The catches were taken from March to June 2017. The samples of the traps were periodically verified with relative replacement of the attractive. Honey bee colony strength was checked from June to November in each apiary and the results were compared with data from two apiaries around which *V. velutina*'s queens had not been trapped. The results showed significant differences of colony strength between the two areas in favor of the apiaries where hornet queens were captured. In the same period the number of *V. velutina*'s colonies significantly decreased in the trapped area.

P084 Can sublethal pesticides exposure in honeybee colonies with subclinical infections by *Paenibacillus larvae* favour the development of American foulbrood in clinical form?

Bassi S.[1], Lavazza A.[2], Palamara Mesiano M.[3], Perez Garcia F.[3], Lupi D.[3]
[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione di Modena Italy; [2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy; [3] University of Milan, Department of Food, Environmental and Nutritional Sciences, Milano, Italy

Stress factors may interact with additive or synergic effects and a multi-stress condition is the most probable cause for honeybee decline. We evaluated how the combination of different stresses (sublethal dose of pesticides, and electromagnetism) affect survival and make bee susceptible to *Varroa* attack and viral diseases. Experimental hives, from the same origin and health status, were equally and randomly placed on 5 April 2017 in 2 exposure sites (ES1-2) and in 1 control site (CS) The ESs were inside an experimental farm where chemical treatments are applied in orchards (ES1 sublethal exposures to pesticides), and where there is also a high-voltage electric line (ES2 chemicals as in 1 plus electromagnetism). The CS was far from agricultural fields and from human settlements. Clinical inspections and debris collection from the hives for mite checkup were weekly performed. Monthly, sugar treatments have been also made to check the mite infestation level. During a routine check in mid-June, unexpectedly, one case of American foulbrood (AFB) caused by *P. larvae* genotype ERIC II was diagnosed in ES1. In the following days AFB was diagnosed again in 1 colony in ES1 and in 2 colonies in ES2. No hive in CS resulted affected. After the diagnosis of AFB, sugar and debris previously harvested and stored have been examined for P. larvae detection (culture method). The sugar collected at the end of May in ES ranged from 174,000 to 5,000,000 CFU/g in diseased colonies and from 60 to 17,000 CFU/g in the asymptomatic ones. In CS it was <20 CFU/g (detection limit). The debris of three diseased colonies collected on 28 April showed already a high number of CFU. Since the bacteriological examination always underestimates the number of spores, we hypotheses that low contamination was present at the beginning of the trial in all the colonies. The presence of stressors in ES1-2 has probably favoured the development of the infection by sporadically giving rise to the AFB disease.

P085 Towards an electronic nose for American foulbrood: identifying volatile biomarkers for *Paenibacillus Larvae*

Moran J.[1,2], Melonek J.[2], Putrino G.[3], Small I.[2], Leyland D.[4], Grassl J. [1,2]
[1] Honey Bee Health Research Group; CRC for Honey Bee Products, University of Western Australia, Perth, Australia; [2] ARC Centre of Excellence for Plant Energy Biology, University of Western Australia, Perth, Australia; [3] Advanced Sensing Technologies Group, School of Engineering, University of Western Australia, Perth, Australia; [4] Western Australian Farmers Federation Inc.

American foulbrood (AFB), caused by *Paenibacillus larvae,* is the most economically and biologically devastating bacterial disease of honey bees (*Apis mellifera*). AFB is lethal to honey bee larvae, reducing them to a foul-smelling, glue-like mass, and subsequently causing colonies to die out. Early detection of AFB is crucial to prevent outbreaks from spreading to nearby beehives. However, current AFB field diagnostics rely on beekeepers first identifying symptoms, which can be as discreet as a single symptomatic larva in an apiary with hundreds of hives. Furthermore, the process of opening and inspecting hives can spread disease via equipment and is incredibly time-consuming, particularly for commercial beekeepers. Consequently, the apicultural industry requires rapid and non-invasive diagnostics for AFB. In recent decades, odour sensor systems ("electronic noses") have been used in the non-invasive detection of bacterial outbreaks in horticulture, food, and clinical settings. We are working to develop an electronic nose that can detect early infections of American foulbrood in the hive air from volatile organic compounds. This technology will be a valuable tool for the global beekeeping industry, providing a biosecurity device for screening hives for disease and allowing beekeepers to manage and respond to disease more effectively.

P086 Impact of essential oils at low doses on *Varroa destructor*

Bruneau E., Simon Delso N.
CARI asbl, Louvain-la-Neuve, Belgium

Some essential oils have been used for many years in the fight against *Varroa destructor*. The drugs on the market for the treatment of honeybee colonies have very high doses. Yet in nature, essential oils act at a very low dose. In addition, the concentrations used in aromatherapy are much lower than those used in existing veterinary drugs.
The approach of this preliminary study was to analyze for three seasons the effect of different combinations of essential oils used at low doses on varroa populations and bees.

Various combinations of essential oils: combination of four essential oils (1 application of 4 drops in a feeding syrup), 3 essential oils (3 applications of 4 drops in syrup) and mixture of 13 essential oils (3 application of 5 drops in syrup) were tested on respectively 44, 36 and 36 bee hives. In all the cases, the results are very variable and we cannot in any case consider this type of application as a treatment, however there is an overall reduction in the evolution of varroa populations. No negative effects were observed at brood level or colony harvesting capacity. In view of the results, it would seem interesting to analyze the impact of these oils alone or in mixture on the viruses present and on the immune system of bees.

P087 Bee pathogen occurrence in commercial and traditional beekeeping

Tarić E.[1], Glavinić U.[1], Vejnović B.[2], Aleksić N.[1], Dimitrijević V.[1], Stanimirović Z.[1], Stevanović J.[1]
[1] Department of Biology, [2] Department of Economics and Statistics, [3] Department of Animal Breeding and Genetics, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Beekeepers' practices typical of commercial way of beekeeping significantly influence the fitness and health of honey bees. By many usual practices in commercial apiculture beekeepers prevent natural selection which is to improve the bees' innate resistance. This work was aimed at the detection of differences in the occurrence of 8 bee pathogens between bee colonies kept in a commercial and traditional way. The research was conducted on apparently healthy 120 commercially kept colonies in DB hives (group C) and 24 traditionally kept colonies in primitive, so-called trmka hives (group T) on the Pešter plateau. Samples of brood and adult bees were taken from all of them to assess the occurence of bee brood disease agents (*Paenibacillus larvae*, *Melissococcus plutonius*, *Ascosphaera apis* and sacbrood virus - SBV) and adult bee disease agents (deformed wing virus - DWV, chronic bee paralysis virus - CBPV, acute bee paralysis virus – ABPV, and *Nosema* sp.). The identification of these was done using PCR-based methods. The species of *Nosema* was determined simultaneously with the microsporidial detection.
Concerning bee brood disease-producing agents, in group C *P. larvae* (16.67% samples), A. apis (13.33%) and the SBV (96.67%) were confirmed, whilst in samples in group T the SBV was the only one which was detected (33.33%). *M. plutonius* was not found in any sample.
As for adult bee diseases, in both C and T groups all three viruses monitored were detected (DWV, ABPV and CBPV), but their occurrence in the former (100.00%, 100.00% and 83.33%, respectively) was significantly higher (<0.001) than in the latter (33.33%, 33.33% and 33.33%, respectively). *Nosema* sp. was also detected in samples from both groups, with significantly higher occurrence (<0.001) in group C (61.67%) than in group T (29.17%). In all Nosema-positive samples only N. ceranae was confirmed. In group C no colonies were free from all monitored disease causes, whilst in group T there were 58.33% such colonies.
It can be concluded that traditional, natural way of beekeeping provides significantly better conditions for maintenance of bee health and their resistance to pathogens.

P088 Chronic bee paralysis: An emerging issue in honey bee health

Budge G.E., Holder P., Rushton S.P.
Newcastle University, School of Natural and Environmental Sciences, Newcastle upon Tyne, UK

Chronic bee paralysis virus (CBPV) is an unclassified bipartite RNA virus that until recently caused a rare but severe chronic paralysis disease in honey bees, with very characteristic symptoms including abnormal trembling, flightlessness and shiny, hairless abdomens. Infected symptomatic individuals die within a week leading to mounds of dead bees outside affected colonies, which sometimes collapse or are too weakened for pollination or honey production.
Historic disease prevalence on the UK fluctuates, with an estimated incidence in 1965 of 1%, however, the last three years have seen a dramatic increase in disease incidence with up to 46% of professional UK bee farmers experienced problems with chronic paralysis in the last 2 years. Many reported re-occurring problems within apiaries and colony losses of >40% of affected colonies, which is uncharacteristically severe for this disease.
Emerging infectious diseases, either newly appearing or rapidly increasing in incidence/geographic range in a population, have a history of causing large impacts on honey bee populations. RNA viruses are over-represented causative agents of emerging infectious diseases due to their rapid evolution. Here we will review the literature on chronic bee paralysis and assess the potential for this disease to re-emerge. We will also share the latest results from a new research project focussing on improving our understanding of the epidemiology of this damaging honey bee disease.

P089 Multiple virus and microsporidian infections in individual honey bee workers (*Apis mellifera*)

Retschnig G.[1,2], Williams G.R.[1,2,3], Chejanovsky N.[1,4], Kellermann L.A.[1], Mehmann M.M.[2], Oddie M.A.[1], Yañez O.[1,2], Winiger P.[2], Neumann P.[1,2]
[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Agroscope, Swiss Bee Research Centre, Bern, Switzerland; [3] Department of Entomology & Plant Pathology, Auburn University, Auburn, USA; [4] Institute of Plant Protection, Entomology Department, The Volcani Center, Bet Dagan, Israel

Multiple infections are inevitable in managed honey bee colonies (*Apis mellifera*), but pathogen-pathogen interactions within individual hosts are rather poorly understood. Especially concurrent microsporidian and virus infections are common. Interactions between these two groups of pathogens can range from positive to negative ones, but may be altered if hosts are infected by many different pathogens simultaneously. Here, we investigated bees infected with *Nosema apis*, *Nosema ceranae*, black queen cell virus [BQCV] and deformed wing virus B [DWV-B]). Freshly emerged workers from four local colonies with natural BQCV and DWV-B infections were individually marked, mass fed with *N. ceranae* and *N. apis* spores (~100'000 spores per worker) or not (controls) and introduced into three 2-frame colonies (N=200 workers per treatment and hive). After 14 days, the workers were collected and individual pathogen loads measured with qPCR (N=300, N. ceranae, N. apis, BQCV, DWV-B). The average recovery rate of the workers was 38 % (N=228) in the Nosema-inoculated and 43 % (N=258) in the control group. Significant negative correlations were observed between BQCV and DWV-B (GLMM, $P < 0.05$). Significant positive correlations were found between *N. apis* and *N. ceranae* (GLMM, $P < 0.05$) and between BQCV and *N. apis* (GLMM, $P < 0.05$). In contrast, no significant effect was observed for the two viruses in association with N. ceranae (GLMM, all $Ps > 0.05$).
The data confirm the positive association of *N. apis* and BQCV, but not the one between N. ceranae and DWV-B. The *Nosema*-virus interface may vary due to differential host cell apoptosis during infections or due to the order of infection. The negative correlation between DWV-B and BQCV suggest antagonistic interactions, e.g. competition for host cells. The positive correlation between the *Nosema* species does not support within-host competition. Instead, the results support that some hosts are more susceptible to both *Nosema* species. A closer insight into such complex host-pathogen systems requires extensive individual bee level analyses.

P090 Comparative tests of in-hive traps for diagnosis and control of *Aethina tumida* infestations

Cardaio I.[1], Nanetti A.[1], Cilia G.[1], Cornelissen B.[2], Ellis J.D.[3]
[1] CREA-AA Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy; [2] Wageningen Plant Research, Business Unit Biointeractions and Plant Health, Wageningen, The Netherlands; [3] Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

Aethina tumida (Small Hive Beetle = SHB) is a generalist western honey bee (*Apis mellifera* L.) parasite native to sub-Saharan Africa and regarded as a severe threat to European honey bee colonies. The presence of beetle larvae (the most destructive stage) is believed to depend on various factors and be associated with established infestations, which makes adults more suitable to detect in early diagnostic programs and in infestation quantification. Various tools are commercially available to trap adult SHBs inside a colony, but few comparative data exist to assess their efficacy and correlate the number of trapped adults to colony infestation levels.
We conducted this study with the aim of testing in-hive traps as sampling devices that can be used to predict SHB infestation levels in colonies. Two replicates were made in summer 2017 in Florida (USA) on Apis mellifera colonies reared in Langstroth hives and carrying natural SHB infestations. The following traps were maintained in each experimental colony for eight days: West Beetle Trap (WBT), Better Beetle Blaster (BBL), Beetle Barn (BBRN) and microfiber sheets towels (TWL). Chemical lures were avoided as they are not allowed in many countries. Vegetable oil, oil and vinegar, and fluffy microfibers were used to bait WBT, BBL and BBRN respectively.
WBT captured the largest number of adults and was the only trap where larval stages were also captured. Low proportions of infesting SHBs were captured in TWL and BBL traps. In microfiber-baited BBRN, adult SHBs were only occasionally found. For all trapping systems, the correlation between total and captured adults was insufficient to predict the actual infestation reliably.
The low diagnostic reliability of the tested systems should be considered in SHB monitoring and containment plans. Improvements are necessary in order to reach higher predictive accuracies of in-hive trapping tools.
Study conducted within the AETHINET project, financed by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF).

P091 Prey (honeybee) predator (yellow-legged hornet) spill-over of the bee pathogen DWV

Cilia G.[1], Mazzei M.[1], Forzan M.[1], Sagona S.[1], Giusti M.[1], Bortolotti L.[2], Felicioli A.[1]
[1] University of Pisa, Department of Veterinary Science, Pisa, Italy; [2] CREA-AA, Consiglio per la ricerca in Agricoltura e l'analisi dell'economia agraria, Centro di Ricerca Agricoltura e Ambiente, Bologna, Italy

Vespa velutina newly-emerged (females and males) and hunters (hornet workers caught during their predatory activity in front of the apiaries) were sampled during the beekeeping season of 2017 in Liguria region (Italy) in order to check the possible infection of deformed wing virus (DWV).
The total RNA extraction was performed from each specimen to evaluate the presence of replicative form of DWV. Presence of DWV genome was evaluated and quantified by qPCR and a strand specific RT-PCR was performed to confirm the presence of replicative form of the DWV genome.
Replicative DWV was detected in both hunters and newly-emerged specimens proving that DWV can infect the alien Asian hornet.
Sequence analysis on DWV genome, performed on positive samples, indicates high similarity (99%) with the world-wide distributed genetic variant, DWV type A.
The infection rate of DWV in Asian hornets ranges from 33% (hunters) to 50% (newly-emerged males and females). The infection of DWV in *V. velutina* suggests a role of this RNA virus in a possible natural re-equilibrium of the relationship between the prey (*A. mellifera*) and the alien predator (*V. velutina*).

P092 Influence of *Nosema ceranae* infection on semen characteristics in honeybee drones

Borsuk G.[1], Kozłowska M.[2], Anusiewicz M.[2], Paleolog J.[3]
[1] Institute of Biological Basis of Animal Production; University of Life Sciences in Lublin, Poland; [2] Department of Botany and Mycology; Institute of Biology and Biochemistry; Maria Curie-Skłodowska University in Lublin, Poland; [3] Department of Zoology, Animal Ecology and Wildlife Management; University of Life Sciences in Lublin, Poland

Spores in the reproductive tissues and ejaculate of older drones infected with *N. apis* have been detected. This may cause deterioration of reproduction performance (Peng et al., 2015). In the present study, the hypothesis that *N. ceranae* damages sperm DNA in honeybees was investigated. For this purpose, drones were individually infected with *N. ceranae* spores, and then effects of the infection on the semen volume, sperm concentration in the semen, and sperm DNA fragmentation relative to that of uninfected drones were examined. A total of 120 one-day-old drones were marked with queen bee marking numbers and equally divided into two groups. Drones in the infected group were individually given honey syrup containing 200 000 *N. ceranae* spores per 1 μL, while those in the uninfected (control) group were given honey syrup without spores. The groups were then placed and maintained in two separate colonies. On day 14 after emergence, the drones were captured and their semen was collected using a 1 μL calibrated micropipette. The semen volume was measured using an electronic caliper following the method of Czekońska et al. (2013). The semen collected from each drone was divided into two samples of equal volume. One sample was used to determine the sperm concentration, and the other sample was used for DNA fragmentation analysis.
The sperm concentration was determined using a flow cytometer and a Muse Count and Viability Kit from Merck. To quantify the sperm DNA fragmentation, the Sperm DNA Fragmentation (SDF) test from Halosperm® was used. This assay is based on sperm chromatin dispersion.
A significantly higher volume of semen was collected from the uninfected drones (1.18 μL) compared to the *N. ceranae*-infected drones (0.37 μL). The concentration of sperm in the semen was higher in the uninfected drones (10.06 × 10^6/μL) compared to the *N. ceranae*-infected drones (4.93 × 10^6/μL). A markedly higher percentage of DNA fragmentation was found among sperm cells from the *N. ceranae*-infected drones (48.82%) compared to the uninfected drones (13.56%).

P093 Effect of winter stores on *Nosema Ceranae* infection and on colony fitness

Papežíková I., Syrová E., Zachová A., Rädischová K., Palíková M.
Department of Ecology and Diseases of Game, Fish and Bees, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

This work presents the results of two-year field trial which was aimed at testing the effect of various feeds on course of *Nosema ceranae* infection. In August 2015, four experimental groups of bee colonies were established. After the last honey harvest, each experimental group was provided 20 kg of feed – honey, sugar solution, an inverted sugar syrup made of sucrose, or a syrup produced by a hydrolysis of wheat starch. Samples of living bees from each beehive were taken in August (before feeding), in November and in May. The following year, feeding and sampling was performed in the same way. Bees were examined microscopically to estimate the percentage of *Nosema*-infected individuals in the sample and the spore numbers per one bee. Fitness parameters were measured in all colonies.
None of the colonies died during the experiment. In all beehives, presence of *N. ceranae* was confirmed by PCR. *Nosema apis* was not detected in the apiary. Significant differences in condition and in nosematosis prevalence/intensity were observed among the experimental groups. The best results were found with honey, followed by inverted sugar syrup and by pure sugar solution. The worst results were found with group fed with starch syrup.
This work was supported by IGA VFU Brno, grant No 231/2018/FVHE.

P094 Dietary supplementation protects honey bee from immunosuppression caused by *Nosema ceranae*

Glavinic U.[1], Stevanovic J.[1], Tarić E.[1], Draskovic V.[1], Ristanić M.[1], Lakic N.[2], Stanimirovic Z.[1]
[1] Department of Economics and Statistics, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; [2] Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Previous studies demonstrated that microsporidium *Nosema ceranae* exerts negative impact on honey bee health, including down-regulation of immune-related genes. The aim of this study was to evaluate if dietary amino-acid and vitamin supplement "BEEWELL AminoPlus" may protect honey bees from immunosuppression induced by *N. ceranae*. For that purpose we set up six groups with 40 bees in each. In four groups, we infected bees with N. ceranae and applied supplement at different moments after emergence (on first, third, sixth and ninth day after emergence). Two control groups were established, one with infected bees and fed without addition of supplement, and the other one with bees that were neither infected nor supplemented. Expression of genes for immune-related peptides (abaecin, apidaecin, hymenoptaecin, defensin and vitellogenin) was compared among groups. The results revealed significantly lower *Nosema* load in groups supplemented with "BEEWELL AminoPlus" than in control group (infected with *N. ceranae* but not treated with supplement), especially on 12th day post infection. On 12th day post infection, the expression levels of apidaecin, hymenoptaecin, defensin and vitellogenin were significantly higher in bees that received the supplement suppressed compared to control bees. These results suggest that tested supplement "BEEWELL AminoPlus" may protect honey bees from induced immunosuppression. The supplement exerted the best efficacy when applied simultaneously with *Nosema* infection suggesting early spring as the best moment for supplement application because *Nosema* spore load in hive is highest during that period.

P095 Evaluation of Varromed® performances in winter treatment of honey bee colonies (*Apis mellifera*) after brood interruption in a temperate area

Rivera-Gomis J., Pietropaoli M., Bubnic J., Formato G.
Apiculture Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy

Varroa destructor is one of the most important pests of honey bees. The management of this pest is carried out by using acaricides, which usually present a high variability in efficacy and side effects due to variable factors like mite resistance, climatic or colony conditions. The development of new products based on "soft" acaricides, which have a low risk of residues and not known mite resistance is an asset to beekeeping. In temperate areas, the natural absence of brood in winter is not always guaranteed. This is the first study to evaluate the performances of the organic acids based product Varromed® combined with a queen caging period during winter in a temperate area on honey bee colonies (*Apis mellifera*). The study was carried out in Central Italy (Rome) by quantifying the mite fall in two homogeneous experimental groups of nine hives (treated and control) with similar varroa infestation levels. Both groups were in broodless conditions provided by caging the queen during the whole period of the trial. The natural mite fall observed in the control group was 23.4% ±14.2% (n=9), while the Varromed® treatment recorded a mean acaricide efficacy of 96.1% ± 3.5% (n=9). No toxicity on adult or queen bees was observed. No abnormal behaviour of the bees was noticed. Further studies should be carried out in order to evaluate the performances of this product in different climatic conditions and in presence of brood.

P096 Use of traps containing biocides to diagnose and control SHB

Rivera-Gomis J.[1], Maroni Ponti A.[2], Schäfer M.O.[3], Grandinetti G.[4], Federico G.[5], Barca L.[5], Carullo G.[6], Formato G.[1]
[1] Apiculture Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy; [2] Ministero della Salute, Rome, Italy; [3] National Reference Laboratory for Bee Diseases, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Greifswald, Germany; [4] Task Force per le Attività Veterinarie, Regione Calabria, Reggio Calabria, Italy; [5] Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy; [6] Reggio Calabria ASP, Reggio Calabria, Italy

The Small Hive Beetle (SHB), *Aethina tumida*, is an invasive pest of honey bee colonies that is settled in Calabria (southern Italy, EU) since 2014.
In the surveillance, eradication and control activities against SHB, traps may play a crucial role. So far, no veterinary medicines are registered for honey bees against SHB in EU. An external use of already registered biocides placed into traps located under the anti-varroa net, under the bottom board (non in contact with the honey bees, outside the net), could be supposed. Development of similar traps containing active biocides against SHB could be an asset for the diagnosis, eradication and eventual control of this invasive pest, for example, traps containing acetamiprid, an active principle similar to fipronil.
The use of these traps would eliminate the risk of bee toxicity or contamination of bee products, while offering a highly efficient diagnosis and control of SHB.
The integration of these traps in an integrative program, including its placement inside trap hives and apiaries in at risk zones could be both a useful tool against the expansion of SHB and an effective control instrument in areas with an already stablished SHB population.

P097 Monitoring of Small Hive Beetle (*Aethina tumida Murray*) in Calabria (Italy) from 2014 to 2016: practical identification methods

Rivera-Gomis J.[1], Gregorc A.[2], Maroni Ponti A.[3], Artese F.[4], Zowitsky G.[1], Leto A.[1], Della Marta U.[1], Formato G.[1]
[1] Apiculture Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy; [2] Mississippi State University, Poplarville, Mississippi, USA; Agricultural Institute of Slovenia, Ljubljana, Slovenia; [3] Ministero della Salute, Rome, Italy; [4] Italian Beekeeping Federation (FAI) Calabria, Gioia Tauro, Italy

The Small Hive Beetle (SHB), *Aethina tumida*, is an invasive pest of honey bee colonies that causes significant damage to the beekeeping sector. SHB was detected in southern Italy (EU) in 2014 and despite the adopted eradication measures, it is still present there. After three years of observations of SHB in Calabria (2014-2016), we provide here some practical tips for improving control measures based on clinical inspection:
-use of a lateral divider as SHB trap;
-focus the inspection on areas with higher probability of finding SHB's;
-use of tight fitting latex gloves for examination, handling and sampling of beetles.
A new time-saving colony examination method, including the use of a lateral divider to be placed in the hive reduced the time needed for hive inspections by 31.86 % on average. Prioritizating the inspection of pollen and honey combs rather than brood combs is advised.
Moreover, concerning the sentinel apiaries used to monitor SHB's arrival in free areas, no more than five colonies without supers are suggested for each location in order to attract and to monitor the early appearance of SHB. The colonies should be strong, healthy, queen right, as these are more attractive to the parasite. Inserting protein candy or protein substrates into the hives to feed the bees could ease SHB detection, as both adult and immature stages of the SHB are attracted to protein substrates.
Integrative diagnose measures are essential to detect SHB, implementing sentinel apiaries in at risk areas and performing inspections and other diagnosis methods as detection of SHB DNA from hive matrices. An early detection and eradication is essential in free areas, as once it is stablished, this pest is extremely difficult to eliminate from the territory. The use of these methods will enable early detection and prompt eradication measures activation before this destructive pest can spread in a region where it is not present.

P098 Reactive oxygen species and nitric oxide in honey bee gut epithelial immunity

Piskláková B., Dostálková S., Janků M., Danihlík J., Petřivalský M.
Palacky University, Department of Biochemistry, Olomouc, Czech Republic

Epithelial immunity represents an ancient mechanism comprising local immune responses in barrier epithelia occurring at the site of pathogen infection. The epithelia provide both physical and chemical barriers against invading microbes. Epithelial cells are competent of constitutive and inducible expression of antimicrobial peptides (AMPs) as effector molecules in early chemical defence on the epithelium surface. Reactive oxygen species (ROS) are signalling molecules involved in development and stress responses of organisms across all kingdoms. Increased ROS levels as an early event after pathogen recognition are linked to the activation of the Toll pathway which regulates AMPs production, and which is also essential for activation of antioxidants enzyme to counterbalance oxidative stress caused by elevated ROS. Cross-talk of ROS signalling pathways with nitric oxide (NO) dependent signalling has been uncovered in multiple mechanisms both in animals and invertebrates. A role for NO signalling in activation of AMP expression was proposed in gut epithelial immunity in insect model species, where tissue-specific expression of NO synthase in the Malpighian tubules induced by the pathogen was found associated with increased AMP expression. We combined various biochemical, immunochemical and microscopic methods to study levels of ROS, NO and enzymes of their metabolism in isolated bee guts exposed to bacterial lipopolysaccharide (LPS). Changes of ROS and NO levels in early phase of gut response to LPS treatment were followed by induction of ROS-catabolizing enzymes. We also addressed the potential sources of ROS and NO in epithelial cell by a pharmacological approach using specific inhibitors of potential ROS and NO-producing enzymes. Our results contribute to understanding the roles of ROS and NO signalling in activation of the gut epithelial immunity in honey bees.

This research was supported by Czech Ministry of Education, Youth and Sports through grant LTAUSA17116.

P099 Effect of protoporphyrin IX amide derivatives on *Nosema ceranae* development in *Apis Mellifera carnica*

Buczek K.[1], Trytek M.[1], Borsuk G. [2], Deryło K.[3], Gromada A.[1], Rybicka-Jasińska K.[4], Gryko D.[4]
[1] Department of Industrial Microbiology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland; [2] Institute of Biological Basis of Animal Production, Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences, Lublin, Poland; [3] Department of Molecular Biology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland; [4] Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland

Until now, no effective drug has been developed against microsporidia from the genus *Nosema*, which are particularly devastating to honeybees. Our recent study has shown that porphyrins, which had been dubbed "pigments of life" due to their role in essential life processes, are very promising agents for combating microsporidiosis [1]. In the present work, we describe the biological activity of amphiphilic protoporphyrin IX amides conjugated with either lysine [$PP(Lys)_2$] or glutamic acid [$PP(Glu)_2$] moieties, against *Nosema ceranae*. Differences in the bioactivity of these porphyrins were determined *in vivo* by measuring the number of spores developed in living honeybees during a cage test experiment as well as *in vitro* by counting spores incubated with porphyrin in a 0.5% sucrose solution. We found that both porphyrins (at a 100 μM concentration), administered to the honeybees in a sucrose syrup supplement, substantially reduced spore numbers (from 1.45 to 10.8 fold) in infected honeybees compared to control honeybees, which had not been treated with the porphyrins. Confocal microscopic images of the midguts of porphyrin-treated and untreated *Nosema*-infected honeybees showed distinct differences in the number of spores and their tendency to aggregate. Importantly, the introduction of porphyrins into the honeybee feeding regimen did not affect the mortality of uninfected insects. Among *Nosema*-infected honeybees, bees treated with $PP(Lys)_2$ and $PP(Glu)_2$ porphyrins had a longer lifespan compared to control bees. When tested *in vitro*, both porphyrins had a direct impact on the microsporidia beyond the mechanism of photosensitization. The average reduction in spore counts after 8- and 24-h incubation of *N. ceranae* microsporidia with porphyrin in the dark was 25 and 55%, respectively. Moreover, light microscopic images revealed morphological

changes and higher amounts of cellular debris in porphyrin-treated microsporidia compared to untreated spores. The efficiency of porphyrins in combating *Nosema* pathogens depends on several factors such as duration of porphyrin administration to honeybees and the kind of amino acid moieties conjugated to the protoporphyrin IX molecule.
This work was financially supported by the National Science Centre, Poland (2015/17/B/NZ9/03607).

P100 VarroMed - field trial data and lessons learned from the first centrally approved next-generation veterinary medicinal product against *Varroa*

Hintersteiner M., Hohl D.
BeeVital, Obertrum, Austria

Despite a plethora of research of the past decades *Varroa* is still the number one beekeeping problem worldwide. While the first generation of synthetic anti-Varroa products, such as Cumaphos, Flumethrin, and increasingly also Amitraz show clear signs of resistance, the more ecocompatible organic acids remain a preferred choice for many beekeepers.
In February 2017 the European Commission authorized the next generation anti-Varroa Product VarroMed. VarroMed thereby becoming the first veterinary medicine for honey bees to have passed through the Pan-European registration process.
VarroMed is a new fixed combination product based on the two organic acids Formic acid and Oxalic acid. Historically, products based on one of these acids, were either temperature and weather (moisture) dependent (Formic acid) or limited to single applications in broodless periods due to brood toxicity (oxalic acid). With the fixed combination of both acids, formulated in a matrix of propolis and and other excipients, VarroMed overcomes this limitation and hence has been approved by the authorities for both, broodless conditions and repetitive treatments in presence of brood after harvest and in spring. Furthermore, the specific formulation of VarroMed was shown to increase the duration of effect and reduce bee toxicity.
The presentation/poster will give a summary of the latest scientific results obtained from the controlled field trials with VarroMed at different study sites. Furthermore, aspects such as correlation of dose-response-efficacy parameters across hives with different population sizes will be shown. Finally, the lessons learned from the trial set-up and the utility of some of the established methods of quantification of efficacy will be critically discussed using VarroMed´s registration studies as show-case.

P101 First report of the booklice *Liposcelis* spp (order: psocoptera) associated with honey bee hive

El-Niweiri M.A.A.[1,2], Ghramh H.A.[1]
[1] Department of Biology, King Khalid University, Abha, Asir Region, Saudi Arabia; [2] Department of Bee Research, National Centre for Research (NCR), Khartoum ,Sudan

Several types of insects may inhabit bee hive and cause considerable damage to honey bee's colonies. Some of these insects are described as harmless, but their presence can be cause for concern in some cases. The importance of *Liposcelis* has grown in recent years due to greater recognition of their presence in stored products and the lack of control options Here we report for the first time the encountering of the booklice *Liposcelis* spp (Order: Psocoptera) inside bee hive. A number of about 300 traditional bee hives were inspected for the presence of Liposcelis in Asir region south of Saudi Arabia. 70 percentages of the inspected hives were found to be infested with Liposcelis spp. Although the beekeepers have great concern about this high infestation, we did not found any evidence indicating noteworthy quantitative losses associated with their presence in honey bee hives. However their mere presence in honey bees products my reduce their quality and marketing value since psocids are known to be potential of transmitting fungi and bacteria. Moreover one of *Liposcelis* spp was recently shown to harbor *Rickettsia felis*, the causative agent of flea-borne spotted fever (rickettsiosis) in humans. Our findings suggest the necessity of eliminating *Liposcelis* from bee hive to insure high quality and healthy honey bee products.

P102 Antimicrobial activity of *Bifidobacterium* spp. isolated from *Apis mellifera jemenitica* against drug multi-resistant human pathogens

Ansari M.J.[1,2], Al-Ghamdi A.[1], Vaish A.[2], Kumar P.[2]
[1] *Engineer Abdullah Baqshan for Bee Research, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, KSA;* [2] *Department of Botany, Hindu College Moradabad, Uttar Pradesh, India*

Bifidobacteria were isolated from the intestinal tract of the Saudi Arabian honeybee race, *Apis mellifera jemenitica*, and investigated for potential application as a probiotic agent against *Paenibacillus larvae*, the causal agent of American foulbrood (AFB), based on the findings of in vitro inhibition assays. A total of 5 bifidobacteria strains (designated as KSUB-1-KSUB5) were isolated using a culture-dependent method and their 16S rRNA gene sequences were analyzed. The KSUB isolates belonged to two distinct bifidobacterial phylotypes that were similar to those found in the Japanese honeybee *Apis cerana japonica*. Although the Saudi Arabian and Japanese honeybees are distinct species with different traits and habits, the observation that they share highly similar bifidobacterial phylotypes suggests that bifidobacteria are conserved among honeybee species. Despite having extremely high 16S rRNA gene sequence similarities, the KSUB isolates had markedly different carbohydrate fermentation profiles. In addition, in vitro growth inhibition assays revealed that the cell-free supernatants of all KSUB isolates exhibited antagonistic effects on *P. larvae* growth. These results indicate that the bifidobacteria isolated from the gut of Saudi Arabian honeybee could potentially be employed as a new biological agent to control AFB.

P103 *Lactobacillus plantarum* from the gut of indigenous honeybee of Saudi Arabia inhibit the growth and biofilm formation of *Candida albicans*

Ansari M.J.[1,2], Al-Ghamdi A.[1], Vaish A.[2], Kumar P.[2]
[1] *Engineer Abdullah Baqshan for Bee Research, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, KSA;* [2] *Department of Botany, Hindu College Moradabad, Uttar Pradesh, India*

Introduction: Lactic acid bacteria (LAB) are also considered as probiotic microorganisms, which inhibit pathogenic and spoilage bacteria. Strain of *Lactobacillus plantarum* was isolated from the intestinal tract of the indigenous honeybee of Saudi Arabia, *Apis mellifera jemenitica*, and investigated for its in vitro inhibitory activity against pre-formed biofilm and its interference with the biofilm formation of *C. albicans*.
Methods: The XTT reduction assay and scanning electron microscopy (SEM) were employed to determine the inhibitory effect of *Lactobacillus plantarum* on *C. albicans* biofilm. Changes in the infrared spectrum after treatment with *Lactobacillus plantarum* were also determined by Fourier transform infrared (FTIR) spectroscopy.
Results: *Lactobacillus plantarum* affects biofilms by decreasing the size of mature biofilms and by disruption of their structure. The SEM results indicated that this bacteria affected the cellular morphology of *C. albicans* and decreased biofilm thickness.
Conclusions: The present findings show that *Lactobacillus plantarum* from the gut of indigenous Saudi Arabian honeybee has antifungal properties against *C. albicans* and has the ability to inhibit the formation of *C. albicans* biofilms and disrupt established biofilms.

P104 Antibacterial properties of Saudi Arabian Sidr honey against Paenibacillus larvae, the causal organism of American foulbrood

Ansari M.J.[1,2], Al-Ghamdi A.[1]
[1] Engineer Abdullah Baqshan for Bee Research, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, KSA; [2] Department of Botany, Hindu College Moradabad, Uttar Pradesh, India

Objective: Honey is known as an antibacterial agent and has been employed for therapeutic uses, especially against multi drug resistant human pathogens. On this basis, the possibility to verify a honey role in the prevention of the AFB diseases was considered. The aim of this study was to evaluate the antibacterial activity of Saudi Arabian sidr honey (*Ziziphus spina-cristi*) against (*Paenibacillus larvae* bacteria, causative agent of American foulbrood - AFB), isolated from the bee hive. During 2012, 20 sidr honey samples from different geographical locations were collected and Manuka honey from New Zealand used as reference for antibacterial activity. Method: The agar well diffusion assay was performed on honey samples in order to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Results & Discussion: The results show that all honeys show moderate antibacterial effect against honeybee pathogenic bacteria, *Paenibacillus larvae*. This was the first study of Saudi Arabian Sidr honey against *Paenibacillus larvae* bacteria.

P105 The action of honeybee venom on drug multi-resistant human pathogens

Ansari M.J.[1,2], Al-Ghamdi A.[1]
[1] Engineer Abdullah Baqshan for Bee Research, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, KSA; [2] Department of Botany, Hindu College Moradabad, Uttar Pradesh, India

Objective: This study was undertaken to evaluate the antimicrobial activity on drug multi-resistant Gram-negative and Gram-positive bacteria of the venom from *Apis mellifera jemenitica* from Saudi Arabia. Method: This activity was investigated against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus vulgaris*. Inhibitory halos values and reduction of colony-forming unites (CFU) by dilution plate technique were determined. Results: The bactericidal activity was higher for *S. aureus* and *E. coli*, in comparison to that for *P. aeruginosa*. *P. vulgaris* was the most resistant organism tested to the action of venom solutions. Conclusion: The demonstrated bactericidal property of *A.m.jemenitica* venom highlight it as a promising candidate for further studies according to detect which component, or components, are responsible of this activity, in order to then investigate their potential use as antimicrobial agent.

P106 Bacterial strains of the *Lactobacillus* and *Fructobacillus* genera isolated from the gastrointestinal tract of honeybees for the use in the control and prevention of bee diseases and for probiotic preparations based on such bacterial strains (Patent application No. P.423363)

Ptaszyńska A.A.[1], Pachla A.[2], Wicha M.[2], Grzęda M.[2], Małek W.[3]
[1] Department of Botany and Mycology, Maria Curie-Skłodowska University, Lublin, Poland; [2] Biowet Puławy Sp. z o.o., Puławy, Poland; [3] Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Lublin, Poland

Lactic acid bacteria (LAB) play a crucial role as intestinal microflora in the nutrient assimilation, the modulation of immune response as well as in the mitigation and prevention of diverse intestinal disorders. The antimicrobial potential of these bacteria comprise, inter alia, the production of lactic acids, acetic acid, short-chain-volatile-fatty acids, H2O2, and bacteriocins-like molecules. Fructofilic LAB (FLAB) are a special group of lactic acid bacteria, which, under anaerobic conditions, prefer D-fructose as carbon and energy source and exhibit very weak growth on the glucose. FLAB microbiota of honey bees may play an important role in the health of these insects by inhibiting pathogens and promoting the

digestion of carbohydrates. Honeybee populations still decline worldwide, mainly due to the presence of various pathogens (*Paenibacillus larvae*, *Nosema apis*, *N. ceranae*, *Melissococcus plutonius*), pesticides, industrial agriculture, and climate change. The aim of this work was to isolate and phenotypically as well as genomically characterize and identify LAB associated with honeybees' intestinal track.
The bacteria were isolated from intestines of healthy honeybees (*Apis mellifera*). Biochemically, all isolated lactic acid bacteria showed typical fructophilic features and grew well on fructose under anaerobic conditions, but poorly on glucose. A good growth on glucose was noted in the presence of oxygen and in the presence of fructose as an electron acceptor. Residents of the honeybee gut were classified as heterofermentative lactic acid bacteria. From glucose, they produced almost equimolar amounts of lactic acid and acetic acid, and trace amounts of ethanol. Based on 16S rDNA and recA gene sequence analyses, twelve lactic acid bacteria were classified as Lactobacillus kunkeei and two as Fructobacillus fructosus. Isolated FLAB bacteria inhibited the growth of major honeybee pathogen, Paenibacillus larvae, meaning that investigated FLAB may show health-conferring properties of probiotics. Therefore, one strategy to reduce the disappearance of honeybee populations may involve using probiotic lactic acid bacteria so as to prevent infections in honeybees.

P107 European foulbrood disease: host-pathogen interactions and the impact of secondary invaders

Lewkowski O., Erler S.
Martin-Luther-University Halle-Wittenberg, Institute of Biology, Molecular Ecology, Halle (Saale), Germany

The causing agent of European foulbrood, *Melissococcus plutonius*, is a widespread pathogen of honey bees and locally leading to significant colony losses. The brood is mainly infected in early larval stages and usually dies before pupation. Other Gram-positive bacteria have been identified as characteristic secondary invaders co-occurring in diseased larvae but their role in the course of the disease is poorly understood.
Using *in vitro* larval infection experiments, we studied the interaction of different host and pathogen genotypes and the impact of combined *M. plutonius* and secondary invader (*Enterococcus faecalis* or *Paenibacillus alvei*) infections.
We found a host type independent high virulence of *M. plutonius* strain 49.3, with a distinct course of disease in hosts with different genetic background. A secondary infection did not affect larval survival or weight.
Our results suggest that although the virulence of *M. plutonius* is mainly depending on pathogen genotype, the host genetic background is contributing to disease dynamics. Secondary invaders following *M. plutonius* infection do not increase larval mortality and therefore may be a colonisation of immunocompromised or dead hosts.

POSTERS

P108 Effects of Deformed wing virus (DWV) on honey bee

Mookhploy W.[1,2], Chantawannakul P.[1,3]
[1] Department of Biology, Chiang Mai University, Chiang Mai, Thailand; [2] Graduate School, Chiang Mai University, Chiang Mai, Thailand; [3] International College of Digital Innovation Chiang Mai University, Chiang Mai, Thailand

Honey bees are the most important commercial pollinators. Recently, they are considered to be threatened globally by several pathogens. Deformed wing virus (DWV), Sacbrood virus (SBV) and Black queen cell virus (BQCV) have been reported in European honey bee (*Apis mellifera*) in many countries around the world. The virus infections lead to the death of honey bee. In European honey bee colonies in Thailand, DWV infection could be detected more frequently than SBV and BQCV. To understand pathological effects of DWV, DWV was directly injected to the white-eye pupae stage of honey bee (*A. mellifera*) and monitored viral loads and specific gene expressions by using real-time PCR technique. It was found that honey bee pupae which were injected by the highest concentration of viral loads (~107 copies number of DWV) showed higher mortality rates and abnormalities compared with control groups. Also, gene expression levels of immune genes were different when compared to that of the control groups.

P109 Sulforaphane is also present in bee pollen

Ares A.M.[1], Valverde S.[1], Bernal J.L.[1], Martín-Hernández R.[2], Nozal M.J.[1], Higes M.[2], Bernal J.[1]
[1] I.U. CINQUIMA, Grupo de Química Analítica (TESEA), Universidad de Valladolid, Valladolid, Spain; [2] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Spain

Nowadays, a large body of research on functional foods, especially in anticarcinogens, has focused on a single bioactive isothiocyanate, sulforaphane (SFN); this is of interest on account of its potential role not only in the prevention of cancer, but also in that of chronic and degenerative diseases such as diabetes, atherosclerosis and cardiovascular disorders. The present study investigates the possibility of SFN existing in one of the most widely consumed food supplements, bee pollen. This matrix was selected because SFN has been previously detected in honey, and because nowadays natural products from insects, especially bees, are receiving attention in the food industry. Consequently, it may be surmised that SFN are also present in other bee products, such as bee pollen. An analysis of SFN could be of significant interest for verifying its presence as since, to the best of our knowledge, it is the first research devoted to analyze the potential presence of this compound in bee pollen. In addition, it might help to determine the existence of SFN in products to be consumed by humans, who would be positively affected by their beneficial health properties. It will therefore be necessary to develop analytical methodologies to assess its potential presence in this bee product. In this study, a new liquid chromatography-tandem mass spectrometry method has been developed for the determination of potential residues of SFN in bee pollen from local markets and from organic apiaries. Several samples of bee pollen were analyzed, and SFN was detected in most of the bee pollen samples at low concentration levels. The presence of SFN in bee pollen is an interesting finding, and it could increase the nutritional and bioactive value of this food supplement.
Acknowledgements: Authors gratefully acknowledge the INIA-FEDER founding (National public research project; RTA2015-00013-C03).

P110 *Paenibacillus larvae*: genetic diversity and susceptibility to European and Asian honey bees and antagonistic activity of microflora in bee hives on American foulbrood pathogen

Krongdang S.[1,2], Evans J.D.[3], Chen Y.[3], Pettis J.S.[3], Mookhploy W.[1], Chantawannakul P.[1,4]
[1] Bee Protection Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; [2] Interdisciplinary Program in Biotechnology, Graduate School, Chiang Mai University, Chiang Mai, Thailand; [3] USDA-ARS, Bee Research Laboratory, Beltsville, Maryland, USA; [4] Environmental Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Paenibacillus larvae is a Gram positive bacteria cause of the virulent larvae disease in honey bee; American foulbrood (AFB). In our studies, a total of thirty three independent *Paenibacillus larvae* isolate from various geographical origins in North America and five reference strains were investigated for genetic diversity using multilocus sequence typing (MLST) compared with ERIC and BOX DNA fingerprinting. The results showed that almost all represented the ERIC I strain and extensive resistance to tetracycline and the first records of resistance to tylosin. Our data highlight the intraspecies relationships of *P. larvae* and the potential application of MLST methods in enhancing our understanding of epidemiological relationships among bacterial isolates of different origins. Moreover, to understand *P. larvae* infectivity and pathogenicity between European (*Apis mellifera*) and Asian (*Apis cerana*) honey bees, susceptibility, larval mortality, survival rate and expression of antimicrobial peptide genes (AMPs) in *A. mellifera* and *A. cerana* when infected with *P. larvae* were investigated. Our results showed all AMPs of infected bee larvae showed significant upregulation compared with non-infected bee larvae in both honey bee species. However, larvae of *A. cerana* were more susceptible than *A. mellifera* when the same larval ages and spore concentration of *P. larvae* were tested. Furthermore, we determined the potential of bacteria isolated from hives of Asian honey bees (*Apis cerana*) to act antagonistically against *P. larvae*. Isolates were sampled from different locations on the fronts of *A. cerana* hives in Vietnam. A total of 69 isolates were obtained through a culture-dependent method and 16S rRNA gene sequencing showed affiliation to the phyla Firmicutes and

Actinobacteria. Out of 69 isolates, 15 showed strong inhibitory activity against *P. larvae*; *Bacillus pocheonensis* (VN101) showed the largest zone of inhibition (26 ±1 mm). In this study, the diversity and richness of antagonistic isolates indicated that *Bacillus* spp. are the most promising as inhibitors of *P. larvae*. These finding suggested that certain bacterial isolates can act as antagonist to control *P. larvae* and may have other biotechnological applications.

P111 Healthy bee: monitoring of Belgian honey bee health (2016-2017)

Matthijs S.[1], De Waele V.[2], Vandenberge V.[1], Brunain M.[3], Verhoeven B.[4], Evers J.[4], De Winter P.[4], Hooyberghs J.[4], de Graaf D.C.[3], Roels S.[1]
[1] National Reference Lab on Bee Diseases, Infectious diseases in animals, Sciensano, Brussels, Belgium; [2] Veterinary epidemiology, Sciensano, Brussels, Belgium; [3] Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium; [4] Federal Agency for the Safety of the Food Chain, Brussels, Belgium

The Belgian Federal Agency for the Safety of the Food Chain (FASFC) started the Healthy Bee project in 2016 as a surveillance program for honey bee health, in co-operation with the Laboratory of Molecular Entomology and Bee Pathology (Ghent University) and the Belgian National Reference Lab on Bee Diseases (Sciensano). A total of 193 beekeepers (mainly hobbyists) and 865 colonies distributed all over Belgium were selected for this project to assess the vitality of the colonies, the number of Varroa mites and the winter and summer mortality. The beekeepers filled in a questionnaire about their colonies and the treatments applied.

Half of the selected honey bee colonies showed a "normal" vitality in autumn, while 39% of the colonies seemed stronger than average and only 11% seemed weak. During the visits in autumn, *Varroa* mites were found in 87% of the colonies (on average 2.97 mites per 100 bees) even though most had already received a treatment against *Varroa* (mostly based on thymol, oxalic acid or formic acid). The average winter mortality in 2016-2017 was 27,9%, much higher than the 10% winter mortality that is considered normal at the European level. The average summer mortality in 2017 was 3,72%. A significant relationship was found between the number of *Varroa* mites and honey bee mortality: the higher the *Varroa* mite infestation in autumn, the higher the chance that the colony would not survive winter. While more than 70% of the colonies were infected with *Nosema* sp. in spring, no significant correlation between Nosema infection and mortality was found.
The FASFC will continue the monitoring of honey bee health in the coming years.

P112 Nosemosis in Estonian and Latvian apiaries: difference between countries, persistence over years and species distribution

Naudi S.[1], Jürison M.[1], Tummeleht L.[2], Karise R.[1]
[1] Plant health department, [2] Veterinary Institute, Estonian University of Life, Tartu, Estonia

In the last decades, beekeepers have observed increased colony mortality of honeybees (*A. mellifera* L.). One of the reasons for bee decline is believed to be the proliferation of diseases in bee colonies. Previously it was considered that honeybees can only be infected by *Nosema apis* (Zander), however, studies made in Europe since 1996 show that there are two species of the microsporidians causing the disease – *N. apis* and *N. ceranae* (Fries et al). The symptoms of infection by these two pathogens are very different, as are the virulence, spread, and pathogenicity, which is why it is important to know the species distribution.
Until the beginning of the 2000's, *N. ceranae* was originally considered to be restricted to *A. ceranae*, which is distributed mainly in Asia – but several studies show that, *N. ceranae* is nowadays a parasite of *A. mellifera* across much of the world, however, it seems to be prevailing in regions with a warmer climate.
As a result of acute *N. apis* infection, the lifespan of infected bees is reduced, diarrhea may occur in the bees. *N. ceranae* has no clinical signs. It has been thought to be a factor in the increased mortality of colonies detected across the year.

Since bees can pick up the spores even from flowers where they forage, the problem seems to be persistent in infected regions.
Five years ago, the EPILOBEE (2012-2014) study was conducted to map the spread of bee diseases in European member states. Then clinical symptoms of nosemosis were detected only in spring in Estonia, whereas from Latvia no clinical symptoms were sampled. However, no species determination of *Nosema* was done. Nosemosis is not allowed to be treated with medical preparations in Europe, instead, hygienic beekeeping practice is used to fight the disease out. Now the aim of present study is to find out, which *Nosema* species infects honey bees in Estonia and Latvia, and is the disease still present in formerly positive apiaries. The results of resampling of former positive apiaries, the species distribution and spore quantities in Estonian and Latvian apiaries will be discussed.

P113 Ectoparasitic mites *Varroa underwoodi* in Eastern and Western honey bees

Wang S.[1], Lin Z.[1], Dietemann V.[2], Neumann P.[2,3], Hu F.[1], Zheng H.[1]
[1] College of Animal Sciences, Zhejiang University, Hangzhou, P.R. China; [2] Swiss Bee Research Center, Agroscope, Bern, Switzerland; [3] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

The ectoparasitic mite genus *Varroa* is known for the key role played by *Varroa destructor* in *Apis mellifera* colony losses throughout the world. *V. destructor* was originally confined to the Eastern honey bee *Apis cerana* and switched hosts to *A. mellifera*. All other species of the genus are poorly studied mainly because they have not caused damage and are, with very few exceptions, still confined to their original hosts and native distribution areas. Here, we focused on *Varroa underwoodi*, because this mite shows a broad host range including *A. cerana*, *Apis nuluensis* and *Apis nigrocincta*. Together with its detection in Western honey bee colonies in Papua New Guinea, this indicates a high potential for host shifts and threat to *A. mellifera* by this mite. Screening 43'400 brood cells of 101 *A. cerana* and 28 *A. mellifera* colonies for infestations in several regions of China, we collected data on the occurrence, prevalence, morphology, reproductive abilities and phylogenetics of *V. underwoodi*. *V. underwoodi* was not found infesting *A. mellifera* colonies and only reproduced in *A. cerana* drone brood. Genetic population structuring was detected, but was not associated with differences in morphology or reproductive ability. Comparative studies on the so far neglected *Varroa* spp. not only provide new insights into host-parasite interactions in the *Apis* spp. – *Varroa* spp. system, but also inform on the possibilities for novel host shifts, thereby enabling a risk assessment for *A. mellifera* populations and global beekeeping.

P114 Spatio-temporal variation of honeybee pathogens prevalence in wild bees in semiarid areas

Martínez-López V.[1,2], Ruíz C.[1,3], Muñoz I.[1], Ornosa C.[4], Higes M.[5], Martín-Hernández R.[5], De la Rúa P.[1]
[1] Departamento de Zoología y Antropología Física, [2] Departmento de Ecología e Hidrología, Murcia, Spain;
[3] Departamento de Biología Animal y Edafología y Geología, Universidad de la Laguna, San Cristóbal de la Laguna, Spain; [4] Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense, Madrid, Spain; [5] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental, Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (CIAPAIRIAF), JCCM, Marchamalo, Guadalajara, Spain

Interspecific transmission of pathogens from managed to wild pollinators is one of the main sources of Emerging Infectious Diseases (EIDs) and stands among the different factors related to the worldwide decrease of pollinator communities. However, most research has been focused on particular species (mainly honeybees and bumblebees), and there is a lack of studies at the community level. To overcome this, we present spatio-temporal analyses of pollinators density (honeybees and wild bees) and pathogen prevalence in a protected area of the Southeast of the Iberian Peninsula (Parque Regional de Sierra Espuña) during late winter and spring 2017. Nine sampled sites of 100x100 metres were chosen according to a gradient of beekeeping intensity to determine the spread of pathogens (*Nosema apis* (Zander, 1909) and *Nosema ceranae* (Fries, Feng, Silva, Slemenda & Pieniazek, 1996)) from managed honeybees

(*Apis mellifera Linnaeus*, 1758) on the wild bee community. Individuals were captured through active sampling (entomological nets) while bees were foraging.
Density of honeybees remarkably decreased along the study period (it was six time higher at the beginning of the study), whereas wild bee density slightly increased at the beginning of spring. Interspecific transmission of pathogens from managed honeybees to more than 15 genera of wild bees was detected. *N. ceranae* was by far the most common pathogen while *N. apis* occurrence was residual and only detected in two individuals (one *A. mellifera* and one *Eucera* sp.). The prevalence of *Nosema* spp. in *A. mellifera* decreased along the study period in parallel to the reduction of honeybee density. However, the prevalence of pathogens in the wild bee community increased in spring at the end of the sampling. Preliminary results did not show any spatial relationship between honeybee densities and the prevalence of pathogens in wild bee communities.
In conclusion, our results highlight the impact of managed honeybees on the wild bee community by promoting pathogen spill-over in the ecosystem. Furthermore, such fact seems to remain and even increase when the main host species (i.e. honeybees) decrease. Our findings can help to design conservation strategies to protect the health status of local pollinators.

P115 Cement honey - effects of the trisaccharide melezitose on honey bees

Seeburger V.C.[1,2], Shaaban B.[3], Schroeder A.[1], Lohaus G.[3], Hasselmann M.[2]
[1] Apicultural State Institute, [2] University of Hohenheim, [2] Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany; [3] Molecular Plant Research/ Plant Biochemistry, University of Wuppertal, Wuppertal, Germany

Cement honey is a major issue for beekeepers, representing a special kind of honeydew honey with high levels of the trisaccharide melezitose. Honeydew honey is the result of insects (Hemiptera) sucking phloem sap of trees and excreting the sugars in honeydew droplets. Subsequently honey bees take up the honeydew and process it into honeydew honey. Honey with high amount of melezitose crystallizes and obstructs the combs, leading to an economical loss. Precise analyses of the conditions under which melezitose occurs have not been realized, although they could be an important tool to avoid cement honey. Furthermore, it is not known which impacts the trisaccharide has on the digestion and health of honey bees.
In order to determine the influence of environmental variables for the emergence of melezitose, honeydew droplets were analyzed. To obtain the impact of melezitose on honey bee health, additional feeding experiments and comprehensive 16S rRNA sequencing of the microbiota have been realized.
We found remarkable differences in the amount of melezitose between the honeydew of different honeydew producer and host tree species, their geographical provenance and weather conditions. Feeding experiments with melezitose displayed increased food uptake and higher mortality. Results of the sugar spectrum in the honey vesicles show that honey bees digest melezitose into smaller-molecule sugars. Gut microbial community analyses evidence that the composition of intestinal bacteria changes when bees are fed with melezitose.
Our study provides that the amount of melezitose in honeydew is influenced differently by multiple factors. Additionally, feeding experiments have shown the high effort of honey bee's degradation process of the large-molecule melezitose and higher intake of food amount, which could explain intestinal diseases and higher mortality. The comprehensive analyses of honey bee microbiota can explain the impact of melezitose on honey bee's digestion and health.

P116 Oxybee® (containing oxalic acid) in the treatment of varroosis in honey bees under field conditions in Germany

Braun G.[2], Lohr B.[2], Dany N.[1], Schneider C.[2], Marsky U.[3], Hellmann K.[2]
[1] Dany Bienenwohl GmbH, Munich, Germany; [2] Klifovet AG, Munich, Germany; [3] Vetopharma, Villebon-sur-Yvette, France

A clinical field study in honey bees naturally infested with *Varroa destructor* was conducted to evaluate the efficacy and safety of the product Oxybee® in Germany from November 2012 to April 2013. Oxybee® is a veterinary medicinal product containing oxalic acid, for trickling application to control varroosis in honey bees. A total of 45 colonies were enrolled at 2 study sites, one in Southern and one in Northern Germany.
Safety evaluation was based on: Bee mortality, colony and queen survival until the following spring, colony strength in the following spring, and area of open/sealed/drone brood in the following spring.
The results showed that Oxybee® was highly efficacious and safe in the treatment of Varroosis in honey bees caused by Varroa destructor under field conditions in Germany.
Oxybee® is one of the first Varroa medicine for honey bees to receive a positive opinion regarding a centralizedauthorization in Europe. It is distributed since starting starting of 2018 by Véto-pharma, the French pharmaceutical company 100% dedicated to honey bee health, and manufacturer of Apivar.

P117 A study of local adaptation in the Iberian honeybee (*Apis mellifera iberiensis*) using a reciprocal translocation experiment

Lopes A.R.[1], Neves C.[1], Ventura P.[2], Vilas-Boas M.[1], Rodrigues P.J.[3], Garnery L.[4], Biron D.G.[5], Pinto M.A.[1]
[1] Mountain Research Centre, Polytechnic Institute of Bragança, Bragança, Portugal; [2] Apis Ventura S.U. Lda, Bragança, Portugal; [3] Polytechnic Institute of Bragança, Bragança, Portugal; [4] Laboratoire Evolution, Génomes et Spéciation, CNRS, Gif-sur-Yvette, France; [5] Laboratoire Microorganismes, Génome et Environnement, Université Clermont-Auvergne, Aubière Cedex, France

In Europe, several translocation experiments suggested that native populations of *Apis mellifera* are adapted to local climate and flora. However, so far, no study has been conducted on the Iberian honeybee, *Apis mellifera iberiensis*. The goal of this study was to assess the existence of genotype-environment interaction (GEI), and consequently local adaptation, in the Iberian honeybee. In 2015 two apiaries were set up, each one with 36 colonies (18 of the origin Bragança and 18 of the origin Vila do Bispo), in two latitudinal extremes of Portugal: Bragança (north) and Vila do Bispo (south). Several traits of the 36 colonies were measured for almost 2 years, including: number of brood and pollen cells, honey yield, survival, and Varroa destructor infestation. The analyses were performed using t-Student and Mann-Whitney tests to compare those traits between the two origins in the same apiary and the same origin between the two apiaries. The survival analysis was performed using the Cox proportional hazard model in R. Colonies of the southern origin Vila do Bispo showed a tendency to collect more pollen and consequently they produced a higher number of brood cells, had a higher varroa infestation level and a lower survival rate than colonies of the origin Bragança in both locations. Honey yield was the only trait that showed existence of GEI, and therefore local adaptation, since the local honeybees had a higher honey production in their apiary of origin. Additionally, the differences between the two origins were sharper in more favourable environments where the honeybees can better express their genetic potential. Our findings highlight the importance of protecting local honeybee diversity in a period of increasing selection pressures such as climate change, agricultural land overuse and novel pathogens and parasites.
This research was funded through the 2013-2014 BiodivERsA/FACCE-JPI Joint call for research proposals, with the national funders FCT (Portugal), CNRS (France), and MEC (Spain).

P118 Introgressive hybridization and latitudinal admixture clines in honeybees in East-Central Europe

Oleksa A.[1], Abonyi T.[1,2], Chybicki I.[1], Kusza S.[2], Meyza K.[1], Nawrocka A.[3], Sztupecka E.[1], Tofilski A.[3], Ulaszewsk B.[1]
[1] Department of Genetics, Institute of Experimental Bioogy, Kazimierz Wielki University, Bydgoszcz, Poland; [2] Institute of Animal Husbandry, Biotechnology and Nature Conservation, University of Debrecen, Hungary; [3] Department of Pomology and Apiculture, Agricultural University, Kraków, Poland

One of the threats to the sustainable use of honey bees is the loss of local genetic variability as a result of the mixing of gene pools originating from different evolutionary lineages. In Central Europe, this results mainly from the introduction of *A. m. carnica* (AMC) into the natural range of *A. m. mellifera* (AMM). In our study, we aimed to recognize the level of introgression of AMC genes into the AMM gene pool in the geographical gradient from southern Hungary to northern Poland. One worker was collected from each the sites arranged along two transects with a length of approximately 900 km each (17.5 °E and 23 °E, N = 171 and N = 223). The bees were genotyped using 13 SSR loci, and for each individual, the probability of assignment to AMM was estimated using the STRUCTURE method. In addition, the probability of assignment to AMM was estimated based on the analysis of the geometric morphometrics of wing venation. Generalized linear model (GLMs) and generalized additive models (GAMs) were used for modelling of the relationship between dependent and environmental variables to find the most parsimonious set of variables that explained the observed pattern of admixture. As explanatory variables, we included sample coordinates, altitude, climate (BIOCLIM data) and intensity of land use. No combination of independent variables described the observed admixture better than latitude alone. The best-preserved gene pool of AMM subspecies was found north of 54 °N, where the average proportion of nuclear genes from AMM accounted for about 70%, and individuals that can be classified as pure AMM accounted for approximately 25% of all studied bees. To the south of 52 °N, no pure AMMs were found, while there were many hybrids between subspecies. We observed no significant differences between transects. The obtained results may be important for the development of an optimal strategy for the protection of AMM in Poland, including the indication of areas for conservative breeding.

P119 A comparative study of colony performance, hygienic behaviour and parasite and disease infection in the endemic honeybee *A. m. ruttneri* and the introduced *A. m. ligustica* in Malta

Meixner M.D.[1], Mifsud D.[2], Zammit-Mangion M.[3], Cutajar S.[4], Galea T.[5], Uzunov A.[1,6]
[1] Landesbetrieb Landwirtschaft Hessen - Bieneninstitut Kirchhain, Kirchhain, Germany; [2] University of Malta, Rural Sciences and Food Systems, Institute of Earth Systems, Msida, Malta; [3] University of Malta, Department of Biochemistry and Physiology, Msida, Malta; [4] Greenhouse Malta, Marsaskala, Malta; [5] Breeds of Origin, Zebbug, Malta; [6] Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, Macedonia

Apis mellifera ruttneri, the honey bee subspecies endemic to Malta, must be regarded as seriously endangered. However, there is a critical need for scientific data to support and guide conservation measures, since only two scientific papers concerning this subspecies were published since its original description in 1997.
To this end in June 2017, a first systematic study was initiated to compare colony development, performance, hygienic behaviour and infection levels of honey bee diseases of the endemic honey bee with introduced colonies of *A. m. ligustica*. A total of 33 colonies (*A. m. ruttneri*, n=15 and *A. m. ligustica*, n=18, headed by sister queens) were evenly distributed across two locations on Malta, at a central site UNI (n=17) and a site in the Southern region SIGG (n=16). After an initial treatment against Varroa destructor, no further chemical treatment was performed.
Standard methods are used to assess colony productivity and behaviour (number of adult bees, number of brood cells, number of visible cells with pollen) in regular intervals. Hygienic behaviour is assessed using the pin test method; *Varroa* infestation is monitored using powdered-sugar and natural mite fall methods. Assessment of infection levels with *Nosema* spp. and the most common honey bee viruses is also being carried out. The selected commercial stocks of *A. m. ligustica* remain consistently less defensive and calmer on the combs. However, by spring 2018, the *A. m. ruttneri* colonies in general showed higher numbers of adult bees, brood cells and pollen cells. Early seasonal drone production and significant swarming behaviour were observed in the colonies of the endemic bee, but not in *A. m. ligustica* colonies.

The baseline data on the performance of native and introduced genotypes under Maltese environmental conditions provided by this study will contribute to guiding beekeepers in their decision on queen purchases, and ultimately, support conservation measures for *A. m. ruttneri*.

P120 Using exuviae as a non-destructive sampling method for population genetic analysis of bees

Friedel A.[1], Soro A.[1], Quezada-Euan J.J.G.[2], Paxton R.J.[1]
[1] General Zoology, Martin Luther University Halle-Wittenberg, Germany; [2] Departamento de Apicultura, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida Yucatán, Mexico

Obtaining DNA for genetic analysis often requires sampling methods that are harmful or lethal to the organism. Collection of whole bodies, which is often done in insect studies, may assure high quantity nucleic acid for genetic studies, but it also limits further investigation of the organism, which is especially damaging for species that occur in low numbers. In some cases, the trade-off between collecting genetic material and further investigation of the living animal can be solved by using small body parts, such as the tip of an antenna or tarsal claws as a non-lethal sampling method. These methods do not require destruction of the entire organism, but might however influence its behaviour or survival. We tested the non-destructive approach of using bee exuviae for DNA extraction to avoid difficulties associated with these other, more destructive methods of sampling using females of the Neotropical orchid bee *Euglossa viridissima*. Females built their nests solitarily in small artificial wooden boxes and, after brood emergence, nests can become social, when one or more of the daughters of the foundress stays to help the foundress with further brood cell production. To be able to genotype social nestmates whilst also observing them, we retrieved their exuviae from their brood cells, from which DNA was extracted using two different protocols 1) using Chelex and 2) using a high concentration salt solution. PCR with primers for mt CO1 and subsequent agarose gel electrophoresis of PCR products confirmed successful DNA extraction. DNA extracts of all exuviae sampled amplified at the CO1 locus. Four orchid bee-specific microsatellite primers were additional used to rule out the possibility that the extracted DNA originated from different organisms or was otherwise cross-contaminated with that of other organisms. Tested samples revealed PCR fragments that would be expected for orchid bees. We now test extracts of exuviae with respective adult bees for consistency of genotypes. Nests of *E. viridissima* are usually difficult to obtain and are sensitive to disturbance. Thus, this non-destructive method might facilitate investigation of the population genetics and relatedness structure of orchid bees whilst still permitting detailed behavioural observation of social nestmates.

P121 Genetic characterization of the Italian *Vespa velutina nigrithorax* (*du Buysson*) population

Granato A.[1], Negrisolo E.[2], Bonomi J.[1], Mutinelli F.[1]
[1] Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Beekeeping, Legnaro, Italy; [2] Department of Comparative Biomedicine and Food Science, University of Padova, Agripolis, Legnaro, Italy

Vespa velutina Lepeletier 1836 (Hymenoptera: Vespidae), the yellow-legged hornet native to tropical and subtropical areas of South-East Asia, has been unintentionally introduced in southwestern France in 2004. In particular, all the specimens collected until now in Europe belong to the subspecies nigrithorax (du Buysson, 1905). This wasp has spread rapidly across Europe and overseas. In *Italy V. velutina nigrithorax* has been officially reported in June 2013 and is now well-established in the western part of Liguria region and occasionally reported in Piedmont region. More recently, it has been detected only occasionally in Veneto (2016), eastern Liguria, Lombardy and Tuscany (2017) regions. In order to investigate the phylogenetic relationships and the origin of Italian *V. velutina nigrithorax* specimens, twenty-four samples, collected in 2012, 2016 and 2017 from different sites (Veneto, Piedmont and Liguria regions), were analysed after amplification of a 710 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene (Folmer et al., 1994) and sequencing of PCR product. The sequences of cox1, obtained from the Italian specimens, resulted identical to those

obtained from the European specimens sequenced to date (French and Great Britain) as well as from Chine specimens of Jiangsu and Zhejiang. To better understand the placement of Italian samples, we performed a phylogenetic analysis according to the Maximum likelihood method implemented in the IQ-TREE. Finally, the cox1 haplotypes were analysed using the Network 5 package. The phylogenetic analysis as well the haplotypes study confirmed the placement of Italian specimens within a cluster containing the other European samples plus Chinese specimens collected in Jiangsu and Zhejiang.
All our results strongly support the view that the Italian specimens of *V. velutina nigrithorax* derived from an invasion of *V. velutina nigrithorax* population originating from France.

P122 *Varroa* selection criteria: how can beekeepers use it?

Guirao A-L.[1,2], Béguin M.[1,2], Nozière E.[1,2], Mondet F.[2,3], Gaillard C.[1,2], Sudan A.[1,2], Basso B.[1,2]
[1]ITSAP-Institut de l'abeille, Avignon, France; [2]UMT PrADE, Avignon, France; [3]Abeilles et Environnement, INRA, Avignon, France

Selection of *Varroa* resistant honeybees is a high priority for all queen breeders. Nowadays, breeding methods are based on scientific experiments that have studied the resistance mechanisms and behaviors of honeybees to identify relevant selection criteria.
Several different approaches to measure the resistance traits have been developed. The SMR (Suppressed Mite Reproduction) and the VSH (Varroa Sensitive Hygiene) protocols as well as the measurement of the growth rate of the varroa population are among the most important methods . These selection criteria have shown their effectiveness throughout different scientific experiments. and beekeepers legitimately want to apply these methods in their own selection method. However, the application of these methods in the practical conditions of beekeeping faces many operational limitations in terms of material, available time, dedication and proficiency for the beekeepers. Furthermore, a wrong or faulty implementation of the protocols are counterproductive: beside the lack of efficiency, the wasted time and energy may discourage motivated beekeepers.
This study therefore offers a comparison of the effectiveness of these protocols under realistic apiary conditions. At a beekeeper level, with a breeding programseveral dozen of colonies have to be measured in the shortest time possible to limit the influence of environmental factors on breeding colony values. Under this premis this study focuses on the comparison of three important points that can induce errors: the material (measuring tools and biological material), the skills needed and the working time induced..
Without debating the real effectiveness in term of the selection of the varroa resistance trait, of these criteria, it appears extremely difficult to ensure an efficient selection based on theSMR, VSH or varroa groth rate criteria, which have to be reliably measured over a large number of colonies. The prospects of new tools allowing a simplified but still reliable measurement are therefore essential to develop an efficient varroa resistance selection in the beekeeping industry.

P123 Smartbees - sustainable management of resilient bee population

Dahle B.[1,2], Bienefeld K.[3], Bowman, A.[4], Bendixsen C.[5], Buechler R.[6], Kryger P.[7], Ljung M.[8], Meixner M.[6], Thiery R.[9], Vingborg R.[10]
[1] Norweegian Beekeepers Association, Kløfta, Norway; [2] Norwegian University of Life Sciences, department of animal and aquacultural sciences, Ås, Norway; [3] Länderinstitut für Bienenkunde, Hohen Neuendorf, Germany; [4] University of Aberdeen, Aberdeen, UK; [5] Aarhus University, Department of Agroecology, Aarhus, Denmark; [6] Landesbetrieb Landwirtschaft Hessen Bieneninstitut, Kirchhain, Germany; [7] Aarhus University, Department of Molecular Biology and Genetics, Aarhus, Denmark; [8] Swedish University, of Agricultural Sciences, Skara, Sweden; [9] ANSES, Sophia-Antipolis laboratory, Maisons-Alfort, France; [10] Eurofins Genoskan, Aarhus, Denmark

Smartbees is a collaborative research project between 16 partners from universities, research institutions and companies across Europe. We are working on solutions to prevent colony losses caused by the Varroa mite and viruses and to counteract the systematic replacement of many native European honeybee subspecies with only two specific subspecies which is observed over the last years.
Smartbees has introduced selective breeding in Europe's neglected honeybee races to increase their popularity among bee breeders and beekeepers because conservation is best achieved through utilization. *Varroa* resistance is one of the selected characters that aim for a long term sustainable solution.
Through observational studies and genetic analyses of honeybees, we have identified genetic markers for the ability to detect and remove brood infested with *Varroa* mites. We have collected samples of Europe's honeybee subspecies both to supplement the existing morphometric collection and database, but also to develop genetic markers specific for each subspecies. These findings are now used to develop a new genetic tool for breeders that can verify a colony's subspecies affiliation and indicate its level of *Varroa* resistance.
We have studied how honeybee susceptibility towards parasites and pathogens can be influenced by their diet. One aim is to be able to give recommendations on optimal diet to reduce honeybee susceptibility.
Varroa and the viruses it vectors is a deadly combination. We have studied the role of *Varroa* saliva and the relationship between *Varroa* and DWV and found it to a symbiotic relationship.
Dissemination of project results are made through various channels. In addition to scientific dissemination, we target beekeepers and bee breeders through our Smartbees newsletter and articles in Europe's beekeeper magazines. Since we would like to make lasting changes in beekeeping we have developed an extension tool box in beekeeping that will be available online.

P124 How to best control for differences in microsatellite loci variability when comparing genetic diversity across populations

Wild C., Soro A., Theodorou P., Paxton R.J.
Martin-Luther-University Halle-Wittenberg, Department of General Zoology, Halle (Saale), Germany

Comparing genetic diversity among populations is of general interest for population genetics and conservation, including that of bees, because genetic diversity is an indirect measure of viability and evolvability of populations. In such analyses, genetic diversity, predominantly measured as expected heterozygosity or allelic richness of neutral and highly polymorphic microsatellite markers, is compared between populations using a wide range of non-parametric and parametric statistical approaches. Here, we ask which the best way is to test for variation in genetic diversity among populations using microsatellite loci that are inherently different in their variability within a population. After performing a literature review, we identified the majority of studies comparing genetic diversity between populations of animals and plants to use non-parametric tests (i.e. Kruskal Wallis, Mann-Whitney test). Parametric tests such as analysis of covariance (ANCOVA) and linear mixed models (LMMs) were both represented in only 12% of studies. We then evaluated the suitability and performance of all these statistical methods using our own empirical datasets of bees and wasps as well as computer simulations. Non-parametric tests were poor for such comparisons since they average genetic diversity across loci within each population, conflating locus variability with variation between populations. Regarding parametric tests, the assumptions of the ANCOVA method were rarely met in empirical datasets. However analyses using our simulated datasets showed that ANCOVA was quite robust to violations of assumption and outperformed the LMM statistical approach. We conclude that non-parametric tests should be avoided and that ANCOVA is the most powerful method when comparing genetic diversity across populations of bees or any other organism.

P125 Genome-wide analysis of structural and single nucleotide variation at candidate loci for behavioural traits in Carniolan honeybee (*Apis mellifera carnica*)

Zorc M., Dovč P.
Department of Animal Sciences, University of Ljubljana, Domžale, Slovenia

The honeybee (*Apis mellifera*) is regarded as a valuable model organism for genetic studies of social behavior. Among behavioral traits, aggressiveness plays an important role and significant variability of this trait was found among races but also among individual colonies. Carniolan bee is known for its calm and non-aggressive behavior, making it suitable for honey production in densely as well as sparsely populated areas. Our preliminary bioinformatics analysis showed that many genes involved in shaping of behavioral traits in bees are highly conserved among different species of social insects, however, the most prominent differences could be expected in the regulatory regions of these genes. Since subspecies of bees were subjected to different selection pressures, it is possible that binding sites for transcription factors, which were shaped by cis-regulatory evolution were modified and so they represent molecular basis for certain adaptive traits. Among most prominent candidate genes for behavioral traits are genes involved in biosynthesis of juvenile hormone genes coding enzymes for its degradation. Juvenile hormone esterase is the enzyme that degrades juvenile hormone and contributes to the regulation of hormone amount in hemolymph. In the current study, publicly available whole genome sequences of *Apis mellifera carnica* were compared with reference genome of *A.m. ligustica* and analyzed for structural and SNP variation within the candidate gene regions, related to behavioral traits. Several SNPs were found in genes coding juvenile hormone modifying enzymes: juvenile hormone esterase (406066), juvenile hormone epoxidase (406152), juvenile hormone methyl transferase (724216). In addition, SNPs were also found in the coding region of the dopamine receptor gene (406133) in exons and introns of farnesyl diphosphate synthase gene (107964026) and in introns of alpha glucosidase gene (406131). In the majority of candidate gene regions for behavioral traits structural variants could not be found. However, in the coding region of the odorant receptor gene (OR37), which is involved in organoleptic perception, extensive structural variation has been observed.

P126 Applying reduce SNP assays for inferring C-lineage introgression patterns in Iberian honeybee populations of the Azores archipelago

Lopes A.R.[1], Neves C.[1], Ferreira H.[1], Henriques D.[1], Quaresma A.[1], Martín-Hernandez R.[2], Azevedo J.[1], Pinto M.A.[1]
[1] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal;[2] Centro de Investigación Apícola y Agroambiental de Marchamalo, Spain

The genetic composition of the honeybee populations of the Macaronesian archipelago of the Azores is poorly known. Until now, only honeybee populations of the island of São Miguel have been surveyed for genetic variation through the use of the $tRNA^{leu}$-cox2 intergenic mitochondrial DNA region and microsatellites. Here, we combine data from the mtDNA obtained with the DraI test (intergenic region) and from the nuclear DNA obtained with newly developed reduced SNP assays to provide a complete picture of introgression patterns in the Azorean honeybee populations at both mitochondrial and nuclear compartments. The sampling was carried out in 2014 and 2015 and comprised 474 colonies widely distributed across the 8 islands populated by honeybees. Our cyto-nuclear results show that C-derived introgression varies across the archipelago ranging from virtually pure populations of the Iberian honeybee in the island of Santa Maria (Q-values <5%) to highly introgressed populations in the island of Graciosa (Q-values>30%). The introgression levels are alarming and contrast with those of the Iberian honeybee populations of the mainland in Iberia, which are still virtually free of C-derived introgression, despite frequent importation of commercial queens.

P127 Developing reduced SNP assays from whole-genome sequence data to estimate C-lineage introgression in the Iberian honeybee (*Apis mellifera iberiensis*)

Henriques D.[1], Parejo M.[2], Vignal A.[3], Wragg D.[4], Wallberg A.[5], Webster M.T. [5], Pinto M.A.[1]
[1] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal, [2] Agroscope, Swiss Bee Research Centre, Bern, Switzerland, [3] GenPhySE, Université de Toulouse, INRA, INPT, INP-ENVT, Castanet Tolosan, France; [4] The Roslin Institute, University of Edinburgh, Edinburgh, UK; [5] Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

The honeybee has been subject to a growing number of threats. In Western Europe one such threat is large-scale introductions of commercial strains (C-lineage), which is leading to introgressive hybridization and even the local extinction of native populations (M-lineage). Here, we developed reduced assays of highly informative SNPs from 176 whole genomes to estimate C-lineage introgression in ;M-lineage subspecies *Apis mellifera iberiensis*. We started by evaluating the effects of sample size and sampling a geographically restricted area on the number of highly informative SNPs. We demonstrated that a bias in the number of fixed SNPs (F_{ST}=1) is introduced when the sample size is small (N≤10) and when sampling only captures a small fraction of a population's genetic diversity. These results underscore the importance of having a representative sample when developing reliable reduced SNP assays for organisms with complex genetic patterns. We used a training dataset to design four independent SNP assays selected from pairwise F_{ST} between the Iberian and C-lineage honeybees. The designed assays, which were validated in holdout and simulated hybrid datasets, proved to be highly accurate and can be readily used for monitoring populations not only in the native range of *A. m. iberiensis* in Iberia but also in the introduced range in the Balearic islands, Macaronesia, and South America, in a time- and cost-effective manner. While our approach used the Iberian honeybee as model system, it has a high value in a wide range of scenarios for the monitoring and conservation of potentially hybridized domestic and wildlife populations.

P128 Polymorphisms in cytochrome P450 versus cline distribution of evolutionary lineages in Apis mellifera iberiensis

Muñoz I.[1], Henriques D.[2], De la Rúa P.[1], Pinto M.A.[2]
[1] Departamento de Zoología y Antropología Física, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain; [2] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus Santa Apolónia, Bragança, Portugal

Honey bees (*Apis mellifera*) are the most prominent and economically important pollinator species worldwide. However, the reported decline of its populations in several regions of the world over the last decades is of concern. The causes are manifold, including the spread of pathogens and parasites, malnutrition and habitat loss, climate change and xenobiotics, especially pesticides. Among the main mechanisms used by insects to cope with the adverse effects of xenobiotics is the metabolic resistance mediated mainly by three superfamilies of enzymes: the cytochrome P450 monooxygenases, the glutathione transferases and the carboxylesterases.
We hypothesize that the genetic background influences the sensitivity to pesticides or detoxification capacity of different honey bee populations, ecotypes and subspecies. The Iberian Peninsula provides an interesting scenario to study the genetic variability of the cytochrome P450 genes given the co-occurrence of two clinally distributed evolutionary lineages, as a result of secondary contact.
In this study, the genetic variability of six genes of the cytochrome P450 superfamily (CYP6AS3, CYP6AS4, CYP6AS5, CYP6AS7, CYP6AS12 and CYP6AS17) was analyzed in the Iberian honey bee (*Apis mellifera iberiensis*) to provide more information on the mechanisms of resistance to xenobiotics and to identify the genetic variation involved in local adaptation. Genomic signal of selective sweeps was detected in three genes, of which CYP6AS5 presents the highest number of point mutations under selection, being proposed as a candidate gene to perform gene expression studies. We discuss the correlation between the variability of P450 genes and the distribution of the evolutionary lineages in the Iberian Peninsula. The identification of polymorphisms in these genes promises to shed light on the relationship between diversity and xenobiotic tolerance of *A. m. iberiensis*.

P129 Contribution to the characterization of the genetic diversity of the honeybee *Apis mellifera*: case of the sex determination locus csd

Canale-Tabet K., Catays G., Vignal A.
GenPhySE, INRA, Castanet Tolosan, France

Sex determination in the honeybee is under the control of the csd gene composed of 9 exons, the seventh of which being hypervariable. A bee will be a female worker or a queen only if it is heterozygote at this specific locus and individuals having a single allele for the exon 7 of csd will develop as males, which is typically the case of the normal haploid males. Diploid individuals, homozygotes for the locus will also develop as male larvae, that will be killed by the workers. Within the framework of the INRA-ITSAP SeqApiPop project, we focused here on the diversity at csd by Sanger sequencing PCR products of the hypervariable exon. By amplifying DNA from haploid males, the direct sequencing of PCR products was possible without a sub-cloning step. We analyzed a total of 183 individuals from the 3 subspecies *A. m. mellifera* (n=86), *A. m. ligustica* (n=29) and *A. m. carnica* (n=37), in addition to bees from French beekeepers (n=51), which are a mixed type.
A total of 77 DNA and 73 amino acid haplotypes were observed, 25 of which are new. Polymorphisms include a variation of length of a short tandem repeat-like sequence, causing the length of csd exon 7 to varie between 73 and 101 amino acids, small indels and SNPs. Most mutations observed at the DNA level translate into amino acid changes. Across all the studied population, the mean number of individuals in which one specific amino acid haplotype is detected is 2.5 ± 2.1. When excluding the 51 mixed type bees, amongst 33 amino acid haplotypes observed in at least two individuals, 8 (24 %) could be detected in both M-type and C-type samples. Such a high level of allele sharing between otherwise genetically distant sub-species is surprising. This suggests either pressure for high allele diversity acting against loss by drift which could be due to the sex determination mechanism, or the convergent appearance of alleles in different populations due to a high mutation and fixation rate.

P130 Applying molecular tools for conservation of wild and managed black bees in Ireland

Browne K.A.[1], Henriques D.[2,3], Hassett J.[4], Geary M.[4], Moore E.[4], Pinto M.A.[2], Native Irish HoneyBee Society[5], McCormack G.P.[1]
[1] Department of zoology, National University of Ireland, Galway, Ireland; [2] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal; [3] Centre of Molecular and Environmental Biology (CBMA), University of Minho, Braga, Portugal; [4] Department of Applied Science, Limerick Institute of Technology, Limerick, Ireland; [5] www.nihbs.org

Apis mellifera mellifera (Amm) is threatened over much of its natural range. However, in Ireland microsatellite and mitochondrial data have shown that a significant population of this subspecies exists in pure form and is spread over a large geographical region on the Island. Black bees have been managed and protected by beekeepers on the island, some of whom formed the Native Irish Honeybee Society (NIHBS)in 2012 and a breeding programme was initiated for Amm in 2014/2015.
The application of a SNP panel that detects hybridization between M and C lineages clearly supports other data showing that the majority of beekeepers included in the breeding programme indeed have bees with very low to no introgression from the C lineage.
Furthermore, SNP data has also been applied to the first feral bee colonies located in Ireland subsequent to the introduction of Varroa. Here we will present on the use of molecular data as an aid to manage and conserve honeybees in Ireland, and to elucidate patterns in colour variation and honeybee subspecies purity in wild and managed bees with a view towards improving conservation approaches in the face of a potential hybridization threat.

P131 The transcription of ecdysteroids related genes in *Apis mellifera* workers and drones brood

Giurgiu I.A.[1], Dezmirean S.D.[1], Moritz F.A.R.[2], Aurori-Pacurar A.[1]
[1] University of Agricultural Science and Veterinary Medicine Cluj Napoca, Romania; [2] Martin-Luther-University Halle-Wittenberg, Institute of Biology/Zoology, Halle an der Saale, Germany

Background: The Halloween genes coding for the enzymes controlling the biosynthesis of ecdysone are highly conserved in arthropods. Whereas the *Apis* genome comprises the full gene set its parasitic mite *Varroa destructor* lacks four Halloween genes, and can most likely not produce ecdysone. Female mites may, therefore, depend on the intake of ecdysone or intermediate compounds from the host hemolymph to activate its reproductive cycle. We here compare the transcript abundance of the Halloween genes in drones and workers at specific time points before and after brood cell capping when the female mite needs to activate its ovaries.
Material and methods: RNA was extracted from the entire body of honeybee drone and worker larvae sampled at different time points just before and after cell capping (0, 8, 12, 16, 24, 30 hours). The transcript abundance of the Halloween genes shroud (*sho*), neverland (*nvd*), disembodied (*dib*), phantom (*phm*), spook (*spo*), shade (*sad*) and shadow (*shd*) were quantified by qPCR in addition to Cyp18a1, another key gene of the ecdysteroid pathway.
Results and conclusions: *Shd* (in drones) and Cyp18A1 (in workers) were down-regulated after capping. All other genes showed a swift upregulation within 24 hrs with a significantly higher transcript abundance in drones compared to that of the workers. This may explain the higher reproductive success of *Varroa* mites in drone brood and its preference for infesting drone brood.
Acknowledgements: We would like to thank to the GRAL project PN-III-P4-ID-PCE-2016-0637 contract number 162 for funding.

P132 *Varroa* mite reproduction, hygienic and grooming behavior of Ethiopian honeybee (*Apis mellifera jementica*)

Ngussie H.G.[1,3], Bezabh A.[2], De Smet L.[1], de Graaf D.C.[1]
[1] Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium; [2] Holeta Bee Research Center, Ethiopia; [3] Tigray Agricultural Research Institute, Ethiopia

Beekeepers in developed countries use several chemicals to minimize the impact of *Varroa destructor*. In Africa, the mite co-exist with the local bees without beekeepers' intervention and has no impact. However, little is known about the factors that enable African bees to co-exist with the mite without beekeepers' intervention. The study was designed to investigate the contribution of the mite's reproductive ability, hygienic and grooming behavior of Ethiopian bees (*Apis mellifera Jementica*) against the mite. The study was conducted at the Tigray Agricultural Research Institute, Ethiopia. The hygienic behavior was examined using the pin-killing test. The mite's reproductive ability was determined on worker broods about to emerge. The grooming behavior was evaluated by calculating the percentage of damaged mites. The hygienic behavior of the local bees in 24 hr was 92.2% and had a negative association with varroa mites' infestation in adult bees ($r = -0.57$, $p<0.01$) and worker bee brood cells ($r=-0.72$, $p<0.001$). The grooming behavior of the local bees during the active and dry season was 34.1% and 42.1%, respectively. However, the grooming behavior of the local bees had no association with the level of varroa mite in adult bee and brood cells ($p>0.05$). In our study, seven kinds of *Varroa* mite damages due to the grooming behavior of the local bees were identified. Of the seven kinds of damages identified, leg damage was the most frequent kind of damage recorded. The fertility of *Varroa* mite in the local bees was 60.15%, however only 18.80% of the mites produced viable female offspring. Thus, the mite has low reproductive success in the local bees. However, our result did not find any evidence regarding the contribution of grooming behavior to the tolerance of the local bees against the mite since the grooming behavior of the local bees had no association with the level of varroa mite. Hence, it is recommended to maintain colonies which have high hygienic behavior.

P133 High sample throughput genotyping for estimating C-lineage introgression in the dark honeybee: an accurate and cost-effective SNP-based tool

Henriques D.[1], Browne K.A.[2], Barnett M.W.[3], Parejo M.[4], Kryger P.[5], Freeman T.C.[3], Muñoz I.[6], Garnery L.[7], Highet F.[8], Jonhston J.S.[9], McCormack G.P.[2], Pinto M.A.[1]
[1] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal; [2] Department of Zoology, Ryan Institute, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland; [3] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Edinburgh, Midlothian, Scotland, UK; [4] Agroscope, Swiss Bee Research Centre, Bern, Switzerland; [5] Aarhus University, Department of Agroecology, Slagelse, Denmark; [6] Área de Biología Animal, Dpto. de Zoología y Antropología Física, Universidad de Murcia, Campus de Espinardo, Murcia, Spain; [7] Laboratoire Evolution, Génomes et Spéciation, CNRS, Gif-sur-Yvette, France; [8] Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh, Scotland, UK; [9] Department of Entomology, Texas A&M University, College Station, USA

The natural distribution of the honeybee (*Apis mellifera* L.) has been changed by humans in recent decades to such an extent that the formerly widest-spread European subspecies, *Apis mellifera mellifera*, is threatened by extinction through introgression from highly divergent commercial strains in large tracts of its range. Conservation efforts for *A. m. mellifera* are underway in multiple European countries requiring reliable and cost-efficient molecular tools to identify purebred colonies. Here, we developed four ancestry-informative SNP assays for high sample throughput genotyping using the iPLEX Mass Array system. Our customized assays were tested on DNA from individual and pooled, haploid and diploid honeybee samples extracted from different tissues using a diverse range of protocols. The assays had a high genotyping success rate and yielded accurate genotypes. Performance assessed against whole-genome data showed that individual assays behaved well, although the most accurate introgression estimates were obtained for the four assays combined (117 SNPs). The best compromise between accuracy and genotyping costs was achieved when combining two assays (62 SNPs). We provide a ready-to-use cost-effective tool for accurate molecular identification and estimation of introgression levels to more effectively monitor and manage *A. m. mellifera* conservatories.

P134 A collection status of the world biogeography and population genomics of *Varroa destructor* project

Techer M.A., Mikheyev A.S.
Okinawa Institute of Sciences and Technology, Ecology and Evolution Mikheyev Unit, Tancha, Onna-son, Kunigami-gun, Okinawa-ken, Japan

Globalization has provided opportunities for species to cross geographical barriers and establish outside their native range. The Western honey bee previously geographically isolated from all other *Apis* species was introduced in Asia for more than a century. One unfortunate consequence of this new sympatry was the successful host switch of *Varroa destructor* from the Asian honeybee to the Western honeybee. While the Asian honeybee, original host, co-evolved with *Varroa* mites and developed defense strategies, the Western honeybee was naïve toward this parasite leading to important colony damages. Following beekeeping and honey bee movements, *V. destructor* spread nearly worldwide and is considered as the major destructive force behind colony global collapse. Despite several efforts to track accurately *Varroa* invasion since its host switches, the ancestral origins and pathways of introductions remain unclear. In order to better understand the world biogeography of this successful biological invader, we started to build a *Varroa* mites world collection since 2017. Whole genome sequencing of *V. destructor* collected from different continents, countries, and honey bee subspecies will be used i) to reconstruct the demographic history of the parasite, and ii) study the genetic diversity and connectivity of invasive populations. The current collection contains female mites collected from 560 colonies of *Apis mellifera* from 22 countries resulting from a huge collaborative effort from the honey bee research and beekeeping community. Efforts on improving collection size and coverage are ongoing. A subset of samples has been selected for preliminary sequencing to assess the genetic diversity level within and among apiaries. The results from world population genomics will be valuable to identify the demographic key factors behind the global success of Varroa invasion.

P135 Identification of honey bee populations from the azores: insigths from wing geometric morphometrics

Ferreira H.[1], Machado C.[2], Costa C.[3], Francoy T.[4], Pinto M.A.[1]
[1] Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal; [2] School of of Philosophy, Sciences and Letters, University of São Paulo, Ribeirão Preto, Brazil; [3] School of Medicine, University of São Paulo, Ribeirão Preto, Brazil; [4] School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, Brazil

The geometric morphometrics of the wings has been an important method for the identification and evaluation of honey bee diversity patterns around the world. Honey bee populations of the Macaronesian archipelagos of Canaries and Madeira have been intensively surveyed for diversity using a variety of genetic markers. In contrast, honey bee populations inhabiting the Azorean archipelago have been largely undersampled. To fill this gap, we sampled 473 colonies from across the Azores and assessed diversity patterns using a geometric morphometrics approach. A total of 5 forewings were collected per colony, mounted in a slide and photographed with a stereomicroscope. Additionally, the forewings representing 711 colonies of *A. m. iberiensis*, 11 *A. m. ligustica*, 15 *A. m. carnica* and 12 *A. m. caucasia* were used as reference samples. To extract shape information, 19 anatomical landmarks were plotted across the veins' intersections in the wing structures of all individuals. The analyses of wing shape were performed in MorphoJ using the Procrustes superimposition method. Shape differences were investigated through multivariate statistical analysis and Mahalanobis and Procrustes distances were used to construct a dendrogram of the morphological proximity. Results revealed the power of landmark-based methods to discriminate different honey bee populations from the Azores, and also to distinguish them from the subspecies of the reference collection. The wing geometric morphometrics patterns showed that while, overall, populations from the Azores exhibited a closer relationship with *A. m. iberiensis*, some populations, especially those from the islands of Graciosa, but also Terceira and Pico tended to cluster closer to *A. m. ligustica*, *A. m. carnica*. Several non-mutually exclusive factors can contribute to the observed wing patterns such as the recent human-mediated introductions of subspecies from Eastern Europe, and the founder effect resulting from honey bee introductions in historical times. Moreover, the particular insular environment and the barrier to gene flow due to geographical isolation possibly shaped the diversity patterns currently observed in the Azores.

P136 Integrative approach apply to three Belgian species (*Thoracobombus*) involving DNA sequences and male marking secretions

Brasero N., Martinet B., Michez D., Valterova I., Rasmont P.
Université de Mons, Laboratoire de Zoologie, Mons, Belgium

Recent betterment in taxonomy consider multiple operational criteria. The integrative taxonomy provides a methodological framework merging these multisource approaches. Bumblebees are considered as a complex group where their classification remain one of the most difficult. Here, we investigate the taxonomic statuses inside a monophyletic group including six taxa (*B. inexspectatus*, *B. mlokosievitzii*, *B. ruderarius*, *B. sylvarum*, *B. velox* and *B. veteranus*) in the most diverse subgenus of bumblebees: *Thoracobombus*. We used an integrative approach based on mitochondrial and nuclear genetic makers and eco-chemical traits commonly used in bumblebee taxonomy. For all species, our genetic analyses demonstrate and confirm clear differentiation in our genetic analyses and species-specificity in the eco-chemical traits. However, based on their unique haplotypes and CLGS differentiation, we conserve the subspecific status of *B. ruderarius simulatilis* and *B. sylvarum daghestanicus* from the east of Turkey and Iran.

P137 Genetic architecture of honey bee virus susceptibility

Bhatia S., Baral S., Wagoner K., Rueppell O.
Department of Biology, University of North Carolina at Greensboro, USA

Recently, honey bees have experienced major population declines throughout the world, including 30% annual losses within the US. Viruses play a major role in causing a high mortality rate in honey bees. Isareli Acute Paralysis Virus (IAPV), one of the 22 known bee viruses, is transmitted by Varroa mite and is responsible for some of the collapses of honey bee colonies. Through this study, natural variation to IAPV resistance in honey bee workers of different genetic stocks was assessed. We present preliminary results from a large-scale survival comparison among multiple queens from different stocks in the U.S., with a final assessment of this data, a screen of viruses present in these stocks. Honey bee queens representing different US genetic lines of bees were obtained from multiple sources in the US as follows: USDA-Pol, USDA-Russian, Italian Californian, Minnesota Hygienic and Italian and Carnolian Hawaiian bees. Worker offspring from these queens; upon emergence were inoculated with IAPV by topical applications. A total of 5,500 worker bees were analyzed for this part of the study. Survival probability among these different genetic stocks was computed and although no significant differences were found among stocks, but a significant variation to IAPV survival within the stocks was observed. Based on this data, 10 most and least susceptible colonies were selected. To validate the above findings, fold expression changes in certain immune genes in bees collected prior and post inoculation was quantified. This is the first systematic study on honey bee virus susceptibility comparing workers from different colonies of genetic stocks and how they vary in their susceptibility levels. Overall, our results should inform beekeepers and queen breeders about these important properties of the different genetic stocks and help mitigate the ongoing honey bee health crisis.

P138 The conservation of honey bee subspecies depends on beekeepers' involvement

Kryger P.[1], Papoutsis L.[2], Montes I.[3], Bouga M.[2], Estonba A.[3], Meixner M.[4]
[1] Department of Agroecology, Aarhus University, Slagelse, Denmark; [2] Lab of Agricultural Zoology and Entomology, Agricultural University of Athens, Athens, Greece; [3] Lab. Genetics, University of the Basque Country (UPV/EHU), Leioa, Bilbao, Spain; [4] LLH Bee Institute Kirchhain, Kirchhain, Germany; All authors are part of the SmartBees consortium

Honey bees are native to most parts of Europe with a rather stable population size. The activities of beekeepers since centuries, have resulted in a large fraction of the species being particularly well protected. Managed colonies that do swarm, may establish as feral colonies, live on for generations while producing further swarms. The wild and the managed populations are thus interconnected, also due to the mating structure of honey bees.

Some ten subspecies of honey bees in Europe can be differentiated. During the last decades, conservation efforts were initiated across Europe in order to preserve the genetic diversity of honey bees. In some cases these efforts have led to the establishment of conservation areas ranging from isolate and small islands, to entire countries like Slovenia and Croatia. The need for conservation areas is caused by the more than a century old trade in honey bees. Trade in bees and migratory beekeeping practice is an ongoing activity, with the consequence that a considerable proportion of the honey bee population is hybridised, and several subspecies are endangered in Europe.

Based on a questionnaire circulated via the SMARTBEES FP7 project (KBBE 613960), with around 6000 beekeepers participating across Europe, we note a need for further educational efforts with an emphasis on conservation activities. Beekeepers are often unfamiliar with basic biological concepts, including nomenclature, introgression and adaptation. We must seek to create the knowledge based awareness needed to initiate and sustain a broad movement among beekeepers towards the conservation of subspecies diversity.

P139 The impact of solid state fermentation on bee pollen out layer and phenolic compounds

Urcan A.[1], Mărghitaș L.[1], Dezmirean D.[1], Criste A.[2], Bobiș O.[1], Bonta V.[1], Mărgăoan R.[3], Campos M.[4]
[1] Department of Beekeeping and Sericulture, [2] Department of Microbiology, [3] Department of Horticulture, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; [4] Coimbra University, Faculty of Pharmacy, Coimbra, Portugal

Natural fermentation of bee pollen in the hive, done by bees, results in a well-known product - beebread. Classical understanding of the beebread is that pallets are result of pressing together bee pollen loads inside of the bee-comb-cell. Bees add glands' secretions and honey that provide a proper microenvironment for the fermentation. Many studies speculate that prior to consumption by bees, stored pollen undergoes long-term nutrient conversion, becoming more nutritious beebread as microbes predigest the pollen. The aim of this study was to perform solid-state fermentation of bee collected pollen and assessment the impact of fermentation to the pollen out layer as well as on the biochemical composition, total phenolic content and total flavonoid content of fermented and non-fermented bee pollen. This study was the first attempt to produce artificial beebread. Three different bee pollen samples were fermented, a polifloral bee pollen sample and two samples of monfloral bee pollen pellets, under laboratory conditions using as inoculum beebread extracted from combs of Apis mellifera. Each sample was placed in a sterile bottle, with pollen and inoculum to which it was added a thin layer of honey to prevent the entry of air and thus avoid losses of oxidation and fermentation aerobic putrefactive. Pollen samples were analyzed at the optical microscope before and after fermentation process to observe the outer layer, the exine and to identify the botanical origin. Total phenolic and total flavonoid content were analyzed by spectrophotometric methods; phenolic profile was evaluated by HPLC. Concerning the chemical composition and the total phenolic content, the fermented pollen samples have preserved the characteristics of the pollen with small changes in carbohydrates and protein amount. Fermentation revealed positive impact on the total flavonoid content (amount of flavonoids increased by 10%). The results for the fermented pollen were comparable with the results reported from other authors for natural beebread.

P140 Alpha-mangostin and apigenin induced the death of BT474 breast cancer cells via necrosis involving with autophagy and inflammation

Ittiudomrak T.[1], Puthong S.[2], Palaga T.[3], Roytrakul S.[4], Chanchao C.[5]
[1] Program in Biotechnology, [3] Department of Microbiology, [5] Department of Biology, Faculty of Science, [2] Institute Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, Thailand; [4] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani, Thailand

Breast cancer has been widely found worldwide. Although therapeutic strategies have been discovered, new compounds from natural products are still required to overcome the mainstay of metastatic breast cancer. Here, alpha-mangostin and apigenin was focused. Doxorubicin, a recent chemotherapeutic drug, was used as positive control. Ductal carcinoma (BT474) and normal mammary epithelial fibroblast (MCF-10A) were used. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the half maximal inhibitory concentration (IC50) which showed that alpha-mangostin and apigenin were more cytotoxic to BT474 cells unlike doxorubicin. By observing the morphology change under the light microscope, the longer exposure to three compounds caused more floating and less density of cells. Also, more vacuoles could be revealed in formed colonies of cells except in doxorubicin treated BT474 cells. However, the change could not be seen in alpha-mangostin treated- and apigenin treated MCF-10A cells. After flow cytometric analysis of annexin V and propidium iodide (PI) stained cells, both alpha-mangostin (4 ug/mL) and apigenin (10 ug/mL) had caused necrosis to BT474 cells since 24 h. However, small amount of early apoptotic cells could be detected at 24, 48 and 72 h. For doxorubicin (0.25 ug/mL), it caused early apoptosis to BT474 cells at 24 h. Caspase-3 was obviously involved in the death of BT474 cells treated by three compounds. At the same concentrations, after flow cytometric analysis of PI stained cells, cell cycle was arrested at G1 subphase by alpha-mangostin and apigenin while it was arrested at G2/M subphase by doxorubicin. The data from flow cytometry analysis of annexin V and PI stained

cells was supported by the assayed activity of caspases3, 8 and 9. In addition, those three compounds could cause the change in gene expression of 1) inflammation-associated genes, 2) proto-oncogene, 3) autophagy-associated gene and 4) apoptosis-associated genes. In conclusion, alpha-mangostin and apigenin may be a new source for antiproliferation of BT474 breast cancer cells in term of more cytotoxic to the cancer cells, cell cycle arrest at the G1 subphase and change in gene expression of some cancer relating genes.

P141 Monocyclic aromatic hydrocarbons in urban honey bee larvae and pollen

Szentgyörgyi H.[1], Zięba K.[2], Moos-Matysik A.[2], Kozaczka K.[2], Czekońska K.[1]
[1] Department of Pomology and Apiculture, Faculty of Biotechnology and Horticulture, Agricultural University in Kraków, Kraków, Poland; [2] Department of Environmental Chemistry, Faculty of Chemistry, Jagiellonian University, Kraków, Poland

Aromatic hydrocarbons are environmental pollutants, which are often carcinogenic, mutagenic, and teratogenic and may have significant health implications for all taxa. Bees can be exposed to monocyclic hydrocarbons by atmospheric dust pollutants and contact contamination when gathering pollen and nectar from flowers. The aim of our study was to compare the BTEX (Benzene, Ethylobenzene, Toluen, o-Xylene and p-Xylen) contamination levels in collected pollen and in honey bee larvae developing on such pollen. Bees were exposed to various levels of pollution on five sites located in the city and the surroundings of Kraków, where the emission of BTEX, especially during the heating season, is a well-documented phenomena. Samples of pollen and honey bee larvae were taken three times during the season from three hives on each site: in May, June and July.
In May BTEX levels of pollen were the highest reaching 817,98 μg/g. In June on all sites levels were below 114,84 μg/g, while in July below 62,66 μg/g. In May BTEX levels in bee larvae was reaching 367,13 μg/g. In June, BTEX levels in all samples were below 56,21 μg/g, while in July below 52,99 μg/g. Levels of BTEX contamination in bee larvae raised on contaminated pollen showed no or even slightly negative correlation with pollen contamination levels.
This result is contrary to our expectations and suggests, that bees might have a mechanism protecting them from accumulating aromatic hydrocarbons from their food source. Further studies are conducted in the following two years to explain this phenomenon. High BTEX levels in May were found to be the result of high levels of o-xylen on all sites both in pollen and bee larvae, while the rest of the BTEX were on fairly similar levels during the whole season. We suggest, that these high levels during the first sampling are the result of heating still ongoing in the surrounding households.
The study is supported by the Polish National Science Centre (UMO-2016/21/B/NZ9/01163).

POSTERS

P142 First scientific studies of beehive air composition

Recklies K., Kuhn F., Speer K.
Dresden University of Technology, Food Chemistry and Food Production, Dresden, Germany

Within the last few years, "beehive air inhalation" is becoming more and more popular in apitherapy. Especially respiratory illnesses, such as bronchitis, or asthma, can be improved by beehive air treatment. So far, clinical and scientific studies are almost completely absent. Therefore, the beehive air therapy is not recognized as an alternative cure in Germany. Scientific investigations are still required.
In the bee season May to August 2017, volatile organic components (VOCs) of beehive air were analyzed at the Dresden University of Technology using various "air sampling" techniques. For that purpose, the established GC-MS method for the analysis of honey-flavoring substances could be adapted to the new matrix "beehive air" [1]. The optimized air sampling techniques manual solid phase micro-extraction (SPME) and thermal desorption tubes (TD) - proved to be suitable systems for extracting the VOCs from beehive air. Over fifty different VOCs were detected with the two systems (SPME & TD). The assignment of the VOCs to the individual bee products from the hive showed that the substances identified in the beehive air predominantly originated from propolis and beeswax. In pollen, honey, drone brood, and royal jelly the lowest number of VOCs was detected.

A first quantification of ten VOCs using TD-GC-MS revealed very low concentrations ranging from 0.08 to 4.57 ng/L beehive air. For some of the detected VOCs, air guideline values for indoor air from the Federal Environment Agency exist in Germany. Exceeding the guideline values may cause some people to react with headaches and irritation of the mucous membranes [2]. However, all quantified VOCs in the beehive air which patients inhaled directly via a breathing mask during treatment were clearly below the specified indoor guideline values.

P143 Authentication of honeydew honeys by analyzing non-volatile components

Recklies K., Bengler T., Kölling-Speer I., Speer K.
Dresden University of Technology, Food Chemistry and Food Production, Dresden, Germany

Honeydew which is excreted by aphids feeding on conifers in Germany and Central Europe is an important food source for bees in late summer. The honeys produced from it are of a dark color and have an especially spicy, malty taste. Many consumers like these properties and accept the higher prices.
So far, honeydew honeys are distinguished mostly by their sensory properties. The classical microscopy pollen analysis failed due to the missing pollen. In order to protect the quality and the authenticity of these rare expensive honeys, a project called "BoogIH" (botanical, zoological, and geographical identification of honeydew honey) was launched and funded in Germany by The Federal Ministry of Food and Agriculture (BMEL). The aim of the German "BoogIH" project is to provide an accurate definition of honeydew honeys by means of objective chemical-analytical methods in order to promote their marketing and to discover food fraud.
For this purpose, our group has developed a multimethod for the determination of various non-volatile honey components. The detection of individual honey substances was carried out by SPE-(U)HPLC-PDA-MS/MS.
The chromatographic profiles of a total of 42 authentic fir, spruce, and pine honeys were compared, and marker substances were identified by using chemo-metrics. Employing a discriminant analysis based on the best specific markers, a differentiation of the botanical origin of honeydew honeys was achieved. Afterwards, it was possible to classify more than 20 commercial honeydew honey samples from the market. Consequently, honeydew honeys can be tested for their purity objectively for the first time.
Acknowledgement: The BoogIH project (No. 2816500314) is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

P144 Quality and variety of Ukrainian honey

Adamchuk L.
National University of Life and Environmental Sciences of Ukraine, Department of Horse-Breeding and Beekeeping, Kyiv, Ukraine

In recent years, Ukraine has taken a leading position in exporting honey to EU countries. According to Ukrainian statistics, about 50 thousand tons in 2017. In most cases honey is exported as raw material for further processing, and our state loses the value added of production. This is due to the imperfect system of honey quality and not sufficiently investigated both geographic and botanical origin of honey. The research is aimed at studying and improving the quality of Ukrainian honey, establishment of their origin and original species characteristics is an actual scientific direction for increasing the economic value of the beekeeping industry in Ukraine.
Our main goal was to research composition and properties of the local Ukrainian honey.
Highly productive plant species are identified for obtaining original varieties of honey. These are 9 of annual herbaceous and 2 of biennial herbaceous species plants, 25 of perennial herbaceous species plants; 10 bushes and 11 of trees species. The botanical composition of honey obtained from the Reserve and the National Natural Parks of Ukraine has been

researched. It has been established that honey contains pollen from rare plants listed in the European Red List: *Silene lithuanica*, *Tragopogon ucrainicus*, *Chamaecytisus podolicus*, *Pulsatilla grandis*, *Salvia cremenecensis*, *Schivereckia podolica*. Also discovered plant species protected by Annex I of the Berne Convention: *Cypripedium calceolus*. *Jurinea cyanoides*, *Carlina onopordifolia*, *Fritillaria montana*, *Pulsatilla patens*. The honey also contains pollen from more than 50 species of plants that are part of the Red Book of Ukraine. Physical, chemical, biologically active and safety parameters of unifloral Ukrainian honey from *Hyssopus*, *Echium*, *Salvia*, *Epilobium*, *Onobrychis*, *Phacelia*, *Silybum* were investigated. The qualitative and quantitative pollen analysis of unifloral Ukrainian honey is carried out. The first atlas of pollen grains of honey plants of Ukraine was created.

P145 Effect of honeybee race and season changes on propolis composition

Kekeçoğlu M.[1,2], Kolayli S.[3]
[1] Faculty of Art and Science, Department of Biology, [2] Beekeeping Research Development and Application Center (DAGEM), Düzce University, Duzce, Turkey; [3] Karadeniz Technical University, Trabzon, Turkey

Variations of seasonal and bee races on propolis composition was investigated in this study. The chemical profile of the propolis were characterized by total phenolic content, total flavonoid contents, total antioxidant capacity (ferric reducing/ antioxidant power (FRAP)), free radical scavenging activity (DPPH) and phenolic profiles. Propolis samples were collected from May to September in Düzce city of Turkey. Five different indigenous honeybees consist of three races (*A. m. caucasica*, *A. m. syriaca*, and *A. m. carnica*) and two ecotypes (Yigilca and Mugla ecotypes) were used in Central Anatolia. The results were revealed that genetic differences of bee colonies and gathering time effected on phenolic compositions, and antioxidant properties of the raw propolis. And higher quality propolis could be obtained from the bees lived in suitable habitats.

P146 Microbeekeeping protocol to design a quantitative and qualitative properties of natural honey

Duangphakdee O., Rod-im P.
King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand

Microbeekeeping protocol to design a quantitative and qualitative properties of natural honey as taste, aromatic quality, colour, sugar contents as well as some medicinal properties by manage a honeybee flora within the foraging radius of the bees was performed. The aims of this project is feasibility testing of a model in an economically scale. *Cucurbita* sp. and *Cosmos sulphureus* were two types of flora that were used as signature in honey of stingless bees (model code: SP1C10) .Stingless bee (SP1C10) yield results 3.5 kg/rai which was 45.39 % lower than expected yield of original model. Produced honey is qualified the phycio-chemical properties standard of Thailand Food and Drug Administration standard demonstrated that the protocol is highly feasible for beekeepers. The analysis of highly volatile compounds in honey using GC-MS techniques revealed total 34 compounds in Stingless bee (SP1C1) consisting of 52.5 % similarity to original model honey. Regards to the Social Enterprise concept, this project has been conducted a feasibility study in production methodology and evaluated a potential traction channels. Three traction has been tested as social enterprise shop, facebook and Line@ application. The products are now on shelf of social enterprise shop of Chaipattana foundation under the royal project of H.M. the late King Rama IV as the most promising traction channel. We have collaborated with 25 beekeepers from Ratchaburi, Kanchanaburi and Lopburi provinces that join our projects and potentially improved their quality of life - sufficiency and economic status.

P147 *Fallopia japonica* honey: an antimicrobial candidate against methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococus aureus*

Bobis O.[1], Niculae M.[2], Dezmirean D.[3], Mărghitaş L.[3], Bonta V.[1], Paşca C.[3], Domokos T.[3], Urcan A.[3], Moise A.[3]
[1] Life Science Institute "King Michael I of Romania", [2] Faculty of Veterinary Medicine, [3] Faculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

Previous studies indicated the complex ability of bee products (honey, propolis) to inhibit several bacterial species; while certain products such as propolis and Manuka honey have been extensively studied, others less known may represent valuable sources of antimicrobial agents. Japanese knotweed (*Fallopia japonica*) honey, originates from a plant from *Polygonaceae* family, with a high production of nectar and an elevated percentage of resveratrol extracted in large amounts from its roots for commercial purposes. This particular type of honey has a fluid dark black aspect and it is recognized for its sweet flowering taste, but also for valuable nutritional characteristics such as high amount of minerals (28.77-46.09 mg/kg Ca; 58-81-68.88 mg/kg Na; 1187-6196 mg/kg K), proteins (0.64 – 1.05%) and lipids (0.1 – 0.5%). Its high content of polyphenols (150-190 mg/100g) is responsible for important antioxidant properties (60.54-77.73% inhibition of DPPH radical). This study was aimed to determine the chemical composition of this type of honey and to investigate in vitro the antibacterial activity of locally available *Fallopia japonica* honey against *Staphylococcus aureus* both methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) strains using an agar-well diffusion method. A microbroth dilution assay was also performed to determine the minimum inhibitory (MIC) and bactericidal concentrations (MBC). The results pointed out interesting antibacterial potential against all *S. aureus* strains and the bactericidal effect was strongly dependent on the honey concentration especially for MRSA isolates. To the best of our knowledge, this is the first study aimed to evaluate the antibacterial activity of Romanian *Fallopia japonica* honey and given the promising results, further in vitro and in vivo studies are intended to recommend the clinical use of this bee product as an anti MRSA agent.

P148 Bee products in Thailand: their medicinal properties

Pattamayutanon P.[1], Angeli S.[2], Thakeow P.[3], Abraham J.[4], Disayathanoowat T.[1], Peng C.[5], Sinpoo C.[1], Chantawannakul P.[1]
[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; [2] Faculty of Science and Technology, Free University of Bozen-Bolzano, Bolzano, Italy; [3] Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand; [4] Department of Entomology and Wildlife, School of Biological Sciences, University of Cape Coast, Ghana; [5] Department of Biotechnology, National Formosa University, Yunlin, Taiwan

Bee products have been determined by scientific approaches indicating their medicinal properties which are defined by their plant origins. In this research, the impact of floral source, honeybee species, and post-collection processing on antibacterial and antioxidant activities, and other biochemical compositions of different Thai honey and bee products were analysed. Honey samples from three honeybee species (*Apis mellifera*, *Apis cerana*, and *Apis dorsata*) were obtained from nine floral sources (longan, wild flower, lychee, coffee, sunflower, sesame, bitter bush, para-rubber, and manuka as a control) in different regions of Thailand. These samples were evaluated for both their total and nonperoxide antibacterial activities against ten human pathogens by agar incorporation technique. Honey samples were further analyzed to evaluate the capacity for free radical-scavenging activity, total phenolic content, and the total flavonoid contents by the 2,2-diphenyl-1-picrylhydrazyl assay, Folin–Ciocalteu method, and aluminum chloride colorimetric assay, respectively. Findings of this study suggest a strong differences in their medicinal properties of honeys collected from different floral origin and honeybee species. We also assessed the impact of pollen feeding from common floral sources in Thailand (e.g. tea, coffee, and bitter bush) on royal jelly properties (i.e protein pattern, (E)-9-hydroxydec-2-enoic acid (9-HDA) and (E)-10-hydroxy-2-decenoic acid (10-HDA) contents and antibacterial activity). The protein patterns from three different pollens were different, however, they showed no effect on protein on royal jelly samples derived from bee colonies fed by different pollen species. Nevertheless, royal jelly samples from bee colonies fed by bitter bush and coffee pollens

possessed the higher 10-HDA levels than royal jelly collected from bee colonies fed by tea pollen. The 9-HDA was found in lower amount than 10-HDA in every sample. Even though the antibacterial activities of pollen were varied, however, royal jelly samples exhibited similar antibacterial properties. This is the first report showing that different pollen feeding affected 10-HDA contents, but not affected overall protein content and antibacterial properties.

P149 Gbaya - beekeeping and honey hunting

Gruber M., Mazi S., Brückner D.
Department of Biology, University of Bremen, Bremen, Germany

This ethnographic film depicts different activities related to traditional beekeeping and honey hunting as practiced amongst the Gbaya in the area of Ngaoundere, Central Cameroon. The film first shows the highly sophisticated construction of a traditional hive by a beekeeper, who later demonstrates the harvest of honey from such a hive in a savannah habitat. The same beekeeper then demonstrates the practise of honey hunting. The film was produced in 2015 by the anthropologist Martin Gruber and the bee biologist Dorothea Brückner, both University of Bremen, in collaboration with members of the University of Ngaoundéré.

P150 Physicochemical and sensory properties of different types of honey from Serbian market

Pisinov B.[1], Roković N.[1], Radović M.[1], Mladenović M.[2], Lončar D.[3], Rašić S.[4]
[1] Institute of Veterinary Medicine of Serbia, Belgrade, Serbia; [2] Faculty of Agriculture, University of Belgrade, Belgrade, Serbia; [3] Institute SUPERLAB, Vrčin, Serbia; [4] Faculty of Ecological Agriculture, University EDUCONS, Sremska Kamenica, Serbia

Objectives: The main objective of this study was to evaluate the quality and sensory of different honey samples collected from markets in Serbia and verifying their compliance with national and international regulations.
Material and Methods: Physicochemical properties and sensory of 112 different honey samples collected during September and November 2017, arranged in six honey types acacia, blossom, linden, sunflower, honeydew and bakery honey were studied. Methods established by the International Honey Commission (IHC) were used. High performance liquid chromatography (HPLC) was used to determine the sugar content and 5-hydromethylfurfural (HMF). Sensory analysis was carried out using quantitative descriptive analysis method.
Results: The ranges of physicochemical properties were: reducing sugars 57.7–84.4 %, sucrose <0.4–6.4 % and HMF content <0.4-66.5 mg/kg for acacia honey, reducing sugars 60.0–88.0 %, sucrose <0.4-12.1 % and HMF content 0.8-250 mg/kg for blossom honey, reducing sugars 60.1–86.5 %, sucrose <0.4–0.7 % and HMF content 1.0-55.7 mg/kg for linden honey, reducing sugars 60.0–83.0 %, sucrose <0.4–1.1 % and HMF content 0.9-45.2 mg/kg for sunflower honey, reducing sugars 50.7–80.0 %, sucrose <0.4 % and HMF content 1.9-3.1 mg/kg for honeydew honey, reducing sugars 30.1–50.0 %, sucrose 11.0-25.2 % and HMF content 0.9-90.0 mg/kg for bakery honey. In this work, evaluated color, taste, smell and viscosity of honey meet characteristics for a particular kind of honey.
Conclusion: This paper gives an overview of current established quality criteria and the methods used for their determination. The aim was to compare the results of our analysis with reference values taken from national and international regulations and dealt with the different aspects of such analysis in detail. It has been observed that the honey produced and sold in Serbia is of very good quality with the exception of a very small number of false samples.

P151 Development of liposome containing bee venom extract from *Apis dorsata* for anti-aging

Somwongin S.[1], Chantawannakul P.[2,3], Chaiyana W.[1,4]
[1] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand; [2] Bee Protection Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; [3] International College of Digital Innovation, Chiang Mai University, Chiang Mai, Thailand; [4] Research Center of Pharmaceutical Nanotechnology, Chiang Mai University, Chiang Mai, Thailand

The aims of the present study were to investigate the antioxidant activity of bee venom extract from Apis dorsata and develop the liposome formulation for anti-aging.

Bee vonom extracted from *A. dorsata* was investigated for the melittin content by high performance liquid chromatography- and the antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl and 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay. Liposomes were prepared by using thin film method. Various factors, including effect of condition preparation (shaking time and sonication time), as well as liposome's compositions (cholesterol and surfactant) were investigated. The formulations were characterized by means of particle size, polydispersity index, zeta potential, and stability under heating-cooling cycles.
Melittin was detected as the major component of bee vonom extract from *A. dorsata* since the extract contained up to 95.8 ± 3.2% of melittin. In addition, bee venom extract possessed high antioxidant activity with the inhibition against DPPH● of 41.1±2.2% and Trolox equivalent antioxidant capacity of 10.21±0.74 mM Trolox/mg.
The most suitable liposome was prepared by using thin film method with 20 min of shaking and followed by 20 min of sonication. The compositions which produced the smallest internal droplet size of liposome included 45% w/w lecithin, 5 %w/w cholesterol, and 50 %w/w deionized water. The internal droplet size of the liposome was 442±24.42 nm with the polydispersity index of 0.49±0.06 and zeta potential of -39±0.31 mV. Additionally, the liposome was stable after the heating-cooling stability study.
In conclusion, bee venom extract from *A. dorsata* possessed high antioxidant activity which could be useful for using as the active ingredient for anti-aging. Besides, liposome containing bee venom extract from *A. dorsata* was suggested to be used as anti-aging cosmetic products.

P152 Increased fluctuating asymmetry in honey bee drones exposed to neonicotinoid pesticides

Friedli A.[1,2], Bruckner S.[1,2,3], Williams G.R.[1,2,3], Neumann P.[1,2], Straub L.[1,2]
[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Swiss Bee Research Centre, Agroscope, Bern, Switzerland; [3] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

Fluctuating asymmetry (FA) of morphological traits can be influenced by environmental stress. Therefore, FA can be used as a proxy for developmental homeostasis. One example of an environmental stressor could be neonicotinoid pesticides, which have been shown to yield clear sublethal effects on honey bees. Given that FA can be used as a reliable measure for sublethal effects, its use as a proxy could serve as a tool for enhancing risk assessment efforts. Currently no data exist on how neonicotinoids may affect drone developmental stability. Here, we examined effects of two neonicotinoids (thiamethoxam and clothianidin) on forewing size and shape of newly emerged drones (N=152). Local, queenright colonies of equal strength (N=20) were randomly allocated to two treatments (1. Field relevant concentrations of both neonicotinoids or 2. No neonicotinoids), and exposed for eight weeks in early spring 2015. Individual drone forewings were carefully dissected post emergence and were mounted on a cover slide so that digital images could be taken. Using MorphoJ software and the Procrustes superimposition method, 16 individual landmark coordinates were measured to enable shape characterization and to assess asymmetry. Our results revealed no significant effects of neonicotinoid pesticides on wing size or wing size FA ($F1 = 0.46$, $p = 0.49$); however, a significant treatment effect on wing shape and wing shape FA was observed ($F28 = 4.6$, $p < 0.001$), whereby drones exposed to neonicotinoids demonstrated increased wing shape FA. These results represent novel evidence of a sub-lethal effect on the development of a male

insect, likely due to applied neurotoxins acting upon factors involved in developmental stability of individual drone larvae. FA appears to be a valuable tool for detecting sublethal environmental stress in honey bees and other non-target organisms, and so could serve as a inexpensive and highly sensitive bio-indicator for future risk assessments.

P153 Drone's endophallus pigment: comparative study of absorption spectra of four *Apis* species

Khongkhuntian T.[1], Phiancharoen M.[1], Chomphuphan S.[1], Koeniger N.[2], Koeniger G.[2]
[1] *King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand;* [2] *University of Würzburg, Behavioral Physiology and Sociobiology (Zoology II), Biocenter, Am Hubland, Würzburg, Germany*

The cornua gland's orange color secretion of drone's endophallus is commonly found in all Apis species. Though its function still unknown, the color must have some effect to mating behavior, as the drone put resources into it. In this study we compared the absorption spectra of the pigment extracted from endophallus of four Apis drone species(*A.florea*, *A. cerana*, *A. dorsata* and *A. mellifera*) in order to test the differences which might lead to understand its function.In contrast with the quantity of the pigment, the uniformity of absorption spectra of the four species can be seen. The first and the second prominent peaks of all species were in the UV-range spectrum, from 230-300nm. However, the third peak at 450 nm of the pigment in A. florea set itself apart from other three species which was found around 385nm. Though the third peak differed in A. florea, all the peaks were in bee-visible range. (Menzel and Blaker, 1975). It is possible that labelling the sting chamber of the queen with cornua gland's orange color secretion of drone's endophallus made the drone recognized the queen easier, thus the successful multiple mating as suggested by Koeniger(1990), which demonstrated that drone use visual differences between the queen and drones for final guidance to the queen.

P154 Honeybees (*Apis mellifera*) and bee pollen as bio-indicators of heavy metal pollution in different geographic areas

Pașca C.[1], Dezmirean D.[1], Mărghitaș Al.L.[1], Bobiș O.[2], Bonta V.[2], Aurori A.[1]
[1] *Department of Apiculture and Sericiculture,* [2] *Life Science Institute "King Michael I of Romania", University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*

Honeybees (*Apis mellifera*) may forage over a distance of several kilometres from the hives for nectar, pollen and even water and they effectively sample the environment for contaminants in plants, soil and atmosphere. If present, these contaminats can be detected in the hive products or directly in the honeybee`s bodies. The search of metal concentrations has become a priority for metal pollution monitoring allover and also in Romania.
The aim of this study was the screening of honeybees and bee collected pollen from different geographic areas with different levels of pollution. The studied metals were Al, Cd, Cr, Cu, Fe, Mn, Na, Ni, Pb, and Zn. Mineralization of the samples was performed in a microwave furnace, Berghof digestion system MWS-2 and the quantitative analysis was done using an Aanalyst 800 Atomic Absorption Spectrometer from Perkin-Elmer.
Different samples (honeybees and multifloral bee pollen) from distinctive areas of Romania: Mureș, Sălaj, Cluj, Dolj, Maramureș counties were collected. Samples of bee pollen were separated by color and microscopically analyzed for identification of the flower of origin, palinologically identified to have monofloral pollen. Lead concentration in two samples of bee pollen was 0.168 mg/kg (Mureș) and 0.287 mg/kg (Dolj), the rest of the samples being free of this heavy metal.
These results suggest the usefulness of honeybees and bee pollen as biological indicators for pollution.

P155 Multi-stress approach for the assessment of decline causes for honeybee

Tremolada P.[1], Palamara Mesiano M.[2], Parolini M.[1], Giacchini R.[3], Benocci R.[3], Marino G.[3], Parenti P.[3], Lavazza A.[4], Bassi S.[4], Colombo M.[2], Lupi D.[2]
[1] University of Milano, Department of Environmental Science and Policy, Milan, Italy; [2] University of Milano, Department of Food, Environmental and Nutritional Sciences, Milan, Italy; [3] University of Milano-Bicocca, Department of Earth and Environmental Sciences, Milan, Italy; [4] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy

Honeybee decline is a problem of high concern since a complex pathology (Colony Collapse Disorder) brought to worldwide events of colony losses. Many adversities may be responsible of this decline: recrudescence of old and new pathologies, contamination from pesticides and emerging contaminants (e.g. nanoparticles) and environmental stresses. Stress factors may interact among them with additive or synergic effects and, currently, a multi-stress condition is accepted as the most probable decline cause for honeybees. Experimental hives were placed in two experimental sites in Northern Italy: an exposure site ES and a control site CS (14 km far from ES, agricultural field and significant human settlements). ES is located inside an experimental farm where a high-voltage electric line is present together with a complex and controlled pesticide application schedule for orchards. In the ES, two experimental area were set up (one just below the electric line, with the combined presence of electromagnetic fields and sublethal pesticide exposure SPE, and one exposed only to SPE). Honeybees were sampled from April to October 2017 weekly for health status (mites, virus, bacteria spores and fungi), and population parameters (queen and brood status and food stokes, together with daily mortality), and monthly for biomarker analyses (acetylcholinesterase, catalase, glutathione S-transferase and alkaline phosphatase activity, amount of reactive oxygen species, lipid peroxidation, and DNA fragmentation). Preliminary analysis revealed that population parameters and biomarkers were both affected by stresses. Effects on biomarkers were registered in relation to pesticide applications. Multi-stress position showed the most severe effects, leading to colonies death. Colony losses were related to the development of different pathologies. Reduced immunity defences and social disorders by frequent queen replacement seem to be the main effects related to the multi-stress condition.

P156 The brood survival rate of a colony predicts the adult emergence rate of its larvae reared in vitro

Mortensen A.N.[1,2], Ellis J.D.[1]
[1] Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; [2] Pollination & Apiculture, Plant & Food Research, Hamilton, New Zealand

Rearing honey bees, *Apis mellifera* L., in vitro can eliminate many uncontrollable factors (e.g. colony strength, weather conditions, food availability, etc.) that bias field studies on honey bee development and health. There have been significant improvements in the methodology used to rear honey bee larvae artificially, and high survivability can now be achieved in the laboratory. However, variable in vitro survival rates are still reported within and between laboratories. We conducted a comparative study of the survival rates of larvae in two different rearing environments: their parental colony (hive-reared) and in the laboratory (in vitro-reared). There was no statistically detectable difference in the percent survival to adult emergence of hive-reared and in vitro-reared bees. Furthermore, hive-reared brood survival percentage at day 11 (the prepupal stage) was predictive of the survival percentage to adult emergence of bees reared in vitro. We suggest that the 11 day brood survival percentage should be used when selecting suitable source colonies for in vitro-rearing risk assessments. Based on our results, colonies with brood survival percentages of ≥80% are suitable colonies from which to source larvae for in vitro-rearing risk assessments.

P157 The adverse effects of nanosilver on *Apis mellifera carnica*

Jemec Kokalj A., Glavan G., Drobne D., Kos M.
Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Nosema is regarded as one of the causes of colony collapse disorder and there is a great need to provide efficient treatment techniques. Among these, colloidal and nanosilver particles are increasing being used by some honeybee keepers. However, up to date few data regarding potential side effects on honeybees exists. In this study, we investigated chronic (9 days) effects of silver nanoparticles on survival of summer honeybees, activities of enzyme involved in cholinergic nerve transmission (acetylcholinesterase; AChE) and activities of detoxification enzyme glutatione S- transferase (GST). Honeybees were fed with sucrose solution containing nanosilver (2, 10, 50, 250 and 500 mg/L). Some of these concentrations (12.5 and 25 mgL nanosilver) were previously found to have an effect on decreasing *Nosema* spp spores (Borsuk et al. 2013. Med. Weter, 69, p.730). We observed increased mortality of honeybees at the highest exposure concentration, however signs of detoxification as measured with GST activity were evidenced already at 2 mg/L and increased significantly in dose-response manner. The activity of membrane AChE in the head of honeybees was significantly increased at all exposure concentrations in comparison to controls. This indicates that cholinergic transmission was affected. In line with this we also observed difficulties in locomotion (staggering) of honeybees at high exposure concentrations. We conclude that nanosilver presents a potential hazard to honeybees and should not be used without caution.

P158 Effects of a potential natural acaricide carvacrol on carnolian honeybee (*Apis melifera carnica*)

Jemec Kokalj A., Božič J., Novak S., Glavan G.
Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Beekeepers mostly use different synthetic acaricides to keep *Varroa* mite populations under control. Due documented adverse impact of these substances on honey bees, there is a growing research effort to imply alternative treatments with naturally derived acaricides. Monoterpenoid carvacrol, the major compound of many oregano essential oils, possess a high acaricidal potential. The mode of action through which carvacrol exerts its insecticidal and acaricidal activities is the inhibition of acetycholineesterase (AChE), a marker of cholinergic neurotransmission in honeybees. However, the impact of carvacrol on honeybees was never investigated before. Here we studied the effects of long term 7-day consumption of carvacrol on survival and on activities of AChE and detoxifying enzyme glutathione S-transferase (GST) in honey bee head and thorax. First, a range finding test with 0.05%; 0.5% and 5% (w/w) of carvacrol was done. In this case, 21±7 % mortality was found at 0.5% and 100% of honeybees died at the highest exposure (5%) of carvacrol. Subsequently, an experiment with 0.05 and 0.5% (w/w) carvacrol was done. In this case, 30±7 % mortality was observed at 0.5% and 19±1 % at 0.05% of carvacrol. The activity of the indicator of detoxification process GST was elevated in the head and thorax. The activity of AChE was elevated in the head at 0.05 and 0.5 % showing that carvacrol does not inhibit AChE in honeybees as anticipated from literature. Nevertheless, our results showed that activity of honeybee AChE was affected by carvacrol indicating that it could have adverse effects on honey bee nervous system. We conclude that the use of natural essential oils containing carvacrol in acaricidal purposes may be limited by its negative impact on honeybees.

P159 Hemolytic activity of pathogenic bacteria, erythrocyte membrane protection, and immunostimulatory effects of Saudi honeys

Khan K.A.[1,2], Ibrahim E.H.[2,3], Ghramh H.A.[1,2,4]
[1] Unit of Bee Research and Honey Production, Faculty of Science, King Khalid University, Abha, Saudi Arabia; [2] Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia; [3] Department of Blood Products Quality Control and Research, National Organization for Research and Control of Biologicals, Cairo, Egypt; [4] Research Center for Advanced Materials Science (RCAMS), King Khalid University, Abha, Saudi Arabia

Bacterial pathogens challenge mankind by spreading numerous diseases and have become serious threats for immunocompromised patients in recent years. The emerging resistance in bacterial pathogens has drawn attention toward natural agents with medicinal and immunostimulatory effects. Honey is a well-documented natural substance with high medicinal properties and these benefits are due to the presence of polyphenols and flavonoids.
Objectives: The present study was designed to investigate (i) the hemolytic activity of pathogenic bacteria, to measure (ii) the red blood cell (RBC) membrane protection effect, and to investigate (iii) the immunostimulatory effects of Saudi honeys.
Materials and methods: Hemolytic activity of pathogenic bacteria and erythrocyte membrane protection effect was measured by liquid hemolysis assays while, the immunomstimulatory effect of honey sample was tested in murine spenic cells by measuring cell viability after incubation with honey samples for 24 h.
Results: All the studied bacteria exhibited hemolytic activity on cow RBCs greater than 10%. Hundred percent erythrocyte membrane protection was observed for each tested honey sample. Both diluted (20% v/v), and concntrated honey samples (Majra, Dharm, and Sider honeys) stimulated the proliferation of splenic cells on dose dependent manner.
Conclusions: All the tested bacteria had hemolytic activity and were pathogenic. Saudi honey samples exhibited 100% erythrocyte membrane protection effects. Saudi honey has immunostimulatory effect and could boost the immune system.

P160 Field effects of two neonicotinoid pesticides on honey bee drones, *Apis mellifera*

Straub L.[1,2], Kolari E.[1,2], Villamar-Bouza L.[1,3], Bruckner S.[1,2,4], Vidondo B.[5], Chantawannakul P.[6], Maitip J.[6,7], Williams G.R.[1,2,4], Neumann P.[1,2]
[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Swiss Bee Research Centre, Agroscope, Bern, Switzerland; [3] EFSA European Food Safety Authority, Pesticides Unit, Parma Italy; [4] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA; [5] Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [6] Bee Protection Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; 7 Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Rayong Campus, Rayong, Thailand

There is clear evidence for field effects of neonicotinoid pesticides on female diploid honey bee, *Apis mellifera*, queens and workers. However, field effects on drones have yet to be investigated, despite their importance as male sexuals and the possibility of higher susceptibility as predicted by the haploid susceptibility hypothesis. In light of previous cage experiments, reduced drone longevity and reproductive capacity as well as imparired behavior are expected. Here, we examined effects of two neonicotinoids (thiamethoxam and clothianidin) on reproductive capacity traits (sperm viability & quantity (N=347)), drifting behavior (N=2952) and longevity (N=3272) of individual adult drones in the field. Local, queenright colonies of equal strength (N=30) were randomly allocated to one of two treatments (1. Field relevant concentrations of both pesticides or 2. No pesticides) for eight weeks in early spring 2016. Newly emerged drones were colony-specific labeled and reintroduced into their maternal colonies to monitor drone lifespan, drifting behavior and reproductive traits. The data show that drones originating from pesticide treated colonies had a reduced sperm viability (meglm, $p < 0.001$) and total living sperm quantity (meglm, $p = 0.03$) as well as reduced longevity (mstreg, $p < 0.001$) compared to the controls. There was no significant difference between treatment groups for sperm quantity (meglm $p = 0.34$). Drones originating from pesticide treated colonies drifted more often (xtmixed, $p < 0.01$). The results

clearly demonstrate that thiamethoxam and clothianidin can negatively affect drone longevity, behavior and reproductive capacity in field colonies, thereby confirming previous laboratory studies. Since mating is the only significant function of drones, the compromised reproductive capacity resulting from of colony exposure to neonicotinoids may play a key role in increased queen failures reported across the northern hemisphere. Laboratory studies appear to be sufficient for future risk assessments of the effects of pesticides on male honey bees. The results support that the widespread prophylactic use of neonicotinoids may have previously overlooked contraceptive effects on non-target insects, thereby limiting conservation efforts.

P161 The ups and downs of hops (*Humulus lupulis*) beta acids in *Varroa* control

Moskric A., Bubnic J., Smodis Skerl M.I., Presern J.
Agricultural Institute of Slovenia, Animal Science Department, Beekeeping, Ljubljana, Slovenia

Parasitic mite *Varroa destructor* (*Varroa*) is one of the major threats to health of honey bee colonies worldwide. In search for natural substitute product that suppress the mite in honey bee population without negative impacts on honey bees, their products and environment, hops (*Humulus lupulus*) beta acids (HBA) were suggested to have potential in suppression of the mite and are safe to be used in treating honey bee colonies that are infected with *Varroa*.

Cardboard impregnated with HBA for insertion into the infested hive is commercially available and claims to has sufficient efficiency in *Varroa* control without negative impact on honey bees and environment. However the research on HBA influence on honey bees and the mechanism of action on suppression of the mite are scarce. There is no record on the effect of HBA on behavior of honey bees as a potential sign of stress. Therefore we were interested in influence of HBA on the behavior of workers.

For observation purposes, two observation hives were set-up. The allogrooming dances were video-documented before, in-between, and after the insertion of cellulose carrier containing 40 % HBA, and mite fall during the experiments was counted in parallel. The number of allogrooming dances after insertion of cellulose carrier with HBA almost doubled on the first day and dropped sharply the next day. A peak in mite fall and a quick drop afterwards was also observed. No such change was observed neither in number of fallen mites nor in number of grooming dances in the control hive.

This is the first observation of the influence of HBA on behavior of honey bees in hive. Although preliminary this study suggests that HBA inserted in the hive may present a stress for honey bees and stimulate them into performing allogrooming dances as a mechanism to eliminate the stressor. Thus to answer the question of suitability of HBA to fight *Varroa*, the next step is to determine the mechanism of its action on honey bees on molecular level.

P162 The impact of in hive pesticide contaminations on honey bee mortality

El Agrebi N., Saegerman C.
Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine of Liège, University of Liege, Liege, Belgium

In Europe, colony success depends strongly on the management of the ectoparasitic bee mite, *Varroa destructor*, which historically has been achieved using acaricides that have an impact on honey bee health. Despite honey bees being managed as a domestic pollinator, the impacts of the use of veterinarian pesticides has often been overlooked as a possible factor influencing bee mortality. Honey bees are at risk of being exposed to a broad set of chemicals originating from their environment, inside and outside the hives, from agriculture and from beekeeping practices. In an attempt

to better understand the in hive pesticide burden, a pesticide screening (N=186) on the presence of 293 substances was realised in foundation wax by LC–MS/MS and GC–MS/ MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS method and a hazard quotient was calculated. The significant levels of pesticides that are found preserved in wax can be explained by the lipophilic properties of these; the majority of the contaminants are stable and remain unchanged during the process leading to the re-use of the wax by most beekeepers. The pesticide burden in wax was correlated to bee mortality using a logistic regression model. The contamination level found in foundation wax raise our concern. Nevertheless, no correlation was found between pesticide burden in wax and bee mortality. The benefit of pesticides in controlling *Varroa* infestations should be considered as regard to their toxic effects on bees. Proper diagnosis of Varroa infestation rates should be generalised before using acaricides with parsimony. More efforts are needed in research to characterise the total pesticide burden, which bees and larvae are confronted to in wax, beebread, pollen, nectar, water, honey and propolis.

P163 Enhancement of chronic bee paralysis virus levels in honeybees acute exposed to imidacloprid: a Chinese case study

Diao Q.[1,2], Li B.[1,2], Zhao H.[3], Wu Y.[1,2], Guo R.[4], Dai P.[1,2], Chen D.[4], Wang Q.[1,2], Hou C.[1,2]
[1] Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; [2] Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Beijing, P.R. China; [3] Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou, P.R. China; [4] College of Bee Science, Fujian Agricultural and Forestry University, Fuzhou ,P.R. China

Though honeybee populations have not yet been reported to be largely lost in China, many stressors that affect the health of honeybees have been confirmed. Honeybees inevitably come into contact with environmental stressors that are not intended to target honeybees, such as pesticides. Although large-scale losses of honeybee colonies are thought to be associated with viruses, these viruses usually lead to covert infections and to not cause acute damage if the bees do not encounter outside stressors. To reveal the potential relationship between acute pesticides and viruses, we applied different doses of imidacloprid to adult bees that were primarily infected with low levels (4.3×105 genome copies) of chronic bee paralysis virus (CBPV) to observe whether the acute oral toxicity of imidacloprid was able to elevate the level of CBPV. Here, we found that the titer of CBPV was significantly elevated in adult bees after 96 h of acute treatment with imidacloprid at the highest dose 66.9 ng/bee compared with other treatments and controls. Our study provides clear evidence that exposure to acute high doses of imidacloprid in honeybees persistently infected by CBPV can exert a remarkably negative effect on honeybee survival. These results imply that acute environmental stressors might be one of the major accelerators causing rapid viral replication, which may progress to cause mass proliferation and dissemination and lead to colony decline. The present study will be useful for better understanding the harm caused by this pesticide, especially regarding how honeybee tolerance to the viral infection might be altered by acute pesticide exposure.

P164 The effects of tau-fluvalinate and tebuconazole on honeybee (*Apis mellifera*) queens

Raimets R., Karise R., Naudi S., Mänd M.
Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia

Honeybee (*Apis mellifera*) queen is the most important member of the colony. The main aim of a mated honeybee queen is to assure colony development and survival via laying eggs (Milchreit et al. 2016). As a feed, the queens are receiving royal jelly excreted by young nurse bees (Böhme et al. 2018). Several conducted studies show that honeybee food, nectar and pollen, may be contaminated by various pesticides (Chauzat & Faucon 2007; Mullin et al. 2010). However, a study conducted by Böhme et al. (2018) shows that nurse bees, who were fed on contaminated food, still produce pure royal jelly for honeybee queens. Despite the fact of being fed with clean feed, there is still always persis-

tent threat to pesticide exposure. Different lipophilic pesticide residues have been found from honeybee wax (Chauzat & Faucon 2007; Ravoet et al. 2015). Developing larval honeybee queens may be exposed to pesticides via contaminated wax of queen cell cups. In addition to agricultural routes, the contamination may originate also from apiculture itself. The aim of this study was to investigate whether the field realistic concentrations of lipophilic pesticides (tau-fluvalinate and tebuconazole) mixed to the wax of queen cell cups are affecting honeybee queens development and maturation. During the three consequtive experimental years (2016-2018) queen cell cups were made of honeybee wax, which was purchased from an organic beekeeping operation. Pesticides tau-fluvalinate and tebuconazole were mixed into wax in lower and higher field realistic concentrations. The treatment groups were following: 1) tau-fluvalinate alone 2) tebuconazole alone 3) tau-fluvalinate and tebuconazole mixture. 1-2 days old honeybee worker larvae were grafted into treated and untreated queen cell cups and placed into queenless colonies. The parameters measured were: grafted larvae acceptance or rejectance by bees; queen hatching; newly hatched queen weight; queen mating success. This study helps to fill the gap in knowledge, whether the pesticides in honeybee wax are affecting honeybee queen development or not.

P165 Thyme nectar and pollen terpenes potentially improving honey bee health

Erler S.[1], Fischer J.[1], Heidler J.[1], Lewkowski O.[1], Degenhardt J.[2], Wiese N.[2]
[1] Molecular Ecology, [2] Pharmaceutical Biotechnology, Martin-Luther-University, Halle (Saale), Germany

Host-plant-parasite interactions of pollinating insects and their foraging target plants are mainly studied on the host-parasite or host-plant but rarely on plant-parasite level. However, for pollinating insects foraged products present an additional tool for fighting against parasites and pathogens. Especially secondary plant metabolites in nectar, pollen and honey are known for their antibiotic effects which are used by individual worker bees and are transmitted via trophallaxis to the whole colony. Here, we show the antimicrobial potential of nine Thymus vulgaris secondary metabolites against bacteria associated with European foulbrood disease in the honey bee *Apis mellifera*. A high-through put cell growth inhibition assay was used to estimate substance specific IC50 values for each bacterial strain. Comparing the results across all tested strains, carvacrol and thymol showed to have the highest antimicrobial activity. Synergistic effects may increase the inhibitory effects observed for single substances, as T. vulgaris essential oils are mixtures of monoterpenes and acetates. All substances could be detected in floral nectar and pollen of six different thyme chemotypes, by means of GC-MS and GC-FID. Electroantennography revealed that young and old worker honey bees' antennae perceive the different thyme terpenoids. It seems that honey bees can forage for highly antibiotic plant secondary metabolites present in floral nectar and pollen of *T. vulgaris*.

P166 The Norwegian bumblebees in the face of climate change

Ahuja I.[1,2], Michez D.[3], Dahle S.[4], Martinet B.[3], Brasero N.[3], Bones A.M.[1], Rasmont P.[3], Ødegaard F.[4]
[1] Department of Biology, Norwegian University of Science and Technology (NTNU), Realfagbygget, Trondheim, Norway; [2] Norwegian Centre for Organic Agriculture (NORSØK), Gunnars veg, Tingvoll, Norway; [3] Laboratory of Zoology, Biosciences Institute, University of Mons (UMONS), Mons, Belgium; [4] Norwegian Institute for Nature Research (NINA), Trondheim, Norway

The bumblebees (*Bombus*spp.) are one of the most eye-catching insects in this World. They comprise an important part of our ecosystems through their significant role as pollinators for a variety number of plants, especially in the temperate, boreal and arctic ecosystems. From the 250 species of bumblebees, which exist globally, 35 species have been found in Norway (i.e. 14% of the global diversity). In latitudes of Norway, bumblebeesare widely distributed in natural habitats from coasts to mountains. Some of the most common species are: *Bombus-hypnorum*, *lucorum*, *terrestris*, *hortorum*, *soroeensis*, *lapidarius*, *pascuorum*, *pratorum*, and *sylvestris*. Many bumblebees species declined during the last 100 years. Climate change, for which the clear evidence is there, is probably one of the main factors explainingshifts in the

distribution of bumblebees across Europe. Besides other European countries, Norway is also expected to be highly impacted by global warming. In this country, we have beenalready observing that the mountain bumblebees occur more than 100 meters further up than just a few decades ago. If these changes occur faster than the vegetation manages tofollow, the access to the resources must be a problem. The examples, the bumblebee species that would be affected by the global warming are: *B. alpinus*, *B. cingulatus*, *B. consobrinus*, *B. humilis* and *B. polaris*. In this communication, we will present the distribution and mode of living of the bumblebees recorded in Norway. Additionally, we will highlight the first results from the heat-shock(34°C) trial-experiments that have been conducted with the Norwegian bumblebees under controlled conditions. The bumblebees were collected from different parts of Norway in 50ml tubes (containing BioGluc solution and flowers), and were kept in the growth cabinet for 48h. Some bumblebees died within 24h of heat-shock, but many of them like *B. hypnorum*, *B. soroeensis*, and the *Bombus* sp. of the subgenus *Thoracobombus* survived 48h of heat-shock. This pilot study canprovideus the basic information about how the bumblebees will respond towards a heat-wave, which is becoming quite usual in a country like Norway, like in July this year, the day/night temperatures have been recorded >30°C/20°C.

P167 BPRACTICES and Hivelog web application for honey bee products traceability

Pietropaoli M.[1], Vejsnæs F.[2], Kilpinen O.[2], McCabe P.[3], Jannoni-Sebastianini R.[3], Jørgensen A.S.[2], Lietaer C.[4], Formato G.[1]
[1] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri", Rome, Italy; [2] Danish Beekeepers Association, Sorø, Denmark; [3] International Federation of Beekeepers' Associations, Rome, Italy; [4] Tecnologies and practices for small agricultural producers (TECA) platform of the Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

BPRACTICES (www.izslt.it/bpractices) is a project funded from the H2020 ERA-Net SusAn – European Research Area on Sustainable Animal Production Systems, that aims to develop a sustainable beekeeping breeding system by implementing innovative management practices (Good Beekeeping Practices). The Hivelog web application (www.hivelog.dk) is a free application for smartphones, tablets and personal computers, able to record the most important apiary management data like colony strength, queen's performances, feedings, honey harvest, varroa situation (treatments), colony behavior, sanitary status, developed by the Danish Beekeepers Association to improve the general data collection within danish beekeeping. The backbone of the program is to keep it simple and easy to use. The program is already translated into 8 languages. In the future the program will be open source, so that beekeepers groups are expected to continue the development. During the 36 months of BPRACTICES project, an innovative traceability system will be set up to inform beekeepers on the innovations proposed with the new management system . The traceability system will be integrated into the Hivelog program with an interface to be used during the hive products processing to help beekeepers to maintain product traceability thanks to QRCode/RFID technology (from flower to bee colony to extraction to filling to consumer). Users will be able to record harvest data (lot number, quantity), attach analytical results, and to know all details about the colonies that produced those products. Consumers, accessing the application directly from the jar, will be educated to responsible consumption and will be made aware of the benefits of consuming a product deriving from an environmentally-friendly management, increasing the development of local productions. The traceability system will be implemented thanks to a consumers' panel during the second and third year of the project through a social research technique.

P168 Possible side effects of sugar supplementary nutrition on honey bee health

Frizzera D., Annoscia D., Del Fabbro S., Nazzi F.
Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy

Food shortage, along with biotic stressors (e.g. parasites and pathogens) is a leading factor of winter honey bee colony losses. To support honey bee colonies, beekeepers normally supply homemade syrups which could contain compounds (e.g. hydroxymethylfurfural, HMF) with possible negative side effects. However, literature on this subject is unclear; in

particular, both the toxicity of HMF for bees and its concentration in homemade syrups are still unknown.
In this study, we tested the survival of uninfested and mite infested bees fed with different doses of HMF as estimated according to the literature. We then quantified the concentration of HMF in homemade syrups produced with different temperature, pH and storage methods and supplied these syrups to newly emerged bees to assess their survival.
We show that doses of HMF similar to those reported as sublethal in the literature appear to be non-toxic also for mite infested bees; however, the amount of HMF that can be found in homemade syrups, which increases with temperature and acidity, can be much higher and cause significant bee mortality.

P169 Fat body evaluation of wintering bees with NIR (near infrared spectroscopy)

Cabbri R., Giaretta E., Galuppi R., Isani G., Formigoni A.
University of Bologna, Department of Veterinary Medical Sciences, Ozzano dell'Emilia, Italy

Near infrared spectroscopy (NIR) is a technique widely used in insect research. It has successfully determined sex, age and spotted parasitized insects. However, its applications, has not been investigated in apidology: to our knowledge only one study, focused on differentiating mated and unmated queens has been conducted on Apis mellifera. Given the importance of the physiological condition of the bees composing the cluster for wintering success of the colony, we considered the idea of using NIR to estimate the lipid content of the fat body in order to quickly assess their nutritional status. To do so we sampled 100 bees from 4 different colonies in December 2017; each bee was freeze killed and then its spectra was acquired with the FT-NIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) first with the whole bee and then with the intestine removed from the gastrum. The reference assay used for lipid quantification was a modified sulfo-phospho-vanillin colorimetric method. Fat content obtained with the chemical analysis resulted in 346 ± 147 μg/bee. Spectral data were analysed using the Partial Least Squares (PLS) statistical method by OPUS spectroscopy software. The automatic optimization method of OPUS software was used to determine the useful spectral ranges and the best pre-processing methods. The coefficient of determination (R2), the ratio performance deviation (RPD) and the root mean square error of prediction (RMSEP) were used to evaluate the regression equations. The best predictive equation was obtained in test-validation (with 10 test samples), using the spectra obtained from the bees without intestine (R2 = 86.68, RPD = 2.81). Nevertheless, all the calibration models showed high RMSEP and low R2, both in cross-validation and in test-validation (with 30 or 50 test samples), suggesting us that more samples, obtained from different seasons, have to be added to the model in order to try to increase its range and its accuracy.

P170 Influence of pH on stability and structure of major royal jelly proteins

Mureşan C.I.[1], Buttstedt A.[2]
[1] Universitatea de Ştiinţe Agricole şi Medicină Veterinară, Departamentul de Apicultură şi Sericicultură şi Biotehnologii, Cluj-Napoca, Romania; [2] B CUBE - Center for Molecular Bioengineering, Technische Universität, Dresden, Germany

Royal jelly (RJ) is a secretion of the hypopharingeal and mandibular glands of young worker bees that take care of the growing brood in the hive. RJ is fed to the larvae for the first three days after hatching and to the queen bee for her entire life. About 50% of the dry weight of RJ is composed of proteins from which up to 90% are the major royal jelly proteins (MRJPs). These proteins are produced in the hypopharingeal glands at a pH value of 7.0 and after being secreted they mix with the acidic mandibular gland secretion composed mainly of fatty acids giving to the final product a pH of 4.0. After the publishing of the complete genome of the honeybee, mrjps genes were proposed to occur in various tissues, including the bee brain. The pH is well known for influencing the behaviour of proteins. Since MRJPs must be stable over a broad pH range starting from their site of synthesis (the endoplasmic reticulum with pH 7.0), until their area of action (the RJ in the queen cell with pH 4.0 or the bee brain), we estimated the pH dependent stability of MRJP1, MRJP2 and MRJP3 purified from three different RJs with the help of differential scanning fluorimetry which allows measuring

thermal unfolding of proteins in the presence of a fluorescence dye (SYPRO® Orange). The fluorescence intensity of MRJPs at a 2 μM concentration and SYPRO® Orange at a dilution of 1:1000 in 50 mM Na2HPO4/citric acid pH 2.5-8.0 or 50 mM Na2CO3/NaHCO3 pH 9.0-11.0 was measured using the FRET channel of the Real-Time System (excitation: 450-490 nm, detection: 560-580 nm). All the proteins were most stable at pH 4.0 compared to pH 7.0 with MRJP3 being the most stable at pH 4.0.

P171 Molecular mechanism study of honeybee caste differentiation regulated by the comformation change of major royal jelly protein 1

Tang J., Chen C., Chen X., Shi W.
Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

Caste differentiation is an important foundation of insects sociability formation. In its natural state, royal jelly major protein 1 has two forms of monomer and polymer without mutually transformation, royal jelly major protein 1 (MRJP1) monomer can activate epidermal growth factor receptor (EGFR) in honeybee's fat body and regulate the emergence of honeybee's caste differentiation. But the reports about the effect of MRJP1 conformational change on caste differentiation and the molecular mechanism of EGFR activated by MRJP1 are limited. In this project, we plan to determine the three-dimensional structure of MRJP1 monomers by X-ray diffraction and analyze the conformational difference with the polymers. Then, the phenotypic differences of
one-day old larvae after feeding different MRJP1 are analyzed to explore the pivotal role of MRJP1 conformational change in caste differentiation, combined with the detection of EGF transcription/translation level by real-time PCR and western blot. GST pull-down and peptide array technology were used to verify the interaction between MRJP1 and EGFR, to locate the interaction region and the key residues. Meanwhile, we will confirm the key residues by site-directed mutagenesis to illuminate the mechanism of MRJP1 interaction with EGFR. At present, we have successfully obtained Apis mellifera recombinant protein of MRJP1 and EGFR by High Five insects cell line respectively and screened the protein crystals of MRJP1 .

P172 Colonies and queen replacement strategies: a systemic experiment

Kouchner C.[1,2,3,4], Basso B.[1,4], Decourtye A.[1,4,5], Tchamitchian M.[2], Ferrus C.[1,4], Le Conte Y.[3,4]
[1] ITSAP-Institut de l'abeille, Avignon, France; [2] ECODEVELOPPEMENT, INRA, Avignon, France; [3] Abeilles et Environnement, INRA, Avignon, France; [4] UMT PrADE, Avignon, France; [5] ACTA, Avignon, France

Despite annual colony losses that can be high, professional beekeepers have to keep enough healthy colonies to ensure the sustainability of their farm. Creation of artificial swarms, requeening, weak colonies management and other practices contribute to colony replacement and losses management. As these different practices interact with each other, they should be considered together as overall strategies rather than as independent practices. These strategies may have impacts on the availability of a sufficient number of productive and healthy colonies and may have other consequences on the farm sustainability.
To assess the consequences of different management strategies, we settled in 2016 a systemic experiment that compares two strategies that were defined according to French professional beekeepers' practices, as identified through a survey conducted among professional beekeepers. Each strategy is defined as a consistent set of practices and decision rules for colonies and queen replacement, with requeening as a focus of the compared strategies. No queen replacement and no introduction in artificial swarms are the main rules of the first strategy. The second includes yearly requeening and introduction of bred queen in artificial swarms. Two sets of colonies are managed according to these strategies, and a minimal number of 60 colonies per modality is kept by creating artificial swarms to replace losses. Within each strategy, two different honeybee strains are used to assess potential interactions with management strategy.
Performances of the tested strategies are compared through a multidimensional analysis that includes the possibility

of maintaining a minimal colony number, which was different between the four sets of colonies. Such a systemic experiment requires an adapted analysis but allows to study the consequences of an overall replacement strategy, that includes several management practices and their possible interactions.

P173 Sustainability of beekeping farms: a definition proposal

Kouchner C.[1,2,3,4], Ferrus C.[1,4], Blanchard S.[1], Decourtye A.[1,4,5], Basso B.[1,4], Le Conte Y.[3,4], Tchamitchian M.[2]
[1] ITSAP-Institut de l'abeille, Avignon, France; [2] ECODEVELOPPEMENT, INRA, Avignon, France; [3] Abeilles et Environnement, INRA, Avignon, France; [4] UMT PrADE, Avignon, France; [5] ACTA, Avignon, France

Through apiculture, honeybee colonies provide both bee products (honey, royal jelly, propolis, pollen, queen and swarm production) and pollination services. Despite the facts that professional beekeepers manage most of the honeybee livestock and that honeybee is a well-studied social insect, there is still little work on honeybee farm management and on their sustainability, which includes social, economic and environmental dimensions.
Through a participatory methodology, we adapted the concept of sustainability, already commonly used in other agricultural sectors, to the specificities of French beekeeping farms. Various points of views on sustainability of beekeeping farms were collected through individual interviews: professional beekeepers, beekeeping advisors, research and development experts and other stakeholders from apicultural sector were involved. The numerous suggestions for sustainability components that resulted from these interviews were then collectively discussed and organized into a consistent framework.
This framework includes technical, economic, social and environmental items that are distributed into six main themes: economic viability, quality of life, environmental impact, local development, contribution to current stakes of the value-chain and production security. It partly covers common components of sustainability at farm level but also emphasizes some specificities of beekeeping, as the ability to face uncertainties and to ensure a production under environmental uncertainties, in an agricultural system that mainly depend on non-managed resource.
Our study resulted in the first sustainability framework adapted to beekeeping farms, which is the first step for development of an assessment tool at farm level. It also provides a definition of sustainability for beekeeping farms that allows some technical issues to be situated in a larger framework at farm level.

P174 Laurel (*Laurus nobilis*) extract induces gene expression of antioxidative enzymes in honeybees

Aurori A.[1], Bobiş O.[1], Dezmirean D.S.[1], Sisea C.R.[2], Diaconeasa Z.[3], Mărghitaş L.A.[1], Erler S.[4]
[1] University of Agricultural Science and Veterinary Medicine, Department of Apiculture and Sericulture, Cluj-Napoca, Romania; [2] University of Agricultural Science and Veterinary Medicine, Faculty of Horticulture, Cluj-Napoca, Romania; [3] University of Agricultural Science and Veterinary Medicine, Faculty of Food Science and Technology, Cluj-Napoca, Romania; [4] Martin-Luther-University Halle-Wittenberg, Institute of Biology, Department of Molecular Ecology, Halle (Saale), Germany

Objectives: Many plant extracts are known to be beneficial for health in general, and can be effective by reducing infections of different origin. Previously, we have shown that treatment of honeybees with laurel ethanolic extract (LEE) significantly decreased virus load and replication of the black queen cell virus (BQCV) in forager bees. Extracts in water (LWE) did not have antiviral effect. Very often antibiotic effects of plant extracts correlate with their antioxidant activity due to the polyphenols they contain. Indeed, LEE showed to have higher antioxidant activity in vitro, which was interpreted as indicator of its antiviral potency. However, laurel can have either antioxidant or pro-oxidant activity depending on the experimental system. Here, we assess its role on the oxidative status in honeybees.
Material and Methods: For this, the bees were fed with both LEE and LWE extracts. The relative gene expressions of antioxidative enzyme genes: catalase, CuZn superoxide dismutase 1 (SOD1), glutathione peroxidase (Gtpx-1), glutathione

S-transferase (GstD-1), Mn superoxide dismutase 2 (mtSOD2), thioredoxin peroxidase (mtTpx-3), were measured at different time points to quantify the oxidative status of the bees.
Results and Conclusions: Expression levels of all genes were generally enhanced, being higher for the LEE than for LWE. A higher transcription suggests a pro-oxidative effect in vivo, especially for the LEE. Higher antioxidative enzymes levels correlate well with the pro-apoptotic and antiviral activity of the extract, as well as the higher in vitro antioxidant activity. Mechanistically it is still unclear and the next step is to understand how increased consumption of high quality antioxidants (polyphenols) is interacting with the activation of antioxidative enzyme genes expression. Moreover, other compounds from the LEE besides polyphenols have to be considered, leading to its antiviral activity.

P175 New probiotic preparation for honey bees

Mudroňová D.[1], Kuzyšinová K.[2], Maruščáková I.[1], Schusterová P.[1], Bielik B.[2], Toporčák J.[2]
[1] Department of microbiology and immunology, [2] Clinic for birds and exotic animals, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

During the last years we developed a probiotic preparation based on autochtonous strain *Lactobacillus brevis* B50 Biocenol™ (CCM 8618) isolated from guts of healthy adult honey bees. As the best carrier for the probiotic lactobacilli was selected pollen which is a natural part of bee nutrition. The aim of our first experiments with this preparation was to study its influence on gut microbiota composition including possibility to prevent *Paenibacillus larvae* infection, and on clinical status of bee colonies. In these trials we noted disappearance of *P. larvae* from digestive tracts of honey bees within 2 weeks after the first application of probiotics, positive effect on gut microbiota (increased numbers of lactic acid bacteria and decreased counts of enterobacteria and total aerobes), higher grooming activity, higher honey yields, better condition of colonies, and interestingly, also significantly lower (around 70%) incidence of Varroa mites. Based on these findings, we wanted to study the mode of action of the probiotic preparation. Therefore in the subsequent experiment we tested not only the influence on gut microbiota, but also gene expression for immunologically important molecules. The results showed that probiotic lactobacilli on pollen carrier have significantly increased expression of genes encoding Abaecin, Defensin-1, toll-like receptors, Cactus (an equivalent of IκB in mammals), and peptidoglycan recognition proteins. Positive effects on gut microbiota and clinical status of bee colonies were confirmed again. These results indicate that probiotic lactobacilli together with pollen can modulate gut microbiota composition as well as immune response in honey bees, and thus positively influence health and productivity of bee colonies. The probiotic preparation has been registered for the protection in the Industrial Property Office of the Slovak Republic (PP50081-2016).
Acknowledgement: This work was supported by VEGA project 1/0358/16.

P176 Pollen as a key factor for the response of honey bees to mite parasitization

Annoscia D.[1], Zanni V.[1], Galbraith D.[2], Grozinger C.[2], Bortolomeazzi R.[1], Nazzi F.[1]
[1] Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italy; [2] Department of Entomology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University, Pennsylvania, USA

Honeybee colony losses are triggered by multiple stress factors, among which the parasitic mite *Varroa destructor* and the associated Deformed Wing Virus (DWV) play a key-role.
To counteract the proliferation of diseases within the colony, bees collect and use different substances, such as plant resins with antibiotic properties; however, little is known about the therapeutic effect of other products that are normally used as a foodstuff by the colony. In particular, the potential of pollen to mitigate the adverse effects of parasitization and the related viral infections, is still largely unexplored.
In this study, we tested the hypothesis that pollen can be beneficial for honey bees challenged with the *Varroa* mite

and found that a pollen rich diet can actually mitigate DWV viral proliferation and the negative impact of parasitization, increasing the lifespan of mite infested bees.
We characterized the compounds responsible for the recorded positive effects and observed that the apolar components of pollen contribute to reduce the mortality of parasitized bees.
Subsequently, to gain insight into the possible function of the identified compounds, we compared the transcriptome of uninfested and mite infested bees fed with pollen or not and developed a comprehensive framework to better understand the favourable effects of pollen on honey bee health.
Further studies will fully elucidate the potential role of pollen and the active components of this complex stuff, in order to identify possible ingredients for food supplements to be tested under field conditions.

P177 Opportunities and challenges for sustainable apiculture on the mountain Avala and surrounding region (Belgrade area, Serbia)

Zatezalo A.[1], Rašić S.[2]
[1] Institute for Nature Conservation of Serbia, Department of Biodiversity, Belgrade, Serbia; [2] Univesity EDUCONS, Faculty of Ecological Agriculture, Sremska Kamenica, Serbia

Mount Avala belongs to the Belgrade City area and it is located only 16 km of downtown. Regarding to its natural and cultural values this area is protected as Landscape of Outstanding Features. Availability of more than 600 plant species, local climate conditions, experience and practices of beekeepers and socio-economic value of honey bee products are opportunities for sustainable apiculture in this area. The main challenges are presence of pests and pathogens, pesticide poisoning, climate changes and lack of knowledge and experience. Data for this survey were collected from the private apiary during the period 2014-2018. Apiary is placed about 1,5 km south-east from the peak of the mountain Avala and contains 20 honey bee colonies. Also the members of the local beekeeping society were interviewed. Climate parameters, phenology and condition of honey bee colonies were monitored.

P178 The Scientific Veterinary Medical Association for Apiculture (SVETAP)

Rivera-Gomis J.[1,3], D'Ascenzi C.[2,3], Mortarino M.[4,3], Barca L.[5,3], Brajon G.[1,3], Cerrone A.[5,3], Marino A.[6], Formato G.[1,3]
[1] Beekeeping Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy; [2] University of Pisa, Pisa, Italy; [3] SVETAP – Società Scientifica Veterinaria per l'Apicoltura, Roma, Italy; [4] University of Milan, Milan, Italy; [5] Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy; [6] Cosenza ASL, Cosenza, Italy

On October 2016 it was founded in Pisa (Italy) the first Scientific Asociation for Veterinarians in Apiculture. SVETAP is the acronym (Società Scientifica Veterinaria per l'Apicoltura).
SVETAP is a non-commercial and non-profit scientific association, which has technical-scientific, cultural and educational scopes.
The main objectives of SVETAP are:
1. To promote and to enhance the veterinarians professional skills applied to the field of integrated protection of beekeeping patrimony, from the perspective of bee health, food safety and public health, helping beekeepers to produce respecting the Good Beekeeping Practices (GBPs), ensuring the quality of honeybee products;
2. To cooperate with the view "One Health".
3. To promote the study and research within the veterinary disciplines applied in beekeeping;
4. To facilitate the technological upgrading and the development of innovation in beekeeping;
5. To establish working groups and committees for the study of specific topics in the field of beekeeping;
6. To set up awards and scholarships;
7. To promote research activities related to the field of beekeeping;
8. To ensure the continuing education of its members;

9. To promote and organize training activities (e.g. conferences, workshops, seminars, courses of all types including undergraduate, master, round tables, etc.), both at national and international level;
10. To promote the activities of communication, dissemination and correct technical and scientific information of the beekeeping topics, also to public opinion.

P179 BPRACTICES: first attempt of definition of Good Beekeeping Practices (GBPS)

Rivera-Gomis J.[1], Bubnic J.[1], Cersini A.[1], Chabert M.[2], Chauzat M.P.[2], Eggenhoeffner R.[3], Erat S.[4], Gregorc A.[5], Haefeker W.[6], Higes M.[7], Jannoni-Sebastianini R.[6], Lietaer C.[8], Lubroth J.[9], McCabe P.[6], Moosbeckhofer R.[10], Muz D.[4], Muz M.N.[4], Ozdemir N.[4], Pietropaoli M.[1], Ribarits A.[10], Riviere M.P.[2], Smodis Skerl M.I.[11], Tiozzo B.[12], Formato G.[1]
[1] Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Roma, Italy; [2] ANSES, Honeybee pathology unit, European Union Reference Laboratory for bee health, Sophia Antipolis, France; [3] University of Genova, Biophysic Section of Department of Surgery Sciences and Integrated Diagnostics (DISC), Genova, Italy; [4] University of Namik Kemal, Tekirdag, Turkey; [5] Mississippi State University, Center for Costal Horticulture Research, Poplarville, MS, USA; [6] International Federation of Beekeepers' Associations, Roma, Italy; [7] Centro de Investigacion Apicola y Agroambiental de Marchamalo, Marchamalo, Spain; [8] Tecnologies and practices for small agricultural producers (TECA) plattform of the Food and Agriculture Organization of the United Nations (FAO), Roma, Italy; [9] Animal Health Service, Animal Production and Health Division, FAO, Roma, Italy; [10] Austrian Agency for Health and Food Safety, Vienna, Austria; [11] Agricultural Institute of Slovenia, Ljubljana, Slovenia; [12] Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy

Nowadays, beekeeping faces numerous challenges, and numerous disorders that affect honeybee colonies, including the potential introduction and spread of bee diseases, the effects of pesticides and climatic change.
In this context, the "BPRACTICES" project, funded from the European Union's Horizon 2020 research and innovation programme aims to develop a system of sustainable apiculture by implementing innovative management practices (Good Beekeeping Practices - GBPs).
Good beekeeping practices(GBPs) are those integrated and sustainable activities which beekeepers apply for the hive management to obtain an optimal health for honeybees, positive socioeconomic impacts (e.g. beekeepers and consumers health protection) and to ensure environmental protection.
The application of GBPs results in a positive effect on the wellbeing of honeybee colonies, on food safety and environmental protection, thus guaranteeing high production standards.
An essential part of the Good Beekeeping Practices (GBP) are the preclinical indicators, which allow to diagnose infection or infestation before symptoms appear, representing an essential tool for prevention. These preclinical indicators will be identified and interpreted using innovative laboratory diagnostic methods and matrices from the hive. Examples are the preclinical diagnosis from powder sugar for American Foulbrood (*Paenibacillus larvae*, AFB) or European Foulbrood (*Melissococcus plutonius*, EFB), the preclinical detection of the SHB from bottom hive debris by Real-time PCR, or the yeast *Kodomaea ohmeri* as an indicator for the presence of SHB.
The risk of residues in honeybee products due to chemical treatments is reduced through the application of GBPs, thus guaranteeing quality and safety. GBPs also avoid productivity losses.
Preventive GBPs represent an opportunity to ensure the improvement of honeybee health and consequently increase the performance of honeybee colonies, the profitability of the beekeeping operation and the pollination service provided by honeybees.
Resilience of the beekeeping sector, its sustainability and the income of beekeepers increase when sanitary problems are prevented and costs (e.g. for treatments, colony losses, production decrease) are reduced. The implementation of GBPs provides a direct benefit to beekeepers, supporting the sector.
In conclusion, by improving beekeeping management through GBPs, honeybee health, bee products safety, and the competitiveness and resilience of the apicultural sector are improved at all levels.

P180 Three species of native Thai honey bees exploit overlapping pollen resources: Identification of bee flora from pollen loads and midguts from *Apis cerana*, *A. dorsata* and *A. florea*

Suwannapong G.[1], Maksong S.[2], Yemor T.[2], Junsuri N.[2], Benbow M.E.[3]
[1] Department of Biology, Burapha University, Chon Buri, Thailand; [2] Program of Biological Science, Faculty of Science, Burapha University, Chon Buri, Thailand; [3] Department of Biology, Universty of Dayson, Ohio, USA

In this study, we investigated the bee flora utilised by the Thai honey bees, *Apis cerana*, *A. dorsata* and *A. florea* in Nan province, northern Thailand, through the identification of pollen grains from their pollen loads and midguts. We compared the pollen grains morphologically to match with local flowering plants and determined their protein concentration using a Bradford assay. The results showed that 8 families and 15 species were found by pollen load analysis whilst 12 families and 25 species were found from pollen grains of the bee midguts. The greatest number of bee flora was found from pollen loads of
A. cerana, (11 species), while there were fewer for *A. florea* (10 species) and the fewest with *A. dorsata* (6 species). The highest number of bee flora species identified from pollen was found in the midgut of *A. cerana*, with 19 species, while *A. florea* had 13 and *A. dorsata* with only 11. The results show that the major pollen source plants of the three native honey bee species of Thailand were *Mimosa pudica* L., *M. pigra*, *Celosia argentea* L., *Zea mays* L., *Wedelia trilobata* L. and *Syzygium malaccense* L. The protein content ranged from 31.85 ± 0.83 to 48.44 ± 0.81 mg/100 mg pollen. The most abundant pollen source was from *M. pudica* L., perhaps because of the flower structure, shape, size, long blooming season, wide distribution, high protein concentration (43.31 ± 0.79 mg/100 mg pollen) or a combination of these characteristics.

P181 Pollen consumption by adult solitary bees: amount, frequency, and species composition

Dobson H.E.M.
Whitman College, Department of Biology, Walla Walla, WA, USA

Recent studies show that adult females of the solitary bee *Nomia melanderi (*Halictidae) consume pollen regularly for their own sustenance in addition to collecting it to provision their nests, but studies of more bee species, including both oligolectic and polylectic species, are needed to establish the generality of these findings to solitary and semi-social bees. To address this gap in our knowledge of pollen consumption by adult bees, males and females of over eight bee species belonging to several families were collected at different times of day and across flight seasons and dissected to determine the fullness of each gut region with pollen in relation to both daily and seasonal periods, as well as to establish the identity of pollen consumed and how it compared to pollen collected for nest provisioning. Overall, females of each species fed regularly across the season, but their patterns across the day varied among species and appeared to depend mainly on local climatic conditions and the daily temporal availability of floral resources. Oligolectic females consumed the same pollen that they collect for nest provisioning, suggesting that that the term oligolecty be extended to include specialization in pollen consumed by adults; polylectic females tended to consume a greater diversity of pollens than carried in their scopae, raising questions of how females select pollen plants for each purpose. Male bees of all investigated species differed from females in consuming pollen in much lesser quantities and mainly at the beginning of the flight season. The widespread evidence of pollen feeding by adult solitary and semi-social bees, which is especially extensive in females, suggests that pollen fills an important nutritional role in adults as well as larval stages. It also calls attention to the need of incorporating adult feeding when estimating the amount of pollen resources required to sustain bee populations.

P182 BDA (beekeeping database) in Italy: an achievement in the management of bee health

Bressan G.
Simevep (Italian Society of Preventive Veterinary Public Medicine), Rome , Italy

The national database for beekeeping - BDA is regulated by the law of 4 December 2009 and by the regulation of 11 August 2014. The database regulates the data of beekeepers, identifies the position of the beekeepers and obliges the operators to enter all movements: nomadism, pollination, buying and selling bee queens, nuclei, hives. Furthermore, at the end of each year all beekeepers or their delegates must enter a census of their hives. Veterinarians are responsible for checking the correct use of the application. The results of 4 years of experimentation are examined and discussed and the limits and advantages of this new management of Italian beekeeping are reported
Objectives : analyze the merits and defects of a law whose main purpose is to identify the number of beekeepers and hives currently present in Italy and their movements
Results and conclusions : the application of the law was not easy especially by beekeepers not able to use the pc. The trade associations helped the software development and its application. The development of the database made it possible to identify the real consistency of beekeepers and hives (even if many beekeepers still lack the appeal). Thanks to this law it has been possible to provide data to the European community, both in order to access contributions and to facilitate the monitoring of diseases by veterinary services. There is still a lot to do, but the road is the right one.

P183 Quality of honeybee nutrition originating from proteins is key factor of honeybee health

Bilikova K.[1], Dudekova J.[1], Yamaguchi Y.[2], Yamaguchi K.[2]
[1] Institue of Forest Ecology, Slovak Academy of Sciences, Laboratory of Molecular Apidology, Bratislava, Slovakia; [2] JRJ Institute of Apimedical Science, Tokyo, Japan

Nutrition plays one of the most important roles in development and health of honeybee colony. Floral resources of honeybee food are nectar or honeydew, collected by foragers from plants, as the major source of carbohydrates, and pollen, as the main source of proteins and lipids naturally available to honey bees. Both pollen and nectar are also rich of micronutrients and phytochemicals - the secondary plant metabolites with broad range of antioxidant and antimicrobial properties. However, pollen and nectar of different floral origin varied in nutritive and defensive components. From this point of view, the plant diversity is important factor for rich and properly balanced honeybee food. During processing of nectar to honey and pollen dust to bee bread and royal jelly, honeybee enriches these products by secretions of cephalic glands that contain authentic honeybee proteins and antimicrobial peptides. Apalbumins, the major proteins of royal jelly, are not only source of nutrition, but they play a key role in larval development, and participate in defense of honeybee against microbial pathogens. Royal jelly proteins are regular components of honey, royal jelly and bee pollen, while the compounds of floral origin can vary significantly. We present here the main nutritional factors of honeybee health, with focus on the proteins and peptides of honeybee origin, as markers of quality of honeybee products.

P184 Heavy metal pollution levels in honey bee larvae depending on pollen and environmental pollution

Czekońska K.[1], Zięba K.[2], Miśkowiec P.[2], Pałka K.[2], Szentgyörgyi H.[1]
[1] Department of Pomology and Apiculture, Faculty of Biotechnology and Horticulture, Agricultural University in Kraków, Kraków, Poland; [2] Department of Environmental Chemistry, Faculty of Chemistry, Jagiellonian University, Kraków, Poland.

Much is known about the effects of heavy metals on various living organisms, but bees were largly omitted in such studies. Directly, the main source of pollution for bees is the deposition of contaminated dust particles on their food source: on pollen and nectar. The aim of our study was to compare the contamination levels with Zn, Cd and Pb in pollen collected by honey bees, and in honey bee larvae developing on such pollen. Bees were exposed to various levels of pollution on four sites located in the vicinity of a zinc smelter in Southern Poland. Samples of pollen and honey bee larvae were taken three times during the season from three hives on each site: in May, June and July. Also samples of soil were taken on each site during the last sampling period to determine general environmental pollution levels.
Zinc levels in soil were ranging between 114,59 - 2976,29 μg/g, contamination of pollen was between 35,03– 104,54 μg/g, similarly as in the body of bee larvae: 44,94– 64,08 μg/g. Cadmium levels in soil were ranging between 0,99 - 13,35 μg/g, contamination of pollen was between 0,42 – 0,85 μg/g, and somewhat lower in the body of bee larvae: 0,22 – 0,45 μg/g. The level of lead was ranging between 49,94 - 931,75 μg/g in soil samples, while undetectably low in most of the pollen samples and were also below detection level in bee larvae samples.
Contrary to our expectations there was no correlation between the contamination levels in pollen and bee larvae. This result is suprising in the light of other studies showing clear correlations between these levels in solitary nesting bees. We suggest that young honey bee larvae may be protected from pollution thanks to feeding on royal jelly after hatching instead of pollen, like solitary bees do. Further studies are conducted in the following two years to explain this phenomenon
The study is supported by the Polish National Science Centre (UMO-2016/21/B/NZ9/01163).

P185 Sustainable beekeeping and advisory service: a critical review

Ljung M.[1], Fabricius Kristiansen L.[1], Kristiansen P.[2]
[1] Swedish University of Agricultural Sciences, National Competence Centre for Advisory Services, Skara, Sweden; [2] Apinordica, Tjällmo, Sweden

Advisory services are key element of a Knowledge and Innovation System supporting Beekeeping (B-KIS) (Ljung, 2016). One core function is to bridge between best available knowledge and existing beekeeping practices. Such process of knowledge development for and with the beekeepers, has become even more crucial in the implementation of measures to decrease winter losses and improved bee-health.
The specific requirements, practices and innovative actors of the many different beekeeping traditions across Europe pose many challenges to the advisory system. A major challenge is related to the need to manage both the diversity of bees and people. This implies that advisory services must manage different needs for knowledge and services. Nonetheless, there are some universal principles that are known to decrease the implementation gap through advisory services. Best practices exist not only in beekeeping but also in advisory services. However there is still some knowledge gaps about the range and use of existing tools and techniques among advisors and other actors working to implement more sustainable beekeeping.
This presentation is a critical review on how to understand the challenges that advisory service within the beekeeping sector face over the next decade, acknowledging the diversity of available theories and concepts. It is intended as a resource to inform ongoing discussions on the transition towards more sustainability practices as well as the future of the bee-sectors knowledge and innovation system in an European context. We argue that there is a need to build a strategic and collaborative knowledge system and critically analyse the different pathways where knowledge development in beekeeping, active or non-active, are performed. Strengthened advisory services will help identify new ways on how to reach a sustainable beekeeping with improved bee health, lower winter losses and better apicultural practices.

The study is partly carried out within the EU FP7 Project Smartbees. One purpose of the Smartbees-project is to contribute to the development of a tool-box for extension and advisory services within the European B-KIS, in order to make available significant and reliable knowledge for beekeepers and other relevant actors.

P186 Comprehensive study of midgut/pyloric bacterial population in European and Japanese honey bees

Stoic M., Yoshiyama M., Ogihara M., Morimoto N., Kimura K.
Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, Tsukuba, Japan

Bacterial communities situated in digestive system are strongly connected to their host's health and metabolism. Midgut represents the anterior segment of the honey bee gut, where only few bacteria can thrive as a result of its dynamic medium. The goal of this study was to get a more detailed insight into the status of midgut bacterial population in two sympatric host bee species, European and Japanese honey bee, which shared the same apiary in different geographical locations in Japan. Samples were taken in spring and autumn to determine whether seasonal change, along with the factors of host bee species and locality, could also result in microbial fluctuations. Possible variations in those symbiotic bacteria were examined by using metagenomic analysis of 16S rRNA gene and bioinformatics. Regardless of certain variety in bacterial species, our results showed that three members of previously identified core gut microbiota (*Gilliamella apicola*, *Snodgrassella alvi*, and *Frischella perrara*) remained abundant in both bee species. Same apiaries expressed different trends, however sympatric location and changes in season did not cause any general significant shifts in microbial population. Nevertheless, differences in bacterial trends were noticed between two bee species, and their distinct varieties additionally occurred in every tested location and season. Likewise, some bacterial species remained particularly connected to their bee hosts. Core gut member *F. perrara* was more present in European, whilst a non-core member Apibacter adventoris was specific to Japanese honey bee samples. In conclusion, the factor of host bee species can affect the presence and abundance of particular bacteria. This type of experiment can offer a step in future research in defining the possibility of interactions between sympatric bee species, along with the influence of other factors in location, such as possible stressors, that may significantly affect bacterial members.

P187 The influence of beekeepers' management practices and pesticides on bee health in Chile's Central Valley

Doorn M.[1], Rodríguez S.[1], Maus C.[2]
[1] Fundación Fraunhofer Chile Research, Center for Systems Biotechnology,, Santiago, Chile; [2] Bayer AG, Bee Care Center, Monheim, Germany

Economically important crops depend on pollination by insects in order to guarantee the quality and quantity of fruits or seeds produced. However, the intensification of agriculture can negatively impact pollinators and thus on the competitiveness of farmers. In order to understand this multifactorial problem there is a need to understand the most important issues related to agriculture's most important pollinator, Apis mellifera. Therefore, Fraunhofer Chile Research in collaboration with Bayer AG developed a pilot project in Chile's Central Valley to monitor bee health, measuring management capacities of beekeepers, honey bee colony strength, presence of pathogens in beehives and pesticides residues in bee bread. The following data were collected from the apiaries of 60 small, medium and large beekeepers: General information about the beekeeper and hive management practices; Hive strength of three randomly selected hives; and bee bread to measure the occurrence of pesticide residues to determine pathogens relevant for Chile. The presence of bee pathogens Varroa destructor and Nosema spp. were determined with the standard methods for bee research established by Coloss. Beebread samples were analysed for pesticide residues by multi residue analysis (GC-MS and LC-MS/MS). The beekeepers' hive management was very diverse. The colony strength varied strongly, the majority of the hives being weak before winter explaining the high mortality rates reported by the beekeepers. Varroosis occurred with greater frequency than nosemosis. Significant differences in infestation were found related to the beekeeper's size, hive

management, and hive strength among others. A total of 15-19 active ingredients were found in the beebread samples with the organophosphorus insecticide coumaphos most frequently detected. In general, the residue concentrations of each individual compound were below levels that would be hazardous to bee colonies.

P188 Proline enriched diet effects on haemolymph amino acid composition in *Osmia bicornis* (L.) females and males

Tafi E.[1], Sagona S.[1], Meucci V.[1], Nepi M.[2], Felicioli A.[1]
[1] *University of Pisa, department of Veterinary Sciences, Pisa, Italy;* [2] *University of Siena, department of Life Sciences, Siena, Italy*

Nectar is the main food resource of adult bees and it fuels their flight. Amino-acids are the most abundant solutes present in nectar, second only to sugars. All the essential amino-acids have been found almost ubiquitously in nectar. Among them, the protein amino acid proline has received more attention because of its role in the insect flight metabolism and because it is the most abundant amino-acid found in the honeybee haemolymph.
Amino-acids affect bee physiology thereby their pollination activity. Bee amino acids requirements are still to be fully investigated, especially for wild bees. Mason bees are important pollinators of fruit trees and they are managed in several countries for orchards pollination. Due to its solitary life-cycle and the possibility to be easily kept in cages, *Osmia bicornis* (L.) is a suitable species for laboratory experiments.

In order to investigate haemolymph amino-acids composition and the effect of a proline enriched diet on *O. bicornis*, both females and males newly emerged specimens have been reared separately in laboratory conditions. Each sex genre was divided in two groups of 15 mason bees and fed with a control sugar syrup and a proline-enriched sugar syrup respectively. Haemolymph was sampled from mason bees after 10 days of rearing. An additional group of 9 bees was used for haemolymph sampling at 0 days of rearing. Haemolymph samples were analysed by gradient HPLC.
Differences in haemolymph composition resulted between the two sex genres. γ-amino butyric acid (GABA) is present in the haemolymph of newly emerged males while it is not present in females.
Proline is present in high concentration in both newly emerged females and males, suggesting the occurrence of a storage mechanism of this amino-acid ingested by bees during their larval development. After 10 days of rearing, proline haemolymph concentration appeared not to be affected by a proline-enriched diet, while GABA haemolymph concentration increases in females fed with proline. The increase of GABA concentration in the haemolymph of mason bees fed with a proline enriched diet, allows us to hypothesise the existence of a metabolic pathway leading to the proline conversion into GABA.

P189 Reducing the risk of honey bee colony loss through beekeeping management practices

Steinhauer N.[1], Saegerman C.[2], vanEngelsdorp D.[1]
[1] *Department of Entomology, University of Maryland, College Park, MD, USA;* [2] *Faculty of Veterinary Medicine, Université de Liège, Liège, Belgium*

Over the past 10 years, a survey of honey bee (*Apis mellifera*) mortalities in the US revealed an average of one in three colony dying over the winter (Seitz et al., 2016 J. Apic. Res. 54(4): 292-304; Kulhanek et al., 2017 J. Apic. Res. 56(4):328-340). Honey bee health, and ultimately, colony loss, is affected by multiple stressors acting concomitantly and sometimes interacting. Those stressors include pests and diseases, forage availability and pesticide exposure (Potts et al., 2010, Trends in Ec. & Ev. 25(6): 345-353; vanEngelsdorp & Meixner, 2010, J. Inv. Path. 103:S80-S95). Management practices have the potential, when used judiciously, to alleviate some of those stressors (Giacobino et al., 2014, Prev. Vet. Med. 115(3-4): 280-287; Molineri et al., 2017, Prev. Vet. Med. 140: 106-115). Using long term observational data obtained from the Bee Informed Partnership monitoring of honey bee colony losses and management practices in the US,

we were able to summarize management information into a quality index, based on experts' opinion, and confirmed the association between management practices quality and overwintering colony loss.
Further, we ranked individual practices based on their associated potential reduction in colony mortality. The top management criteria were identified in various subsets of respondents, resulting in different set of regionally and operation-size specific recommendations. In particular, we will develop the topic of varroa management and how it differed between small-scale and commercial operations. The disparity of top influencing criteria between operation types illustrates the divergence in the beekeeping industry and the need of extension programs to address backyard and commercial beekeepers independently.

P190 Perception of risk factors affecting bee colonies (*Apis mellifera*) health and mortality in Belgium

El Agrebi N.[1], Danneels E.[2], de Graaf D.[2], Saegerman C.[1]
[1] Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine of Liège, University of Liege, Belgium; [2] Laboratory of Molecular Entomology and Bee Pathology, Department of Biochemistry and Microbiology, University of Gent, Ghent, Belgium

Understanding beekeepers' perceptions of risk factors impacting bee health and mortality is essential to analyze the reasons for adopting or rejecting some beekeeping management practices. To date, to the knowledge of the authors, no study on how beekeepers perceive and manage these risks has been carried out. For beekeepers, adopting strategies that mitigate risk to health and bee mortality is an action involving behavioral changes. In order to better understand the factors that determine changes in management practices, as well as the decision-making and action process, in Belgian apiaries, a perception survey was designed and launched online, based on the Health Belief Model (HBM) commonly used in human medicine. This sociological survey concerns 355 randomly distributed beekeepers all over Belgium. A first descriptive analysis of the data shows that beekeepers tend in general to take little risk, their perception of climate change, Varroa destructor and management practices is acceptable. On the other hand, their perception of pesticides use in beekeeping and agriculture, is confusing. Their main motivations are the production of a quality honey, bee health and environment. A Welch test (mean test for samples with unequal variance) comparing beekeepers' perceptions in function of mortality rates, indicates that beekeepers (N=213), with mortality rates <10% (rate considered acceptable in Europe) have a significantly better perception of risk factors for their colonies and apply more measures limiting these factors. Despite a real perception of risk, the constraints of investing time in the execution of these actions and the lack of feeling of the financial impact that the loss of a colony entails, are the main obstacles to the implementation of measures to limit the risk. The results of this study highlight the importance of taking socio-economic determinants into account in any strategy aimed at mitigating the risks associated with bee mortality.

P191 Natural comb honeybee management in frame hives for professional beekeepinga

Fontana P., Malagnini V., Zanotelli L.
Centro Trasferimento tecnologico, Fondazione Edmund mach, San Michele all'Adige, Italy

The increasing of problems connected with the pesticide residues in commercial wax foundations, highlights the necessity to change the concept of wax production in particular in organic beekeeping. Moreover the beekeeper is in trouble in finding insured no contaminated wax foundations and they are generally sold at constant high price. On the other hand and in the same time, natural beekeeping is involving day by day more beekeepers, focusing on the role of natural combs for the general fitness of bee colonies. In both case natural combs, built totally by bees, are the only available solution for Natural beekeeping, but can be a solution even for professional and conventional beekeeping, allowing to reduce costs and increasing the value of the products obtained by bees.

For this reasons a trial have been conduct during the 2018 season by the Ecotossicology and honeybees decline group of Edmund Mach Foundation (Italy) to evaluate the speed and the structure of comb building in frame hives. The trial took place in Maso Franch (46° 08′ 35′′N 11° 07′ 12′′ E), Giovo, TN, Italy
3 different treatments were tested and challenged:
1. 7 Dadant-Blatt beehives with wax foundations,
2. 7 Dadant-Blatt beehives with empty frames (equator frames) and no wax
3. 7 Italian Natural Hives (a new concept of hive developed in Mach foundation) beehives with small empty frames.
All colonies have been obtained using a bee queen and a bee package of 1,5 kg. Both queens and bee packages were of Apis mellifera carnica. All colonies received the same amount of glucose-fruttose syrup.
The assessments consist in the evaluation of the frame building surface, and the estimation of quantity of brood and food storage twice a months until the trial ending.

P192 First European study of honey production in beehives under an annual "Vita Feed" nutritional protocol

Papachristoforou A.[1], Mielgo P.[2], Watkins M.[2]
[1] Aristotle University, Department of Zoology, Thessaloniki, Greece; [2] VITA EUROPE LTD, Basingstoke, UK

In recent years, climate change, crop spraying and monoculture are some of the factors that have led to a decline in honey production worldwide. Variations in the content of available proteins exerts a serious effect on the needs of bees. Five years of making records and evaluations in Argentina showed that supplementation with VitaFeed Nutri by sprinkling during the honey flow generated an average increase of 2.5 kg of honey per beehive. This led to the creation of an annual nutritional supplementation protocol with the objective to observe the impact on production of those beehives. The 2014/15/16 seasons showed an average increase of 20% of honey for the colonies treated under the protocol, compared with untreated control colonies, so it was decided to repeat the test in order to confirm if we can achieve the same excellent results in Europe.
The tests were conducted in the 2017 season, at one conventional apiary located in Neo Klima town, in the island of Skopelos, Greece.
The apiary was divided into two groups, one group subject to the supplementation protocol and the second comprising control (only sugar syrup).
The nutrition plan was administered to the experimental colonies, consisting of: 300g of "Vita BeeFood", 25 ml of "VitaFeed Power" in sugar syrup and 50g of "VitaFeed Nutri", the latter sprinkled on top of the brood frames.
The measurements were performed in the extraction room. From the field, honey supers were marked to extract honey were carried separately.
In this test in Europe, the honey production obtained was an average of 10.3 kg for the treated colonies and 7.71 for the control hives. This increase of 34% per hive not only shows the necessity of good quality of nutritional products but also show that a good investment has quick positive results. The use of products designed exclusively for bees shows a positive effect, which correlates with the last 5 years of trials carried out in Argentina.

P193 Validation of reference genes for RT-qPCR analysis of thermal stress gene expression in *Bombus terrestris*

Blasco-Lavilla N., García-Reina A., De la Rúa P.
University of Murcia, Department of Zoology and Physical Antropology, Murcia, Spain

Bumblebees provide an important pollination service for both crops and natural ecosystems. Nowadays, however, there is increasing evidence that bees of the genus *Bombus* are decreasing at both local and regional scales. One of the multiple factors advocated as a cause of this decline is climate change, which could be adversely affecting warm- and

cool-adapted bumblebee species simultaneously. Therefore, studies assessing how thermal stress influences gene expression patterns involved in different biological processes are needed to determine how climate change will affect bumblebees. Expression studies require the use of several reference genes known to be stably expressed under the conditions tested, making them acceptable internal controls in the experiments. To date, several reference genes have been validated for RT-qPCR in Bombus terrestris, but no information regarding their stability under thermal stress is available. In this study, six candidate reference genes (Arginine kinase-AK, Elongation factor 1α-EEF1α, Phospholipase A2-PLA2, α-Tubulin-TUB, β-Actin-ACTB and Ribosomal protein L13-RPL13) were selected from the literature and their stability under different temperature conditions was tested using RT-qPCR. Two out of the six genes did not amplify properly (PLA2 and ACTB). The stability for the four remaining genes was analysed with the algorithms GeNorm, NormFinder and BestKeeper. All four genes analysed (AK, EEF1α, TUB, and RPL13) showed appropriate stability values under different temperature conditions. Nevertheless, AK was the least stable gene, while RPL13 and EEF1α presented the most stable values and are thus postulated as the two best reference genes for RT-qPCR studies under thermal stress in *B. terrestris*.

P194 BeeHeal: promoting bee health for sustainable agriculture

Martín-Hernández R.[1], Lopes, A.R.[2], Chevjanovsky N.[3], Dalmon A.[4], Higes M.[1], Le Conte Y.[4], Pinto A.M.[2], Reyes-Carreno M.[4], Soroker V.[3]

[1] Centro de Investigación Apícola y Agroambiental de Marchamalo, Spain; [2] Mountain Research Center, Polytechnic Institute of Bragança, Portugal; [3] Agricultural Research Organization, The Volcani Center, Israel; [4] Centre de Recherche Provence-Alpes-Côte d'Azur Unité: Abeilles et Environnement, France

During the last years there is an alarming increase in the collapse of honeybee colonies (*Apis mellifera*) where bee parasites and pathogens like Varroa destructor mites, the microsporidia *Nosema* ssp. and viruses have played the leading role. Colony decline might compromise not only food security but also present and future income to the growers. Hence, four institutions from Mediterranean area have joined forces to put together a project entitled, "BEEHEAL: Promoting bee health for sustainable agriculture". BEEHEAL is a collaborative research between Centro de Investigación Apícola y Agroambiental de Marchamalo - CAR (Spain), Centre de recherche Provence-Alpes-Côte d'Azur Unité: Abeilles et Environnement - INRA (France), Agricultural Research Organization, The Volcani Center - ARO (Israel) and Mountain Research Center (CMO), Polytechnic Institute of Bragança (Portugal). The aim of this project is to determine the phenology and interaction of the microsporidia *Nosema ceranae* and viruses including acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), Black queen cell virus (BQCV) Chronic bee paralysis virus (CBPV) and Deformed wing virus (DWV), in Spain, France, Portugal and Israel. The findings of this project, which involves an active and unique cooperation among partners representing Mediterranean countries which encompasses a wide range of environmental and beekeeping management conditions, will contribute to ameliorate the damage caused by the expansion of *N. ceranae* through a rational implementation of existing treatments to avoid emergence of synergistic pathogens that accelerate colony collapse compromising food security. This project started at 2017 and it will end in 2020. BEEHEAL is funded through the ARIMNet2 (2016) Call by the following funding agencies: INIA (Spain), ANR (France), MOARD (Israel), and FCT (Portugal). This presentation will detail the tasks that are ongoing in the BEEHEAL project.

P195 Bioactive compouds that could be found in bee pollen

Ares A.M.[1], Valverde S.[1], Bernal J.L.[1], Martín-Hernández R.[2], Nozal M.J.[1], Higes M.[2], Bernal J.[1]
[1] I.U. CINQUIMA, Grupo de Química Analítica (TESEA), Universidad de Valladolid, Valladolid, Spain; [2] Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain

The consumption of apicultural products (honey, royal jelly, propolis, beeswax or bee pollen), which have been used in both phytotherapy and diet since ancient times, is gaining prominence due to their bioactive compounds associated with beneficial properties to health. Particularly noticeable is the consumption of bee pollen, which is a combination of mainly floral pollen with some nectar or honey, enzymes, wax and bee secretion. It consumption has significantly increased in modern-day diets over the last few years because of its wide range of biological functions, such as those of an antioxidant, anti-inflammatory, anticarcinogenic or antibacterial nature. These functions are mainly due to the nutrients and bioactive compounds that are present in bee pollen such as vitamins, lipids, phenolic compounds, minerals, or proteins, which are among the main components of bee pollen, include enzymes and both essential and nonessential amino acids. In fact, bee pollen is referred to as the "only perfectly complete food", as it contains all the essential amino acids needed for the human organism. However, it must be specified that the composition of bee pollen is particularly dependent on plant origin, together with other factors such as climatic conditions, soil type, beekeeper activities, and the different processes or storage treatments in commercial production. Thus, this study aims to give a comprehensive overview of and insight into the analysis of bioactive compounds from bee pollen by analyzing the related literature in the last few years (2011-2017). The structure of the study is in accordance with the different families of bioactive compounds, their main related health-promoting activities, and a brief summary of the extraction procedures together with the analytical techniques employed for their determination.
Acknowledgements: Authors gratefully acknowledge the INIA-FEDER founding (National public research project; RTA2015-00013-C03).

P196 SAMS - International partnership on innovation in Smart Apiculture Management Services

Brodschneider R.
Institute of Biology, University of Graz, Graz, Austria

SAMS is a project funded by the European Union within the H2020-ICT-39-2016-2017 call. SAMS enhances international cooperation of ICT (Information and Communication Technologies) and sustainable agriculture between EU and developing countries in pursuit of the EU commitment to the UN Sustainable Development Goal "End hunger, achieve food security and improved nutrition and promote sustainable agriculture". The project consortium comprises four partners from Europe (two from Germany, Austria and Latvia) and two partners each from Ethiopia and Indonesia. Beekeeping with small-scale operations provides perfect innovation labs for demonstration and dissemination of cheap and easy-to-use open source ICT applications in developing countries. SAMS allows active monitoring and remote sensing of bee colonies and beekeeping by developing appropriate ICT solutions supporting management of bee health and bee productivity and a role model for effective international cooperation. SAMS addresses requirements of end-user communities on beekeeping in developing countries. It includes technological improvements and adaptation as well as innovative services creation in apiculture based on advanced ICT and remote sensing technologies. SAMS increases production of bee products, creates jobs (particularly youths/women), triggers investments, and establishes knowledge exchange through networks. To find out more visit our project website https://sams-project.eu/.

P197 Bee world is celebrating its 100th Anniversary in 2019

Brodschneider R.
Institute of Biology, University of Graz, Graz, Austria

Bee World was founded in 1919 by the *Apis* club and publication was taken over by the International Bee Research Association (IBRA) in 1952. With the exception of a hiatus between 2006 and 2009, it appeared uninterrupted, and is approaching its 96th volume and 100 year anniversary in 2019. So far, more than 6500 articles have been published. In 2015 Taylor and Francis took over the publication of Bee World, which continues to appear in 4 issues a year publishing, among others, Original Articles, Review Articles, Art & Culture, Book Reviews, Obituaries and Conference Abstracts. Bee World is a printed journal, but all articles, including those from all editions going back to the very first issue, are also available online (see www.tandfonline.com/toc/tbee20/current). Bee World is IBRA's popular journal, available free to IBRA members, and acts as a bridge between today's beekeepers and bee scientists, encouraging two way discussions. There are no publication fees. In 2018, an editorial board was established, that seeks to further develop the journal. To point to the 100th anniversary, and in order to promote the journal among young scientists, a one year subscription of Bee World will be given as an additional prize to the best student presentation at Eurbee 8.

P198 Study regarding the content of the various nutrients in the *Crataegus monogyna*, *Brassica* spp. and *Prunus* spp. bee pollen

Mărgăoan R.[1], Urcan A.[2], Zacharias I.[2], Bonta V.[2], Topal E.[3], Varadi A.[4]
[1] Department of Horticulture, [2] Department of Apiculture, UASMV Cluj-Napoca, Cluj-Napoca, Romania; [3] Aegean Agricultural Research Institute Menemen-Izmir, Department of Apiculture, Izmir, Turkey; [4] Association Health with CasaBIO, Cluj-Napoca, Romania

Bee pollen is promoted as a health food with a wide range of nutritional and therapeutic properties. A high concentration of proteins, lipids, essential amino acids, unsaturated and saturated fatty acids, polyphenols, minerals like Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Zn and high K/Na ratio make honey bee pollen very important for human diets. The nutrients content of bee pollen varies greatly, depending on the botanical origin. Knowledge about the botanical source of the bee pollen samples, as well as their chemical composition, it is important to add value to the product. *Crataegus monogyna*, *Brassica* spp. and *Prunus* spp., tree types of spring bee pollen were analyzed regarding nutritional and biological value. The nutritional value was 323.18 Kcal/100g for *C.monogyna*, 348.63 Kcal/100g in the *Brassica* spp. bee pollen sample and 322.38 Kcal/100g for *Prunus* spp. bee pollen. Twenty-six free amino acids were identified in the bee pollen samples of which the 8 essential amino acids are all present. Total free amino acids determinate in the study samples was 3301.81 mg/100g for the C. monogyna, followed by *Prunus* spp. and *Brassica* spp. with values 2615.22 mg/100g and 1460 mg/100g. The most abundant was proline (PRO), 1203 mg/100g, followed by ASN (asparagine) 533.5 mg/100g and HIS (histidine) 122 mg/100g in C.monogyna bee pollen sample.
The higher mineral content was 9471.14 μg/g for *Prunus* spp. and the lowest was 7953.30 μg/g for *Brassica* spp. bee pollen.
The variability regarding the mineral content in the examined bee pollen samples was distributed as follows: the higher concentration for K, Fe, Mn, Na, Zn belonged to the *Prunus* spp.; Ca, Cu, Mg for *Brassica* spp. and Ni for *C. monogyna*.
All these nutritional values give for bee pollen the name of superfood, due to the concentration of the nutrients, but it also raises it to the functional food grade with an essential role in api-nutrition

P199 A multi-stressor analysis of spatio-temporal shifts in Belgian bee community

Rollin O.[1,2], Michez D.[2], Jacquemin F.[1], Marshall L.[3], Rasmont P.[2], Dufrêne M.[1]
[1] BIOSystem Engineering Department, University of Liège - Gembloux AgroBioTech, Gembloux, Belgium; [2] Research Institute for Biosciences, University of Mons, Mons, Belgium; [3] Department of Geography, University of Namur, Namur, Belgium

As recommended by Potts et al (2010), it necessary to address the multiple effects of drivers as interactions to evaluate the supposed role of non additive effects. Species distribution models are increasingly used to predict species distribution shifts under scenarios of future change of environmental conditions. With such models, the ecological niche of the species is captured by a statistical link between presence data and environmental variables.
The suitability of environmental conditions for a species may result from a variety of factors, such as climate, land use, topography or soil properties, and significantly improved the accuracy of the models. The relative importance of each of these factors may vary across some key biological traits (e.g. sociality, size, phenology, habitat specialisation). In addition, climate and land use change are assumed to underlie a multitude of environmental pressures that may have a greater joint impact on biodiversity than when operating in isolation. Moreover, land use changes operate across a variety of spatial scales and time horizons.
Here, based on biogeographical records of ~650,000 specimens, we aim to perform modelling of wild bee species' probability of presence per landscape unit in Belgium, taking into account possible land use and climate change, to facilitate decision-making with regards to these species' conservation. We considered different group of species showing different biological traits.
Using the techniques of meta-analysis, we performed a comprehensive comparative analysis to identify the specific or different driver combination roles, taking into account spatial and temporal covariates, and biological traits.

P200 Colony performance of *Apis mellifera* feeded with *Lactobacillus johnsonii* AJ5 metabolites

De Piano F.G.[1,2], Lora Marchetti D.A.[2], Mitton G.[1], Ramos F.[1], Maggi M.D.[1], Audisio M.C.[3], Ruffinengo S.[1,2]
[1] Centro de Investigación en Abejas Sociales (CIAS), Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM - CONICET - CIC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes Mar del Plata, Buenos Aires, Argentina; [2] Cátedra de Apicultura y Calidad de Miel, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Buenos Aires, Argentina; [3] Instituto de Investigaciones para la Industria Química (INIQUI)/Facultad de Ingeniería, Universidad Nacional de Salta, Salta, Argentina

Several stressors affect *Apis mellifera* with an important impact over the insect development and consequently, in colony performance. *Varroa destructor* and *Nosema ceranae* are the main parasites of honey bees and together with nutritional deficiency produce an immune depression, with a consequent weakening of colonies. In order to overcome this situation, natural and non-contaminant alternatives have been incorporated to strengthen the colonies. In this context, bacterial metabolites of beneficial microbiota of bees emerged in recent years. The aim of this study was to assess the effect of the oral administration of the metabolites produced by *Lactobacillus johnsonii* AJ5 on the performance of *A. mellifera colonies*. Assays were carried out in an experimental apiary located in the EEA-INTA Balcarce, Argentina (37°45'42.5''S 58°18'04.3''W), between November 2016 and February 2017. Eighteen colonies standardized in population were divided in three groups of six. Colonies were fed with the following treatments: i) CFS: cell-free supernatant of *L. johnsonii* AJ5 (CFS) at 40% v/v in sugar syrup; ii) MRS (control): MRS broth at 40% v/v in sugar syrup; and iii) JYR (control): sugar syrup (2:1). Four applications of 500 ml of different treatment were done every 7 days. The following parameters were registered and considered as estimators of colony evolution: combs covered with (a) adult bees, (b) open brood, (c) sealed brood and (d) honey reserve. *N. ceranae* prevalence and intensity, and V. destructor prevalence were registered at the beginning (T1) and at the end of the experiment (T6). Along the assay, population parameters (a, b and c) did not show statistical differences between groups. However, a significant increase in honey reserves was evidenced in the group CFS at T6 ($p < 0.05$). Nosema prevalence and intensity did not show changes between treatments, though

V. destructor prevalence showed a trend to reduction between T1 and T6, in the CFS group. These bacterial metabolites emerge as an alternative for parasite control and increase honey reserves, however it is necessary to deepen the knowledge that leads to its application.

P201 Bee *Varroa* scanner

Bassignana D.[1], Francini G.[1], Ghirardi M.[1], Varesio A.[1], Porporato M.[2], Martino F.[2], Laurino D.[2], Cuttini D. [2]
[1] Apisfero A.P.S., Turin, Italy; [2] University of Turin - Department of Agricultural, Forest and Food Sciences (DISAFA), Turin, Italy

Varroa destructor and the viruses it transmits are considered the first cause of honey bee colony losses. The control of this parasite is a difficult task for beekeepers and requires repeated determinations of infestation level throughout the year to decide if and how to treat honey bee colonies. Counting the number of varroa fallen on the hive bottom board is a reliable and not invasive way to do so, but is rather time consuming and prone to observer's errors.
The Bee *Varroa* Scanner (BeeVS) is an integrated and really innovative system that will allow to reduce the time required to carry out the checks and that highly improves the precision of the determination. This highly technological prototype tool is able to recognize and count the number of *Varroa* on the "fall tray" of the hive using a sophisticated algorithm of artificial intelligence fully automating the count. Furthermore, the system with an online database that allows geo mapping of the mite distribution in the land and accurate hot spot analysis of the state of *Varroa destructor*.
The University of Turin tested in 2017 the tool BeeVS in his experimental apiaries and also some of the main Italian beekeepers associations have been able to verify its operation. The trials made it possible to verify precision, accuracy, and reliability of the instrument.
BeeVS allows timely *Varroa* control treatments, thus reducing the damages caused by the mites and the use of acaricides. Its use by beekeepers will allow saving bee colonies from collapsing due to varroa and virus infestation adopting an integrated approach, moving towards a more sustainable future of beekeeping.

P202 Creating overwintered nucleus colonies for early spring research

Lamas Z.[1,2], Palmer M.2
[1] Department of Entomology, University of Maryland, College Park, USA; 2 French Hill Apiaries, St Albans City, VT, USA

We used a method of nucleus colony production to make experimental colonies with a high degree of standardization. Traditionally, nucleus colonies are small hives with five frames of brood, bees and a laying queen. They are often available for sale during the spring months, along with honey bee packages. Michael Palmer has been keeping nucleus colonies in an alternative method in Saint Albans, Vermont. The nucleus colonies are initially established during the spring honey flows, and then expanded to grow vertically by adding an additional box. The colonies are managed prior to the winter. 240 nucleus colonies were made in Clinton Co. New York during June and July of 2016. Each colony was started with two frames of brood and a queen cell. They were grown out to 10 frames or 5x5 configuration. 127 colonies were treated, equalized and moved to Columbus Co. North Carolina. In January 2017, 48 colonies were used for a migratory study where colonies were sent from the coast of North Carolina to California almonds. We believe overwintered nucleus colonies are ideal for early spring research. All colonies met minimum contract requirements for the almond broker. Colonies had a high level of standardization amongst the group. Queen quality and overall colony health was well observed. Outliers were omitted from selection for the experiment. Instead of purchasing packages or nucleus colonies in the spring, this project exemplifies how Palmer style management of nucs can be used to establish quality colonies for research efficiently and cost effectively.

P203 An assessment of the Belgian *Halictids* species, with an overview of the endangered species in other countries in Europe

Pauly A., Boevé J-L.
Royal Belgian Institute of Natural Sciences, OD Taxonomy & Phylogeny, Brussels, Belgium

The poster presents the status of 84 bee species of the Halictid bee family in Belgium. 38 species are threatened or extinct, 46 are stable or expanding north. The situation of endangered or extinct species in Belgium is examined in other European countries. For many of them, it seems that the plains populations have become extinct or are disappearing everywhere in Central Europe, while some populations still remain in the mountains of southern Europe (Pyrenees, Alps, Mount Olympus in Greece). The poster presents the different models of maps used in the countries concerned to illustrate this regression. It is currently necessary to join efforts at the European level to analyze the situation, understand the causes of these regressions and develop an atlas of distribution of European Apoidea. The development of an "European Apoidea Fauna", with species identification keys and a barcodes library (COI) would also be desirable to obtain reliable data.

P204 Improvement of the almond production using bumblebees

Marqués A.[1], Herrera C.[1], Juan A.[2], Ruíz M.[3], Traveset A.[4], Leza M.[1]
[1] Laboratory of Zoology, Department of Biology, University of the Balearic Islands, Palma, Balearic Islands, Spain; [2] Servicio de Agricultura, Conselleria de Medi Ambient, Agricultura i Pesca, Palma, Balearic Islands, Spain; [3] Geographic Information Systems (GIS) and Remote Sensing, University of the Balearic Island, Palma, Balearic Islands, Spain; [4] Mediterranean Institute for Advanced Studies (IMEDEA), Esporles, Balearic Islands, Spain

The pollination of almond trees strongly depends upon insects, as it is a self-incompatible species and thus requires cross-pollination to set fruits. The current decline of honeybees and wild bees' populations due to multifactorial causes (such as the use of pesticides, diseases caused by parasites, viruses or bacteria) is leading to the search of alternatives to increase the production of almond, in particular, the use of commercial pollinators. In this study, we examined the effect of introducing bumblebees (*Bombus terrestris*) on almond production. We performed the study during three years (2015-2016-2017) in two different orchards of Mallorca (Balearic Islands, Spain) where almonds represent one of the most important crops. We compared an orchard in which bumblebees were introduced with another one without bumblebees and that was used as control. In each orchard, we marked c. 200 trees from which we monitored fruit set and pollinator visitation rate during the flowering period. We modelled the visits of the main pollinators, *B. terrestris* and *A. mellifera*, under different climatic conditions, and also assessed, for the first time, the spatial distribution of fruit set in the orchard with bumblebees. We found that fruit set was significantly higher where *B. terrestris* had been introduced compared to the control field. The proportional increment varied across years, ranging from 27.6% to 57%. Bumblebees showed to preferentially forage towards a southwest orientation and close to their colony. The model showed that the activity of both *B. terrestris* and *Apis mellifera* is significantly influenced by environmental conditions; specifically, *B. terrestris'* activity is conditioned by temperature, whereas that of *A. mellifera* is more influenced by global radiation and relative humidity. It is the first time large scale study confirms that the use of *B. terrestris* increases almond production and determines foraging activity and behaviour of *B. terrestris* in Mediterranean conditions.

P205 Ensuring access to high quality flower resources can reduce impacts of climate change on bumblebee colony development

Martinet B., Vanderplanck M., Rasmont P., Barraud, A., Renaudeau C., Carvalheiro L.G., Michez D.
Université de Mons, Laboratoire de Zoologie, Mons, Belgium

Pollinators are experiencing declines globally. Well known drivers of such decline include climatic stress and nutritional stress (e.g. due to transformation of naturally diverse landscapes to large monocultures). Understanding potential synergies between these two important drivers is needed to improve predictive models on future effects of climate change on pollinator decline. Here we performed bioassays on 117 colonies of *Bombus terrestris* to evaluate the potential for interactived effects of heat stress, loss of resource quality and colony size. When acting isolated, both nutritional and climatic stress led to change in colony development, more specifically investment in male production is substantially reduced. Additionally, when acting together climatic and nutritional stress lead to accentuated reductions in colony development. Small colonies were much more sensitive to heat and nutritional stresses than large ones, possibly because the number of workers buffers environmental stresses and helps maintain homeostasis. Overall, our study suggests that in the context of current global warming, ensuring access to high quality flower resources could reduce impacts of climate change on bee decline.

P206 Hyperthermic stress resistance of bumblebees: what about of sub-boreal Belgian species

Zambra E., Martinet B., Michez D., Rasmont P.
Université de Mons, Laboratoire de Zoologie, Mons, Belgium

Global warming can result in gradual changes with modifications of main climatic parameters (humidity, temperature, etc.) but also in an increase in the frequency of extreme and localized weather events (e.g. heat waves). These heat waves are hyperthermic stress which have been associated with direct physiological perturbations, which are suspected to dramatically increase insect mortality. Many insect pollinators are experiencing a global decline of their populations. Climate change including heat waves have been pointed out as one of the main drivers of pollinator decline. Here, we assessed the hyperthermic stress resistance through their time before heat stupor (THS) at 40°C of ten Belgian species including sub-boreal species particularly threatened by climate change. We also investigated ethological aspects to define the behavioral time-line to heat stupor. Our results show that heat stress resistance is significantly different among species. Our results highlighted a heat resistance gradient: the heat stress resistance of sub-boreal species is weaker than the hyperthermic resistance of widespread and ubiquitous species.

P207 Foraging in the cities: ecological niche breadth and overlap of Euglossini bees

Oi C.A.[1], Nascimento Lima I.[2], E.A [3], López-Uribe M.M.[4], Del Lama M.A.[5], Freitas B.M.[6], Silva C.I.[2,6]
[1] KU Leuven, Department of Biology, University of Leuven, Leuven, Belgium; [2] Universidade Federal do Ceará, Departamento de Zootecnia, Fortaleza, Brazil; [3] Universidade Estadual de Santa Cruz, Departamento de Ciências Biológicas, Ilhéus, Brazil; [4] Pennsylvania State University, Department of Entomology, University Park, USA; [5] Universidade Federal de São Carlos, Departamento de Genética e Evolução, São Carlos, Brazil ; [6] Universidade de São Paulo, Departamento de Ecologia, São Paulo, Brazil

Bees of the tribe Euglossini are considered important pollinators. Both males and females visit different species of plants for the collection of floral resources to feed themselves or offspring. Due to the broad diversity of plants used for the collection of floral resources, euglossine bees are characterized as polylectic. The aim of the present study was

to obtain information on the trophic niche of *Euglossa cordata* and *Eulaema nigrita* through an analysis of pollen on specimens collected in urban areas to characterize the ecological niche of these euglossine bees. Grains of pollen from *Solanum paniculatum* and *Tradescantia zebrina* together represented 63% of the diet of *Eg. cordata*. Grains from *S. paniculatum* and *Psidium guajava* together represented 87% of the diet of *El. nigrita*. *Euglossa cordata* had significantly more diverse diet in comparison to *El. nigrita* (H'*Eg. cordata* = 1.974; H'*El. nigrita* = 1.270; $t = 75.41$; $p < 0.001$). The two species shared half of the floral resources, but only pollen from *S. paniculatum* was abundantly collected by both species. The model of potential distribution for both bees showed that all the AUC training values were higher than 0.900. *Eulaema nigrita* had a greater number of suitable areas than *Eg. cordata*, mainly in the southeastern region of Brazil. In the present study, the Ecological niche models show that the distribution of *Eg. cordata* and *El. nigrita* overlaps throughout most of the areas of occurrence in the Neotropical region, as demonstrated by pollen collected from bees in urban areas. Ecological niche modeling is important to gaining knowledge with regard to priority areas for the conservation of native species. Thus, the present results on the trophic niche of *El. nigrita* and *Eg. cordata* provide decision makers with knowledge so that they can decide what species should be used in urban landscaping programs.

P208 Foraging activity of honey bees and wild pollinators on fruit trees and berry shrubs

Bevk D.
Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

The results of the monitoring of pollinators' foraging activity on apple trees, blackcurrants, strawberries, blueberries and raspberries will be presented. Foraging behaviour was monitored in an orchard in central Slovenia in years 2017 and 2018 during the whole flowering period and in different weather conditions. The number of pollinators was determined by counting at the sample sites from 5 or 7 a.m. to 8 p.m. Furthermore, we compared the pollination speed of different bumblebee species and honeybees. Great differences in pollinator communities between different plants were found. In general, the most numerous pollinators of all plants except blueberries were honeybees. Bumblebees were the main pollinators of blueberries and frequent pollinators of blackcurrants. Other numerous pollinators were hoverflies, solitary bees and wasps (only in strawberries). Differences in pollinator communities among days were observed. In most cases, bumblebees and wasps became active earlier in the morning than other pollinators. They were the most active in the morning and also at low temperatures and in rainy and windy weather. Other pollinators were generally the most active in the middle of the day. Bumblebees were two to four times faster than honeybees; therefore, the importance of bumblebees is much greater than could be concluded on the basis of their numbers. The complexity and dynamics of pollinator communities and activities show the great importance of maintaining pollinator diversity.

P209 Effects of some behavior characteristics of honey bee (*Apis mellifera l.)* and bumble bee (*Bombus terrestris*) on cherry pollination (production, quality, phenology and yield) and climatic temperature change

Topal E.[1], Yucel B.[2], Margaoan R.[3], Kosoglu M.[1], Karaca Ü.[1]
[1] Aegean Agricultural Research Institute, department of Apiculture, İzmir, Turkey; [2] Ege University, department of Animal Science, İzmir, Turkey; [3]UASMV Cluj-Napoca, department of Horticulture, Cluj-Napoca, Romania

In this study,the effect of environmental temperature on cherry production, quality, plant phenology and yield was investigated on 2015 and 2016 in Kemalpasa District by using honey bee and bumble bee. For this purpose, the effect of visit frequency for honey bees and bumble bees and the foraging activity on pollination were examined in the cherry. Also, effect of the visits of the honeybees (*Apis mellifera* L.) and bumblebees (*Bombus terrestris*) to cherry blossoms on their fruit fertility and quality were examined. The experiment was carried out as closed application (3.8 mm x 3.8 mm porous tulle) and free practice in 24 trees with three plots and 8 trees per parcel. As well as the honey bees and the bumble bees, other insects were counted during the blossoming. In order to identify the effects of the implementations on the fruit set and quality, pomological analysis was done on the harvested fruit. The honey bee pollination produced a

fruit set of 14.1% in open implementation and 4.5% in enclosed implementation. A statistically significant effect of the pollinator implementation on seed weight and stem length was, which are pomological features of the fruit, was not found. In terms of fruit breadth, length and weight, however, a difference between implementations existed ($P<0.05$). In the experiment, the temperature change in 2016 affected the first flowering of cherries, the phenological periods and the variety of pollinator in the application garden. According to years, indicating that the honey bees have more pollination activity than the bombus and other natural pollinators. It was determined that the farming activities of honey bee and Bombus terrestris have reached the highest level at 12.00 hours in accordance with the climatic conditions, and it was significantly decreased after 15.00 hours ($P<0.05$). As a result of the experiment, the highest fruit attitude was obtained in areas visited by honey bees and bumble bees ($P<0.05$). Where sudden climate changes occur, when also other pollinator insect varieties are protected, not only honey bees and bumble bees, the loss of fruit yield will prevent.

P210 Genetic analysis on recently found *B. veteranus* specimens in Belgium, does the supposed extinct species returned or just never left?

Maebe K.[1], D'haeseleer J.[2], Teepe A.[2], Ronse A.[2], Smeets P.[2], Meeus I.[1], Smagghe G.[1]
[1]Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium; [2]ACULEA, De bijen en wespenwerkgroep van Natuurpunt, Natuurpunt, Mechelen, Belgium

The decline of pollinator species is an emerging threat gaining worldwide attention. Generalist foragers like bumblebees, which are essential pollinators in natural and managed ecosystems, are also undergoing decline. A good example of a bumblebee species in decline in Belgium is *Bombus veteranus* (Thoracobombus) (Fabricius, 1793). *B. veteranus* was one of the most abundant bumblebees in Belgium one century ago, but it started to decline in the 1950s and this species was even considered as extinct in Belgium. However, in July 2016, during an Aculea bee sampling, a few *B. veteranus* workers were collected. The newly discovered specimens were genotyped with 16 microsatellites for species identification. In addition, we investigated if these recent discovered specimens were representatives of the original Belgian *B. veteranus* population or if these specimens are emigrants from nearby *B. veteranus* populations in France. A better understanding of this species biology and origin is crucial for their conservation, and in turn to avoid a new or actual extinction in the near future.

P211 Spatio-temporal floral resource shifts in Belgium

Jacquemin F.[1,2], Violle C.2, Folshweiller M.[3], Drossart M.[3], Michez D.[3], Rasmont P.[3], Dufrêne M.[1]
[1] Université de Liège-Gembloux Agro-Bio Tech, UR Biodiversité et Paysage, Gembloux, Belgium; [2] CEFE, Ecologie comparative des organismes, des communautés et des écosystèmes, Montpellier, France; [3] Université de Mons, Laboratoire de Zoologie, Mons, Belgium

For the last decades we have been facing up a large and worldwide bee decline. Land use changes, such as agricultural intensification and urbanization, are largely pointed out to be responsible for this decline as they are known to have induced global biodiversity losses and a change in the nesting and floral resources for wild bees. Our study aims to investigate the link between changes in floral resources and the decline of wild bees in Belgium. To reach this objective, we compiled a large historical dataset of plant species occurrences (almost 7 million data) at the country scale and we reviewed floral resources lists at the plant genus level for five declining bumblebee species (*Bombus humilis*, *B. jonellus*, *B. ruderatus*, *B. soroeensis*, *B. sylvarum* and *B. lucorum*). We splitted our resulting databases in two historical periods (1930-1970 and after 1970) corresponding to large contrasted landscape contexts in Belgium. We corrected sample bias for plants using Hurlbert rarefaction method. The resulting dataset allowed us to observe the spatial and temporal dynamics of the floral resource diversity (number of genus identified as resources) and richness (% of genus identified as resources on the total number of plant genuses) for each bumblebee species for an area covering Belgium with a

meshing of 4x4 km. Land use data were used to interpret these dynamics. We highlighted major changes in resource diversity for most declining bumblebees. Some squares observed a significant loss of genus richness (> 25% decrease). These dynamics were concomitant with the expansion of cities and the agricultural landscape homogenization. Our results contribute to a better understanding of the bee decline causes. Extended to other pollinator species, they can be used to improve the effectiveness of targeted conservation measures in floral resource deficient areas.

P212 Exotic and native plant species and their role attracting native pollinators

Giovanetti M.[1], Fico G.[2,3], Giuliani C.[2,3], Palamara Mesiano M.[4], Boff S.[4], Lupi D.[4]
[1] Centre for Ecology, Evolution and Environmental Changes, Faculty of Sciences, University of Lisbon, Lisbon, Portugal; [2] Department of Pharmaceutical Sciences, University of Milan, Milan, Italy; [3] Ghirardi Botanical Garden, Department of Pharmaceutical Sciences, University of Milan, Toscolano Maderno, Brescia, Italia; [4] Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy

Facing continuous losses of natural habitats, botanical gardens seem to pop up as important centre to sustain insect communities. Actually, what distinguishes many of these areas is the fact that the preserved plants are often exotic and that, in the case of Angiosperms, there is a wide variability of flower anthesis and morphology in a restricted area. The role of exotic plant species in attracting floral visitors in such environments, is little known yet. We addressed the question of how successful native and exotic plant species within the same environment were in attracting insects. In summer 2016 and 2017 we performed pollinator surveys at all flowering plants species at the Ghirardi's botanical garden at Garda Lake (N 45°38′ 21,61′′, E 10° 36′ 40,33′′), Brescia, Italy. Network analysis of plants and native pollinators were performed by distinguishing the origin of plants in two groups, native and exotic. Thus, we assigned the role of each plant group in attracting the bee community. Furthermore, we grouped all bees by functional traits (tongue size) to address the question if bee-plant interactions were driven by plant origin (native/exotic) or by compatibility between tongue size and floral traits. Our results added a novel paradigm in bee-plant interactions in urban areas where, according to our results, exotic species seem to play an important role in network interactions.

P213 Pollination efficiency of thirteen bee species visiting *Cajanus cajan* in Cameroon

Sanda M.[1], Paxton R.J.[2]
[1] Department of Biological Sciences, University of Ngaoundéré, Ngaoundéré, Cameroon; [2] Institute for Biology, Martin-Luther-University Halle-Wittenberg, Germany

Bee species and thought to vary in the efficiency with which they act as pollinators, yet rarely are they compared in their efficiency and in the role they therefore play in the pollination of any crop plant. We determined the role of 13 bee species (the native honey bee and 12 other wild bee species) when visiting pigeon pea (*Cajanus cajan*) flowers across two years in Cameroon. Observations were made on the duration of flower visitation and the speed with which bees moved between flowers. In addition, an experiment was performed in which the fruit and seed set of flowers visited once by a single bee were compared to those open to flower visitors and those permanently bagged; these data were used to quantify single-visit pollination efficiency of the 13 bee species. In the Open treatment, 93% (in 1st season) and 96% (in 2nd season) of flowers set pods, whilst in Bagged treatment only 63% (in 1st season) and 70% (in 2nd season) of fruits were produced compared to the open treatment. Pigeon pea benefited from insect visitation. Bee species varied in the speed with which they visited flowers; *Ceratina* sp. visited the least flowers whilst *Chalicodoma rufipes* visited the most per minute. They also varied in their single-visit efficiency, with *Ch. rufipes* the most efficient, *Megachile* sp.1 the least efficient and the honey bee intermediate between the two. Clearly, multiple bee species are of importance in their role in *C. cajan* pollination, highlighting the importance of maintaining a diverse pollinator community for food security.

P214 Status and trends of wild pollinators in Belgium and North of France

Folschweiller M.[1], Jacquemin F.[2], Drossart M.[1], Dufrêne M.[2], aMichez D.[1], Rasmont P.[1]
[1] Laboratoire de Zoologie, Université de Mons, Mons, Belgium; [2] UR Biodiversité et Payasage, Université de Liège – Gembloux AgroBioTech, Gembloux, Belgium

Pollinators play a very important role in terrestrial ecosystems. Indeed, by contributing to the pollination of most of our wild and cultivated flowering plants, they provide an essential ecosystem service. The main goal of the SAPOLL project is to elaborate an action plan for the conservation of wild pollinators in Belgium and north of France. In order to do so, prior assessments are needed. Here we present our first review of wild pollinators situation at global and regional level. This report, made by regional experts, addresses the decline of wild pollinators, the associated factors and also the consequences of this decline.

P215 Adaptation to a changing world: how wild bees cope with climate change

Folschweiller M., Lecocq T., Dufrêne M., Rasmont P.
Université de Mons, Laboratoire de Zoologie, Mons, Belgium

The current climate change affects living systems, especially by inducing shifts in species phenology (the time of the year at which a seasonal activity is performed). First fragmented available results strongly suggest that the phenology of wild bees (Hymenoptera : Apoidea) is changing. However, this is still poorly understood since few researches have focused on this phenomenon. According to the key role of wild bees in ecosystem service, the understanding of their phenology changes is thus a biological conservation priority.
In this project, starting in January 2015, we will perform the first comprehensive study of the apoïds (i.e. wild bees) phenological shifts in Europe since the 19th Century. Our main goal is to determine if the phenology shifts are triggered by the meteorological parameters and/or species life history traits by comparative statistical and modeling analyses. In this study, we will also investigate the interpopulational and intergenerational adaptations to climate change in a model species (*Bombus terrestris*) through comparative bioassays. These bioassays will focus on the effect of temperature on diapause termination in this species. It will bring more understanding on how this model species adapts to the changes in climate through adaptation and phenotypic plasticity.
This research is innovative as it focuses on a topic of great ecological importance but still very scarcely studied. Thus, this work will be pioneering and it will bring to light results with significant applications in conservation ecology as well as for the study of ecosystem services and climate change.

P216 May regulations against thistles threaten bumblebees?

Vray S.[1,2], Lecocq T.[1,3], Roberts S.P.M.[4], Rollin O.[1], Rasmont P.[1]
[1] Laboratory of Zoology, Research institute of Biosciences, University of Mons, Mons, Belgium; [2] Department of Geography, University of Namur, Namur, Belgium; [3] Research Unit Animal and Functionalities of Animal Products (URAFPA), University of Lorraine – INRA, Vandoeuvre-lès-Nancy, France; [4] Visiting Research Fellow, Reading University, Reading, UK

Bumblebees (*Bombus* genus), which play a widely recognized and essential role for the pollination ecosystem service, are undergoing a strong decline in Europe due to, amongst other things, the reduction of floral resources. Leguminous plants (Fabaceae) are considered to be one of the main pollen sources of bumblebee colonies, but thistles (Asteraceae, tribe Cardueae) have been suggested to be important for male diet. Yet, several European countries apply strict regu-

lations against thistles since they are considered weeds in agriculture. Here, we assess the importance of thistles (*i.e.* Cardueae) in the diet of each caste of bumblebees, and if the legislations against thistles could be a threat for their conservation. For this, we use field observations across European countries where a legal regulation against thistles is in effect, including Belgium. Our results confirm the great importance of thistles (mainly *Cirsium* spp. and *Carduus* spp.) in the diet of male bumblebees. We show that a high number of species, many of which are rare in Belgium and Europe, depend largely on thistles and on the four species for which the destruction is legislatively mandatory. Such laws could therefore threaten bumblebee populations, already greatly weakened by global environmental changes. We argue for the abolishment of these legislations in favor of alternative measures that reconcile the conservation of biodiversity and agricultural needs.

P217 Beekeeping potential in the red dwarf honeybees, *Apis florea*

Rod-im P., Duangphakdee O.
King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand

Apis florea is a honeybee species which is widely distributed through the warm climate of Asia. It usually builds a single comb approximately 20 – 30 cm width in shrubs, bushes and small trees. This bee is highly adapted to build their nests in many kinds of habitats such as disturbed areas, urban areas, intensive agricultural areas, and savanna ecotopes. This study suggested beekeeping with *A. florea* can be developed for sustainable beekeeping particularly for Thailand. This research projects aims 1) to investigate *Apis florea* bee flora; 2) to study comb developmental cycle of them; 3) to develop a low cost equipments to facilitated *A. florea* beekeeping; 4) to develop technique producing honey 5) to compare the cost effectiveness of developed method and conventional honey hunting method. The total yield of honey from beekeeping and natural honey hunting was 1,229.56 ± 230.26 g and 270 ± 89.92 respectively. The beekeeping technique developed in this project yield 4.5 time higher than honey collected from natural honey by the conventional hunting method. There was a distinctly difference between the cost per year gained for conventional honey hunting than developed beekeeping technique. In the same investment of timing, conventional honey hunting lose 16,743.8 bath per year whereas developed beekeeping technique gained 57,445.61 bath per year. In conclusion, *Apis florea* is highly recommended potential for economically beekeeping.

P218 Honey bees (*Apis mellifera* L.) use pollen from the biogas crop Sorghum bicolor and increase the seed-set

Siede R.[1], Freyer C.[2], Büchler R.[1]
[1] Landesbetrieb Landwirtschaft Hessen, Bieneninstitut Kirchhain, Kirchhain, Germany; [2] Justus Liebig Universität, Institut für Allgemeine und Spezielle Zoologie, Tierökologie; Gießen, Germany

Global climate change is a fundamental threat. Fueling biomass can mitigate global warming. In Central Europe maize is the key-crop for biogas production. Maize farming is supposed to have a poor ecological value as seen by e. g. low insect prevalences in maize fields. Diversifying bioenergy cropping systems by growing Sorghum bicolor could putatively counterbalance these negative impacts. A field trial was conducted to test, whether a) the pollen of *Sorghum bicolor* has a nutritive value for bees and whether b) foragers of honey bees improve the seed-set of *S. bicolor*. At an experimental farm in Groß-Gerau (Germany) maize (M), phacelia (P), sorghum hybride (SH) and sorghum line (S) were grown under insect proof net tunnels. Shortly before onset of the simultaneous flowering of all crops at the end of July 2017, small shook swarms of 0.5 kg bees and egg-laying sister queens were placed inside the tunnels. Swarms were supplied regularly with syrup (ApiInvert ®), but not with proteinaceous supplements. Once a week from end of July to beginning of October colony performance data were collected. The means of bee mass were 278 g (M), 350 g (P), 367 g (SH) and 339 g (SL). Differences were not statistically significant (p=0.369, gen. lin. model). The colonies in the phacelia tunnels had significantly more eggs, more brood cells and more bee bread compared to the sorghum and maize colonies.

Differences between sorghum and maize were not significant. The resp. means for eggs were 477 (M), 665 (P), 385 (SH) and 380 (SL), for open brood 179 (M), 431 (P), 160 (SH) and 160 (SL) and for sealed brood 244 (M), 859 (P), 282 (SH) and 312 (SL). Further 16 SH-tents were established at Groß-Gerau and at a climatically stressful site in Germany. Honey bee nucs were placed in four tents of each variant. The bees increased significantly the seed setting at the stressful site (t-test, p=0.0002).
Conclusions: Diversifying bioenergy crop rotations with sorghum can provide utilizable pollen sources for bees. Seed-setting of *S. bicolor* can be improved by bees.

P219 Impacts of honey bee density on crop yield: a meta-analysis

Rollin O., Garibaldi L.A.
Instituto de Investigaciones en Recursos Naturales, Agroecologia y Desarrollo Rural (IRNAD), Universidad Nacional de Río Negro (UNRN), San carlos de Bariloche, Argentina

There is increasing recognition that pollination deficits are limiting crop yields worldwide. However, management strategies for optimal pollination are still unclear for most crops. Current management focuses on providing high densities of honey bees, but recommended densities are highly variable, even within single crops and varieties.
We performed an extensive literature search to record honey-bee densities (colony density and/or flower visitation rates) and crop productivity (fruit set, seed set, fruit weight, and/or yield). Effect sizes represented the difference in crop productivity between the two most extreme levels of honey-bee densities.
Surprisingly, out of 795 reviewed studies, only 22 analyzed the effect of at least two levels of honey-bee densities on crop productivity (reporting 60 effect sizes in total). Thus, most recommendations for crop pollination management are not based on proper experimental designs.
We found that both colony density and visitation rates increased all the productivity variables. However, effects were non-linear for visitation rates, suggesting that there is an optimum (mean of 8-10 visits per flower) beyond which more honey bees are not beneficial (or even detrimental) for crop productivity.
Effect sizes for visitation rates were greater than those for colony densities, suggesting that visitation rates are a more direct measure of the pollination process. Data on the relation between colony density and visitation rates are lacking. Interestingly, effect sizes of visitation rates were greater for crops with separate sexes than those with hermaphrodite flowers; therefore, the benefits from honey-bee pollination varies according to the crop biology.
Synthesis and applications. Current practices for crop pollination assume that more honey bees are always better for crop yield. However, our analyses suggest that there is an optimum of honey-bee densities. Despite the importance of honey bees and pollinator-dependent crops worldwide, there is a lack of studies designed for finding such an optimum level of crop pollination. Our analyses further suggest that visitation rates could be used as a proxy to guide management recommendations such as colony density and spatial arrangement.

P220 Effects of landscape structure and floral border on pollination services provided by honeybees and native bees in avocado orchards of central Chile

Rodríguez S.[1], Perez L.[1], Jaramillo J.[2]
[1]Agriculture, Food and Ingredients, CSB, Fraunhofer Chile Research, Chile; [2] Bayer Bee Care Center, Monheim, Germany

Different spatial and temporal configurations of the landscape surrounding the crops provide feeding and nesting resources to bees, influencing the diversity of potential pollinators. This study aims to establish the relationship between landscape heterogeneity, pollination by *Apis mellifera* and native bees, and avocado production in three commercial orchards located in central Chile, since avocado represents one of the most important crops throughout the country. If the heterogeneity of the landscape increases the diversity of native bee species and this leads to greater fruit production through the pollination process, then the yield will be higher in avocado orchards that present a more heterogene-

ous landscape and a larger diversity of native bees. The main pollination management practices implemented by beekeepers and farmers during the flowering period of the avocado were registered. Satellite images of the surrounding landscape of each of the three orchards were obtained and analysed, defining 30 points of random sampling of plants and bees, distributed in a radius of 500 m, that allow to estimate the diversity of *Apoidea* associated with native and introduced plants. In a next season, in each orchard avocado trees will be randomly selected in a gradient distance from the floral border to the interior of the orchard to measure the frequency of visit and the pollen load of the bees as an indicator of the efficiency of pollination The accuracy and precision of each floral visitor will be evaluated by filming the different visits in the field. The pollen of the avocado flowers will be dyed and its location in the body of the visiting bees will be studied. Preliminary results indicate that the avocado orchards with the largest planted area (223ha) have a high percentage of introduced flora, a smaller number of native bees and a higher abundance of honeybees, compared to the landscapes with a smaller planted area (25ha).

P221 The effect of pest management in orchards on honey bee welfare

Soroker V.[1], Kamer Y.[1], Goldenberg I.[1], Hezroni A.[1], Zaadi E.[1], Shoker S.[1], Rave E.[1], Knol Y.[1], Sarig S.[2]
[1] *Agricultural Research Organization, The Volcani Center, Israel;* [2] *Katif R&D Center, Sdot Negev, Israel*

Annual losses of honeybee colonies in Israel are about 25%, with majority occulring during summer months. The agrochemicals are often blamed for the phenomena. Indeed, intensive agriculture is based on use of variety of agrochemicals and especially pesticides. As some of the latter may pose a significant risk to some none target organisms such as pollinators we have focused on evaluation on impact of pest management in vine and citrus orchards on honeybees. For three years we regularly monitored the condition of honeybee bee colonies. In addition, residues of agrochemicals in live and dead honeybees was assessed by a combination liquid chromatograpy mass spectrometry (LCMS)and gas cheomatography and mass spectrometry (GCMS). The analysis revealed the presence about 30 different chemicals including, herbicides, fungicides, acaricides and insecticides. Presence of 15 plant protection agrochemicals was accompanied by extensive honey bees' mortality. Although these are only circumstantial evidence, the results call for adoption of more pollinator friendly agricultural practices.

PARTNERS AND EXHIBITION

Partners

We gratefully acknowledge the support of our partners and do thank them sincerely for their engagement.

Local Academic Partners

GHENT UNIVERSITY

HoGent

Scientific partners

fwo Research Foundation Flanders Opening new horizons

Belgium – Flanders - FWO

FWO supports – inter alia - researchers with the organisation of scientific conferences where the international and inter-university dimension is a central element of the programme. International scientific conferences are an important means of stimulating scientific debate, disseminating knowledge and developing informal networks.

Eva Crane Trust

Eva Crane Trust

The Trust was formed by Dr Eva Crane. It was enhanced by the residue of her estate bequeathed to the Trust on her death in 2007. Its aim is to advance the understanding of bees and beekeeping by the collection, collation and dissemination of science and research worldwide as well as to record and propagate a further understanding of beekeeping practices through historical and contemporary discoveries.

Belgium – Wallonia - FNRS

fnrs LA LIBERTÉ DE CHERCHER

The mission of the Scientific Research Fund - FNRS is to develop fundamental scientific research within the context of initiatives put forward by the researchers. It encourages the production and development of knowledge by supporting, on the one hand, individual researchers and by financing, on the other hand, research programmes carried out within the laboratories and departments which are mainly located in the Universities of the Wallonia-Brussels Federation.

Partners in the public sector

DEPARTEMENT LANDBOUW & VISSERIJ

EurBee 8 in collaboration with the
Government of Flanders, Department of Agriculture and Fisheries

Provincie Oost-Vlaanderen
Voor ieder van ons

With the support of the **Belgian Province of East-Flanders**

federal public service
HEALTH, FOOD CHAIN SAFETY AND ENVIRONMENT

With the support of **the Federal Government of Belgium**

Other Partners & Exhibitors

Gold Partner

Véto-pharma
Committed to apiculture

Others

VITA beehealth

SATT NORD

ALVEIS By Chemicals Laif

46th APIMONDIA International Apicultural Congress
MONTRÉAL, 8-12 SEPTEMBER, 2019
QUÉBEC - CANADA

ROTARY CLUB WETTEREN

BEEKEEPING NEEDS ! River Wood Inc.

BIJENHOF

biobest SUSTAINABLE CROP MANAGEMENT

AT ONE WITH NATURE MELI

QUADRI

Koninklijke Vlaamse Imkersbond

AVI vzw


AUTHORS LIST