The 7th European Conference of Apidology

7-9 September 2016

Cluj-Napoca, Romania

Edited by Daniel S. Dezmirean

Descrierea CIP a Bibliotecii Naţionale a României
EUROPEAN CONFERENCE OF APIDOLOGY (7 ; 2016 ; Cluj-Napoca)
Seventh European Conference of Apidology : 7-9 September 2016, Cluj-Napoca, România / ed. by Daniel Severus Dezmirean. - Cluj-Napoca : AcademicPres, 2016
ISBN 978-973-744-536-0

I. Dezmirean, Daniel (ed.)

595.799

Director editură – Sef lucr. dr. **Dan VODNAR**

Editura AcademicPres

Universitatea de Ştiinţe Agricole şi Medicină Veterinară Cluj-Napoca

Calea Mănăştur, nr.3, 400372 Cluj-Napoca
Tel. 0264-596384

Fax. 0264-593792

E-mail: eap@usamvcluj.ro

President of EurBee
Daniel Severus Dezmirean

Secretary
Otilia Bobiș, Rodica Mărgăoan, Adriana Urcan

Scientific Committee
Jean Christophe Sandoz, Silvio Erler, Lars Chittka, Pilar de La Rua, Ingolf Steffan-Dewenter, Otilia Bobiș, Liviu Al. Marghitas, Laura Stan, Dirk de Graaf.

Local Organizing Team
Liviu Al. Mărghitaș, Adriana Criste, Adriana Urcan, Andreea Bunea, Claudia Pașca, Florina Copaciu, Georgeta Diniță, Laura Stan, Marius Zăhan, Rodica Mărgăoan, Silvia Pătruica, Victorița Bonta.

Photographs and cover design by

Radu Pădurean (Radupadurean Photography), Sorina Darjan (USAMV Cluj), Silviu Samsodan (Suprema Advertising)

Volunteers:
Bogdan Butilcă, Demetra Dezmirean, Mara Dezmirean, Patricia Fedor, Cristian Mătea, Bogdan Onica, Lelia Pop, Demetra Rakosy, Alexandra Sicoe, Tania Someșfălean, Thomas Vezeteu.

Acknowledgements:
We are grateful to our collaborators: Robert Paxton, Robin Moritz, Christina Grozinger, Christian Pirk, Emilio Caprio and Silvio Erler.

Table of Contents

Tuesday - 06.09	Wednesday - 07.09	Thursday - 08.09	Friday - 09.09
15:00-18:00 Registration	8:00 – 9:00 - Registration	**9:00 – 9:45 Plenary lecture - Matthew Webster**	**9:00 – 9:45 Plenary lecture – Olav Rueppell**
	9:00 – 9:15 - Welcome Adress	**9:45 – 10:30 Plenary lecture – Marcelo Aizen**	9:45 – 10:30 - Session Pathology - Aula Biological activity of bee products – Amf. Verde SMARTBEE
	9:15 – 10:00 Plenary lecture Christina Grozinger	**10:30 – 11:00 Caffee Break**	**10:30 – 11:00 Caffee Break**
	10:00 – 10:30 Caffee Break	11:00 – 13:00 - Session	11:00 – 11:15 EurBee Meeting **11:15-12:00 Plenary lecture - Jean Christophe Sandoz**
	10:30 – 11:15 Plenary lecture Adrian Horridge 11:15 - 13:00 - Session Population Genetics – Amf. Albastru Genomics – Amf. Verde Pathology- Aula Pollinators ecology – A6 Behaviour - Bibilioteca	Biological activity of bee products – Amf. Verde Pathology - Aula Behaviour - Bibilioteca Population Genetics – Amf. Albastru Pollinators ecology – A6	12:00 – 13:00 - Poster Session
	13:00 – 15:00 - Lunch break	**13:00 – 15:00 - Lunch break**	**13:00 – 15:00 - Lunch break**
	15:00 – 15:45 Plenary lecture- Franco Mutinelli **15:45 – 16:30 Plenary lecture - Simon Potts**		**15:00 – 15:45 Plenary lecture - Maria Campos** 15:45 – 16:30 – Session Pathology - Aula Biological activity of bee products – Amf. Verde
	16:30 – 17:00 Caffee Break 17:00 – 18:00 - Session Population Genetics - Amf. Albastru Genomics - Amf. Verde Pathology - Aula Pollinators ecology- A6 Neurobiology – Biblioteca	**Social Event – Trip to Salt Mine Turda and Romanian evening at Ciumbrud winery**	**16:30 – 17:00 Caffee Break** 17:00 – 18:00 - Session Pathology - Aula Biological activity of bee products – Amf. Verde SMARTBEE
	18:00 – 18:30 - Poster Session		20:00 - Banquet at Hotel Napoca
19:00 Welcome Party			

Wednesday – 07.09.2016

	Aula "Mihai Şerban"	Green Amphitheater	Blue Amphitheater	Library Hall	A6 Hall
09:00-09:15	Welcome Adress				
09:15-10:00	**Plenary Christina Grozinger**				
09:45-10:30	**Coffee Break**				
10:30-11:15	**Plenary Adrian Horridge**				
11:15-11:30	Anneleen Parmentier	Aumer Denise	Ayça Özkan Koca	James Makinson	Rakosy Demetra
11:30-11:45	Jessy Praet	Lewkowski Oleg	Meral Kekecoglu	Dreyersdorff	Laura Moquet
11:45-12:00	**Pathology** Pierre Giovenazzo	**Genomics** Vignal Alain	**Population Genetics** Gayaunan Danailu	**Behaviour** Christian Pirk	**Pollinator ecology** Gherardo Bogo
12:00-12:15	Tine Descamps	Silva Sara	İrfan Kandemir	Stephan Wolf	Yael Mandelik
12:15-12:30	Marie-Pierre Chauzat	Regan Tim	Diego Cejas	Adam Tofilski	Mazi Sanda
12:30-12:45	Marion Laurent				Çiğdem Özenirler
12:45-13:00	Marco Porporato				Pierre Ouvrard
13:00-15:00			**Lunch**		
15:00-15:45	**Plenary Franco Mutinelli**				
15:45-16:30	**Plenary Simon Potts**				
16:30-17:00	**Coffee Break**		**Coffee Break**		**Coffee Break**
17:00-17:15	**Pathology** Moses Chemurot		**Population Genetics** Denis Michez,		**Pollinator** Alon Ornai
17:15-17:30	Qiang Huang		Silvio Erler		Aniko Kovács
17:30-17:45	Laurianne Paris		Laura Jara		Alice Fournier
17:45-18:00	Jevrosima Stevanovic		Beaurepaire Alexis		Pierre Rasmont
18:18:30			**Poster Session**		

Thursday 08.09.2016

	Aula "Mihai Șerban"	Green Amphitheater	Blue Amphitheater	Library Hall	A6 Hall
09:00-09:45	**Plenary Matthew Webster**				
09:45-10:30	**Plenary Marcelo Aizen**				
10:30-11:00	**Coffee Break**	**Coffee Break**	**Coffee Break**	**Coffee Break**	**Coffee Break**
11:00-11:15	Marie-Pierre Chauzat	AnjaButtstedt	Letitia	Manuel Tritschler	Robert Brodschneider
11:15-11:30	Häussermann Claudia	Saorla Kavanagh	Papoutsi	Seltzer Rya	Lina De Smet
11:30-11:45	Ziegelmann Bettina	Janko Božič	Delatte Hélène	Melissa Oddie	Reet Karise
11:45-12:00	Mendoza Yamandú	Annely Brandt	Alice Pinto	Fanny Mondet	Laura Jones
12:00-12:15	Paul Page	Christina Kast	Dora	Meral Kekeçoğlu	Oraw.Duangphakdee
12:15-12:30	Nanetti Antonio	Carmen Muresan	Henriques	Monika Kos	Stavrinides Menelaos
12:30-12:45	Bettina Ziegelmann	Mohammad Javed	Melanie Parejo	Manuel Tritschler	Arnon Dag
12:45-13:00	Michele Mortarino	Ansari			
13:00-15:00	**Lunch**				

Pathology | **Bee products** | **Population Genetics** | **Behaviour** | **Pollinator ecology**

From 15:00 **Social Event – Trip to Salt Mine Turda and Romanian evening**

Friday - 09.09.2016

	Aula "Mihai Șerban"	Green Amphitheater	Blue Amphitheater
09:00- 09:45	**Plenary Olav Rueppell**		
09:45-10:00	Joachim de Miranda	Ahmet Guler	Kaspar Bienefeld
10:00-10:15	Anne Dalmon	Sang Mi Han	Desiderato Annoscia
10:15 –10:30	Karel Schoonvaere	Meral Kekecoglu	Adrian Siceanu
10:30 – 11:00	**Coffee Break**	**Coffee Break**	**Coffee Break**
11:00 – 11:15	EurBee Meeting		
11:15-12:00	**Plenary Jean Christophe Sandoz**		
12:00 – 13:00		**Poster Session**	
13:00 – 15:00	**Lunch**		
15:00 – 15:45	**Plenary Maria Campos**		
15:45-16:00	Robert J. Paxton	Thomas Vezeteu	Ralph Büchler
16:00-16:15	Ibrahim Youssef	Adriana Aurori	Gennarod i Prisco
16:15-16:30	Philippe Bulet	Llorens-Picher M.	Ewan Campbell
16:30 – 17:00	**Coffee Break**	**Coffee Break**	**Coffee Break**
17:00-17:15	Hatjina Fani		Wegener, J
17:15-17:30	Fabio Sgolastra		Marina Meixner
17:30-17:45	Geoffrey R. Williams		Alex Uzunov
17:45-18:00	Ales Gregorc		

Pathology | **Bee products** | **SmartBee**

From 20:00 **Banquet at Hotel Napoca**

Preface

EurBee 7 brings together in Cluj researchers from around the world concerned with apidology fields. The Conference Sections capture current topics of great interest and actuality from genomics, population genetics, behavior, pollinator ecology, neurobiology, the study of the biologically active potential of bee products and last but not least, bee pathology, which is extremely complex in present days.

Consistent with the objective for which this conference was designed, over 300 enrolled participants, PhD students or senior researchers coming from all over the world will present and discuss their research results. As always, the reason is one and the same: the continuous challenge of studying the behavior of the fascinating bee family as well as the concern for the health maintenance in their living conditions nowadays , given the fact that the bees come up against decreased habitat and biodiversity, global warming, not to mention the excessive use of insecticides and pesticides.

At the seventh edition, EurBee manages to achieve something else that is entirely new. This year, in Cluj Napoca at our university, the conference becomes the bond base around which the largest European research programs on apiculture meet together during an entire week, to show and discuss their achievements. Certainly, colleagues from COLOSS, Super-B and SmartBee, will enjoy a maximum efficiency and will consider our university and city a permanent partner for cooperation.

Cluj Napoca will give you the chance to live a few days in the middle and spirit of Transylvania, living new experiences, in novel locations, restaurants with traditional food and unforgettable pubs.

Welcome to Cluj Napoca !

Daniel Severus DEZMIREAN

President of EurBee

Plenaries

The inputs to vision of colour in the honey bee

Adrian Horridge
E-mail: horridge@netspeed.com.au

I started work on the compound eye in 1960 and most of my students worked on insect vision. We had a wonderful run, but others in the field almost destroyed the subject.

In the case of bee colour vision, Karl von Frisch (1914) believed he had proved it when bees trained to come to a colour would select most colours from all shades of grey, even though they could not select mid-grey when trained on mid-grey, as shown here.

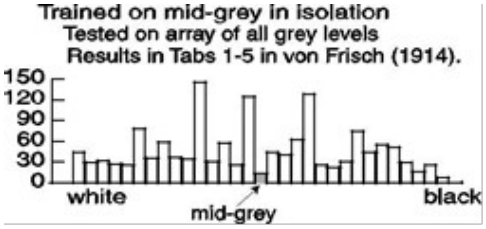

Fig. 1. How could von Frisch use grey levels when bees could not recognise mid-grey?

There were many anomalies in the 188 pages + plates, which were bitterly defended by the author despite being disproved by Carl von Hess, Mathilde Hertz and a sequence of researchers in Germany before 1939, and more anomalies in the 1990s.

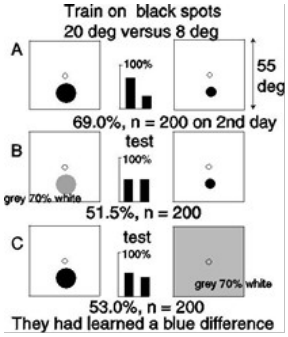

Fig 2 Bees trained on a large versus small black spot failed to distinguish a large grey spot from a small black one, or a large black spot from a plain grey target. A and B. From Ronacher 1998. Biol. Cybern. 79, 477-485.

The new theory was anticipated when it was shown that a target of equal

yellow and blue panels could not be distinguished from its mirror image in the absence of green contrast at the boundary, even though each panel subtended 25° × 50°.

A concentrated effort starting in 2012, training bees to plain colours and coloured patterns, followed by repeated tests of the trained bees, revealed the new results. The strongest input was green receptor modulation. Modulation is a measure of the total length of vertical edge multiplied by the contrast at each part of the edge and the spectral sensitivity of the receptor. Colour and pattern structure are inseparable in the same two receptor channels, blue and green. Bees detect, locate and measure the blue content in the display before them with the blue receptor in each ommatidium, and also detect, locate and measure green receptor modulation. They learn coincidences between these two inputs at different places on the eye, and the angle between them. Blue receptor modulation is also detected. In colour, they see only monochromatic blue. Grey levels are shades of blue, white is a very bright blue, and black is not a stimulus. Therefore, there are no bee colours, no true colour vision or achromatic vision. The ultraviolet receptors appear not to participate in colour vision because UV inhibits the perception of blue. So far as is known, bee vision is similar to that in fly (*Drosophila*).
For recent papers see my web page:-Adrian.Horridge.org

Bee nutrition: from genes to landscapes

Christina M. Grozinger
Department of Entomology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University
E- mail: cmgrozinger@psu.edu

Populations of honey bees, bumble bees, and other pollinators are in decline globally due to the effects of multiple biotic and abiotic stressors. Together with our collaborators, we have examined the impacts of several of these stressors (pathogens, parasites, and pesticides) on bees at the genomic level to determine if they perturb common or distinct pathways, and if these pathways are related to particular physiological functions or behaviors. Both abiotic and abiotic stressors modulate expression of metabolic and nutrition-related pathways, and improvements in diet quality can mitigate the impacts of these stressors on bees. We further demonstrated that bees' pollen foraging preferences are driven by the macronutrient ratios and nutritional quality of pollen, allowing bees to selectively

forage in complex landscapes to obtain their optimal nutrition. Finally, we show that survival of bee colonies is strongly influenced by the quality of the forage available in their landscapes. Overall, our results provide a framework for improving nutritional resources for bees and supporting resilient bee populations.

The impact of invasive bees on agriculture

Marcelo A. Aizen
Laboratorio Ecotono-CRUB, Universidad Nacional del Comahue and INIBIOMA, (8400) San Carlos de Bariloche, Río Negro, Argentina
E- mail: marcelo.aizen@gmail.com

Management of crop pollination is mainly based on a relatively few domesticated bee species, most notably *Apis mellifera*, that have been transported across the world and introduced in regions outside their native ranges. A few of these species have become phenomenal invaders in several continents; the most impressive examples are the Africanized honeybee (i.e. a hybrid between the African *Apis mellifera scutellata* and the European honeybee subspecies) in the Americas and the European bumble bee *Bombus terrestris* in Southern South America, New Zealand, Tasmania and Japan. Because of their high abundances, these invasive bees can change the nature, from mutualistic to antagonistic, of many of the flower-pollinator interactions they usurp, with negative consequences even for crop pollination. This is exemplified with a study of the effect of *B. terrestris* on raspberry pollination in southern South America, where it has been recorded that fruit quality decreases above a few bumble bee visits because of increasing style breakage. Also, nectar robbing of raspberry flower buds by bumble bees decreases nectar availability for more legitimate pollinator. At a continental scale, evidence on a supposedly positive effect of the invasion of the Africanized honeybee in coffee yield is put into question, showing that temporal trends in yield vary greatly among countries and that yield declines have occurred in some Neotropical countries during the last decades. It is concluded that invasive bees can have several direct and indirect negative effects on pollination services and further introductions should be discouraged.

Small Hive Beetle in Italy

Franco Mutinelli
Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università, 10, 35020 Legnaro (PD), italy
E-mail: fmutinelli@izsvenezie.it

Aethina tumida Murray, the small hive beetle (SHB), adults and larvae were firstly identified in Italy in September 2014 in honey bee nucleus colonies near the Gioia Tauro port in the Calabria region (southern Italy). In November 2014, a single infested apiary was found in Sicily. Genetic analyses revealed the African origin of SHB introduced into Italy. Early reaction measures adopted in Italy required immediate notification of SHB detection to the local veterinary services, movement restriction of the concerned colonies and apiaries, destruction of infested apiaries and ploughing and pyrethroids treatment of the surrounding soil. In Calabria region 89 positive sites and one in Sicily were detected and destroyed between 2014 and 2015. The Ministry of Health granted compensation to beekeepers according to the law in force. Furthermore, a protection (20 km radius) and surveillance (100 km) zone were established. The surveillance zone includes the whole territory of Calabria and Sicily region. Compulsory visits to all apiaries in the protection zone with georeferentiation and visual colony inspection according to 5% expected prevalence (95% CI) are applied. In the surveillance zone, apiaries are selected according to a risk analysis or randomly and colonies are inspected according to 2% expected prevalence (95% CI). Sentinel honey bee nucleus colonies were installed to improve SHB detection in the protection zone and around it. Furthermore, a national SHB surveillance program was carried out in spring and autumn 2015 and 2016. Until now, no SHB has been detected outside the two concerned regions. Future perspectives of containment are discussed.

Understanding the potential for multi-trait selection in the honey bee

Olav Rueppell
University of North Carolina, Department of Biology, 321 McIver Street, Greensboro, NC 27403, USA
E-mail: o_ruppel@uncg.edu

In light of the continuing honey bee health crisis, artificial selection is widely advocated as one of the most promising approaches to improve honey bee health and ensure the long-term sustainability of apiculture. Here, I discuss the genetic architecture of the pollen hoarding syndrome, a well-studied model system for understanding the prospect of artificial selection in apiculture. Importantly, trait correlations may impede simultaneous optimization of multiple economically important traits. The long history of selection in managed populations of *Apis mellifera* may in fact have contributed to the disease susceptibility of our managed honey bee populations. However, some trait correlations can be broken down, which is particularly true for the highly-recombining honey bee genome. Therefore, understanding meiotic recombination is important and I will discuss some efforts to identify causes and consequences of recombination in *Apis*. In conclusion, the prospects for artificial selection to improve honey bee health depend on our understanding of 1) the genetic architecture of the various traits in question, including identified genetic markers, 2) the population structure of the commercial honey bee populations, and 3) the recombinational landscape of the honey bee genome.

Bee pollen Standard Methods |State of the art

Maria G. Campos, Lídia Barreto and Ofélia Anjos
Coimbra Chemistry Centre (CQC, FCT Unit 313) (FCTUC) and, Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Santa Coimbra, Coimbra, Portugal
E-mail: mgcampos@ff.uc.pt

Bee pollen is a crude material that can be sold as fresh or dry product, as food or dietary supplement. Nevertheless until now, no normalized quality control parameters were harmonised, all over the international markets, for this natural product. The “Bee pollen working group” from “International Honey Commission”

(http://www.ihc-platform.net) is now finishing a proposal of "Standard Methods for pollen analysis" that will be published in Coloss Bee Book (http://www.coloss.org/beebook) Volume III in a very near future. From the data collected in the research group and from the bibliography a good amount of taxa are now identified as good sources of bee pollen for commercial transactions and the characterization is almost completed. Microscopic photos, DNA analysis, and the quantification of the main metabolites as proteins, aminoacids, glucids, lipids, vitamins, minerals are some of the compounds that were object of the evaluation for a standard methods to be proposed. Producing Bee Pollen from harvest to the storage, loads authentication by means of computer vision and machine learning, classification of corbicular pollen morphology, phenolic and polyphenolic profiles as fingerprints of floral origin, determination of phenolic profile of bee pollen extracts by LC and LC/MS, identification of bee pollen mixtures through meta-barcoding, pyrrolizidine alkaloid profiling in pollen by UHPLC-HRMS are also object of the publication.

How do bees perceive the olfactory world? A behavioural and neurophysiological account

Jean-Christophe Sandoz
Evolution Genomes Behavior and Ecology, CNRS – University Paris-Saclay, 1 avenue de la Terrasse, 91190 Gif-sur-Yvette, France
E-mail: Jean-Christophe.Sandoz@egce.cnrs-gif.fr

For many years, the honeybee *Apis mellifera* has been an important model for the understanding of the neuroethological basis of olfactory perception and learning. Olfaction is a crucial sensory modality for bees, both within the colony for intraspecific communication and outside the colony during foraging. Through different processing steps, olfactory systems create evolving internal representations that differently represent odors' chemical characteristics and/or biological value. In addition, experience modifies how odors are represented in the brain. We study olfactory perception and learning using a range of behavioural protocols associated with neurophysiological and neuroanatomical techniques. In bees, odors are detected by sensory neurons on the antennae, which project to a primary processing centre in the brain, the antennal lobe (AL). Then two main tracts of projection neurons convey odor information to higher brain centers, the mushroom bodies (MB) and the lateral horn (LH). Using olfactory conditioning

and *in vivo* calcium imaging, I will describe olfactory processing and the representation of floral and pheromonal odors at these different levels of the olfactory pathway. We will discuss the implications of these findings for understanding how the bee brain may classify odorants with different biological meanings, eventually giving rise to adaptive behaviours. I will then show how such neuroethological knowledge on olfaction can be applied for evaluating potentially deleterious effects of pesticides on honeybee olfactory behaviours, or for developing control methods against a new bee enemy in Europe, the invasive hornet *Vespa velutina*.

Supply and demand of Pollination Services

Simon Potts
Reading University, UK
E-mail: s.g.potts@reading.ac.uk

Pollinators have rapidly risen up the scientific, political and public agendas over the last decade which has stimulated intense research efforts to help inform policy and practice. In my talk I aim to synthesise our current knowledge on the sustainable management of pollination services. First, I explore the multiple values pollinators provide society and then answer the questions: who actually pollinates our crops? and does current pollinator supply match crop demands? Along the way I bust a well-established myth, and go on to show how wider landscape management and local farm management practices both effect pollinators and service delivery. Finally, I give an overview of some of the land management tools which have been shown to be effective for safeguarding pollinators and services.

The genetic basis of local adaptation in *Apis mellifera*

Matthew T. Webster
Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala Sweden
E-mail: matthew.webster@imbim.uu.se

Uncovering the genetic basis by which species adapt to their local environments is a major goal of evolutionary biology. The honeybee *Apis mellifera* inhabits a huge geographic range across temperate and tropical regions and is adapted to local conditions across this range. We are using population-scale genome sequencing to uncover the evolutionary history and the genetic nature of local adaptation in populations of honeybees from around the world. Recently, we have focussed on adaptations in two populations of African honeybees. The first are the Cape bees of South Africa, in which worker bees are able to lay diploid eggs and engage in social parasitism. The second are high altitude populations of honeybees that inhabit the mountains of Kenya and are morphologically distinct from bees in surrounding regions. In both cases we demonstrate that these populations are genetically extremely similar to surrounding populations across their entire genomes, but we are able to identify genes with highly divergent patterns of genetic variation that are responsible for their specific adaptations. Our results give insights into the evolutionary history and biological basis of honeybee subspecies.

Spoken presentation

Neurobiology

Coordinator: Jean Christophe Sandoz

Because of its fascinating social behaviors and its impressive cognitive feats, the honey bee has become in the last 50 years an important model for the study of the neurobiological basis of insect behavior, perception, learning and memory. An impressive corpus of neuroanatomical, neurophysiological and neuroethological data has already been obtained making of the honey bee one of the best studied insect models. Recently, however, this line of research has taken a new turn, as it has become increasingly clear that many pesticides used in agriculture provoke deleterious sub-lethal behavioral effects through - sometimes subtle - actions on the bees' neural system. This session will present current research aiming to understand the function of the bees' nervous system both under normal and pesticide-affected condition.

Effects of sublethal doses of thiacloprid on the learning and memory performances of honey bees

Léa Tison[1], Marie-Luise Hahn[1], Sophie Holtz[1], Alexander Rößner[1], Amy Adeoye[1], Önder Kalkan[1],Uwe Greggers[1], Gabriela Bischoff[2] and Randolf Menzel[1]
[1] Institüt für Biologie-Neurobiologie, Freie Universität, Berlin, Germany;
[2] Julius Kühn-Institüt, Federal Research Centre for Cultivated Plants, Berlin, Germany.
E-mail: lea.tison@neurobiologie.fu-berlin.de

There has been growing concern in recent years over the decline of pollinators worldwide. Honeybee foragers may be exposed to neonicotinoids during their foraging flights via pollen, nectar and guttation drops and yet, the colony relies on the foragers' ability to locate food sources and bring pollen and nectar back to the hive.Most studies have focused on three neonicotinoid insecticides - clothianidin, imidacloprid and thiamethoxam – which are currently suspended in Europe. Here we focus on thiacloprid, a cyano-substituted neonicotinoid thought to be less toxic to honey bees and of which use has increased in the last years.In an earlier field experiments, we showed the effects of thiacloprid on honey bees *(Apis mellifera carnica)* exposed chronically for several weeks to a sublethal concentration.The foraging behavior, homing success, navigation performance, and social communication of the exposed honey bees were impaired, and thiaclopridwas shown to add up into the foragers over time. We also showed in the laboratory and with semi-field experiments that the substance does not have a repellent taste for honey bees.
In this new experiment, we performed Proboscis Extension Response conditioning to assess whether thiaclopridhas an effect on honeybees'learning and memory. In order to know on which processes the substance is acting (learning, memory consolidation/retrieval), bees were fed with thiaclopridactive substance or with the formulation Calypso®at 3 different sublethal doses either 1 hour before conditioning, 5 hours after conditioning or 1 hour before the memory test. Honey bees' learning rates were evaluated after 3 conditioned (reinforced) stimulus (CS). The retrieval of the memory was always tested at the same time for the 3 different feeding groups, 24 hours after conditioning.In addition to the CS, bees were exposed to 2 novel odors to determine whether or not they were responding selectively to the CS.
Learning and memory are playing a central role in the behavioral ecology and communication of foraging bees and we have already shown how this substance chronically affects honey bees' behavior in the field. Here we are showing once

more that thiacloprid, as active substance and as formulation,is posing a risk to this important insect pollinator by disrupting their learning and memory functions, helping also to understandprevious results obtained in the field.

Voltage-gated Ca^{2+} channels of honeybee: cloning and characterization

Pierre Charnet, Cens T, Rousset M, Chahine M, Menard C, Thibaud J-B, and Collet C.

Institut des Biomolécules Max Mousseron (IBMM), CNRS UMR 5247, Bat. CRBM, 1919 Route de Mende, 34293 Montpellier cedex 5, France. Université Montpellier, Montpellier, France
E-mail: pierre.charnet@crbm.cnrs.fr

Olfactory memory is a key process by which honeybees detect food, individuals and hives, and is thus crucial for individuals and colony long-term survival. It has been studied extensively over the past decades, but the molecular actors are still poorly identified and characterized. While Ca^{2+} influx has been shown, on living bees, to be involved in memory formation, the nature and biophysics of the underlying Ca^{2+} channels are still unknown. Analysis of the honeybee genome allowed the identification and the cloning of the genes encoding for the 4 types of voltage-gated Ca^{2+} channels Ca_V1, Ca_V2, Ca_V3 and Ca_V4 and their regulatory $\alpha2-\delta$ and $Ca_V\beta$ subunits. The functional properties of the Ca_V gene products have been analyzed after expression in Xenopus oocytes and their expression profiles in various central and peripheral neurons as well as in muscle cells. Recording Ca^{2+} channel activity in these cell types allowed a preliminary characterization of the role of these genes in the Ca^{2+} influx.
Their biophysical/pharmacological characterization identified one LVA and three HVA Ca^{2+} channels and suggests that they could represent a potential collateral target of the widely used pyrethroids insecticides. Interestingly, the LVA channels appear to be poorly expressed in the brain. Our work also provides new tools allowing to anticipate the toxicity of various environmental pollutants for the honeybee *Apis mellifera*.
Financial support: ANR Bee-channels N° NR-13-BSV7-0010

Towards an in vitro screening to better understand the toxicity of insecticides, improve anticipation of risks encountered by pollinators and find alternatives

Collet Claude, Cens T, Rousset M, Gosselin-Badaroudine P, Chahine M, and Charnet P
INRA, UR 406 Abeilles et Environnement, Toxicologie Environnementale, 228 Route de l'aérodrome, Avignon, France
E-mail: claude.collet@avignon.inra.fr

Membrane ion channels are macromolecular complexes responsible for many vital neural activities. For this reason, most neurotoxic insecticides currently used in agriculture target neuronal ion channels. However, mandatory tests required in official guidance documents on the risk assessment of plant protection products refer mainly to classical toxicology procedures, for instance the determination of LD50 values on living organisms. These tests are devoted to identify the 'potential risk of pesticides in causing unacceptable harm to bees'. By contrast, modern human toxicological procedures use in vitro approaches as e.g. the hERG safety bioassay, predictive of an in vivo cardiotoxicity for candidate pharmaceutical drugs. We have recently succeeded in inducing the in vitro expression of the major honeybee ion channels genes targeted by insecticides. We now describe an alternative methodology to evaluate the in vivo and in vitro effects and modes of action of pyrethroids, one of the most popular insecticides class. We tentatively link their effects at the cellular/molecular levels evaluated on the expressed channels, to their sublethal neurotoxicity identified through the mean of physiological tests at the organism level. These integrated approaches may allow us to propose a new strategy to evaluate risks that pesticides pose to pollinators with patented in vitro methods and a possible new screening of toxicants on honeybee ion channels. Alternative pollinator-friendly protection tools (e.g. biocontrol) efficient against pests may also be identified through a differential screening procedure. Financial support: ANR-13-BSV7-0010 Bee channels.

Modelling elemental learning of honeybees by spiking neural networks of the lateral antennal lobe tract

HaDi MaBouDi[1], Hideaki Shimazaki[2] and Lars Chittka[1]
[1]Department of Psychology, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, UK
[2]RIKEN Brain Science Institute, Wako-Shi, Saitama, Japan
E- mail: h.d.maboudi@qmul.ac.uk

The honeybee olfactory system is a well-established model for understanding functional mechanism of learning and memory. Olfactory stimuli are first processed in the antennal lobe (AL), and then transferred to the mushroom body (MB) and lateral horn (LH) through dual pathways termed medial and lateral antennal lobe tracts (m-ALT and l-ALT). Recent studies reported honeybees could perform elemental learning by associating an odour with a reward signal even after lesions in m-ALT or blocking MB [1,2] although such learning has been studied by modelling m-ALT that terminates on MB. To test if the lateral pathway (l-ALT) is sufficient for elemental learning, we modelled local computation within glomeruli with axons of projection neurons (PNs) connecting to a decision neuron (LHN) in LH. The model is further enriched by synaptic plasticity in AL and octopaminergic modulation in AL and LH [4-6]. We show that inhibitory spike-timing dependent plasticity (iSTDP) on synapses from local neurons (LNs) to PNs, a model of non-associative plasticity by exposure to different stimuli, decorrelates PNs' outputs. By additionally modelling octopaminergic effects on synapses among LNs in AL and PNs into LHN using modulated STDP [3,7], the model can discriminate conditioned stimuli, which explains associative olfactory learning by a few stages of odour-processing in the l-ALT. Importantly, by monitoring performance of models with different connectivity caused by non-associative learning, one can describe changes in structural organization of honeybees' AL [8] and their behavioural performances during the first week of their life.

Genomics

Coordinator: Silvio Erler

With upcoming availability of newly sequenced social insects, especially bee genomes, open questions on the origin of sociality and factors driving adaptation to environmental changes can be studied in detail. Population- wide comparative studies provide options to characterize genomic signatures of diseas resistence and selection. Here, we will discuss new techniques and tools in the century of next generation sequencing, but also the importance of gene function and regulation

Reproductive characteristics and genetic analysis of laying *Apis mellifera capensis* workers with different parthenogenetic strategies

Aumer Denise, Allsopp MH, Yusuf AA, Pirk CWW, Jarosch-Perlow A and Moritz RFA
Hoher Weg 4, 06120 Halle (Saale)
E-mail:denise.aumer@zoologie.uni-halle.de

In honeybees worker reproduction is rare in the presence of a queen. Only if the queen gets lost and there is no brood in the colony workers can activate their ovaries and lay unfertilized haploid eggs that develop into males (= arrhenotokous parthenogenesis). An exception are laying workers of the South African Cape bee *Apis mellifera capensis.* Workers of this subspecies are able to produce female progeny via thelytokous parthenogenesis. Thelytoky as well as some other phenotypic traits providing Cape bees with reproductive dominance (high number of ovarioles, early onset of egg-laying and production of a queenlike mandibular gland pheromone) have been suggested to be controlled by a recessive allele at the *thelytoky* locus (*th*). We here report on experiments free of intracolonial selection for reproductive dominance finding the mode of parthenogenesis in laying *A. m. capensis* workers to be dimorphic, segregating in 50% thelytokous and 50% arrhenotokous workers. This Mendelian segregation suggests the queen to be heterozygous at the *thelytoky* locus with all siring males carrying the *th* allele and therefore a single locus controlling thelytoky.

Social isolation and small group sizes induce stress-related transcriptional changes in workers of the western honeybee (*Apis mellifera* L.)

Oleg Lewkowski and Hans-Hinrich Kaatz
Martin Luther University Halle-Wittenberg, Molecular Ecology, HoherWeg 4, 06099, Halle, Germany
E-mail: oleg.lewkowski@zoologie.uni-halle.de

In colonial organisms, social contextis a crucial modulator of colony organisation. Whereas crowding is often associated with a variety of fitness reducing effects in solitary species, social organisms may respond differently. In fact, social interactions are considered to have beneficial effects on social organisms and, in particular, to enhance performance on colony level. In order to derive a systemic stress response of honeybee workers in isolation and in groups of 10, 100 and 1000 individuals, we determined fat body mRNA levels of frequently used stress marker genes from the upstream insulin-like peptide pathway, vitellogenin (*vg*) and the heat shock protein 70family (*hsp70*). We found robust evidence for a group size induced response. Young bees were characterised by an increasing expression of *insulin-likepeptide receptor genes (InR*s*)* with decreasing group size. In 15 days old workers we observed a threshold-like expressional pattern with a distinctly elevated expression of *vg* and *insulin-likepeptide 2* in the group of 1000 bees while *InRs* and two *hsp70* genes showed a reversed pattern. These results point presumably to an elevated stress response in isolated and small groups of workers (1 to 100) compared to more crowded conditions (1000), demonstrating an impact of social context on honeybee workers. Confirming and complementing previous studies, our results highlight the importance of experimental group size when conducting experiments with social insects, investigating their behaviour, physiology as well as the impact of diseases.

Using genomics to understand environmental adaptation of Iberian bumblebees and assess the impact of the use of commercial lineages for crop pollination

Sara E. Silva, Sofia G. Seabra, Vera Nunes, Eduardo Marabuto, Ana S.B. Rodrigues, Francisco Pina-Martins, Telma G. Laurentino, Maria Teresa Rebelo, Joana Martins, Elisabete Figueiredo, Luísa G. Carvalheiro and Octávio S. Paulo
Centre for Ecology, Evolution and Environmental Changes (cE3c), Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
E-mail: saraema.silva@gmail.com

Bumblebees (*Bombus* spp.) are key pollinators with high ecological and economic importance. Due to this and their recent population declines, they have been increasingly studied. With new genomic approaches and the recently available information of complete bumblebee genome sequences (*Bombus impatiens* and *Bombus terrestris*), there is a new open window for more information about bumblebee biology. Coupled with knowledge on species' ecology, this information has the potential to revolutionise our understanding on bumblebee adaptation andresilience. Approximately 42,000 single nucleotide polymorphisms (SNPs) already obtained in this study through Restriction-Site Associated DNA (RAD) sequencing, give new insights about population genetic structure and environmental adaptation of the buff-tailed bumblebee, *Bombus terrestris*, in Iberian Peninsula. We are performing the same analyses for two other bumblebee species present in the same area. We are also applying this genomic approach to study the impact of the introduction of commercial lineages of bumblebees for crop pollination. These nonnative bumblebees could eventually compete with other bees for nesting sites and flower resources, and cause spread of new parasites and pathogens, profoundly impacting native ecosystems. Also, introgressive hybridisation / admixture between introduced and native *Bombus* species / subspecies may alter their genetic composition with unforeseen consequences. In this study we assess the ecological and genetic impact of introduced commercial bumblebees, known to be derived from *Bombus terrestris dalmatinus* (from Greece and Turkey) and *Bombus terrestris terrestri*s (from central/northern Europe), on the native *Bombus terrestris lusitanicus* in Portugal. Our data suggest a signature of potential admixture on natural populations.

Sequence Analysis of the Holobiome of British Honey Bees

Tim Regan, Mark W. Barnett, Stephen J. Bush, Dominik R. Laetsch, Fiona Highet, Giles E. Budge, Mark L. Blaxter and Tom C. Freeman
The Roslin Institute, University of Edinburgh, Edinburgh, EH25 9RG, UK
E-mail: tim.regan@roslin.ed.ac.uk; m.barnett@ed.ac.uk

To better understand the holobiome of honey bees, we have sequenced DNA extracted from hives across the UK and are exploring the use of new methods and tools to analyse these complex data. DNA was sequenced using the Illumina HiSeq 2500 with 125bp paired end read sequencing. Following alignment of reads to the reference genome, *de novo* assembly of the unmapped reads was performed. Assembled contigs were then analysed by BLAST in conjunction with Blobtools, a package which allows visualisation of the taxonomic distribution of such assemblies based on contig size, GC content and coverage. Reads mapping to contigs were binned according to phyla, and the remaining reads reassembled. This iterative process was continued until the orphan reads no longer assembled. Binned reads were then subjected to a final assembly; delivering a comprehensive description of the microbiome composition. We have also used network analyses to visualise assemblies using the tool Miru in combination with the NGS graph generator package. This creates a 3D network plot where each node represents a read, and edges between nodes represent sequence similarity between reads. This is useful to better understand the quality of assemblies, homologies between them and their association with a given sample.

We are currently exploring how the distribution of these species is influenced by location and host genetics. This better understanding of life in UK apiaries has the potential to improve bee health, as well as develop the knowledge base to protect against further threats.

Understanding the French honeybee populations by whole genome sequencing of haploid drones

Dave Wragg, Benjamin Basso, Maxime Beguin, Kamila Canale-Tabet, Cecilia Costa, Aleš Gregorc, Kaspar Bienefeld, Alice Pinto, Melanie Parejo, Laurent Gauthier. Andrew Abrahams, Hélène Delatte, Johanna Clémencet, Jean-Pierre Bidanel, Yves Le Conte and Alain Vignal
GenPhySE, Université de Toulouse, INRA, INPT, INP-ENVT, Castanet Tolosan, France
E-mail: alain.vignal@toulouse.inra.fr

The endemic honeybee subspecies *Apis mellifera mellifera* has gradually been replaced in many beekeeper operations in France by other subspecies and by hybrids between *A. m. ligustica*, *A.m. caucasica*, and *A. m. mellifera*, which were found to be more efficient producers of honey and royal jelly, and also to be less aggressive. In order to understand the impact of these practices on the genome makeup of bee populations, we sequenced over 600 haploid drones, each from one colony belonging to various populations including black bee conservatories, queen breeders (honey and royal jelly production) as well as a few out-groups from various European locations. Results show that sequencing haploid individuals allows high confidence genotyping at a low cost, yielding millions of SNP. Chromosomal regions highly differentiated between populations are observed, presumably as a response to artificial selection on traits such as honey or royal jelly production, but also to other unknown events such as pathogen pressure and other environment variables. Varying degrees of admixture between the local black bee *Apis mellifera mellifera* (M mitotype) and C-type sub-species that were imported by beekeepers are observed.

Behaviour

Coordinator: Lars Chittka

The behaviour section will be opened with a plenary lecture by one of the pioneers on the research on insect vision, Prof Adrian Horridge, whose undoubtedly controversial ideas will surely generate an interesting discussion. Day 2 of the conference will see the first part of the behaviour symposium, with presentations covering radar tracking and spatial orientation of bees, sociobiology, feeding and hygienic behaviour and pollination biology in both honeybees and bumblebees. On Day 3, talks in the behaviour section will focus on honeybee health. Poster presentations will again be on bumblebees and honeybees, and include such exciting topics such as starvation, incest avoidance, fighting behaviour, and the slaughtering of queens by 'balling' by workers.

Bees in Space: lifelong radar tracking of bumblebee foragers reveals extreme variation in exploration vs. exploitation strategies

James C Makinson, Joseph L Woodgate, Ka S Lim, Andrew M Reynolds and Lars Chittka.
Bee Sensory and Behavioural Ecology Lab School of Biological & Chemical Sciences Queen Mary University of London Mile End Road, London E1 4NS
E-mail: j.makinson@qmul.ac.uk

Pollinators such as bumblebees provide crucial ecological servicesfor the habitats in which they live. By studying the spatial movement patterns of these important animals, we can develop a better understanding of the flow of plant genes, pathogens, and the bees themselves through the landscape. We used harmonic radar to track the entire foraging careers of four*Bombusterrestrisaudax* workers, from their first departure from the nest until their disappearance and presumed death. We found that bumblebees produce two distinct types of flight activity; exploration and exploitation flights. Exploration flights involve one or more loops, and where typically produced during the first few flights of a bee, but could occur at any time. Exploitation flights, in contrast, were defined as straight paths to and from a foraging patch. There was a huge amount of variation between the bees in terms of patch fidelity, ratio of exploration to exploitation, as well as the duration and frequency of foraging bouts. Beesexploited a single location at a time, remaining faithful to a particular patch for up to six days. Bees did not switch back and forth between foraging patches, remaining committed to a new foraging patchonce they had abandoned their old foraging location. Our data provides the first ever insight into how bumblebees balance exploration of the environment with exploitation of forage patches, and demonstrate that individuals are extremely variable in their behaviour.

Impact of strawberry production systems on bumble bees foraging preferences

Gerit Dreyersdorff, Reet Karise, Anna Bontšutšnaj and Marika Mänd
Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Fr. R. Kreutzwaldi 5, Tartu 51014, Estonia
E-mail: gerit.dreyersdorff@emu.ee

Strawberry is an important fruit crop grown worldwide.There are twomain strawberry production systems used: open fields and protected cultures such as greenhouses or high tunnels. As quality and yield of strawberry fruits depend largely on adequate pollination, it would be necessary to bring additional pollinators under the tunnels. Bumble bees*Bombus terrestris* L. could be suitable alternatives to honey bees*Apis mellifera* L., although in case of partially open tunnels they may prefer other forage plants over strawberry.
This study was established to estimate i) the number of commercial and indigenous pollinators foraging on strawberry flowers and ii) the proportion of strawberry pollen in bumble bee pollen forage under high tunnels and open field.
A two-year study with commercially produced *B.terrestris* colonies was conducted on strawberryin Estonia.The number of pollinators was counted, bumble bee corbicular pollen was collected and analysed under light microscopy.
We found that the number of indigenous pollinators was very low under high tunnels whereas quite numerous in open field. The mean number of bumble bees under high tunnels was significantly higher than open field. In both years bumble bees collected strawberry pollen at statistically higher rate under high tunnels than in open field despite that high tunnels are partially open and pollinators have free access to outside where the food resources are more diverse. Our results indicate that in case of pollinator-dependent crops like strawberry, it is important to bring additional pollinators under high tunnels in order to obtain higher yield.

Up the social ladder – changing the reproductive potential of honeybee workers

Abdullahi A. Yusuf**,** Robin M. Crewe and Christian W.W. Pirk
Social Insect research Group, Department of Zoology & Entomology, University of Pretoria, Private Bag X20, Hatfield 0028 Pretoria , South Africa.
E-mail: cwwpirk@zoology.up.ac.za

The secretions of the mandibular gland (MG) of honeybees play an important role in regulating reproduction. Workers can become reproductively dominant, so called pseudoqueens, associated with a change in the mandibular gland profile to a more queen-like secretion. Changes in the secretion will trigger a change in the behaviour of other workers towards the pseudoqueen and the more queen-like profile allows the emitter to receive more and higher quality food. The higher quality food allows the worker to activate her ovaries and invest in maturation of eggs. Furthermore, the pheromones of the emitter prevent other workers from becoming reproductively dominant. Pheromonal dominance plays a crucial role in Cape honeybee *Apis mellifera capensis* workers becoming social parasites. This sub-species is endemic to the Cape region of South Africa, usurps colonies of the sister sub-species, the Savannah honeybees *A. m. scutellata*. When colonies are infested by *capensis* social parasites, they are quarantined and killed to avoid transmission of infections throughout the apiary. Here we explore the use of pheromone supplements in management of *capensis* infestations. We introduced workers as mobile pheromone carriers treated with either synthetic mandibular gland pheromones or pheromones extracted from *capensis* parasitic workers. Only pheromone carriers attracted retinues, increased pheromone production, activated their ovaries in transition to a queen like condition. Mandibular gland extracts from natural sources (*capensis*) were more effective than commercially available synthetic queen pheromones. Using this simple mobile pheromone delivery system, we show that providing pheromone substitution can induce pheromone production and can be explored in managing *capensis* infestations through increasing host pheromone production and decreasing ovarian activation in social parasites.

Sex-specific utilisation of subtle cues in foraging bumblebees

Stephan Wolf, Mark Roper and Lars Chittka
Queen Mary, University of London, Mile End Road, London E1 4NS, UK
E-mail: l.chittka@qmul.ac.uk

An animals' sex can markedly affect an animal's utilisation of environmental information. In bumblebees, males need to trade-off mate search and foraging, whereas foraging workers focus solely on flower exploration. Do the fundamentally different life-styles of male and worker bumblebees (*Bombus terrestris*) affect their ability to respond to subtle cues that may indicate reward or predation risk? We hypothesise that multi-tasking males are less acute learners on subtle cues than the "single-minded" workers. In a differential operant learning task we used two saliently coloured feeder types half of which contained a superimposed transparent secondary cue. We tested free-flying males and workers for their ability to identify rewarding feeders using the secondary non-salient cue. Both sexes reached mean choice accuracies of over 80% after 200 visits. However, workers were on average 11% (max. 18.4%) more accurate when the subtle cue indicated reward as compared to the reverse test. Surprisingly, males responded much more cautiously to non-salient cues performing on average 9% better (max. 21.5%) when the reward was predicted by the *absence* of the subtle cue. We discuss the potential evolutionary drivers that may have caused these sex-specific foraging strategies and its implications for our understanding of bee cognitive behaviour.

Observation of wing movements during honey bees' waggle dances with use of a high speed camera.

Adam Tofilski and Sylwia Łopuch
Department of Pomology and Apiculture, Agricultural University, 29 Listopada 54, 31-425 Krakow, Poland.
E-mail: rotofils@cyf-kr.edu.pl

Honey bee foragers returning to the nest perform waggle dances on the comb in order to inform their nestmates about the location of important resources. Nevertheless, it is little known how the information contained in a dance performed

in darkness of the nest is conveyed to the followers. There is indirect evidence suggesting that dancing bees moving their wings produce airborne sounds which transfer the information. In this study, the high speed camera recordings were used in order to directly observe and measure the frequency of wing beats and abdomen wags of dancers. Dancing bees moved their wings by 40.4% time of waggle runs and only 8.1% time of circle runs. Wing movements were performed with folded or unfolded wings in the episodes of one to five wing beats separated by the intervals of motionless wings. The wing beat frequency was 167.0 Hz and it depended on the position of wings and the number of wing beats in one episode. The mean frequency was significantly higher when wings were folded compared with unfolded wings ($p=0.007$) and when the number of wing beats in one episode was three to five compared with one to two ($p<0.001$). The abdomen wag frequency was 14.6 Hz. On average, 7.9 followers observed each dance and significantly more of them gathered around dancers' abdomens than heads and thoraxes ($p=0.001$). Only 55.6% of the followers had direct contact with dancers. Thus, the results of this study confirmed that honey bees dancing moved their wings emitting airborne sounds.

Reversed behavioral fever in a eusocial insect? Deformed wing virus infected honey bee workers prefer cooler temperatures

Manuel Tritschler[1], Jutta Vollmann[2], Anton Stabentheiner[2], Karl Crailsheim[2], Jay D. Evans[3], Orlando Yañez[1] and Peter Neumann[1,4]
1 Institute of BeeHealth, Vetsuisse Faculty, University of Bern, Bern, Switzerland
2 InstituteforZoology, Karl FranzensUniversity, Graz, Austria
3 USDA-ARS BeeResearchLaboratory, Beltsville, MD, USA
4 BeeProtectionLaboratory, Department of Biology, ChiangMaiUniversity, Thailand
E-mail: manuel.tritschler@vetsuisse.unibe.ch

Insects can prefer warmer temperatures to limit disease, which is known as behavioural fever. However, the potential impact of virus infections on temperature affinity is poorly understood in honey bees, *Apis mellifera*. Here, we investigated the temperature affinity ofDeformed wing virus (DWV) infected workers. Given

that affinity to higher temperatures is adaptive, we expect infected individuals to prefer warmer temperatures.
Three groups were investigated in a fully-crossed hoarding cage experiment: 1) 10^7 DWV copies in PBS (Phosphate Buffered Saline) injected in individuals, 2) only PBS injected, 3) Controls; Total N = 15 cages, 750 freshly emerged adult workers). Thermotaxis and behavioural activity were investigated after 8 days using a thermal gradient ranging from 9.5° C to 58.75° C and afterwards, all bees were analysed for DWV using qPCR. While the controls showed low natural infections 5.86 log copies/bee ±0.7s.d., the PBS 11.17log copies/bee±0.17s.d. and DWV 11.6 log copies/bee ±0.08s.d., had significantly highest virus numbers (Kruskal-Wallis ANOVAand post hoc Dunn's test, all $p<0.01$,). Both the DWV and PBS injected bees, significantly preferred colder temperatures compared to the controls (One way repeated measures ANOVAand post hoc Dunn's test, all $p<0.05$).
Our data clearly show that in sharp contrast to other insects, virus infected (DWV) honeybee workers significantly preferred colder temperatures. The resultssuggest that the differences to other insects may have a social basis, e.g. due to individual vs. colony level selection.

Assessing the genetic effects of hygienic behavior: combining methods of classical breeding and artificial insemination

Seltzer Rya[1,2], Paz Kahanov[1,3] , Kamer Yossi[1], Zaydman Ilya[1], Eyer Pierre Andre[2], Malgorzata Bienkowska[4], Hefetz Abraham[2] and Soroker Victoria[1]
[1]Department of Entomology, Agricultural Research Organization, The Volcani Center, Israel
[2]Department of Zoology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel
[3]Bar Ilan University, Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel
[4]Institute of Horticulture, Apiculture Division, 24-100 Pulawy, Poland
Email: rya3683@gmail.com

Hygienic behavior, the recognition and evacuation of damaged pupae in the hive, has been shown to be the main trait contributing to honey bee (*Apis mellifera*) defense against the Varroa mite (*Varroa destructor*). In this study, hives of locally bred bees (*A. mellifera linguistic* bred with locally and sporadically

introduced sub-species) were evaluated to determine the frequency of hygienic behavior, its impact on Varroa mite infestation and the heritability of this trait. The contribution of patrilineal and matrilineal lines was investigated. When comparing naturally mated sister hives, a significant genetic effect was found. There was a negative correlation between hygienic behavior and Varroa infestation rate. To further clarify paternal effects and to assess the value of heritability, hygienic performance of artificially inseminated queens from hygienic and non-hygienic hives was measured. The paternal origin of the queens was confirmed by the genotyping of nine polymorphic markers. Hygienic performance of these hives was compared to that of hives of naturally mated queens.

Recapping behavior in European honey bees surviving *Varroa destructor*

Melissa Oddie, Sandra Lanz, BjørnDahle and Peter Neumann
Institute of Bee Health VetsuisseFaculty, University of Bern, Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland
E-mail: melissa.oddie@vetsuisse.unibe.ch

The ectoparasitic mite*Varroa destructor* has put a widespread and intense selective pressure on European honey bee subspecies (*Apismellifera*ssp.). Several isolated populations are known to survive mite infestations without treatment, but the underlying mechanisms are still not well understood. Here we investigate a possible behavioral mechanism in a Norwegian surviving population. Freshly-capped worker brood was taken from highly-infested susceptible colonies and shared between surviving colonies and susceptible controls. The average fecundity per foundress was significantly lower in surviving colonies (0.87) compared to susceptible ones (1.24), thereby confirming earlier reports of reduced mite fecundity in surviving populations. Moreover, up to 64% of brood cells in the surviving colonies had been chewed open and recapped with wax. In surviving colonies, such cell recapping was specifically targeting mite-infested cells. In susceptible colonies, recapping was uncommon (up to 17%) and not significantly correlated with infestation. Since mites and brood originated from the same source colonies, the results suggest that cell recapping is an adult worker behavior, which could be interfering with mite reproduction. This trait (if as effective as observed) has a very low-energy cost and would allow colonies to survive while retaining energy to cope with other stressors. Understanding the dynamics of this rapid

adaptive change could provide valuable insights into the processes of host-parasite coevolution and help us adapt domestic honey bees to this novel threat.

Specific cues associated with honey bee social defence against *Varroa destructor* infested brood.

Fanny Mondet, Seo Hyun Kim, Joachim R. de Miranda, Dominique Beslay, Alison R. Mercer and Yves Le Conte
INRA PACA Abeilles et Environnement, Domain Saint Paul, Site Agroparc, 84914 Avignon Cedex09, France
E-mail: fanny.mondet@paca.inra.fr

Social immunity forms an essential part of the defence repertoire of social insects. In response to infestation by the parasitic mite *Varroa destructor* and its associated viruses, honey bees (*Apis mellifera* L.) have developed a specific behaviour (varroa-sensitive hygiene, or VSH) that helps protect the colony from this parasite. Brood cells heavily infested with mites are uncapped, the brood killed, and the cell contents removed. For this extreme sacrifice to be beneficial to the colony, the targeting of parasitized brood for removal must be accurate and selective.

The aim of this study was to investigate how adult bees choose which brood to sacrifice when they perform VSH behaviour. Combining a novel behavioural approach in the field with chemical ecology and virology assays, we investigated the characteristics of individual brood targeted or not for removal by VSH behaviour.

We show that unique signatures clearly differentiate targeted from non-targeted brood. This production of uniquely identifiable cues by varroa-infested brood could be used by VSH-performing bees to identify with high specificity which brood cells to sacrifice. This selective elimination of mite-infested brood is a disease resistance strategy analogous to programmed cell death, where young bees likely to be highly dysfunctional as adults are sacrificed for the greater good of the colony.

The control of Varroa mite (Varroa Destructor) in hygienic honeybee colonies

Meral Kekeçoğlu[1,5*,] Pınar Göç Rasgele[2,5], , Merve Kambur[3] and Aykut Burğut[4]
[1]Düzce Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Düzce, Türkiye meralkekecoglu@duzce.edu.tr
[2]Düzce Üniversitesi, Ziraat ve Doğa Bilimleri Fakültesi, Biyosistem Mühendisliği Bölümü, Düzce, Türkiye
[3] Düzce Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Bölümü,
[4] Düzce, Türkiye Çukurova Üniversitesi, Araştırma ve Uygulama Çiftlik Müdürlüğü, Adana, Türkiye
[5]Düzce Üniversitesi, Arıcılık Araştırma Geliştirme ve Uygulama Merkezi (DAGEM), Düzce, Türkiye
E-mail: meralkekecoglu@duzce.edu.tr

We developed a selection and breeding program for local honey bees that display mechanisms of resistance/tolerance to Varroa destructor, by identifying and selecting a line of bees derived from the highly productive Yigilca race of honeybee for hygienic behavior. Firstly, queen bees produced from colonies having hygienic behavior over 95 % were artificially inseminated with marked drones from those colonies. The hygienic behavior of colonies in this first generation was initially be evaluated with a well-tested assay utilizing pin-killed brood. Subsequently, grooming behavior was evaluated using selected superior hygienic and non-hygienic lines by assessing the natural fall of varroa mites. In addition population levels of varroa mite was evaluated in the experimental colonies to investigate the efficacy of breeding and selection programe in hygienic colonies in control of varroa mite.

Acknowledgements: This work, Project No. 2013.01.01.161., was supported by Duzce University Scientific Research Projects Commission. The authors are grateful for support given by Duzce University Scientific Research Projects Commission.

Effect of cerium (IV) oxide nanoparticles on the feeding behaviour and selected enzyme activities of Carniolan honey bee workers *Apis mellifera carnica*

Monika Kos, Anita Jemec, Gordana Glavan, Janko Božič and Damjana Drobne
Department of Biology. Biotechnical faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia
E-mail: monika.kos@bf.uni-lj.si

The use of cerium (IV) oxide ($nCeO_2$) in various industrial applications has increased. The pollution of land by $nCeO_2$ is likely through application of wastewaters biosolids onto agricultural fields and through air pollution since CeO_2 is abundantly used as fuel additive. Honeybees are potentially exposed to $nCeO_2$ via feeding on contaminated plants. In this work we assessed the toxic potential of $nCeO_2$ for *Apis mellifera carnica* workers via 9-days dietary exposure. The honeybees were fed *ad libitum* to various concentrations of $nCeO_2$ in the sucrose solution: 0, 2, 10, 50, 250, and 500 mg $nCeO_2$/L where we tracked feeding behaviour and survival. Additionally we performed a 24-hour group food two-choice assay to test avoidance/preference behaviour for $nCeO_2$ spiked food within the same concentration range. In parallel, we also measured the activity of detoxification enzyme glutathione-S-transferase (GST) and a neurotoxicity biomarkers, the enzyme acetylcholinesterase (AChE) in the head and thorax. Chronic exposure to various concentrations of $nCeO_2$ had no significant effect on survival compared to control, but we observed a significant increase in feeding rate, which was confirmed by evident food preference of honey bees exposed to 250 mg $nCeO_2$/L. Chronic exposure to 250 mg $nCeO_2$/L significantly elevated only soluble AChE form and decreased GST in the heads, while significantly increased activity of the both AChE forms and GST in honey bee thorax. The result indicate that $nCeO_2$ in a given concentration range present a potential risk to honeybees which is reflected in their altered feeding behaviour and detoxification processes.

Population Genetics

Coordinator: Pilar de La Rua

Population genetics remains highly relevant given the present biodiversity crisis, particularly in relation to pollinators decline. Identifying and prioritizing populations for preservation is an imperative task for the protection of ecosystem services such as pollination in both agricultural and wild areas.

In this symposium, we will discuss this subject in relation to honeybee populations as well as solitary bees, bumblebees and stingless bees. We will open the debate for questions on the latest results of techniques such as geometric morphometrics, mitochondrial variation, microsatellites, single nucleotide polymorphism and whole genome sequencing.

Geometric morphometric study on honeybees distributed in Bulgaria, Greece and Thrace

Ayça Özkan Koca, Peter Nentchev, İbrahim Çakmak and İrfan Kandemir
Gastronomy and Culinary Arts, Faculty of Fine Arts, Maltepe University, 34857, Istanbul-Turkey
E-mail: ayca.queenbee@gmail.com

As well as genetic markers, over the last years geometric morphometric has been applied to discriminate honeybee subspecies and populations. The objective of this research was to investigate differences in the shape of forewing and forewing cells of honeybee samples collected from Bulgaria, Greece (including Crete & Skinos) and Thrace. For forewing shape, 20 landmarks were digitized and superimposed by using a GPA. Also, seven cell shapes were studied by using Elliptic fourier analysis. The shape of each cell was described by coefficients and the data obtained from both methods were analysed by using multivariate analysis. Colonies were evaluated in three (Bulgaria-Greece & islands-Thrace) and five (Bulgaria-Greece-Crete-Skinos-Thrace) honeybee groups in statistical analysis. Based on the forewing shape, the differences were found to be statistically significant ($P<0.001$) among three groups with MANOVA. Pairwise test of shape variables were indicated significant differences among groups ($P<0.001$). Although Thrace and Skinos showed significant differences from the other groups, there was no significant differences among Bulgaria, Greece, Crete ($p>0.005$). Similarly, in CVA based on five groups, the colonies from Bulgaria, Greece and Crete showed overlapping clusters, whilst the colonies from Thrace and Skinos formed a nonoverlapping groups. Based on individual cell shapes, the most powerful cell was 2nd cubital cell (75% accuracy). On scatter plot, data obtained from 2nd cubital cell shape displayed similar results with CVA based on landmark data.

A case study, morphometric divergence through loss of original traits of anatolian honey bees: dangerous outcome for turkish beekeeping

Meral Kekecoglu[1], Nazife Eroglu[2] and Merve Kambur[1]
[1]Duzce University, Faculty of Arts and Sciences, Department of Biology, Konuralp, 81620, Duzce, Turkey
[2]The Scientific and Research Council of Turkey, Marmara Research Center, Food Institute, PK. 21, 41470, Gebze, Kocaeli, Turkey
E-mail: meralkekecoglu@duzce.edu.tr

Through genetic pollution owing to largely migratory beekeeping and lack of control mechanisms toward requeening, native honeybees located in Anatolia considered to be threatened with extinction. Hybridization along with or without introgression may tyrannize the presence of naturally-evolved indigenous subspecies and ecotypes. Therefore, the present study accentuate morphological divergence of three Apis mellifera subspecies and two ecotypes of A.m. anatoliaca distributed around Anatolia after which hibridization. After having long-term breeding since 2009 at the controlled conditions of the remote isolated apiary, each honeybee subspecies and ecotypes was collected from their original locations recurrently to investigate potential inequality and variation by time. Among these species. A. m. caucasia from Artvin and Ardahan in northeastern Black Sea region, A. m. syriaca from Hatay in southtern Mediteranean, A. m. carnica from Kirklareli in Thrace region of Anatolia, and A. m. anatoliaca from Yigilca in Western Black Sea and Mugla in Aegean region of Anatolia were represented. Our findings suggested that the honey bees of controlled conditions and uncontrolled conditions form two separate groups based on geometric morphometric techniques, which in agreement of some recent genetic diversity studies on the region. Hence, we strongly urge to have an active monitoring system and banning regarding queen trading and migratory practices as well as periodic testing of recorded apiaries to knowledge ongoing variation on the gene pool.

An analysis of sub-specific variation of populations of honeybee (*Apis mellifera* L.), in West and Central Africa, using geometric morphometrics

Usman H Dukku[1], Gayaunan Danailu[1] and Adam Tofilski[2]
[1]Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi 740004, Nigeria
[2]Department of Pomology and Apiculture, Agricultural University, 29 Listopada 54, 31-425, Kraków, Poland
E-mail: udukku@gmail.com

To improve our knowledge on the sub-specific variability of *Apis mellifera* in West and Central Africa, 179 colonies at 44 locations, were sampled throughout Nigeria and parts of Niger, Cameroon and Chad from different ecological zones. The samples were subjected to geometric morphometric analysis using 19 landmarks on the left forewings of the workers. The analysis allowed to separate wing size and shape. The wing size differed markedly between locations: Bees with smaller wings were present in the southwest and northeast and those with large wings in the northwest and southeast of the area of study. Also wing shape differed significantly between locations and ecological zones. Despite these differences, most of the samples clustered with *A. m. adansonii* in a stepwise discriminant analysis.

Conservation of *Apis mellifera caucasica* and *A. m. anatoliaca* in Turkey

İrfan Kandemir and Ahmet İnci
Ankara University, Faculty of Science, Department of Biology, Tandogan Ankara 06100 Turkey
E-mail: ikandemir@gmail.com

Having a unique geography bridging two continents and being in the intersection of three continents, Turkey harbors several honeybee subspecies distributed in each corner (caucasian, thracian, syrian, agean, and persian) and *Apis mellifera anatoliaca* in the vast area in the centre of Anatolian Peninsula. Two of which were favored for many years by the beekeepers worldwide: Anatolian and Caucasian honeybees. During the course of evolution these subspecies adapted to Turkey's climate and floral diversity, and survived from diseases and parasites for

thousands of years. However migratory beekeeping practices are forcing them to admix and results in loss of genetic diversity. This study reports the efforts started to conserve *Apis mellifera caucasica* in Camili and Posof and *Apis mellifera anatoliaca* in Güdül. For the last two decades many projects and initiative were taken into consideration and with the help of NGO and Ministry of Food, Agriculture and Livestock now, there are at least four apiaries set for the conservation purposes. Three of which are on the Northeast corner for the conservation of Caucasus honeybees and one in Güdül for the conservation of Central Anatolian honeybees, now the apiaries are in good isolation and conservation. They are all registered by the Ministry and many queen breeding enterprises established. Many thousands of queens were produced annually in those operations to market them to the queen producers. Currently the number of pure colonies reached to several thousands and many reciprocal hybrids between Anatolian and Caucasus were anticipated for the future beekeeping practices in Turkey.

Large scale homogenous sperm mixing for custom design colonies?

Silvio Erler, Johanna Pieplow, Job van Praagh and Robin F. A. Moritz
Martin-Luther-Universität Halle-Wittenberg, Molekulare Ökologie, Hoher Weg 4, 06099 Halle, Germany
E-mail: silvio.erler@zoologie.uni-halle.de

Instrumental insemination is a powerful tool for selective breeding of honeybees. The technique is however typically using only semen of few drones to inseminate a queen, much less than the number of drones the queens would mate with under natural conditions. Yet it has been repeatedly suggested that the genetic diversity within the colony is an important factor in colony viability not only affecting honey yield but also traits related to resistance or tolerance towards parasites and pathogens. At the same time it has been shown that worker bees of different patrilines in the colony can specialize in specific tasks. The specialist workers are likely to complement each other to contribute towards an enhanced colony efficiency. We here show that semen mixing of large numbers of drones (>2000) of 10 source colonies in instrumental insemination can establish colonies with reproducible and consistent genotypic composition. Genotyping the offspring,

using highly polymorphic microsatellite markers, revealed an even distribution of all drone donor colonies in the worker offspring generation. The allelic compositions and paternal colony contributions were reliably reproduced in three independent colonies. Consequently, homogenously mixed drone sperm of different genetic backgrounds can be used in instrumental insemination. This opens the path to combining drones of specialist breeding lines at desired ratios to obtain optimized custom design colonies.

Genetic variability of European bees with different level of diet specialization and range size

Denis Michez, Thomas Lecocq, Maxence Gérard and Simon Dellicour
University of Mons, Place du parc 23, 7000 Mons, Belgium
E-mail: denis.michez@umons.ac.be

Past climate change is known to have strongly impacted current patterns of genetic diversity and population structure (i.e. genetic variability) of living organism in Europe. However, ecological factors also have the potential to influence demographic history and thus patterns of genetic variability. So far we are missing studies evaluating the impact of these two factors on the current distribution of European bees. First we investigated the impact of past climate and host plant species abundance, on genetic variability in three related specialized solitary bees of the genus Melitta Second we studied five eusocial species of Bumblebees, but different levels of range fragmentation, range size and food specialization. Our analyses revealed that the solitary species associated with the most abundant host plant displays unusually high genetic variability. Patterns of genetic variability in the two other solitary species indicated lower overall levels of variability. As expected for the Bumblebees, stronger fragmentations of the species range are associated with a stronger geographic differentiation. Furthermore, diet specialization appears to increase population structure at the landscape level, presumably due to the more heterogeneously distributed food resources. On the other hand, no clear association was highlighted between diet specialization or overall range size and genetic diversity.
Surprisingly, the two generalist and co-distributed species investigated displayed widely divergent patterns in terms of genetic diversity and population structure. The analyses of the merged data set showed that the eight

species do not share common hot spot of genetic diversity but their populations are nearly all isolated by distance

Cross-species amplification of microsatellite loci in different species of bumblebees (genus *Bombus* Latreille, 1802).

Diego Cejas, C. Ornosa, P. De la Rúa and I. Muñoz.
Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, 30100 Murcia, Spain.
E-mail:diegomanuel.cejas@um.es

Microsatellites or short tandem repeats (STR) are useful to determine different parameters of evolutionary and ecological interest, like migration rates, bottlenecks, or population isolation coefficients. In the case of bumblebees (genus *Bombus*) STRs are used to analyze their biodiversity in order to conserve their populations. In this study, eleven loci of STR designed originally from the genome of *Bombus terrestris*(B10, B100, B11, B124, B126, B96, B118, B119, B121, B131 and B132), amplified in two multiple reactions, were evaluated in other ten species of the genus *Bombus*: *B. hortorum*, *B. humilis*, *B. lapidarius*, *B. lucorum*, *B. mesomelas*, *B. ruderarius*, *B. soroeensis*, *B. sylvarum*, *B.vestalis* and *B. wurflenii*. The parameters analyzed were quality of amplification of each locus (Q), variation range of the allele size (R), number of detected alleles (Na), and presence of null and private alleles in each species. Eight loci amplified in every species with acceptable values of Q, R and Na; however, six of them did not show allelic variability in some species. Amplification of loci B10, B96 and B118 was negative, or with a low Q in most species. A homocigosis excess was found in two locifor*B. humilis*, five loci for *B. lucorum*, six loci for *B. lapidarius* and in seven loci for *B. ruderarius*which might be due to the existence of null alleles. Ten loci showedprivate alleles, which might be useful to differentiate the species studied. These results are discussed in the context of present needed conservation of bumblebee species.

Effect of transhumance on the prevalence of *Varroa destructor* and the colonies genetic diversity in Iberian honeybees

Laura Jara, Álvaro García-Valero, Pilar Franco, Irene Muñoz, Carlos Ruiz, José Serrano and Pilar De la Rúa
Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, 30100 Murcia. Spain
E-mail: laura.jara@um.es

Transhumance is a widely extended practice in Spain aimed to increase the yield of beekeeping products. However, it represents a factor of homogenization for Iberian honeybee population and also a stress factor for the colonies, affecting their fitness and enhancing the spread and proliferation of diseases. To analyze the extent of these effects, we tracked the genetic composition and the health condition of 20colonies: (i) ten colonies usually staying in our apiary at the University of Murcia; of these, five (UMU-R) remained resident in Murcia (south Spain) while other five (UMU-T) were moved to Soria (north Spain) during the summer period (June - October 2015); (ii) other ten colonies from apiaries of professional beekeepers; five colonies of a resident apiary in Soria (BSO-R) and other five colonies of a transhumant apiary from Murcia (BMU-T), both situated close to our transhumance location and thus sharing the same environmental conditions, but differing in management (resident *vs.* migratory) and with a different genetic background from our colonies. We studied the genetic composition (microsatellites) and the *Varroa* prevalence of the 20 mentioned colonies in four different times: before transhumance (T_0); two weeks after moving the hives from Murcia to Soria (T_1); at the end of the migratory period (T_2), and two weeks after the return of migratory hives to Murcia (T_3). It was found an increasing incidence of *Varroa* infestation in migratory colonies, as well as changes in the genetic diversity of the transhumant colonies when compared to resident colonies

Recombination and genetic diversitydynamics in*Varroa destructor*

Alexis L. Beaurepaire, James D. Ellis, Klemens J. Krieger and Robin F.A. Moritz
Room 125b, HoherWeg 4, 06120 Halle (Saale), Germany
E-mail:alexis.beaurepaire@zoologie.uni-halle.de

Varroa destructor is the most devastating parasite of the Western honeybee, *Apis mellifera*. In the light of the arm race opposing the host and its parasite, genetic diversity is considereda key parameter. However, thelifecycle of *V. destructor* is characterized by extreme inbreeding due to full-sibling mating in its host cells. On the first part of this study, we present a model on the evolution of inbreeding in a purely inbred system and compare our predictions with empirical data base don't he analysis of seven micro satellite markers. We found that our model and empirical data match well early in the season and that multiple inbred mite lineages are present in the honeybee colonies. However, the seline ages recombined towards heend of the season, resulting in an increase in heterozygosity as mites co-infest brood cells. In the second part of this study, we investigated the recombination potential of *V. destructor* lineages due to multiple brood cell infestation. We found that the mites co-infect significantly more frequently a brood cell with a genetically disimilar individual compared to random co-infestation based on the overall mite lineage frequency. Altogether, these intracolonial population dynamics have great relevance for the selection of acaricide resistance in *Varroa* if treatments occur during phases of high inbreeding. The spread of resistant alleles within and between honeybee colonies can be swift as recombination of the different lineages in the colonies is common towards the end of the season and honeybee worker drift homogenises the parasite populations.

Is the possible genetic variability of Varroa destructor associated with this of Apis mellifera?

Laetitia Papoutsi, M. Bouga, M.D. Meixner, P.Kryger and N. Emmanouil
Agricultural University of Athens, Laboratory of Agricultural Zoology & Entomology, 75 IeraOdosStr, Athens 11855, Greece.
E –mail: papoutsilaetitia@aua.gr

The varroa mite, Varroa destructor Anderson and Truemann, is the world's most devastating pest of Western honeybee, Apis mellifera (Hymenoptera: Apidae).There is an open question whether the possible genetic variability of the parasite is associated with this of the host. Adult samples of workers from different subspecies of Apis mellifera and females of the mite, were collected. These samples are being subjected to mitochondrial gene segments sequencing analysis.

More specifically, sequencing analysis of 12sr DNA mtDNA gene segment is performing on females of the mite collected from different subspecies of A.mellifera. Sequencing analysis of COI and ND5 mtDNA gene segments is performing on the respective hosts.

Genetic diversity and structure of Apis mellifera in the South West Indian Ocean islands

Maéva Angélique Techer, Johanna Clémencet, Christophe Simiand, Bernard Reynaud and Delatte Hélène
7, ch de l'IRAT 97410 st pierre la Réunion
E-mail: delatte@cirad.fr

The South West Indian Ocean (SWIO) archipelagos and Madagascar constitute a hotspot of biodiversity with high rate of endemism. In the area, the endemic subspecies A. m. unicolor has been described in Madagascar and belongs to the African lineage. Nevertheless recent studies using genomic approaches have shown that A. m. unicolor was genetically distinguishable from other African subspecies. Honeybees are also found in surrounding archipelagos of the area, yet knowledge on these insular populations is scarce. In order to have a complete understanding of the distribution, genetic diversity and population structure of A. mellifera sp in the SWIO islands, we analysed the genetic diversity of two

mitochondrial regions (COI-COII and ND2) and at 15 microsatellites nuclear loci on samples from the Mascarene (n = 2941), Seychelles (n = 209) and Comoros archipelagos (n = 90). Potential population of origins such as Madagascar (n = 855), continental Africa (n = 84) and Europe (n = 76) were implemented in the analysis to better apprehend the genetic diversity of SWIO populations. Both sequencing of COI-COII and ND2 regions revealed a new mitochondrial African subgroup in the SWIO islands. Mitochondrial diversity patterns supported ancient colonization followed by diversification of A. m. unicolor in 10 islands out of the 11 studied. Nuclear analysis showed that SWIO populations were genetically closer to Madagascar than any other African populations and also exposed to recent European introductions in the Mascarene, yet well differentiated between island or continental populations, suggesting the indigenous status of A. m. unicolor in the area.

Patterns of Iberian honey bee variation inferred from the coding regions of whole mtDNA genomes: comparison with the popular intergenic tRNA^{leu}-cox2 region

M. Alice Pinto, Dora Henriques, Andreas Wallberg, Julio Chávez-Galarza and Matthew T. Webster
Mountain Research Centre(CIMO), Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.
E-mail:apinto@ipb.pt

Iberian honey bees (*Apis mellifera iberiensis*) arewell-known for their complex patterns of variation, which have beenextensively reported by PCR-RFLP data of the intergenic tRNA^{leu}-cox2 region of themitochondrial DNA. This mtDNA marker has revealed a highly structured and diverse subspecies characterized by the presence of western European (M) and African (A) haplotypes belonging to three African sublineages (A_I, A_{II}, A_{III}) forming a cline possibly originated from secondary contact. While the African-derived haplotypes occurin the southwestern half of Iberia, with sublineage A_{III}mostlypresent in the northernAtlantic coast, the northeastern half of Iberia is occupied by haplotypes of M ancestry. Here we report on thediversity patterns inferred from the coding portion of 87 mitochondrial whole genomes of Iberian honey bees and 20 of tworeference

subspecies: the North African *A.m. intermissa* and the western European *A. m. mellifera*.The whole mtDNApatterns werecompared with those obtained with the intergenic $tRNA^{leu}$-cox2 region. As expected, a concordant northeastern-southwestern cline formed bythe two highly divergent lineages A and M was observed. However, the previously grouping of haplotypes into thethree African sublineages is not supported by the entire coding portion of the mitochondrial molecule.This finding suggests that the $tRNA^{leu}$-cox2 region is still a good marker for understanding the big picture of variation patterns.
This research was funded by "Fundação para a Ciência e Tecnologia" and COMPETE/QREN/EU through the project PTDC/BIA-BEC/099640/2008.

Genome-wide detection of signatures of selection in non- synonymous positions of iberian honey bee(*Apismelliferaiberiensis*)

Dora Henriques, Julio Chávez-Galarza, Andreas Wallberg, Cátia Neves, José Rufino, Filipe O. Costa, Matthew T. Webster and M. Alice Pinto
Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.
E-mail: dorasmh@gmail.com

Maternal and biparental genetic surveys of the Iberian honey bee (*Apismelliferaiberiensis*) populations have revealed complex and incongruent patterns of variation which have yet to be completely understood. Complex patternsare expected inregions like the Iberian Peninsulabecause (1) itcomprises a diverse range of habitats and climates,(2) it has served as a glacial refugium during the Pleistocene, and (3) it has functioned as a bridge for populations migrating between Africa and Europe. While the demographic history played an important role in shaping the genome of the Iberian honey bee, selection is an evolutionary force that cannot be discarded. In this study we used Illumina technology to sequence the whole genomes of 87 Iberian honey bees collected across three longitudinal transects in the Iberian Peninsula. The whole-genome dataset was scanned for signatures of selection using two genetic-environment association methods (LFMM and Sam*β*ada). A total of 828 SNPs, spanning 308 genes, were detected by both methods. Of the 308 genes,25 have SNPs in non-synonymous positions which were analyzed for positive selectionusing eight codon-substitution models (four neutral and four under selection) implemented by PAML and

Selectonsoftwares.Of the 25 genes, 13 out show signals of positive selection. Functional annotation indicates that these genes are involved in various biological processes such as sensory perception of smell (2 genes), oxidation-reduction (2 genes), neurogenesis (1 gene) and cellular response to starvation (1 gene). This study represents an important first step into understanding local adaptation of Iberian honey bees.

Selecting highly informative SNP panels for honey bee conservation management

Melanie Parejo [1,2], David Wragg[3], Laurent Gauthier[1], Peter Neumann[1,2] and Markus Neudtischko[1]

[1]Agroscope, Swiss Bee Research Centre, 3003 Bern, Switzerland

[2]Institute of Bee Health, Vetsuisse Faculty, University of Bern, 3003 Bern, Switzerland

[3]Institut National de la Recherche Agronomique, 31326 Castanet-Tolosan, France

Schwarzenburgstrasse 161, 3003 Bern, Switzerland

E- mail: melanie.parejo@agroscope.admin.ch

The native honey bee subspecies of Northern Europe, *Apis mellifera mellifera*, is threatened by introgression from introduced conspecifics. To limit introgression, conservation efforts have been implemented across Europe either using wing morphology or microsatellites to detect and remove hybrids. Here, we aim to characterize the honeybee population structure in Switzerland and the French Alps using whole-genome sequencing and to test whether a limited number of informative SNPs is able to separate and estimate admixture between native and introduced honeybees.

We re-sequenced haploid drones sampled throughout Switzerland and France including *A. m. mellifera* (N=103), *A. m. carnica* (N=34) and Buckfast bees (N=14). To infer fine-scale population structures, we incorporated ancestry proportions inferred by the program Admixture together with a network-based clustering approach (NetView). Based on the network topology, all subspecies can clearly be distinguished from each other. Furthermore, we detected fine-scale population substructures within *A. m. mellifera* suggesting isolation by distance or local ecotypes.

After the assessment of population structure, we selected 100 informative SNPs (most uncorrelated F_{ST}) between introduced and native honeybees. Based upon this panel it was possible to accurately estimate admixture proportions and genetic distances between individuals compared to the whole-genome sequence information. This result suggests that a reduced panel of informative SNPs could be a precise and cost-effective tool to be implemented in conservation management efforts and sustainable honey bee breeding in Switzerland.

Pollinator ecology

Coordinator: Ingolf Steffan-Dewenter

Bees are the most important pollinators of more than 300000 insect-pollinated wild plants and of three quarters of major human crops. Global environmental change including the loss and degradation of natural and seminatural habitats, agricultural intensification, climate change, and species invasions threaten bee diversity and their interactions with wild plants and crops. In the *Pollinator Ecology Section* two keynote lectures, 15 oral presentations and twelve posters will addressed these topics in three sessions focusing on the (1) pollination ecology of wild plants and crops, (2) effects of invasive species and land use on pollinator diversity, and (3) the ecology of honeybees in agricultural landscapes.

How hijacking their pollinator's sexual communication channels drives variation and diversification in a group of Mediterranean orchids.

Rakosy D., Stadler Y., Cuervo M., Paulus H. F. and Ayasse M.
E-mail: metarakosy@yahoo.de

In a complex landscape, such as that of the Mediterranean, male long-horn bees (*Eucera* sp., Eucerini, Apidae) have to rely on a complex combination of chemical, visual and tactile signals for finding and recognizing their mates. However, on occasion these signals may lead them astray and onto the flowers of the sexually deceptive orchid genus *Ophrys* (Orchidaceae). Several species groups within this genus mimic the signals emitted by unmated *Eucera* females in order to lure their males into pseudocopulatingand thus pollinating the flowers. By hijacking their sexual communication system, the orchids have established a species specific relationship with their pollinators. In order to maintain this relationship theory predicts low levels of variation in floral traits. However the genus *Ophrys* is notorious for extreme variation in floral traits, coupled with low genetic divergence. By integrating bioassays, chemical analyses and 3D geometric morphometrics our group has explored the amount and patterns of inter- and intraspecific floral trait variation and its consequence for pollinator-mediated reproductive isolation and speciation. We found that species-specific divergence patterns in floral traits between pollinator-delimited species are maintained despite unusually high levels of variation. In fact the amount of variation differed significantly depending on the specificity of the trait. While floral scent and size were less variable, floral shape varied dramatically, indicating a differential importance of pollinator-mediated selection and neutral factors in shaping these traits.Further the highly fragmented landscape of the Mediterranean has proven an essential factor in shaping the relative role of pollinator-mediated selection and drift in driving the diversification of both the orchids and their pollinators

Pollination effectiveness of different insect visitor species for two ericaceous species

Laura Moquet, Benoît Pirard, Lydiane Bruyère and Anne-Laure Jacquemart
Université catholique de Louvain, Earth and Life Institute – Agronomy, Croix du Sud 2, box L7.05.14 1348 Louvain-la-Neuve, Belgium
E-mail: laura.moquet@uclouvain.be

Heathlands, mainly originating from ancestral practices, are dominated by ericaceous species. These biotopes have been destroyed over the last two centuries in Belgium causing the decrease of several pollinators like the bumblebee *Bombus jonellus*. To determine the possible impact of pollinator decline on reproductive success of ericaceous species, it is necessary to evaluate their pollination effectiveness.

We compared the pollination effectiveness of several visitors of two ericaceous species. *Erica tetralix* is a specialist species for which pollen can be collected only by buzzing performed by a low number of visitor species. *Calluna vulgaris* is a generalist species with a diverse guild of visitor species. We estimated pollination effectiveness by the number of pollen grains deposited on the stigma of virgin flowers in one single visit. We analysed impacts of behaviour, pollen quantity on the body of visitors and time spent per flower on the effectiveness of different visitor species.

Main visitor species transferred similar quantity of pollen on the stigmas of *Calluna vulgaris*. On the contrary, we observed differences in pollen deposition on stigmas of *Erica tetralix* among visitor species and their behaviours. Significantly more pollen grains were deposited by bumblebees than by hoverflies. Moreover, bumblebees were more efficient pollinators when they collected nectar than when they collected pollen. The pollen quantity on the body of visitors and time spent per flower were not valuable estimators of effectiveness. We calculated the importance of each visitor on the reproductive success by combining visitation rates and pollination effectiveness.

Role of non protein amino acids in nectars: effects on bees behaviour

Gherardo Bogo[1,2], Laura Bortolotti[2], Simona Sagona[3], Antonio Felicioli[3], Marta Galloni[1] and Massimo Nepi[4]
[1]Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, Bologna, Italy
[2]CREA-API, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Unità di apicoltura e bachicoltura, Bologna, Italy
[3]Dipartimento di Scienze Veterinarie, Università di Pisa, Pisa, Italy
[4]Dipartimento di Scienze della Vita, Università di Siena, Siena, Italy
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Unità di apicoltura e bachicoltura, Via di Saliceto 80, 40128 Bologna, Italy
E-mail: gherardo.bogo2@unibo.it

Floral nectar mediates interactions that are much more complex than simply alimentary relations. Nectar secondary compounds appear to have a special role in regulating interaction with other organisms. Although very few is known about their ecological roles, recently it was proved that secondary compounds may affect the nectar feeders' behaviour interacting with their neurobiology. We addressed a special focus on the class of non-protein amino acids. Their presence in floral nectar has been reported since long time but their ecological function has not been investigated. Data from several phylogenetically unrelated species indicate that they may represent a consistent part of the total amino acid content of floral nectar (25-45%) and generally the more abundant ones are γ-amino butyric acid (GABA) and β-alanine. The aim of the study was to test the effect of these non-protein amino acids on bees behaviour. We tested an artificial diet consisting of sucrose solutions enriched with GABA and β-alanine at two concentrations on *Bombus terrestris* and *Apis mellifera*. Results show that *B. terrestris* and, only partially, *A. mellifera*, increase their walking activity when fed with the solution enriched with β-alanine at high concentration, while they increase their flying activity with the same solution at low concentration. Moreover, bumble bees greatly increase their survival time when fed with the solution enriched with GABA. These results push us to further consider the role of β-alanine and GABA in increasing the mobility of insects between flowers and their foraging activity and thus their pollination performances.

Watermelon pollinators exhibit complementarity in both visitation rate and single-visit pollination efficiency

Gideon Pisanty, Ohad Afik, Eric Wajnberg and Yael Mandelik
Department of Entomology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
E-mail: yael.Mandelik@mail.huji.ac.il

The concept of pollinator niche complementarity maintains that species-rich pollinator communities can provide higher and more stable pollination services than species-poor communities, due to contrasting spatial and/or temporal pollination activity among groups of pollinators. Complementarity has usually been examined in pollinators' patterns of flower visitation or abundance, while largely neglecting the possibility of complementarity in patterns of single-visit contribution to fruit/seed set (pollination efficiency). However, variability in pollination efficiency can greatly affect pollinators' overall pollination services and may therefore contribute an additional, important aspect of complementarity. In the current study, we investigated the existence of pollinator complementarity in both visitation rates and pollination efficiencies. The study was conducted in 43 watermelon fields in a Mediterranean agro-natural landscape in central Israel. We studied spatiotemporal variation in pollinators' visitation activity and single-visit pollination efficiency. Pollinator species' visitation rates as well as single-visit fruit set efficiencies, but not seed set efficiencies, exhibited significant spatiotemporal variation that contributed to their complementarity. Pollinators' visit frequencies were affected by surrounding land use, location within field, time throughout the season, and time of day. Pollinators' fruit set efficiencies were affected by ovary size and time of day. In sum, we found that crop pollinators exhibit complementarity in both their visitation rates and pollination efficiencies, which can promote the overall level and stability of their pollination services. The finding of complementarity in pollination efficiencies suggests further diversity effects on crop yield, and calls for taking it into account rather than considering it a constant, species-specific trait.

Diversity of Apoidea and their pollination efficiency on the production of cotton (*Gossypium hirsutum*) plants at Dang, Ngaoundéré, Cameroon

Sanda Mazi[1]. and Brückner D[2].
[1]University of Ngaoundéré, Faculty of Science, Department of Biological Sciences, P.O. Box 454 Ngaoundéré, Cameroon
[2]Fachbereich 2, Biologie und Chemie, Forschungsstelle für Bienenkunde, Universität Bremen, FB2, Postfach 330440, 28334 Bremen
E- mail: mazisanda@yahoo.fr

Pollinators, especially bees, are essential to terrestrial ecosystem services. They ensure the maintenance of certain ecological processes, like Angiosperm plants' reproduction. In the past decades, agricultural intensification has caused extensive environmental changes, with major impacts on biodiversity, especially on the pollinators, which reflects the loss of fruits and seeds sets of most agricultural crops. This study was conducted to determine the effects of Apoidea pollinators (*Apis mellifera adansonii, Coelioxys circumscriptus, Crossisaspidia chandleri, Halictus* sp., *Lasioglossum* sp., *Lipotriches blandula, Megachile* sp., *Xylocopa olivacea* , *Xylocopa* sp.) on the productivity of *Gossypium hitrsutm* crop. Two observations were carried out during two seasons for conducting this study. Three treatments were set up each year and a randomized complete block design (RCBD) was laid out with eight blocks. On each block, fifteen plants were choosen and on each plant, three flowers were labelled to set up the treatments (unprotected flowers, Protected flowers from insect visits and opened flowers to a single visit of Apoidea species). Analysis of variance showed that *A. m. adansonii*, *Lasioglossum* sp., *L. blandula,* and *X. olivacea* had a highly significant effect at 5% level of significance on the fruiting rate (seeds set) of *G; hirsutum*. However, *A. m. adansonii*, *Co. circumscriptus, Cr. chandleri, Halictus* sp., *Lasioglossum* sp., *L. blandula, Megachile* sp., *X. olivacea* , *Xylocopa* sp. had shown the same significant effects on normal seeds per pod. Furthermore, this study revealed that studied Apoidea are very efficient pollinators of cotton plants and they have shown highest pollination efficiency on its flowers.

The effects of forest fire buffer zones on pollination webs

Alon Ornai, Gidi Nee'man and Tamar Keasar
University of Haifa - Oranim, Tivon 36006 Israel
E-mail: tkeasar@gmail.com

Pollinating bees are declining worldwide. Their sustainable management, especially in biodiversity hot-spots, requires better understanding of community composition and interactions. Here we studied the effects of forest fire buffer zones on pollination systems.
The Mount Carmel nature reserve, Israel, is a typical Mediterranean habitat, known for its high bee diversity. This fire-prone area has experienced many recurring forest fires, exhibiting a mosaic of post-fire regenerating plant communities. To decrease the impacts of future fires, buffer zones of reduced tree biomass were established. Low plant biomass is maintained by mechanical cutting or sheep grazing. We compared flowering plants and their visitors in cutting, grazing and control (no-maintenance) plots in the buffer zones, during the 2015-2016 spring seasons.
In 2015, mechanical cutting, but not grazing, increased the visits of solitary bees (mainly Chelostoma and Halictus) compared to honey bees. Flowering species composition was significantly affected by grazing, cutting, and the plots' fire history. Pollination webs were most specialized in grazing plots, intermediate in cutting plots and least specialized in no-maintenance plots. Forthcoming results from 2016 will be integrated with these findings to form management recommendations. Future monitoring is needed to assess the long-term effects of this maintenance management on plant-pollinator interactions.

Impacts of Canadian Goldenrod (*Solidago canadensis*) on native pollinator communities in different-aged old-fields

Kovács-Hostyánszki Aniko., Vágási I. C., Beldean M., Földesi R., Kolcsár L. P., Shapiro J.T., Török E. and Fenesi A.
MTA Centre for Ecological Research, Institute of Ecology and Botany, Lendület Ecosystem Services Research Group, Alkotmányút 2–4, H–2163 Vácrátót, Hungary
E- mail:kovacs.aniko@okologia.mta.hu

Invasive plant species might have considerable influence on secondary succession in former arable fields (old-fields), hamper old-fields' potential to become species-rich plant communities and destroy insect assemblages. We studied the effects of Canadian goldenrod (*Solidagocanadensis*) on different aged old-fields (1-20 years since last ploughing) along an invasion gradient in South-Transylvania, Romania. Wild bees and hoverflies were sampled by transect sampling and flower visitation of native plants was observed before and during the flowering of goldenrod. Our results showed negative effect of goldenrod cover before its flowering on the abundance of solitary wild bees and bumblebees independently from the old-field age, while during its flowering the amount of goldenrod flowers correlated positively with the abundance of bumblebees and the species richness and abundance of hoverflies. Therefore, beside the general positive effect of the amount of native flowers, goldenrod might serve alternative foraging resource for late emerging hoverflies and bumblebee species in the second half of summer. However, flower visitation of native plants declined by solitary bees and marginally by both the bumblebees and hoverflies due to the invasive species. It means that pollination success of the native plants might be reduced by the goldenrod along the succession of the old-fields. In the invaded fields the invasive species alters the plant communities and the pollinator assemblages accordingly, having a feed-back again on the plant community, leading to non-desired vegetation stages. Taking actions against the Canadian goldenrod is important challenge of conservation biology to preserve important and unique nature values in Transylvania.

C.S.I. Pollen: Studying pollen diversity available for honey bee colonies in Europe using a Citizen Science approach.

Robert Brodschneider, Janko Božič, Andrejs Briedis, Norman L. Carreck, Robert Chlebo, Karl Crailsheim, Mary Frances Coffey, Bjørn Dahle, Vincent Dietemann, Amelia V. Gonzalez-Porto, Janja Filipi, Dirk C. de Graaf, Alison Gray, Fani Hatjina, Pavlos Ioannidis, Nicoleta Ion, Asger Soegaard Joergensen, Elfriede Kalcher-Sommersguter, Preben Kristiansen, Antoine Lecocq, Jean-François Odoux, Asli Ozkirim, Magnus Peterson, Blaž Podrižnik, Rašić Slađan, Gina Retschnig, Aygun Schiesser, Simone Tosi, Flemming Vejsnaes, Geoffrey Williams and Jozef van der Steen
University of Graz, Institute of Zoology, Universitätsplatz 2, A-8010 Graz, Austria.
E-mail: robert.brodschneider@uni-graz.at

Diverse diets are expected to be more beneficial for honey bees compared to monotonous diets, as there is great variation with respect to the nutritive value of pollen. The diversity of pollen sources is known to be dependent on vegetation, season and land use, but there is no comparable large scale information available on the diversity of pollen collected by honey bee colonies in Europe. Therefore the COLOSS association conducted a pilot study in 2013, followed by a Citizen Science investigation in 2014 and 2015 covering more than 20 countries. Beekeepers collected pollen with pollen traps on three of their colonies on nine predefined times per year. They then determined the number of different (abundant, rare and very rare) colours in a defined subsample (20 gram) and submitted these results online to a database. We received data from 465 participants in 2014 and from 585 in 2015. 300 volunteers participated in both years, 165 in 2014 only and 285 in 2015 only. We received records of a total of 8094 pollen samples in 2014 and a total of 9823 in 2015. On average 69% of the collected samples met the required 20 grams in 2014, and 65% in 2015, with seasonal differences between sampling dates. Preliminary statistical modelling shows seasonal variation in the number of colours found in pollen samples, and a possible effect of landscape composition is currently being investigated. Beyond that, in some countries pollen samples are also being analysed palynologically, to extend the findings of Citizen Scientist beekeepers.

Spatially-explicit analysis of the relation between honeybee health and environmental variables in Belgium.

Lina De Smet[1], Leen Van Esch[2], Peter Jaeken[3], Sofie Vergucht[3], Engelen Guy[2] and De Graaf Dirk[1]

[1] Laboratory of Molecular Entomology and Bee Pathology, University Ghent, Krijgslaan 281 S2 9000 Gent

[2] Unit Environmental Modelling, Flemish Institute for Technological Research

[3] Phytofar Auguste Reyerslaan, 80, 1030 Brussel L-MEB, UGent

E-mail: lina.desmet@ugent.be

The last decades an increased mortality of colonies of the Western honeybee (*Apis mellifera*) has been reported in different countries. Several different drivers such as parasites, disease, insecticides and malnutrition were associated with these losses. In the scientific community there is a consensus that the causes of these big losses are multi-factorial and that environmental factors can also play a role? The way that stressors (biological, chemical and environmental) affect honeybees and contribute to losses in their populations is poorly understood. The underlying mechanisms remain unclear because of the complex nature of the potential combinations and the effects of interactions between them. This spatial-explicit study investigated the potential causal relations between bee mortality and eight groups of explanatory natural and anthropogenic factors, by making intensive use of advanced Geographical Information Systems methodologies. Here we show that the winter mortality in Flanders is related with the frequency of unfavorable factors. Moreover we could show that when beekeepers are travelling with their colonies these relations between winter mortality and the studied factors are less clear which suggest that travelling can neutralize some negative environmental factors. To the best of our knowledge, this is the first study which analyzed the winter mortality in a spatial explicit way taking eight groups of variables into account. These variables include meteorological conditions, urbanization, use of plant protections products, food availability, landscape fragmentation, landmarks availability, food availability, *Varroa* infestation level and the beekeepers profile.

Pesticide contamination in beehives is not depending on agricultural intensity

Reet Karise, Risto Raimets and Marika Mänd
Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Fr. R. Kreutzwaldi 5, Tartu 51014, Estonia
E-mail:reet.karise@emu.ee

The need for insect pollination of field crops is increasing due to increasing demand on high quality fruits and vegetables as well as renewable fuel. However the honey bees are declining in intensively managed agricultural areas so that the deficit is increasing even more. Among insect pollinated crops oilseeds are most common in Northern Europe. In some regions oilseed seems to make a monoculture with regular pesticide application. This poses high risks for honey bees since they prefer large flower-rich foraging areas. The pesticide residues contaminate the hives and increase the stress level for bees. Because of anxiety of pesticide residues many beekeepers prefer not to expose their hives to oilseeds and locate beehives in more natural areas. The aim of this study was to analyse the quantity and composition of pesticide residues in Estonian beehives in landscapes with different proportion of agricultural areas.

We located the hives in landscapes with approximately 0, 20, 40, 60 and 80% of agricultural areas in the radius of 2 or 4 km from the hives. We collected comb honey, beebread, pollen, larval and nurse bee samples and analysed them for 47 contemporary commonly used pesticides.

The most often found active ingredient was tau-fluvalinate which is used both on fields against pest insects and also in hives against Varroa mites. However majority of other pesticide residues found belonged to those connected to oilseed rape cultivation. No correlation between amount of pesticide residues and proportion of arable land in the foraging.

The species diversity and economically utilization of stingless bees in Thailand

Orawan Duangphakdee and Preecha Rod-im
King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand
E-mail: orawan.dua@mail.kmutt.ac.th

The species diversity and economically utilization of total of 32 species of stingless bees in Thailand has been reviewed. The development and current state of Meliponiculture regard to pollination utilization and honey and propolis production have particularly focused. The honey and propolis yields from four common domesticated species of stingless bee of Thailand, *Tetragonula pagdeni*, *Tetragonula laeviceps*, *Lepidotrigona terminata*, *Tetragonula fuscobalteata*, have been compared. The total yields from best to worst for honey production was *T. pagdeni*, *T. laeviceps* , *L. terminata* and then *T. fuscobalteata* and for propolis *L. terminata*, *T. pagdeni* , *T. laeviceps* and *T. fuscobalteata* respectively. The cost effectiveness analysis base on production yield, investment cost and profit-return rate has been carried out. In the same investment of timing, *T. pagdeni* is highly recommended potential for economically Meliponiculture in Thailand.

Biodiversity of wild bees of Cyprus: past, present and future

Varnava Androulla, Stavroulla Louka, Athanasia Mandoulaki and Stavrinides C. Menelaos
Cyprus University of Technology, 30 Archbishop Kyprianos Str., 3036, Lemesos
E-mail: m.stavrinides@cut.ac.cy

Cyprus, an island in the eastern part of the Mediterranean is a biodiversity hotspot. While the biodiversity of wild bees on the island was documented extensively from 1916-1957 by the renowned bee taxonomist G. Mavromoustakis, no analyses were performed on the spatiotemporal distribution of bees. We organized the Mavromoustakis data in an online database with over 1000 entries corresponding to 237 species (wildbeesofcyprus.org). We show that the activity of wild bees peaks in spring and summer, with some species active throughout the year. Bees have been reported from the coasts of the island up to highest altitudes on the Troodos and Pentadactylos mountain ranges. Many of the species reported

on the island are important pollinators of agricultural crops in other parts of the world, but few data exist for Cyprus. Ongoing sampling that begun in 2015 identified more than 11 morphospecies of wild bees from carob flowers, and 41 morphospecies from vineyard margins, two crops considered as high nature value farmland for the island. Important threats to wild bee diversity include the expansion of monocultures, the heavy use of pesticides in agricultural fields and the loss of suitable habitat. Conservation of wild bee populations requires increasing flowering plant diversity in agricultural fields to supply alternative food sources, provision of nesting sites and a reduction in the use of toxic pesticides.

The importance of fatty acids to honeybee nutrition

Arnon Dag, Yael Arien, Dorit Avni, Harmen Hendriksma and Sharoni Shafir
Agricultural Research Organization, Gilat Research Canter, 85280, Israel
E-mail: arnondag@agri.gov.il

Deficiency in essential omega-3 fatty acids has been linked to health problems in mammals, including many mental disorders and reduced cognitive performance. Bees require nectar, their main carbohydrate source, and pollen, which provides proteins, lipids, vitamins, and minerals. However the knowledge on the importance of fatty acids, and especially essential fatty acids (omega-3 and 6) for their nutrition is scant. Malnutrition may be due to low pollen quantity, quality, or diversity, a condition that is aggravated in agricultural monocultures. In the current study we assessed the availability of essential fatty acids in pollen under natural conditions. At the second stage we assessed the importance of essential fatty acids for honey bee learning ability and for brood rearing. In natural, diverse landscapes we found that the two essential fatty acids were with mean (± SE) of 31.6% ± 10.7 for omega-3 and 24.7% ± 9.7 for omega-6 fatty acid. Bees fed artificially either of two omega-3-poor diets, or Eucalyptus pollen (which is poor in omega-3), showed greatly reduced learning abilities in conditioned proboscis-extension assays compared with those fed omega-3–rich diets, or omega-3–rich pollen mixture. Furthermore in a separate experiment, in which we compared 8 artificial bee diets, we found that the one with added essential fatty acids resulted in the highest number of bees produced per gram feeding. In conclusion, our results indicate the importance of essential fatty acids in honeybee diets.

Biological activity of bee products

Coordinator: Otilia Bobiș

Bee products (honey, pollen, beebread, propolis, royal jelly, bee venom) are considerably important for human life and health, having an important nutritional value, but are increasingly used in medicine and pharmacy, due to their biologically active properties. Interactions among groups of active substances or single compounds and bioactive properties of bee products are important for the antibiotic activity against pathogens and parasites. For this reason, modern techniques are used for identifying and quantifying the chemical composition.
The impact of both bee produced gland secretions and plant-derived bee products are reviewed in the present symposium, highlighting the benefits of using them in nutrition as well as in modern medicine.

All the royal makings of a queen – Royalactin does not a honeybee (*Apis mellifera*) queen make

Anja Buttstedt and Robin F. A. Moritz
Martin-Luther-Universität Halle-Wittenberg, Institut für Biologie - Zoologie/Molekulare Ökologie, Hoher Weg 4, 06120 Halle/Saale
E-mail: anja.buttstedt@zoologie.uni-halle.de

Queen determination in honeybees is nutritionally regulated and only larvae that exclusively receive royal jelly during development emerge as queen bees. However, in spite of an intensive search over many decades for the actual compounds in royal jelly triggering queen development, no specific determinator could be singled out. Based on a large body of research testing various compounds of royal jelly, sugars and the fatty acid 10-hydroxy-2-decenoic acid, had been suggested to affect caste development. In the end it seemed clear that queen bee determination is not the result of the absence or the presence of a single determinator but rather the consequence of a discrete feeding regime throughout larval development. Hence claims that a single protein (monomeric major royal jelly protein 1 (MRJP1) or Royalactin) in royal jelly induces by some specific biological activity queen differentiation came to a surprise in light of previous research. Because the issue of caste determination is arguably the most central paradigm for social insect research in general, we here show that this claim is incorrect and not only exclude Royalactin as a determinator for queen caste but also all other royal jelly proteins that could be digested with proteinase K.

Bioactive and physiochemical properties of Irish *Apis* and *Bombus* honeys.

Saorla Kavanagh, Jane Stout and Blánaid White
Dublin City University
E-mail:saorla.kavanagh48@mail.dcu.ie

Honey contains biologically activecompounds with potential antioxidative, antibacterial and anticarcinogenic effects.Various studies have shown the correlation of antioxidant potential of honey and concentration of total phenolics present. These compounds are beneficial not only to human health but also bee health. Irish honey produced by honey bees (*Apis mellifera)* has not been profiled in terms of its bioactive compounds. No bioactive compounds in honey produced by bumble bees (*Bombus spp.*) have been reported until now.
The aim of this research was to identify and quantify biologically active compounds in Irish honeys produced by both bumble and honey bees by measuring their phenolic compound profiles.The physiochemical properties of both honeys are also compared.*Apis* honey samples were obtained from beekeepers across the island of Ireland and the phenolic composition was determined using HPLC-UV.

Functional stratification of bee bread pellets

Janko Božič and Blaž Podrižnik
University of Ljubljana, Biotechnical faculty, Department of biology, Večna pot 111, 1000 Ljubljana, Slovenia
E mail: janko.bozic@bf.uni-lj.si

Classical understanding of the bee bread is that pallets are result of pressing together bee pollen loads inside of the bee-comb-cell. Bees add glands' secretions that provide a proper microenvironment for the fermentation by microorganism and final conservation like a silage products. We look for further valuable information through the investigation of the pallet profile properties from the bee bread comb. We compared profiles from fresh stored chestnut pollen, just covered cells with honey and wax cups and aged covered combs as a mature bee bread samples. In the first step we checked for antimicrobial activity of the different layers of the bee bread as well of the honey stored on the top before covering of the cells. That was compared with total phenolic content. In addition we checked on the selected set of samples also content of lactic acid, gluconic acid as well glucose and fructose content. Results indicate that the processing of the

pollen load into bee bread involves high investment into antimicrobial activity by the bees before closing the cells. Antimicrobial activity didn't correlate with total phenolic content as it is usual for honey. Honey on the top of the bee bread pallet had stronger antimicrobial activity then honey from nearby honey cells. Lactic acid correlated with stronger antimicrobial activity but only in younger upper layer of the beebread pallet. From the results we propose use of fresh stored bee bread, preferably upper layer, without waiting for maturation after covering the cells.
The results suggest that differences in phenolic content exist between honey produced by the bumble bee and honey produced by the honey bee. Finally this study reports a difference in the phenolic composition between *Apis* honeys from predominantly rural areas compared to *Apis* honeys from predominantly urban areas. This has identified, for the first time, the phenolic content of Irish *Apis* and *Bombus* honeys and has enabled individual bioactive profiles of Irish *Apis*honeys to be categorised.

The neonicotinoid clothianidin affects royal jelly composition and reduces hypopharyngeal size and brood development in *Apis mellifera L*.

Annely Brandt, Matthias Schott, Max Sandmann, Marina D. Meixner and Ralph Büchler
LLH Bee Institute, Erlenstr. 9, D-35274 Kirchhain, Germany
E-mail: annely.brandt@llh.hessen.de

Honey bees are highly social insects, with labor division and intensive brood care. Nurse bees produce royal jelly in the hypopharyngeal gland (HPG) to feed larvae, young workers, and the queen. In laboratory experiments exposure of nurse bees to the neonicotinoid imidacloprid leads to a reduction of the HPG size. We wanted to study the effect of the neonicotinoid clothianidin on HPG size, royal jelly composition, and brood development in colonies.

Over a period of 7 weeks, twenty small colonies were chronically exposed to different concentrations of clothianidin (0 µg/L, 1 µg/L, 10 µg/L, or 100 µg/L) in sugar syrup. Every treatment was replicated five times. The clothianidin content was quantified in samples of food and worker bees. Each week all eggs, larvae, or pupae were counted for all colonies, and samples of royal jelly, larvae, and adult worker bees were collected.

The HPG size was reduced in age-defined worker bees after 12 days of exposure to clothianidin, even in the lowest concentration. In addition, the composition of the royal jelly was altered in colonies treated with 10 µg/L or 100 µg/L clothianidin. After three weeks of treatment, the number of larvae and pupae were significantly reduced in colonies exposed to 100 µg/L clothianidin. Moreover, the clothianidin treatment lead to a brood termination rate of 100% in colonies exposed to 10 µg/L or 100 µg/L clothianidin in the second half of the experiment.
Exposure to clothianidin affected HPG size, royal jelly composition, and brood development in small honey bee colonies.

Pyrrolizidine Alkaloids in honey and bee-collected pollen produced at apiaries where *Echium vulgare* is abundant.

Christina Kast, Verena Kilchenmann and Matteo Lucchetti
Agroscope, Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern, Switzerland.
E-mail: christina.kast@alp.admin.ch

Honey and bee-collected pollen can contain toxic pyrrolizidine alkaloids (PAs), when bees collect nectar and pollen from plants containing these alkaloids.We aimed to determine the types of pollen gathered by bees and the time point when bees bring pollen with PAs into the hives. We selected two Swiss apiaries as observation sites near Basel and in the Verzasca valley where *Echium vulgare* was growing abundantly. Beekeepers provided honeys and bee-collected pollen gathered throughout the entire season (years 2010 to 2014). Additionally, we collected daily pollen samples from two to four colonies during the bee seasons of two consecutive years. Honey and pollensamples were analyzed with HPLC-MS/MS, allowing the detection of 18 different PAs and PA-N-oxides (Quality Services International, Bremen). Pollen loads were separated by color and the botanical origin determined by melissopalynology. The PA concentrations in honeys ranged between 2 to 162 µg/kg and from below the LOQ to 586 µg/kg in pollen for commercial use. We detected *Echium*-type PAs (up to 5.6 mg/kg) in the daily collected pollen samples from June until August and *Eupatorium*-type PAs (up to 8.9 mg/kg) in samples from July until August. *Echium*pollen waspresent only in very small amountin samples containing *Echium*-type PAs.Thus, few

Echium pollen loads can lead to asubstantial contamination of the product.Although *Echium vulgare* was blooming near the beehives every year, the PA content in pollen and honey varied substantially between the production years, most likely depending on other simultaneously blooming nectar and pollen plants attractive to bees.

Biochemical characterization of MRJP1 and MRJP2 of *Apis mellifera*

Carmen Ioana Mureşan and Anja Buttstedt
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Faculty of Animal Science and Biotechnologies, Department of Beekeeping and Sericulture,Romania
E-mail: carmen.muresan@usamvcluj.ro

Royal jelly (RJ) is a substance secreted by the mandibular and hypopharyngeal glands of young worker honeybees nursing the growing larvae in the hive. The protein moiety of RJ consits primarily of major royal jelly proteins (MRJPs) which account for approximately 90% of the total water-soluble RJ proteins. In this study, two of the MRJPs, namely MRJP1 and MRJP2, were purified and afterwards their thermodynamic stability was characterized by differential scanning fluorimetry. We thereby found that both MRJPs do exhibit their highest stability at pH 4.0 - 4.5, the native pH of RJ. Furthermore, we prepared MRJP1 and MRJP2 affinity columns using cyanogen bromide-activated sepharose and identified with the help of mass spectrometry binding partners of both proteins whose discovery clearly points to the fact that both MRJPs are certainly more than just food.

Jujube honey improves lipid profile of high fat diet-induced hypercholesterolemia in Wistar rats

Mohammad Javed Ansari[1], Ahmad Al-Ghamdi, Adgaba Nuru[1,] Khalid Ali Khan[1]and Noori Al-Waili[2]

[1]Bee Chair, College of Food and Agricultural Sciences, King Saud University Riyadh, KSA

[2]Chairman and Consultant, New York Medical Care for Nephrology, Queens, NY 11419, USA

E-mail : mjavedansari@gmail.com

Coronary heart disease (CHD) is a major cause of morbidity and mortality throughout the world, including India. One of the risk factors for CHD is hypercholesterolemia. One of the natural remedies to treat CDH is honey. Honey contains antioxidants, free radical scavengers, various vitamins, minerals and some enzyme that is thought to lower cholesterol levels in the blood. This research aims to study effects of jujube honey on levels of total cholesterol, triglycerides, and LDL blood. Twenty-four Wistar rats (*Rattus norvegicus*) males were randomly divided into 4 groups. C1 was the control negative group (given regular feed), C2 was a positive control (fed a high-fat), T1 treated group were fed a high-fat feed for 7 days continued a high-fat plus Jujube honey for the next 7 days, and T2 was a group fed a high-fat feed for 7 days followed the usual plus jujube honey during the next 7 days. After completion of treatment, blood is taken to be checked total cholesterol, triglycerides, and LDL using the CHOD-PAP enzymatic spectrophotometric methods. The results indicated that there were significant differences in the levels of cholesterol, triglycerides, and LDL after the 15th day of the four groups ($P<0.05$). Jujube honey supplementation can lower total cholesterol, triglycerides, and LDL in the blood of Wistar rats compared to positive control group.

Anti-wrinkle effects of purified bee venom on facial wrinkles

Sang Mi Han, In Phyo Hong, Soon Ok Woo, Kwan Kyu Park and Young Mee Nichollos
Department of Agricultural Biology, National Academy of Agricultural Science, Wanju, 565-851, South Korea
E-mail:sangmih@korea.kr

Facial wrinkles are an undesirable outcome caused by extrinsic photodamage and intrinsic aging process. Currently, no effective strategies are known to prevent facial wrinkles. The main aim of the present study was to evaluate the clinical effects of bee venom serum by measuring the clinical wrinkles objectively with a device that converts the wrinkle roughness to numerical values. This is the first clinical study to determine the effects of bee venom containing cosmetics on facial wrinkles in human skin. The main aim of this study was to evaluate the clinical effects of bee venom serum. We analyzed images using replica and a device of Visioline which is an objective technique to reproduce changes in photodamaged skin. The image analysis using replicas was performed at week 12 when significant differences in total wrinkle area, total wrinkle count and average wrinkle depth were observed. This analysis correlated well with the clinical findings. Moreover, the significant difference in visual grade was observed after 8 weeks with bee venom serum indicating the faster effect of bee venom serum on wrinkle improvement. Our findings suggest that bee venom plays an important role in the improvement of skin wrinkles which is used in the cosmetics industry as an anti-wrinkle agent. Bee venom serum provided a greater efficacy in terms of total wrinkle area, total wrinkle count and total wrinkle area in subjects with photodamaged skin. Long-term treatment with bee venom containing cosmetics could be safe because the irritation potential of bee venom is negligible.

Evaluation of therapeutic effects of propolis against alpha amanitine toxicity in rats

Meral Kekecoglu[1,6], Pinar Goc Rasgele[2,6], Ertugrul Kaya[3], Gulzade Ozyalvacli [4], Recep Bayram[4] and Merve Kambur[5]

[1] Duzce University, Department of Biology, Faculty of Science and Letters, 81620 Duzce/Turkey

[2] Duzce University, Faculty of Agriculture and Natural Sciences, Department of Biosystems Engineering, 81620 Duzce/Turkey

[3]Duzce University, Department of Pharmacology, Faculty of Medicine, 81620 Duzce/Turkey

[4]Abant Izzet Baysal University, Department of Pharmacology, Faculty of Medicine, 14280 Bolu/ Turkey

[5] Duzce University, Institute of Science, Department of Biology 81620, Duzce, Turkey

[6]Duzce University, Beekeeping Research Development and Application Centre, Duzce, Turkey

E-mail: meralkekecoglu@duzce.edu.tr

Mushroom poisonings are common all over the world. Amanita phalloides are among the most dangerous natural toxins and alpha amanitine is one of the most substances. Mushroom intoxication and its treatment are major research subjects due to lack of a specific antidote. Silibinin is one of the most commonly used antidotes against to the alpha amanitin toxicity. Propolis is a resinous product collected by honey bees from plant exudates and is known for its anti-toxic and antioxidant properties. The purpose of this study was to investigate the therapeutic effect of different propolis extracts such as ethanol, water, dimethyl sulfoxide, l-lysine and to compare with silibinin against the toxic effects of alpha amanitine by using invitro, invivo and histopatological experiment. In the current study, 96 adult rats (weighing 200-300 gr) were divided into twelve groups of eight rats each. The animals were treated by intraperitoneal (ip) with different doses of propolis in different solvents. Blood samples were taken for determination of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Urea, Blood Urea Nitrogen (BUN) and all of samples were performed by using a spectrophotometer. Potential liver and kidney toxicities were investigated through pathological examinations. The results demonstrated that toxic effect was decreased in the highest concentration of water extraction of propolis. So it is believed that higher concentrations of water extraction of propolis may be more effective in mushroom intoxication.

Acknowledgment: This study was supported by TUBITAK, ARDEB-3001, Project No:114S024

Royal jelly and its growth inhibiting effect on European Foulbrood bacteria

Thomas V. Vezeteu, Otilia Bobiş, Robin F. A. Moritz and Anja Buttstedt
Institut für Biologie, Zoologie – Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06120 Halle (Saale), Germany
E-mail: thomas_vezeteu@yahoo.com

Honeybee colonies depict an attractive domicile for a variety of pathogens providing optimal temperatures, humidity and a plethora of food. Thus, honeybees have to deal with pathogens from the beginning of their lives and are already as larvae affected by severe brood diseases like the European Foulbrood caused by Melissococcus plutonius. Accordingly, it is scarcely surprising that larval food jelly comprises antibiotic compounds. However, although food jelly is primarily consumed by bee larvae, studies investigating the antibiotic effect of this jelly are largely concentrated on bacterial human diseases. We here show that royal jelly fed to queen larvae and added to the jelly of drone and worker larvae, decelerates not only the growth of European Foulbrood associated bacteria but strikingly also that of the causative agent M. plutonius and we are able to trace back a bulk of this effect to the main protein of royal jelly.

Naturally BQCV infected honeybees, a useful model system to screen for antivirals of plant origin

Adriana C. Aurori, Otilia Bobiş, Daniel S. Dezmirean, Liviu A. Mărghitaş and Silvio Erler
Babeș Bolyai University Cluj
E-mail: aaurori2002@gmail.com

Several viruses infect honeybees, leading to a large spectrum of manifestations. Some of them are responsible for deadly diseases while others have a contribution to the poor general fitness of honeybees and yet not lead to any overt symptoms. The Black queen cell virus (BQCV) is one of the ubiquitous occurring pathogens of honeybees which persists typically as an asymptomatic infection. However, under the influence of cumulated stressors (biotic and abiotic) BQCV can lead to disease. Until now, no antiviral agent exists for honeybee virus treatments. The goal of the study was to develop a fast and simple experimental model to screen for plant derived antivirals using natural BQCV infected forager bees. Among the tested extracts (e.g. propolis, *Artemisia absinthium* and *Laurus nobilis* leaf extracts) only *L. nobilis* extract significantly decreased the overall viral load and reduced BQCV replication. Antiviral candidate plant secondary metabolites have now to be verified in field experiments. Nevertheless, the proposed model system presents an innovative, time saving and stress reducing approach for bees.

Is it all pure wax from Africa?

Miguel Llorens-Picher, Aránzazu Meana, Mariano Higes and Raquel Martín-Hernández
Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutensede Madrid
E-mail: migllore@ucm.es

The African continent seem to be a sourceofpure and clean beeswax. Two are the main reasons: anapparently less intensive use of pesticides and the use of beekeeping technology whererecycling the beeswaxis not feasible(i.e. top bar hives).
Beeswax samples (n = 32) fromthirteendifferent locations all over Ghana were obtained during the summer of 2013.Samples were analysed by using ChEChERS method following liquid chromatography and mass spectrometry (LC-MS/MS)(limit of detection 1 ng/g).Traces of 13 pesticides were found and not a single sample was free of residues. The number of residues found per sample ranged from one to nine. Amitraz was the most widely distributed pesticide (75%) and the one detected in higher amounts (194-45890 ng/g), followed by coumaphos(47%; 5-8320 ng/g), chlorpyrifos (34%%; 30-35 ng/g) and fluvalinate (31%; 2-365 ng/g). The rest were rarely present (3-6%) and belonged to a wide range of agricultural or veterinary products including thiabendazole, prochloraz or pyriproxyfen. In a questionnaire to beekeepers, none recognized to use any treatment inside the hives.
The data obtained requires cautious interpretation as none of the samples belonged to recycled beeswax. Thisevidences the high capability of beeswax to accumulate pesticides, encouraging the reduction of its use and urging researchers to clarify the potential side effects on the honey bees, as well as the real purity of African beeswax.

Biological applications of different Romanian honeys, produced by *Apis mellifera*

Otilia Bobiș, Liviu Al. Mărghitaș, Daniel S. Dezmirean, Victorița Bonta, Rodica Mărgăoan, Claudia Pașca and Adriana Urcan
University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Romania
E-mail: obobis@usamvcluj.ro

Honey is a natural product with many attributes and properties useful for humans. Its beneficial biological properties, antioxidant and antibacterial activities, entitle growing consumption of honey worldwide. Because of the varied flora and landscape, in Romania quite a high number of honey types are produced, honeys of high quality and remarkable bioactive properties. Multifloral honey possess high nutritional value, but monofloral honeys are preferred for pharmaceutical or medical purposes.

Melisopalinological analyses are used for uniflorality determination, knowing the fact that bioactive properties are transferred in honey from the nectar and pollen of the plants visited by the bees and used to make the honey. If antioxidant activity is due to the presence of enzymatic antioxidants (glucose oxidase, catalase) or non-enzymatic antioxidants (flavonoids, phenolic acids and some organic acids), the antibacterial activity is correlated with other several parameters, high osmolarity, low pH, production of H_2O_2 and other chemical constituents.

Chemical composition of different types of unifloral honeys were determined (water content, sugar spectrum, diastase, HMF content, total polyphenols, flavonoid content and also individual phenols) and correlated with antioxidant activity determined using different *in vitro* assays. Antibacterial activity against some gram-positive and gram-negative bacteria was determined to establish the most valuable types of honey to be used for specific medical or pharmaceutical purposes.

Pathology

Organizer: Dirk de Graaf

The pathology section with tackle different aspects of bee health. This includes both the pathogenic and non-pathogenic microbes, the parasites and pest species. We have a nice selection of presentations on diagnosis, disease control and host-parasite interactions. This section will also cover the impact of pesticides on bee health and the interactions between different drivers on bee mortality

Microbial communities in bumblebees: exploring the unexplored

Anneleen Parmentier°, Annelies Billiet°,Guy Smagghe, Peter Vandamme and Ivan Meeus
UGent, Coupure Links 653 - 9000 Gent - Belgium
E-mail:anneleen.Parmentier@ugent.be

Insects harbor distinctive bacterial communities with specialized functions to support insect development and survival. As most of these symbiont-host interactions are nutrient-based, microbial communities are particularly prominent in the digestive tract.
Also the gut microbiota of adult bumblebees is widely studied, in contrast to the limited descriptive data on the larval gut microbiota. Using the16S ribosomal RNA Illumina deep sequencing technique, the larval gut microbiota residing from a wild bumblebee nest was characterized revealing a yet unexplored bacterial diversity of mainly *Enterobacteriaceae,Lactobacillaceae*, *Corynebacteriales* and *Bacillales*.
Aside from unexplored life stages, different habitats within theinsects can be colonized by bacteria. The 16S ribosomal RNA sequences of the resident microbial communities in the gut and the fat body of wild foraging bumblebees were studied, showing a distinct set of shared bacterial phylotypes in the adult gut and fat body, but also specific bacterial phyla in the fat body. The unique OTUs (Operational Taxonomic Units) in the fat body, like *Phyllobacterium*, underline a separated functionality. Further the fat body microbiota was correlated with its sampling location and with *Apicystisbombi* infection. *A. bombi* infection disturbs the microbial association network, as positive correlations between the OTUs residing in the gut and fat body increases from 0.18% to 0.69%. Also the OTU identified as *Arsenophonus*, which has a location dependent interaction with *A. bombi*, is a possible candidate to influence the bee health in collaboration with *A. bombi*.

Reversing bumblebee declines: a microbiome approach

Jessy Praet, Ivan Meeus, Guy Smagghe and Peter Vandamme
Laboratory of Microbiology, Department of Biochemistry and Microbiology, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium
E mail:jessy.praet@ugent.be

There is currently great concern about worldwide bee declines which may have a detrimental economic impact and may create an instable ecosystem. These declines are presumably caused by a combination of climate change, changes in agricultural practices, pesticide and insecticide use and pathogen spill over from commercial bees. A good mitigation strategy should therefore include the augmentation of nest and hibernation sites, the reduction of insecticide use and the direct promotion of bee health.

Bumblebees are important pollinators of many commercial crops and wild plants.Their gut microbiota has predominantly been analyzed through metagenomics studies and consists of few and very specific bacterial species including *Snodgrassella alvi*, *Gilliamella apicola* and *Lactobacillus bombicola*. These symbiotic gut bacteria may contribute to bee health by supporting the digestion of pollen and the detoxification of pesticide residues and through pathogen inhibition.

In our study we aim to isolate, characterize and exploit the biotechnological potential of the bumblebee gut microbiota. An extensive isolation and characterization campaign was performed to inventorize the cultivable bumblebee gut bacteria. Their functionality and potential to improve bumblebee health is being investigated by pathogen inhibition assays, a pectin degradation assay and whole genome sequence mining. The present data show that especially lactic acid bacteria including *Lactobacillus bombicola, Weissella bombi, Fructobacillus fructosus* and *F. tropeaoli* inhibit notorious bee pathogens like *Paenibacillus larvae* and *Melissococcus plutonius*. Ultimately, the effects of a set of potentially probiotic strains on bumblebee health in naive conditions and after pathogen exposure will be studied through microcolony experiments.

Sustaining honeybee health with probiotics

Pierre Giovenazzo, Andrée Rousseau, Pierre-Luc Mercier and Nicolas Derome
Département biologie, pavillon Vachon, Université Laval, Québec, Canada, G1V0A6
E- mail: pierre.giovenazzo@bio.ulaval.ca

Maintaining health status of honeybee colonies is a major concern for beekeepers. Various parasites and pathogens are responsible for unsustainable declines of honeybee populations over the past decade. Homologated treatments against disease and pests rely on chemicals and antibiotics that can be harmful for honeybees. For example, antibiotics allow resistant opportunistic pathogens to trigger secondary infections as their natural antagonistic gut bacteria are killed or weakened. Therefore, there is an urgent need to develop new tools to treat and prevent honeybee diseases that are efficient, sustainable and safe for honeybee micro flora. The nutritional probiotic approach is straightforward and it has proved its efficiency in improving health for various farm animals.
Our research team is currently developing bee specific probiotic strains that improve significantly colony survival, performance and disease resistance. Furthermore, we are developing metagenomic tools to monitor gut microbiota homeostasis. Such tools will enable beekeepers to rapidly and cost-effectively monitor health status of their colonies in order (1) to identify colonies healthy colonies and (2) to target those that need personalized probiotic treatments to restore gut microbiota homeostasis, and thus disease resistance.
We have tested survival of caged bees (20 cages per experimental group, 20 bees per cage) with various probiotic candiates/supplements in 2:1 sucrose syrup. Our current results demonstrate that four probiotic candidates improve honeybee survival in caged bee trials, both in prophylactic and curative contexts with experimental infections of the microsporidian parasite Nosema cerana.

Multiple Locus Variable number of tandem repeat Analysis: A molecular genotyping tool for *Paenibacillus larvae*

Tine Descamps, Lina De Smet, Pieter Stragier, Paul De Vos and Dirk C. De Graaf
Ghent University, Laboratory of Molecular Entomology and Bee Pathology, Krijgslaan 281 S2, 9000 Ghent, Belgium
E-mail: tine.descamps@ugent.be

American Foulbrood, caused by *Paenibacillus larvae*, is the most severe bacterial disease of honeybees (*Apis mellifera*). In order to perform genotyping of *P. larvae* in an epidemiological context, there is a need of a fast method with a high resolution. Here we propose Multiple Locus Variable number of tandem repeat Analysis (MLVA) as a new genotyping tool. The tool uses the variation in number of tandem repeats between different strains to classify isolates. Five tandem repeat loci are targeted in a multiplex PCR. The amplicons created by this multiplex PCR give a unique pattern for each MLVA type. This pattern can be visualized on an agarose gel stained with Ethidium Bromide.

MLVA has been used for typing a collection of 189 *P. larvae* ERIC I field strains from which 17 different types could be identified. Within 8 *P. larvae* ERIC II strains, 2 additional MLVA genotypes were identified. Finally, ERIC III and ERIC IV strains each formed a MLVA genotype. The developed methodology not only permits the identification of the four ERIC genotypes, but allows a discriminatory subdivision of ERIC type I and II. Moreover, the present MLVA genotyping protocol also permits to differentiate between the ERIC genotypes by the presence of some discriminatory bands.

A biogeographical study has been conducted showing a significant correlation between MLVA genotype and the geographic region where it was isolated. A difference in prevalence of MLVA types could be noted between isolates from diseased brood and isolates from honey of healthy colonies.

Disease control and beekeeper education are key factors for a better honeybee colony survival to winter in Europe

Antoine Jacques[1,2], Marion Laurent[2], Epilobee Consortium, Magali Ribiere-Chabert[2], Mathilde Saussac[1], Stéphanie Bougeard[3], Giles E. Budge [4,5], Pascal Hendrikx[1] and Marie-Pierre Chauzat[1.2]

[1]Unit of coordination and support to surveillance, ANSES, Scientific Affairs Department for Laboratories, Maisons-Alfort, France.[2]Anses, Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes – CS 20111, 06902 Sophia Antipolis, France.

E-mail: marie-pierre.chauzat@anses.fr

Reports of honey bee population decline have spurred many national efforts to understand the extent of the problem and to identify causative or associated factors. However, our collective understanding of the factors has been hampered by a lack of joined up trans-national effort. Moreover, the impacts of beekeeper knowledge and interventions have often been overlooked, despite honey bees being a managed pollinator. Here, we established a standardisedactive monitoring network for 5 798 apiaries over two consecutive years to quantify honey bee colony mortality across 17 European countries.The EPILOBEE program was co-financed by the participant Member states and the European Commission.Our data demonstrate that overwinter losses ranged between 2% and 32%, and that high summer losses were likely to follow high winter losses. Multivariate Poisson regression models revealed that hobbyist beekeepers with small apiaries and little experience in beekeeping had double the winter mortality rate when compared to professional beekeepers. Furthermore, clinical signs ofdiseaseswere not reported on honey bee colonies kept by professional beekeepers, unlike apiaries from hobbyist beekeepers that had symptoms of bacterial infection and heavy Varroa infestation. Our data highlight,among the recorded factors,apicultural practices and beekeeper background as major drivers of the honey bee colony losses observed. They certainly play an important role in the better survival of honeybee bee colonies to winter. The benefits of conducting trans-national monitoring schemes and improving beekeeper training are discussed.

Evolution of the activities of the European network of National Reference Laboratories in the field of honey bee health since 2011

Marion Laurent[1], Magali Ribière-Chabert, Marie-Pierre Chauzat, Laura Cauquil, and Marie-Pierre Rivière
Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes – CS 20111, 06902 Sophia Antipolis, France.
Email: marion.laurent@anses.fr

The European Commission has designated, in 2011, a European Union Reference Laboratory (EURL) for Bee Health. One of the main EURL duties was to coordinate the network of European National Reference Laboratories (NRLs) by ensuring communication and development of harmonised diagnostic methods within the network.
The first tasks of the EURL were to establish and release every year a questionnaire to gather information on NRLs analytical activities including developed, validated and accredited methods along with their participation in and/or organisation of Inter-laboratory Proficiency Tests (ILPTs). The questionnaires targeted the major honey bee diseases and pathogens (eg. notifiable diseases as the American foulbrood, *Tropilaelaps* spp. and the small hive beetle).
Participation rate to the surveys has always been very high, with more than 92 % of filled questionnaires every year. This allowed giving an accurate overview of the NRL network diagnosis competences.
After five years of survey, a regular and robust improvement in skills and analytical capacities was observed within the network. Consequently, every year, the NRLs enlarged their panel of diagnosis methods by adopting and/or developing new methods. The number of validated and accredited methods has also regularly increased. Finally, the number of NRLs participating to ILPTs has also increased showing the willingness of the network to gain in competence and to participate in harmonising diagnosis methods at a European scale.
This information enables to highlight the analytical capacities of the European network, its strengths and areas of improvement to reach reliable and harmonised honey bee diseases and pathogens diagnosis.

The European Life Project STOPVESPA: first year of activity and most relevant results.

Laurino D., Manino A., Bertolino S., Lioy S. and Porporato M.
Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095, Grugliasco (Turin), Italy.
E mail: marco.porporato@unito.it

The yellow legged hornet *Vespa velutina* Lepeletier 1836 is an Asiatic hornet species introduced in France in 2004 that is rapidly colonizing other European countries. In Italy *V. velutina* was observed for the first time in 2012 and first nests detected in 2013; since then the species is quickly spreading throughout the northwest part of the country, and at the end of 2015 *V. velutina* occupied an area of about 930 km^2.

V. velutina is an invasive alien species in Europe because of its impact on natural ecosystems, apiculture and human well-being. In fact, *V. velutina* could actively prey honeybees, wild bees and other native insects, producing economic and biodiversity losses. In addition nests can be constructed in urban areas and might be considered a risk for human population.

For these reasons, an European Life Project (LIFE14 NAT/IT/001128 STOPVESPA) recently started in Italy. The actions carried out by this project in the first year of activity are: *i)* monitoring the evolution of *V. velutina* populations in Italy; *ii)* controlling the populations by nest-localization and nest-destruction (in 2015 181 nests were observed and 23 collected for nest analysis); *iii)* developing an harmonic radar to track the hornets while flying back to their nests; *iv)* evaluating the impacts of *V. velutina* on natural communities, ecosystems and beekeeping; *v)* establishing of an Early Warning and Rapid Response System at a national level.

In this work we present the most relevant results obtained by the STOPVESPA project in the first year of activity.

New *Nosema* spp detected in honeybee colonies from two highland agro-ecological zones of Uganda: is it an emerging threat to beekeeping?

Moses Chemurot[1, 2], Lina De Smet[1], Riet De Rycke[3] and Dirk C. De Graaf[1]

[1]Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Krijgslaan 281 S2, B-9000 Ghent, Belgium;
[2]Department of Zoology, Entomology and Fisheries Sciences, College of Natural Sciences, Makerere University, P.O. Box 7062 Kampala, Uganda
E-mail: moseschemurot@gmail.com; mchemurot@cns.mak.ac.ug

The microsporidian parasites (*Nosema apis* and *Nosema ceranae)* of honeybees are important threats to beekeeping in western countries. In an effort to provide some insights into microsporidian parasitism in Ugandan honeybees, we sampled adult worker bees in apiaries located in two highland agro-ecological zones of Uganda. We were particularly interested in i) establishing the *Nosema* species present, ii) determining *Nosema* infestation levels along altitudinal gradients and areas of varying land use types and iii) exploring how this variation might affect honeybee colony strength and productivity. We first used light microscopy to establish the presence of microspodian spores and then performed PCR using primers specific for *N. apis* and *N. ceranae,* the known microsporidian parasites of honeybees. Interestingly, several samples with observable microspodia-like spores tested negative for both parasites. We therefore decided to use family primers and transmission electron microscopy. Preliminary results confirm the presence of *N. apis* at low prevalence levels. The results also point to probably a new species of *Nosema* that is smaller in size (Length: 2.36±0.14 µm and width: 1.78±0.06 µm (n=6)) and has fewer polar filament coils **(**10-12 (n=6)) than *N. apis* and *N. ceranae*. This new *Nosema* species was found at high prevalence levels. *Nosema* spore counts in colonies located in protected areas were significantly lower than those in farmlands ($P < 0.01$) and *Eucalyptus* plantations ($P < 0.01$). Finally, the number of frames/top bars with honeybees and amount of honey which was harvested were negatively correlated with *Nosema* spore counts.

Purifying selection and siRNA control of *Nosemaceranae*

Qiang Huang
Institute of Bee Health, University of Bern, Bremgartenstr. 109a, CH-3001 Bern
E-mail:qiang-huang@live.com

To better understand the mechanisms of *Nosemaceranae* parasitism, we deep-sequenced both honey bee host and parasite mRNAs and small RNA throughout a complete 6-day infection cycle. By time-series analysis, 1122 parasite genes were significantly differently expressed during the reproduction cycle, clustering into 4 expression patterns. We found reactive mitochondrial oxygen species modulator 1 of the host to be significantly down regulated during the entire infection period. The expression levels of 17 host microRNAs were significantly regulated. The number of SNP positions per gene and the proportion of non-synonymous substitutions per gene were significantly reduced over this time period, suggesting purifying selection on the parasite genome. Novel microRNA-like small RNAs were identified and confirmed from the parasite genome. I further designed siRNA to target the parasite virulent gene. After treating the host with siRNA, the spore load was significantly reduced. Our data provide new insights into *N. ceranae* pathogenesis and control.

Causes and consequences of reactive oxygen species production in the gut of honeybees exposed to the parasite *Nosema ceranae* and to the insecticide fipronil.

Laurianne Paris, M. Roussel, F. Delbac and M. Diogon
Lmge, Université Blaise Pascal, Campus Universitaire des Cézeaux, 1 Impasse Amélie Murat, 63178 Aubiere Cedex, France.
E-mail: laurianne.PARIS@univ-bpclermont.fr

Many studies suggest that the observed decline of *Apis mellifera* honeybees would be due to the combined actions of multiple stressors, including both pathogens and pesticides. We previously demonstrated that a synergistic increase in honeybee mortality occurred when bees were infected by the gut

parasite *Nosema ceranae* and chronically exposed to a sublethal dose of the insecticide fipronil (Vidau et al. 2011; Aufauvre et al., 2012). Interestingly, it has been suggested that the infection by *N. ceranae* may increase the antioxidant capacity of the bee. We wondered whether the elevation of mortality rate in a context of infection combined with fipronil intoxication could be the result of reactive oxygen species (ROS) production. To assess the impact of these stressors on oxidative balance, we measured soluble ROS and ROS damage through the quantification of protein and lipid oxidation in the midgut of bees experimentally infected by the parasite and/or chronically exposed to the insecticide at sublethal doses (0.014 ng/bee/day) during 22 days. Our results indicated a disruption of the oxidative balance, with an increase of protein oxidation when bees were treated with the two stressors. In contrast, no effect on the oxidative balance was observed in bees only infected by *Nosema*, and the balance was less disturbed with the fipronil alone compared to the double stress. Complementary studies were also conducted in cell culture with different microsporidian species in order to better understand the origin of ROS production, probably generated by mitochondria, during a microsporidian infection coupled with a pesticide exposure.

The earliest record and first report of *Lotmaria passim* in Serbian honey bees

Jevrosima Stevanovic, Ryan S. Schwarz, Branislav Vejnovic, Jay D. Evans, Rebecca E. Irwin, Uros Glavinic and Zoran Stanimirovic
Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Bul. oslobodjenja 18, 11000 Belgrade, SERBIA
E-mail: rocky@vet.bg.ac.rs

In this study, honey bees from Serbia were retrospectively surveyed for the presence of trypanosomatid parasites, *Crithidia mellificae* Langridge and McGhee, 1967 and *Lotmaria passim* Schwarz, 2014. Archival honey bee DNA samples analysed in this study were obtained over a nine-year period (2007-2015). Samples from 162 colonies (18 from each year) originating from 57 different localities were analysed using newly designed species-specific primers for PCR detection of both trypanosomatids. Only *L.* passim was detected and its overall frequency was 62.3%, while *C. mellificae* was not found in any samples. Annual frequencies of *L.*

passim were 72.2% in 2007, 38.9% in 2008, 72.2% in 2009, 61.1% in 2010, 83.3% in 2011, 83.3% in 2012, 38.9% in 2013, 66.7% in 2014 and 44.4% in 2015. These results provide the earliest genetically confirmed record of *L. passim* on the global scale and provide the first long-term survey for both known honey bee trypanosomatid species and first record of *L. passim* in Serbia. All samples were initially collected for microsporidia screening and revealed the presence of only one species, *Nosema ceranae*, while no *N. apis* or mixed *N. apis/N.ceranae* infections were detected. The incidence of *N. ceranae* was very high (with 95.7% overall frequency), and no apiary in Serbia was *N. ceranae*-free. The majority of colonies (60.5%) were co-infected with *L. passim* and *N. ceranae* originating from 43 locations throughout Serbia. However, over the 9 years surveyed, proportions of *L. passim* and *N. ceranae* infections in *A. mellifera* were statistically independent of one another.

The European Union Reference Laboratory for Honey Bee health activities following the detection of *Aethina tumida* in Italy in 2014

Marie-Pierre Chauzat[1,2], Véronique Duquesne[2], Marion Laurent[2], Stéphanie Franco[2], Pascal Hendrikx[1] and Magali Ribiere-Chabert[2],
[1]Unit of coordination and support to surveillance, ANSES, Scientific Affairs Department for Laboratories, Maisons-Alfort, France. [2]Anses, Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes – CS 20111, 06902 Sophia Antipolis, France.
E-mail: marie-pierre.chauzat@anses.fr

Aethina tumida (small hive beetle, SHB) was firstly reported in Italy in September 2014 in a clementine orchard. Thanks to the surveillance implemented since, 61 SHB outbreaks have been detected in 2014 (60 in Calabria and one in Sicily) and 29 outbreaks in 2015 in Calabria (none in Sicily). In 2015, the European Laboratory for Honey Bee health (EURL) associated with five National Reference Laboratories (NRL) have produced guidelines for the surveillance of SHB infestation, presenting methods that could be implemented in the MSs. Subsequently, the EURL has sent a questionnaire to all the NRLs to gather information on actions implemented and to monitor how the actions/tools

developed by the EURL have been used. The results were presented at the fifth Annual workshop of the EURL in October 2015. At analytical level, two methods have been optimized by the EURL to ensure the quality of results. Morphological identification has been developed for primary diagnosis. Specific criteria have been selected to differentiate SHB from other Nitidulidae coleoptera found in honey bee hives and from wax moths larvae. A molecular identification method based on a real-time PCR has been characterized and validated for confirmation. The two methods have been disseminated to European NRLs in order to standardize and ensure reliable diagnosis at European level. The EURL has also been involved in working groups on SHB, with EFSA in particular. A scientific EFSA report and a scientific opinion have been published in 2015.

Course of oogenesis in the honey bee mite *Varroa destructor*

Claudia Häussermann, Bettina Ziegelmann and Peter Rosenkranz
Apicultural State Institute University of Hohenheim, August-von-Hartmann-Str. 13, 70599 Stuttgart
E-mail: Claudia.haeussermann@uni-hohenheim.de

The high reproductive rate of the honey bee mite *Varroa destructor* in colonies of the new host *Apis mellifera* is the main reason why this parasite has become the major threat for apiculture. Reproduction of the female mite exclusively takes place inside the sealed host brood cell. Immediately after the invasion of a brood cell the oogenesis of the foundress mite is activated by volatiles of the bee larva. In the further course the vitellogenesis and embryogenesis of the different mite eggs are obviously triggered by stage specific signals of the host larva. Up to 5 eggs are laid during one reproductive cycle whereby the first egg remains unfertilized and develops due to the haplodiploid sex determination into a male. We here describe in detail the growth rates and morphological changes of the oocytes in short time intervals during the first four days of the mite´s reproductive cycle. We are focusing on the development of the second egg – representing the first fertilized and therefore female egg - with particular attention on the mode and time of its fertilization. We further analysed the egg development in presumably non-reproducing mites in order to better understand the reasons for the often described temporary infertility of *Varroa* females. Such knowledge could be used

to reduce the reproductive success of female mites and therefore limit the growth rate of the *Varroa* population within infested honey bee colonies

Evaluation of different application techniques and duration of action of a mating disruption based on the female sex pheromone of Varroa destructor

Bettina Ziegelmann and Peter Rosenkranz
Apicultural State Institute, University of Hohenheim, August-von-Hartmann-Str. 13, 70599 Stuttgart, Germany
E- mail: Bettina.ziegelmann@uni-hohenheim.de

Mating in Varroa destructor is triggered by a female sex pheromone consisting of 3 fatty acids and the respective ethyl esters. Thereby, the single components as well as the mixture can elicit the male mating behavior. In previous studies we showed that spraying the main component oleic acid to brood combs prior to the Varroa infection leads to reduced numbers of mated females. We here tested different application methods and compositions: (i) oleic acid was added to the melted bees wax before making the comb foundation (ii) oleic acid sprayed directly on the wax foundation (iii) oleic acid was sprayed on capped brood cells and (iv) a mixture of all components sprayed on empty brood combs. The mating success was analyzed by counting spermatozoa in daughter mites from artificially infested brood cells. We could show that the number of transferred spermatozoa is reduced when oleic acid is added to the wax or sprayed on it, and that up to 20% of the daughter mites were unmated. The mixture of all components did not improve this effect. However, no effect was observed when oleic acid was sprayed on capped brood cells. Our tests also revealed that the rate of unmated females decreases slightly with every brood cycle. Furthermore, we present first results of a field test analyzing the mite population growth in honeybee colonies completely equipped with treated combs. The potential use of Varroa sex pheromone as an additional module in integrated Varroa control strategies is discussed. Supported by the BMEL/BLE.

Behavioural resistance of bees is related to tolerance to Varroa destructor and DWV infection level in Uruguay

Yamandú Mendoza, Ivanna Tomasco, Sebastián Díaz-Cetti, Gustavo Ramallo, Cecilia Chambón, Carlos Silva, Karina Antúnez, Belén Branchiccela, Matías Maggi, Pablo Giménez and Ciro Invernizzi.
INIA La Estanzuela, CP: 70006, Colonia, Uruguay.
E-mail: ymendozaspina@gmail.com

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 to the mite (S), collapsing if they are not chemically treated, while bee populations located in the East are tolerant (T), being able to survive without miticide treatment. The aim of this study was to compare the host-parasite interaction in those populations and identify potential factors involved in the tolerance to the mite. One experimental apiary was located in each region, with susceptible or tolerant bee populations (S, N=23 colonies, T, N=21 colonies). No miticide treatment was applied in any of the colonies.

In autumn, *V. destructor* parasitation level was higher in susceptible colonies than in tolerant colonies. No genetic differences were found in *V. destructor* populations from both regions, all of them belong to the K-haplotype.

Regarding to the bee response against the mite, susceptible colonies showed lower grooming (percentage of damaged mites) and lower hygienic behavior than tolerant colonies. Both bees and mites populations were infected by ABPV, BQCV, DWV and SBV; although DWV infection level was significantly higher in bees from susceptible colonies, compared to resistant colonies.

Finally, in winter all susceptible colonies died while only 9% of tolerant colonies died. Those results suggest that resistant behaviour is involved in differential tolerance to *V. destructor* and DWV infection level, as in subsequent colony loss.

The Strength of the Weak: Social Apoptosis in the Asian Honey Bee, *Apis cerana.*

Paul Page, Zheguang Lin, Ninat Buawangpong, Huoqing Zheng, Fuliang Hu, Peter Neumann, Panuwan Chantawannakul and Vincent Dietemann
Agroscope – Swiss Bee Research Center, Schwarzenburgstrasse 161 3003 Bern, Switzerland
E-mail: pagepaul@hotmail.com

The ectoparasite *Varroa destructor* is the keybiotic threat to European subspecies ofthe Western honey bee,*Apis mellifera*. However, colonies of its Eastern sister species and original host of *V. destructor*, *A.cerana*,survive to infestations by theparasitic mite. While Eastern honey bees are better at removing infested individuals together with their parasite, thus preventing mite population growth, the role of the infested brood in this behaviour has to date not been investigated. By excluding the behaviour of adult workers, our experiments show that a larger proportion of infested *A.cerana*individuals die during their development, when compared to *A. mellifera*.Theseresultscounterintuitivelysuggest that susceptible individuals can foster superorganism survival, offering empirical support to theoretical arguments about the adaptive value of worker suicide in social insects. The altruistic suicide of immature bees constitutes a social analogue of apoptosis, as it can prevent the spread of pathogens by sacrificing parts of the whole organism. This newly describedphenomenon constitutes a form of transgenerational social immunity.Taking into account the key role of susceptible immature bees in colony healthcanimprove breeding efforts to mitigate the unsustainably high colony losses of Western honey bees caused by*V. destructor* infestations worldwide.

A citizen science experience on "Varterminator", a new antivarroa medicament

Nanetti Antonio., Bressan G., Barone S., Gardinazzi C., D'Addio E., Obaldi.G.,Giorgio A., Redoglia R., Cremasco S., Ruta V., Marcello P., Massidda A., Cantoni G., Loglio G. and Damiani B., Fe S.
CREA –API - Via di Saliceto 80 – Bologna (Italy)
E-mail: antonio.nanetti@crea.gov.it

This presentation examines the collaboration between Italian veterinaries, researchers, agronomists, technicians and beekeepers in a field trial to test Varterminator, a new antivarroa product containing formic acid. The partners replicated the trial in different Italian regions, on a small number of own colonies, after a shared protocol. Data were recorded online on a database that was made available to all members. In general, variable efficacy and side effects were recorded, but clear correlation with environmental factors could not be detected. We positively evaluate this citizen science experience, that helped respond to a research need by bringing together several counterparts with low individual efforts.

Lithium chloride - a new perspective for managing Varroa destructor?

Bettina Ziegelmann, Stefan Hannus and Peter Rosenkranz
Apicultural State Institute, University of Hohenheim, August-von-Hartmann-Str. 13, 70599 Stuttgart, Germany
E-mail: bettina.ziegelmann@uni-hohenheim.de

Untreated bee colonies usually collapse within 1-3 years due to the infestation with Varroa destructor. Current control methods carry the risk of developing resistances and contamination by residues in bee products. In addition wrong application can result in damages to bee and brood or non-sufficient efficacy. Here we present the extraordinary effect of an alkaline salt on mite mortality with simultaneous low bee toxicity. Remarkably, we could clearly confirm a systemic effect of the compound by feeding caged honey bees the alkaline salt and subsequent monitoring of mite mortality. In brief, we examined the activity on phoretic mites and tolerability by bees in cage tests by feeding different concentrations over 24 h till several days. Every cage contained 50 worker bees and 25 phoretic mites. The bees were fed either with sugar syrup only (control) or a sugar solution containing the alkaline salt in concentrations ranging from 16 µM to 250 mM. Bee and mite mortality were recorded over a period of two weeks. Concentrations of 10mM and higher led to a mite mortality of 90-100 % within 3 days but were well tolerated by the bees. Even a short term application of only 24 hours could eliminate almost all phoretic mites. We here present first data of a completely new miticide with a systemic mode of action. In view of the extraordinary high efficacy and low toxicity on bees our findings open the possibility of a potent, selective and practical alternative to current Varroa treatments.

Effect of Neem (Azadirachtaindica) oil onvarroamite development in field conditions

Michele Mortarino, Mattia Blonda, Sergio Zanzani, Francesca Dell'Orco, Elena Facchini and Rita Rizzi
Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy
E-mail: michele.mortarino@unimi.it

Neem tree (Azadirachtaindica) is a well-known source of natural extracts containing triterpenoids and other active substances with insecticides and miticides effects. In recent years, neem-derived products were tested for tolerability in honeybees and effects against varroa mites, with conflicting results due to the different characteristics of the extracts, dosages, test sites, and methods of application used for in vitro tests and in field trials (Melathopoulos et al., 2000; Gonzalez-Gomez et al., 2012; Anjum et al., 2015). The present study aimed to check, in suitable field conditions, the possible effects against varroaof a commercial preparation of neem oil for veterinary use (RP03 ™, Farmaneem, Italy). The tests were carried out in the years 2013-2014 in the same apiary. The product was applied prior or during the development cycle of the honeybee brood, and the following effects were evaluated: acaricide, repellentfor the mite and / or disturbing the development of varroa. In addition, the tolerability of honeybee families to the productwas assessed. This work allowed to verify in a meaningful way and in field conditions the interaction effects between neem oil and the Varroa mite developmental cycle. The results may encourage further studies in order to confirm the valuability of standardized neem oil preparations as additional tools in the currentstrategies for varroa control.

DWV quasi-species evolution during serial transmission: Radiative bursts, conservative selection, defective genomes and symptom attenuation

Joachim de Miranda, Orlando Yañez, Julio Chávez-Galarza, Christian Tellgren-Roth, M. Alice Pinto and Peter Neumann
Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, 750-07 Sweden
E-mail: joachim.de.miranda@slu.se

There has been strong interest over the years in the pathology of deformed wing virus (DWV), currently the most damaging virus disease of honeybees, and in particular whether the virus has evolved to become more virulent due to its transmission by varroa. We tested this hypothesis by analysing the changes to the DWV quasi-species during serial transmission experiments, and whether these could be linked to the pathology of the resulting adult bees. Although the DWV consensus sequence remained unchanged throughout the experiments, the underlying quasi-species showed strong and distinctive changes that point to a strong, conservative influence of the host on shaping the adult-bee DWV quasi-species, following a burst of irregular variability immediately after inoculation, including evidence of defective-interfering DWV genomes that can be linked to the later appearance of attenuated symptoms among the adult progeny. These results will be discussed with respect to the modern interpretations of virulence and quasi-species functionality.

First evidence of recombination hotspots and positive selection in Deformed wing virus (DWV)

Anne Dalmon, C. Desbiez, M. Coulon, M. Thomasson, Y. Le Conte, C. Alaux, J. Vallon, and B. Moury
INRA, UnitéAbeillesetEnvironnement, F-84000 Avignon, France
E-mail:anne.dalmon@avignon.inra.fr

Varroa destructor spread has contributed to a large increase of viral pathologies. Among the *Varroa*-transmitted viruses, *Deformed wing virus* (DWV, genus *Iflavirus*, family *Iflaviridae*, order *Picornavirales*) is often reported as one of the most damaging for the honey bee colonies. Different genetic variants of DWV have already been described, including recombinant variants between DWV and

the close *Varroa destructor virus-1* (VDV-1). Here, we sequenced the whole genome of two variants, and partially sequenced 42 isolates from colonies with conventional *Varroa*management or from *Varroa*-tolerant honey bee colonies. By combining phylogenetic and molecular evolutionary analyses we identified nine independent recombination breakpoints, and showed that they were not randomly distributed across the DWV genome. Most of the partially sequenced French variants (19/25) clustered with VDV-1 or recombinants in the 5' UTR (untranslated region), but were very similar to DWV variants in the helicase region, suggesting that they must have a recombination breakpoint between the two regions (5' UTR and helicase) and that recombinant isolates may prevail in France. Estimating the dN/dS ratio between non synonymous (dN) and synonymous (dS) substitution rates allowed identifying several codon sites that were under positive selection in the regions coding for structural proteins (leader protein) or for non-structural proteins (polymerase). Such an evolutionary approach provides new insights for understanding which virus proteins or genome regions could be involved in virus adaptation, including virulence properties.

Unbiased metagenomics in social and solitary wild bees detects associations with eukaryote parasites and new viruses

Karel Schoonvaere, Lina De Smet, Guy Smagghe, Andy Vierstraete, Bart P. Braeckman and Dirk C. De Graaf
Krijgslaan 281 building S2, 9000 Ghent, Belgium
E-mail:karel.schoonvaere@ugent.be

Solitary bees and bumble bees constitute a species-rich group of pollinators. However, many species are in decline and among the contributory stressors are bee diseases and the adverse interaction with parasites. In comparison to the well-documented pathosphere of the domesticated honey bee*Apis mellifera*, the parasites and pathogens of wild beesare less well studied. Using an unbiased metagenomics strategy in combination with a dedicated bioinformatics workflow, we explored the subset of eukaryote microorganisms and viruses associated with four common wild bee speciesin Belgium. Among the eukaryote associations were two parasitic protozoa and a parasitic nematode. Among the virus associations were three honey bee viruses (*Black queen cell virus*, *Sacbrood virus* and *Varroa destructor virus 1*), four plant viruses andsix putatively new viruses includingfour negative-sense single-stranded RNA viruses related to bunyaviruses and mononegaviruses, a circular dsDNA nudi-like virus and a positive-sense single-stranded RNA virus related to tombusviruses. The identification of negative-sense RNA viruseswas unexpected andwe only found them in solitary bees. Moreover, preservation of sequence strand specificity after high-throughput sequencing allowed us to discriminate active replication of at least three viruses. In conclusion, unbiased metagenomics proved a powerful approach to identify a wide diversity of eukaryote associations and potentially new virus sequences in wild bees.

Overwinter honey bee loss caused by a more virulent DWV: genotype B (aka VDV-1)

Robert J. Paxton
Institute for Biology, Martin-Luther-University Halle-Wittenberg, HoherWeg 8, D-06120 Halle (Saale), Germany
E mail:robert.paxton@zoologie.uni-halle.de

Though overwinter loss of honey bee (*Apismellifera*) colonies has many causes, the last decade (2005/06 to 2015/16) has witnessed elevated colony mortality in Northern temperate regions. Pests and pathogens are often blamed, particularly Varroa (*Varroa destructor*) mites and associated viruses. Here I review my colleagues and my recent analysis of 14 viral pathogens associated with Varroa mites. Our empirical analysis at four pairs of locations around the world provides clear support for deformed wing virus (DWV) as the most prevalent and most abundant (per bee) virus correlated with Varroa mites. There is equivocal support for black queen cell virus (BQCV) also associated with Varroa. DWV exists as different genotypes, including the originally described and widespread genotype A as well as genotype B, which has been previously named Varroa destructor virus-1 (VDV). Additional colleagues and I now show through structured field sampling and laboratory infection experiments that DWV genotype B is both more virulent than DWV genotype A, is widespread in the environment, and is closely correlated with overwinter loss of honey bees. DWV genotype B likely elevates substantially overwinter colony losses.

Toward a reverse genetic system for Chronic Bee Paralysis Virus molecular studies.

Ibrahim Youssef, Frank Schurr, Adeline Goulet, Nicolas Cougoule, Magali Ribière-Chabert, Hervé Darbon, Michel Bremont, Stephane Biacchesi, Richard Thiery and Eric Dubois
E-mail: Ibrahim.youssef@anses.fr

Chronic bee paralysis virus (CBPV) causes an infectious and contagious disease of honeybees (trembling, crawling and death). Its segmented genome is composed of two major positive single-stranded RNAs, RNA 1 (3,674 nucleotides) and RNA 2 (2,305 nucleotides). Three minor RNAs (about 1,000 nucleotides each) were described in early studies but were not confirmed by sequencing of the CBPV genome (Olivier et al., 2008). In this study, we demonstrate that the CBPV major RNAs are infectious *in vivo*. Honeybees inoculated with 109 RNA copies per bee developed paralysis symptoms within 6 days post inoculation. The number of CBPV RNA copies increased significantly throughout the infection. CBPV particles were visualized by electronic microscopy in inoculated honeybees. These results show that the CBPV major RNAs are sufficient to induce virus replication and produce CBPV particles. In the interest of establishing the first reverse genetic system for CBPV, cDNAs encoding the two major RNAs were cloned into the pcDNA 3.1 plasmid under the control of the CMV and T7 promoters. In order to induce viral replication in emerging honeybees, several conditions using different inoculums were tested. Preliminary results will be discussed.

Identifying stressors and effectors of the honeybee immune response, through mass spectrometry, may represent a promising solution for bee health monitoring: HematoBeeTest Project

Philippe Bulet, Karim Arafah, Sébastien Voisin, Paul-Arthur Sol, Yves Le Conte, Cédric Alaux, Anne Dalmon, Michel Bocquet, Laurent Gauthier, Jozef Simuth and Katarina Bilikova
Université Grenoble Alpes, IAB Inserm 1209 CNRS UMR5309 (Grenoble, France);
E-mail: philippe.bulet@biopark-archamps.org; philippe.bulet@ujf-grenoble.fr

In recent years, populations of western honeybees have declined worldwide. This decline is attributed to many stressors. Up to today, research and methodologies deployed against new challenges (such as use of pesticides, virus prevalence and other pathogens, climate and flora changes) have focused on evaluating and attempting to separately prevent and fight each factor. In addition, most of studies have focused on identifying and quantifying the presence of stress agents, instead of focusing on their impact on the colonies. The aim of the HematoBeeTest project is to address these limitations by establishing robust, effective and sensitive technologies for profiling & deciphering bee immunoproteomes with regards to the host-pathogen interactions. The objective is to deliver practical applications for monitoring and enhance bee immunity for an integrated and adapted health management.
The analyses of bee hemolymph, by hyphenated MALDI-MS and LC-ESI-MS/MS approaches for proteomic characterization of the immunoproteomes, resulted in visually different molecular profiles in function of the bees' infectious conditions (virus, *Varroa* mite, microsporidia *Nosema*). These differences were confirmed by statistical comparison of mass spectrometry profiles and discriminant analysis. We have demonstrated for example that virus-infected bees samples, with or without *Varroa* co-infection, ended up in a cluster of their own inside the overall *Varroa* cluster. This strongly supports the robustness of our monitoring approach in the case of co-infections, its potential as a plausible strategy to monitor honeybees' health, and for a better understanding of the molecular immune response of this social insect, in the context of experimental/natural infections.

Lab vs field - Evaluation of the effects of sublethal imidacloprid doses on nurses bees

Hatjina Fani, Balzola Luigi, Brandt Annely, Bruckner Selina, Laurino Daniela, Manino Aulo, Colli Monica, Krainer Sophie, Hernández López Javier, Porporato Marco, Renzi Maria Teresa, Sgolastra Fabio, Simon-Delso Noa, Tosi Simone and Williams Geoffrey

Division of Apiculture, Inst. of Animal Science, Hell. Agr. Org.'DEMETER', Nea Moudania, 63 200 Greece,

E-mail: fani.hatjina@yahoo.com

It is often recognised the difficulties to extrapolate the effects observed in individual bees kept in laboratory conditions to in-hive bees. In the present study, we studied the impact of sublethal doses of a neurotoxic stressor on the development of hypopharyngeal glands of nurse bees from the same colonies (n=2) both in individual bees kept in small cages under laboratory conditions and in individual bees kept in the original colonies. Bee colonies were fed with 2 ng/kg imidacloprid administered in sugar solution and with 3 ng/kg imidacloprid administered in pollen pasty during one month. Control colonies were kept in the same apiary and fed non contaminated food. After the month, a set of emerged bees were collected at emergence in an incubator, assigned randomly to laboratory cages and were exposed to the same concentrations of imidacloprid in a chronic basis. The laboratory cages were kept in T of 30o C and about 70% humidity. Another subset of emerged bees were marked and kept in the colony. The hypopharyngeal glands of both groups of bees were extracted at 10 and 15 days later and their diameters were visually evaluated using ImageJ free software. Results show a different development of the glands in the laboratory and in the field conditions, as well as different impact of the stressor.

Synergistic effects of a neonicotinoid and an EBI fungicide in honey bees, bumblebees and red mason bees

Fabio Sgolastra[1], Piotr Medrzycki[2], Laura Bortolotti[2], Maria Teresa Renzi[1], Simone Tosi[1], Gherardo Bogo[2,3], Dariusz Teper[4], Claudio Porrini[1] and Jordi Bosch[5]
1 Dipartimento di Scienze Agrarie, Università di Bologna, Bologna, Italy
2 CREA-API, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, *Unità di Ricerca in Apicoltura e Bachicoltura, Bologna, Italy*
3Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, Bologna, Italy
4 Research Institute of Horticulture, Apiculture Division, Puławy, Poland
5 CREAF, Universitat Autònoma de Barcelona, Bellaterra, Spain
Dipartimento di Scienze Agrarie, Università di Bologna, Viale Fanin 42, 40127 Bologna (Italy)
E-mail: fabio.sgolastra2@unibo.it

Bee diversity has been declining worldwide in the last decades. Although many factors have surely contributed to these declines, neonicotinoid insecticides have often been signaled as one of the main factors. Most studies on neonicotinoids tests single compounds. However, in field conditions, bees are exposed to multiple pesticide residues, some of which may interact synergistically. In addition, different bee species have different sensitivities to pesticides, and therefore may respond differently to these synergistic effects. The aim of this study is to assess the possible synergistic effect between a neonicotinoid (clothianidin) and an ergosterol-biosynthesis-inhibiting EBI fungicide (propiconazole) in three bee species of bees with contrasting life histories (*Apis mellifera*, *Bombus terrestris* and *Osmia bicornis*). We orally exposed bees to non-lethal doses of clothianidin (LD_{10} of each species) and propiconazole (7 µg/bee), singly and in combination. There was a strong synergistic effect on mortality in all three species, in particular during the first 48 hours after the exposure phase. The level of synergism and survival rates throughout the experiment varied among species. *Osmia bicornis* was the most sensitive species and *A. mellifera* the least. These results highlight the need to test combinations of pesticides likely to co-occur in agricultural environments and to include several species in risk assessment schemes.

Neonicotinoid pesticides serve as insect contraceptives: effects on male honey bees

Lars Straub[1], Laura Villamar-Bouza[1,2], Selina Bruckner[1], Panuwan Chantawannakul[3],Laurent Gauthier[4], Kitiphong Khongphinitbunjong[3,5], Gina Retschnig[1], Aline Troxler[1],Beatriz Vidondo[6], Peter Neumann[1,4] and Geoffrey R. Williams[1,4,*]

[1] Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

[2] Environmental Science Department, University of Koblenz-Landau, Landau, Germany

[3] Bee Protection Laboratory (BeeP), Department of Biology, Faculty of Science, Chiang MaiUniversity, Chiang Mai, Thailand

[4] Agroscope, Swiss Bee Research Centre, Bern, Switzerland

[5] School of Science, Mae Fah Luang University, Chiang Rai, Thailand

[6] Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland

E-mail: geoffrey.williams@vetsuisse.unibe.ch

Potential sub-lethal effects of neonicotinoid pesticides on the behaviour and physiology of non-target ecosystem service providing insects, such as honey bees, are relatively well-documented. However, their possible impact on insect reproduction is not known. Here we demonstrate that neonicotinoids can significantly reduce reproductive capacity of male (drone) honey bees, *Apis mellifera*. Drones reared using queenright colonies were randomly allocated to either 1.) Neonicotinoid or 2.) Control groups (N=10 colonies per group). Pollen supplements were provided to colonies*ad libitum* for 8 weeks; those fed to the Neonicotinoid group were spiked with 4.5 ppb thiamethoxam and 1.5 ppb clothianidin.Prior to assessment, emerged drones were maintained in laboratory cages for 10 days to sexually mature. While no significant effects were observed for drone teneral body mass (Three-level General Linear Model, P=0.804) and sperm quantity (Three-level Negative Binomial Model, P=0.1375), the data clearly showedreduced drone lifespan (Three-level Survival Model, P<0.0001), as well as reduced sperm viability (proportion living vs. dead) (Three-level Ordered Logistic Model, P=0.0277) and living sperm quantity (Three-level Negative Binomial Model, P=0.049) in the Neonicotinoid group. Our results demonstrate for the first time that neonicotinoid pesticides can negatively affect male insect reproduction, and provide a possible explanation for managed honey bee queen failure and wild insect pollinator declines.

Diet Quality and Effects of Pesticides Coumaphos and Imidacloprid on Honey Bee (Hymenoptera: Apidae) Mortality in Laboratory Experiments

Ales Gregorc[1,2], Patricia R. Knight[1] and John Adamczyk[3]
[1]Mississippi State University, South MS Branch Experiment Station PO Box 193, Poplarville, MS 39470; [2]Agricultural Institute of Slovenia, Ljubljana, Slovenia
E-mail:gregorc.ales@gmail.com

Experiments were performed in order to study comparative effects of protein supplemental additive 'Ultra Bee Dry', (Mann Lake Ltd.), and natural pollen collected by foragers (bee bread), on worker bee mortalities in the Lab. cages experiments. Optimal feeding regime was employed for varieties of coumaphos and imidacloprid treatments in order to find potential synergistic effects on caged bees. We tested coumaphos at 92.600 ppb concentration, in combination with two imidacloprid concentrations, 5 and 20 ppb. Sugar 'Pro Winter patty' (Mann Lake Ltd.) ensured the longest bees survival, similar to that with adding 5% bee bread, whereas adding 5 or higher percentages of Ultra Bee Dry, induced increased bee mortalities ($p<0.005$).
Bees exposed to coumaphos died in higher rates than bees offered coumaphos & imidacloprid 5 ppb or 20 ppb ($p<0.05$) incorporated in sugar patty. Both imidacloprid concentrations had no effect on bees mortalities. Daily intake of coumaphos & imidacloprid contaminated foods of approx 10mg/bee/day, was lower than daily consumption of coumaphos alone (14.3mg) or imidacloprid 5 ppb (18.4mg) or 20 ppb (13.7mg) ($p<0.05$). We determined that Pro Winter patty has good characteristics for feeding Lab caged bees and added protein substitute or bee bread has no positive effect on bees' survival. We also demonstrated that coumaphos alone induced higher bee mortality in comparison to coumaphos and imidacloprid mixtures. Finally we demonstrated that mixture of coumaphos and imidacloprid, induced higher bees mortalities than either imidacloprid concentrations alone. Aspects of using caged bees tests in feeding and toxicological experiments will be discussed.

SmartBee

The Smartbees project

Kaspar Bienefeld
Institute for Bee Research HohenNeuendorf, 16540 HohenNeuendorf, Germany
E-mail: Kaspar.Bienefeld@hu-berlin.de

The diversity of Europe's bee races is the result of natural selection in which each bee has adapted to the climate and diseases of its unique environment. This naturally led to a vast number of different bee races occurring across the continent. Today, the situation has changed drastically. One reason is *Varroa destructor*, which has led to catastrophic losses of European honeybee colonies. In addition we observe a systematic replacement of many native European populations with two races that have been bred for productivity, gentle behavior, and disease resistance. Both of these factors drastically reduce the genetic diversity of honeybees in Europe and endanger sustainable, regionally-acclimated beekeeping. In the EU-sponsored “SMARTBEES” project (http://www.smartbees-fp7.eu/) 16 Institutes from eleven countries cooperate to address this problem. The aim is to analyze the current state of genetic diversity among Europe's bees and to improve it using appropriate methods. Beyond that, the participating scientists will take on the dangerous interrelationship between bees, mites, and viruses to identify which mechanisms allow otherwise innocuous viruses to become so dangerous in combination with Varroa. The reasons for differences in bees' resistance capabilities will also be investigated using the most modern molecular-genetic methods available. Beekeepers' dissatisfaction with the performance of native bees was the fundamental reason for their replacement. Therefore, breeding strategies that have proven to be very successful will be adapted for previously neglected bee races, aiming to suit the needs of local beekeepers. First data on Varroa resistance of different A.mellifera subspecies are presented.

Genetic improvement of European honey bee (Apis mellifera L.) populations

Aleksander Uzunov, R. Büchler and K. Bienefeld
LLH Bee Institute, Erlenstr. 9, 35275 Kirchhain, Germany
E-mail: aleksandar.uzunov@llh.hessen.de

Preservation of local honey bee populations depends on their attractiveness for beekeepers, mainly recognized through their overall performance and vitality. Thus, availability of productive, manageable and vital colonies is a sustainable approach to discourage acquisition of queens of non-local origin. An increase of performance and appreciation of local populations can be achieved by establishing an organized and systematic breeding structure. BEEBREED (www.beebreed.eu) is currently the best example of a well-established sustainable breeding concept for central Europe.

Field testing and selection of European local honey bee populations is a focal point of the SMARTBEES project funded by the European Commission (Sustainable Management of Resilient Bee Populations, www.smartbees.eu). To achieve preservation through utilization we took numerous actions concerning breeders' capacity building and networking, standardization of the performance testing procedure and harmonization to BEEBREED.

More than 200 breeders from 19 European countries were trained for using a standardized protocol for assessment of colony performance concerning the important economic traits, colony development, swarming, gentleness, honey production and, in particular, resistance to Varroa destructor. Based on recommendations laid down in a Performance Testing Protocol, 116 testing apiaries with more than 1200 colonies from 10 subspecies were set up across Europe. For some of the regions, these are initial breeding activities for genetic improvement and preservation of the local populations. The first phenotypic data are already collected and will be used for subspecies specific genetic evaluation. Our goal is to expand the current breeding network for genetic improvement and preservation for other local populations across Europe.

Varroa destructor and Deformed Wing Virus are linked in a mutualistic symbiosis accounting for their major role in honeybee colony collapses

Desiderato Annoscia, G. Di Prisco, M. Margiotta, R. Ferrara, P. Varricchio, V. Zanni, E. Caprio, F. Nazzi and F. Pennacchio
Università degli Studi di Udine – Dipartimento di Scienze AgroAlimentari, Ambientali e Animali – Via delle Scienze 206, 33100 Udine, Italy
E-mail: desiderato.annoscia@uniud.it

Honeybee colony losses are triggered by interacting stress factors, among which the parasitic mite *Varroa destructor* and the associated Deformed Wing Virus (DWV) play a key-role. The vectorial capacity of the mite and the facilitation of viral replication are now well documented features of this association but the possible advantage for the mite have not received so far the due attention.
Here we show that DWV adversely affects the humoral and cellular immune responses by interfering with NF-κB signaling. This immunosuppressive effect by the viral pathogen enhances the reproduction of the parasitic mite.
This unrecognized mutualistic symbiosis between *Varroa* and DWV generates a loop of reciprocal stimulation with escalating negative effects on honeybee immunity and health, largely accounting for the importance of this mite-virus interaction in the induction of honeybee colony losses.

Low Varroa mite reproduction in European honey bees

Büchler, R., Bienkowska, M., Cauia, E., Costa, C., Dahle, B., Kretavicius, J., Kovacic, M., Kryger, P., Lima, A.S., Mondet, F., Panasiuk, B., Parejo, M., Pinto, A. and Wilde, J.
Landesbetrieb Landwirtschaft Hessen, Bee institute, Erlenstrasse 9, 35274 Kirchhain, Germany
E-mail: ralph.buechler@llh.hessen.de

In European honey bees usually 5-20% of the Varroa mites remain infertile after invading worker brood cells. However, Harbo and Harris identified high levels of non-reproduction as an inherited character of colonies selected for Varroa resistance. Increased proportions of non-reproducing mites (40 – 50%) have also been shown as a significant trait of some naturally selected mite resistant *A.*

mellifera populations in Europe. However, the distribution and variability of this interesting resistance trait in different European subspecies has not yet been studied.

In order to investigate the distribution and variability of suppression of mite reproduction (SMR) in European honey bees, infested worker brood containing pupae shortly before emergence were sampled from 335 colonies during July to September 2015. The samples were collected in Croatia, Denmark, France, Germany, Italy, Lithuania, Moldavia, Norway, Poland, Portugal, Romania and Switzerland and represent several subspecies and local hybrids. The brood cells were individually checked for the age of pupae, mite infestation and offspring stages. Single infested brood cells 7-9 days post capping without at least one female deutonymph and one male, and brood cells 10-12 days post capping without at least one adult daughter mite and one adult male were counted as non-reproductive.

On average 28 single infested cells could be evaluated per sample. The rate of non reproductive mites ranged between 0 – 100% with an average of 35.9%. Of those, 28.6% showed no reproduction at all, 48.7% had only offspring stages that were too young, and 31.1% were without male offspring. Significant differences between genotypes encourage further selection for this trait.

Preliminary study regardinginfestation level of Varroa (*Varroa destructor*) correlated with honeybee colonies survival in a case study in Romania.

Adrian Siceanu, Eliza Căuia, Gabriela Oana Vişan and Dumitru Căuia
InstituteforBeekeepingResearch and Development, Bucharest, 42 FicusuluiBlvd, sector 1, code 013975, Romania.
E-mail: adrian.siceanu@icdapicultura.ro

The knowledge regarding the Varroa infestation level in bee colonies is very important in the strategy of Varroa control in order to minimize the number of chemical treatments applications. The studies aimed to apply different methods to evaluate the infestation level in order to establish the threshold that have a negative impact from economic point of view. The experiment involved 30 non-treated bee colonies and 6 evaluations done at 3 weeks interval at the end of the season in the period of August-October 2015 followed by one evaluation at the end of wintering

period – end March 2016. The methods of level infestation evaluation were based on natural mortality counted on screened bottom board, live Varroa counted on bees and live Varroa counted on bee brood. In the same time information about strength of the colony in terms of bees and bee brood were recorded. The results were analyzed in order to establish the impact of infestation levels on the survival rate of bee colonies.Thestudywascarriedout in theframe of EUresearchprogramFP7-KBBE.2013.1.3-02,grant 613960.

Neonicotinoids and immunity

Gennaro Di Prisco, R. Ferrara, M. Iannaccone, E. Caprio, P. Varricchio, D. Annoscia, F. Nazzi, R. Capparelli and F. Pennacchio
University of Napoli Federico II – Department of Agricultural Sciences – Via Università 100, 80055 Portici (NA), Italy.
E-mail:gennaro.diprisco@unina.it

Honey bee colony losses are related to a multifactorial syndrome, associated with reduced immunocompetence and increased load of parasites and pathogens, that can be induced by different stress agents, in particular by the parasitic mite *Varroa destructor* and the Deformed Wing Virus (DWV). Recent studies have demonstrated that neonicotinoids down regulate the immune barriers of honeybees, by negatively modulating a transcription factor in the NF-κBfamily, which is involved in the activation of a number of defense responses. The reduced efficiency of the antiviral barriers under the Toll pathway promotes replication of DWV, which is stably associated to honey bee populations, often with asymptomatic infections. Given the central role of NF-κB in immunity, we wanted to assess the impact of neonicotinoids on both cellular and humoral components, such as encapsulation, melanization and coagulation, which may be relevant not only for pathogen control, but also for their impact on feeding activity of *Varroa* and its fitness. Our results show a negative impact of neonicotinoids on these immune parameters, setting the stage for studies aiming to investigate if and how their sub-lethal doses can have any influence on *Varroa* and other pathogens.
Because NF-κBmodulates a number of conserved pathways in animals, this prompted us to analyze the impact of neonicotinoids on human cell lines, in order to assess any subtle effect on non-target organisms. The transcriptional profiles obtained and the associated immunosuppressed phenotypes indicate the occurrence

of a potential risk, which, however, will have to be carefully assessed at the organism level.

Proteomic analysis of *Varroa destructor* saliva: do factors in mite saliva affect bee immuno-competence?

Ewan M Campbell, D. Stead and A.S. Bowman
University of Aberdeen, Institute of biological end enviromental studies, Aberdeen, Scotland
E-mail: e.m.campbell@abdn.ac.uk

Honeybees have a highly efficient immune system that protects them from a range of infectious agents. When adult female *Varroa* mites feed off the haemolymph of both larval and adult bees they transmit a number of pathogens to the bee hosts, most notably deformed-wing virus (DWV). We hypothesise that, similar to other *Acari*, *Varroa* mites suppress or alter the immune response of the bee host by secreting factors in the saliva. This immune suppression may inadvertently facilitate DWV transmission from *Varroa* to bee and thus allow DWV to establish in the host.

We pioneered a novel micro-collection technique to stimulate and extract nanodrop amounts of saliva from individual reproductive-phase adult mites. In tandem we micro-dissected pairs of salivary glands from feeding mites. Saliva and salivary gland samples were pooled and processed by high resolution, accurate mass Orbitrap LS – MS/MS. Data was cross referenced against our in house *Varroa* transcriptome assembly in tandem with the publically available *Varroa* genome and honey bee databases. Alongside a suite of bee proteins; including hexamerin and Major Royal Jelly Proteins, we identified mite-specific proteins, including those with secretory motifs and proteins homologous to bioactive factors in other *Acari* and hematophagous arthropods. This work represent an important step forward in a deeper understanding of the bee / Varroa / virus axis.

Proteomic analysis of hygienic behaviour in Apis mellifera carnica

Hu, Han[1], Bienefeld, K.[2], Wegener, J.[2], Zautke, F. [2], Hao, Yue[1], Feng, Mao[1], Han, Bin[1], Fang, Yu[1] and Li, Jianke[1]
Institute of Apicultural Research/Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Chinese Academy of Agricultural Science, Beijing 100093, PRChina
E-mail:wegenerj@hu-berlin.de

Hygienic behaviour (HB) is seen as one of the most important breeding traits conferring resistance of honeybees to Varroa destructor. Here we compared the proteome of individual carnica-workers that either expressed or did not express the behaviour, as well as of pupae from colonies from a HB-breeding line and a control line not bred for HB. We concentrated on three tissues, adult antennae, adult mushroom bodies, and pupal hemolymph. In total, 8609 proteins were identified. Pupae from hygienic stock showed a significant enrichment of proteins related to protein and energy metabolism. Worker bees individually observed performing HB showed mushroom body protein profiles indicative of an increased neuronal excitability, and quantitative and qualitative adaptations that may be linked to an enhanced olfactory acuity. While our study shows a surprising degree of overlap with earlier studies, performed on bees of completely different origin, the unprecedented depth of our proteome coverage adds a wealth of details to the mechanistic understanding of HB, and may be useful for marker identification.

Genetic and morphometric variation of *A. mellifera mellifera* across its range

Marina. Meixner, X. Chen, M. Bouga, M.F. Coffey, B. Dahle, A. Estonba, R. Ilyasov, J. Kretavicius, P. Kryger, I. Montes, A.G. Nikolenko, A. Oleksa, L. Papoutsi, A.V. Poskryakov, A.Y. Sharipov, W. Shi, A. Tofilski and R. Vingborg
LLH Bee Institute, Erlenstr. 9, 35275 Kirchhain, Germany
E-mail: marina.meixner@llh.hessen.de

The range of the dark north-western European honey bee, *Apis mellifera mellifera* covers a huge area, from southern France to the Ural Mountains in Eastern Europe, and maybe beyond. While its populations in Western and Northern Europe have been comparatively well characterized based on morphometric and

molecular data, very little is known about populations in the eastern part of this area and the true dimensions of its range.

To assess the status of *A. m. mellifera* variation, adult honey bees were sampled from more than 350 colonies covering an area between Ireland in the west and the Ural mountains in the east. Samples of *A. m. sinixinyuan* in Western China, a recently newly described close relative of *A. m. mellifera*, were also included.

The samples are currently being subjected to standard morphometric analysis. Together with nuclear and mitochondrial genetic sequence information the data will allow, for the first time, a comprehensive overview of the variation of *A. m. mellifera*. The results will significantly expand the current knowledge of the range extension of *A. mellifera* in Eastern Europe and Asia.

Posters

Neurobiology

Differential effects of organophosphate diazinon on membrane and soluble acetylcholinesterase in honeybee head and thorax

Glavan Gordana, Kos Monika, Janko Božič, Drobne Damjana and Jemec Anita
University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, Ljubljana SI-1000, Slovenia
E- mail:gordana.glavan@bf.uni-lj.si

The activity of acetylcholinesterase (AChE) is often used as an important biomarker of neurotoxicity after exposure to xenobiotics. Recently, *in vitro* experiments suggested that the membrane form of AChE is mainly neuronal whereas the role of soluble form is largely unknown, but some suggestions of their protective role against xenobiotics have been given. In this study we investigated *in vivo*effects of 10 days oral exposure to AChE inhibitor diazinon (0.2, 0.5, 1, 2.5 and 5 mg/ml nominal dose) on the activity of soluble and membrane forms of AChE in honey bee head and thorax. The activity of membrane AChE in the head of control untreated honey bees was much higher that the soluble confirming results of*in vitro*experiments showing that the membrane form is probably neuronal. In the thorax this ratio was much lower. The chronic exposure to diazinon diminished the activity of membrane AChE in the head and thorax, but elevated the soluble AChE in both body parts. These differential effects on AChE activity were shown for higher concentrations of diazinon tested. The elevation of the activity of soluble AChE demonstrated in this study might be predictable for its detoxifying function whereas the changes in the activity of membrane AChE could be the result of the compensatory effect of nervous system or direct inhibition by diazinon. However, the role of the soluble AChE needs to be further investigated.

Impact of thepesticides tau-fluvalinate and tebuconazole on honeybee physiology and longevity

Risto Raimets
EstonianUniversity of LifeSciences/ Kreutzwaldi 1/ 51014/ Tartu/ Estonia
E-mail: ristorai@gmail.com

Pollen and nectar of bee for age plants are often contaminated by low doses of pesticides used in agriculture. Tebuconazole is an extensively used fungicide in modern fungal treatments and considered as quite safe to bees. The insecticide tau-fluvalinate is allowed to use on flowering crops. In addition to field application, beekeepers use tau-fluvalinate as an active ingredient of acaricide Apistan against varroa mites. Tau-fluvalinateis a synthetic pyrethroid, which attacks insect nervous system, which in turn playsa key role in controlling automatic functions in anorganism. Despite the relatively low toxicity of tau-fluvalinate on honeybees, Thompson &Wilkins (2003) showed the increasing toxicity of pyrethroids on honeybees when used in interaction with fungicides. Tebuconazoleis EBI-type fungicide which hinders detoxification processes in bees The aim of this study is to investigate the effect and co-effect of sublethal doses of tau-fluvalinate and tebuconazole and their mixture on honeybee physiology and longevity.

Queenless honeybee micro-colonies with 30 foragers were constantly supplied with mixture of honeybee gathered pollen and sugarsyrup. In treatment variants 0.15 µg tau-fluvalinateor 4.12 µg tebuconazole or their mixture per 10 g of the food were added. The micro-colonieswerekept at temperature 30°C and RH 60% in dark. In physiology experiment the metabolic rate and water loss rate were measure dusing flow through respirometry.The two pesticide interaction cause dsynergy, which decrease bees metabolic rate and longevity significantly

Genomics

Identifying the genetic basis of *Apis melifera* drone resistance to *Varroa*

Conlon, Ben.H., Moritz, R.F.A. and Routtu, J.
Molecular Ecology, Institute of Biology/Zoology, Martin-Luther-University Halle-Wittenberg
Hoher Weg 406099 Halle an der Saale, Germany
E- mail: bconlon91@gmail.com

In the relatively short time since *Varroa destructor*'s host switch to *Apis melifera*, it has gone on to devastate honey bee populations globally. The strength of this selective pressure has aided the evolution of *Varroa*-resistance in several natural honey bee populations. The speed with which resistance traits have evolved provides an exciting opportunity to study the evolution of host resistance to a novel parasite. With *Varroa* preferentially infecting drone brood, drone-resistance to *Varroa* can be an effective resistance-trait which appears to have evolved independently in several honey bee populations. We seek to utilise nextgeneration sequencing tools to explore the evolution of drone resistance in *Varroa*-resistant honey bee populations. We will use the next-generation reduced library sequencing method RESTseq which we can tune to suit the required restriction site frequency. In honey bees, ~20,000 fragments is expected to provide enough SNPs to detect patterns of selection. The high recombination rate in the honey bee genome means we anticipate a high resolution when identifying candidate areas. We explore the evolution of parasite-resistance genes using next-generation sequencing methods to identify genes associated with the droneresistance to *Varroa*. The results will be important in understanding the evolution of host defences to a novel parasite as well as in the introduction of *Varroa*-resistant traits to honey bee lineages.

Apis mellifera behaviours identification through brain gene expression

Nicola Fiini and Mario Lauria
Department of Mathematics, University of Trento, via Sommarive 14, 38123 Povo (TN), Italy
E-mail: nicola.fiini@studenti.unitn.it

Apis mellifera shows a wide range of individual and social behaviours. Using some data analysis techniques on a public dataset of honey bees brain gene expression, we improved the original authors accuracy in determining the differences in brain expression between four groups of honey bees (Nest-Recruit, Nest-Scout, Food- Recruit, Food-Scout). Our results show clear difference in honey bees brain gene expression according to the role played at the moment of the study. Even if this separation is evident only on high dimensional spaces, we identified the most statistically significant sequences that are associated with those behaviours.

Clock genes are differentially expressed during the metamorphosis and adult development of honeybees

Fabiano Carlos Pinto de Abreu, Camilla Valente Pires, Vera Lucia Castelo Figueiredo and Zilá Luz Paulino Simões
Faculdade de Medicina de Ribeirão Preto, University of Sao Paulo,FMRP-USP, Ribeirão Preto, SP – Brazil
E-mail: fabiano.biotecnologia@gmail.com

The circadian clock is a conserved system which plays an important role in synchronizing several physiological processes along 24h. In honeybees, the genetic and molecular mechanisms involved in generation of circadian rhythms have been described recently, but not in full details. It`s believed that the circadian clock acts in different ways to modulate honeybee`s development. Here we conducted an expression analysis of the clock genes *period (per), cryptochrome2 (cry2), timeout2 (tim2), clock (clk), cycle (cyc), par domain protein 1 (pdp1)* and *vrille (vri)* using quantitative PCR (qRT-PCR) to evaluate their daily expression patterns along the adult development of *Apis mellifera* reared in a *single cohort colony* and during the metamorphosis. Our results showed that all clock genes are expressed during the metamorphic events, with higher transcriptional levels between the stages of pink-eyed and brown-eyed pupae. In adult workers, young bees (3 and 7

days-old) showed absence or attenuated circadian oscillation for the clock genes *per*, *cry2*, *clk* and *cyc*, which may reflect the nurse activities. Older bees showed a synchronized and strong circadian oscillation between *per* and *cry2* (15 and 25 days-old), as well between *clk* and *cyc* (25 days-old), which may correspond to the intense activity of foragers. Interestingly we observed a strong circadian oscillation for *pdp1* and *vri* in 3-days old and 25 days-old bees. Taken together, our results suggest that the clock genes play important roles in coordinating the metamorphosis and modulating the social synchronization of honeybees and task-related activities. Financial support: FAPESP: 2011/03171-5; FAPESP: 2014/14194-4.

Patrilineal contributions to Varroa sensitive higiene behaviour in the honeybee *Apis mellifera*

David Wragg, Fanny Mondet, Benjamin Basso, Yves Le Conte, Alison R. Mercer and Alain Vignal
GenPhySE, Université de Toulouse, INRA, INPT, INP-ENVT, Castanet Tolosan, France
E-mail: david.wragg@toulouse.inra.fr

In the honeybee *Apismellifera*, hygienic behaviour acts as a defence at the colony level against various brood pathogens, including the parasite *Varroa destructor*. Varroa-sensitive hygiene (VSH) involves two main behaviours, firstly detection and secondly removal of parasitised brood. Some success has been met with regards to colony-level selection for hygienic behaviour and VSH, and it has been demonstrated that by including intracolony selection further improvements to breeding programmes can be made. Queens typically mate with around 12 males, thus a colony consists of multiple distinct patrilines. Detailed patrilineal knowledge, together with VSH phenotypic data for individual bees within a colony, in theory presents the possibility to trace associations with VSH behaviour to a given patriline, assuming the latter can be identified in the first instance. Here we present preliminary results of an investigation into paternal sibship estimates and correlations to VSH behaviour using whole-genome sequence data of 60 worker bees sampled from a single colony. The phenotypes of individual bees were characterised through detailed behavioural observations supporting an earlier transcriptomics study. Our results demonstrate varying contributions of VSH from

the estimated paternal sibships, highlighting the potential to develop an effective selection programme to improve VSH in the honeybee.

Whole genome sequencing and biogeography of the *Varroa* mites

Maéva Angélique Techer and Alexander Mikheyev

Okinawa Institute of Sciences and Technology, Ecology and Evolution Mikheyev's unit,1919-1 Tancha, Onna-son, Kunigami-gun, 904-0495 Okinawa-ken, Japan

Email: maeva.techer@oist.jp, alexander.mikheyev@oist.jp

The domestication of the western honey bee *Apis mellifera* and globalization have facilitated the arrival and spread of new parasites and pathogens such as the Varroa mites. Since the ectoparasitic mite *Varroa destructor* made the host switch to the western honeybee, it has successfully spread almost worldwide and is considered as the most damaging pest to beekeeping and honeybee conservation. Concerns rise as the sister species *Varroa jacobsoni* has emerged as an additional threat to *Apis mellifera* populations after a recent jump from its original host the Asian honeybee (*Apis cerana*). However, major gaps in our knowledge about the host switch processes and world demography history of the *Varroa* pests remain.

Our project aims to i) identify genetic mechanisms associated with the host switches by the mites, ii) determine what genes were under selection after the host switch and iii) reconstruct the pattern and demography of *V. destructor* global spread. To understand what drove these successful host switches we need to quantify the structure of the parasite genomes and to identify which genes have evolved due to recent selection. For that, we plan to sequence whole genomes of both *V. destructor* and *V. jacobsoni* from native and introduced regions. Then, to reconstruct the demographic history of *V. destructor*, the genetic diversity and structure of mite populations will be investigated using genomics. Worldwide sampling will be carried out to better understand the genetic connectivity between populations, and to identify potential source populations along with likely pathways to new invasion.

Behaviour

A preliminary study regardingthe crude protein level in wintering honeybees in colonies with different varroa infestation level.

Eliza Căuia[1], Adrian Siceanu[1], Teodora Colța[1], Roxana Spulber[1], Gabriela Oana Vișan[1] and Silvia Patruică[2]
Institute for Beekeeping Research and Development, Bucharest, 42 Ficusului Blvd, sector 1, code 013975, Romania.
E-mail:eliza.cauia@icdapicultura.ro

The development of bee colonies in spring highly depends on the quantity and quality of wintered bees which depends on many factors, the most important ones being the quantity and quality of bees at the begining of winter, the food supply and health condition. In other words, a succesful wintering means good quality of the bees generations produced in the last season which depends on the availability of good quality pollen, rich in protein. Some studies show that if the wintering bees have good protein storage in their bodies they will be more capable to over pass the winter period, being stronger and healtier. The minimal critic threes hold for the body crude protein was estimated by some authors at 20-25% of total weight (dry matter), under this level the amount of crude protein in the bee body could affect the activity of brood rearing but also the longevity of emerged bees. Another very important factor in the wintering succes and good early spring development is the health status which could be affected by different pathogens. One of these is Varroa (*Varroa destructor*) which threesold infestation level can negatively impact the colony survival. The present study approach an analysis of crude protein level in bees' body collected from colonies with different degree of varroa infestation level and different colonies strength, in August-September 2015.The study was carried out in the frame of national research program ADER 2015-2020, the contract 9.1.1./2015 and the EU research program FP7-KBBE.2013.1.3-02, grant 613960.

The effects of acute starvation of honey bee drone larvae on adult reproductive quality and wing asymmetry

Hajnalka Szentgyörgyi, Krystyna Czekońska and Adam Tofilski
Department of Pomology and Apiculture, University of Agriculture in Kraków, Al. 29. Listopada 54, 31-425, Kraków, Poland.
E-mail:hajnalka.szentgyorgyi@ogr.ur.krakow

Starvation during larval development in honey bees (*Apis mellifera* L.) is negatively effecting adult bees' body size, survival, immunocompetence, ovarian development or even transcription of certain genes . However, much less is known about the changes in the quality of drones starved during larval development.
In the present study we have verified how acute starvation affects body mass, amount of ejaculated semen and forewing asymmetry in drones. Acute larval starvation was achieved by separation of drone larvae of known age from workers with a mash for 10 hours during the 2nd or the 6th day of larval development. Starved drones were smaller than the control group, regardless of the age at starvation. Starvation at later phase of larval development caused more pronounced effects than at earlier phase. On the other hand, volume of ejaculated semen and forewing asymmetry were not affected by starvation. In smaller drones mass of abdomen constituted smaller proportion of total body mass than in larger drones. This suggests that there is a regulatory mechanism maintaining an optimal size of the organs in relation to body size.
This study was supported by the Polish National Science Centre (NCN) grant number UMO-2013/10/E/NZ9/00682 and MSHE grant number DS-3500.

Honey bee drones with a higher body mass live longer

Krystyna Czekońska, Hajnalka Szentgyörgyi and Adam Tofilski
Department of Pomology and Apiculture, University of Agriculture in Kraków, Al. 29. Listopada 54, 31-425, Kraków, Poland.
E-mail: k.czekonska@ogr.ur.krakow.pl

Honey bee drones live approximately one month, but some can live up to three months. Although body mass is an important factor effecting mating success in drones, its effect on drone life span has not been assessed, yet.
The present study body mass at emergence, life span and forewing asymmetry of honey bee drones were measured depending on maternal and/or fostering conditions. Drones were originating from two queens and were reared in their maternal colonies, next, before emergence they were removed and after emergence marked, measured and fostered further in three colonies which were either maternal or unrelated ones. Body mass of emerging drones differed between maternal colonies, however, life span did not. Fostering colonies effected significantly the life span of drones regardless of their origin (originating from the same colony or unrelated to the colony). Drones with larger body mass at emergence lived longer in all three fostering colonies.
Additionally, size and shape asymmetry of drone forewings were compared to life span in each fostering colony, but no correlation between life span and fore wing asymmetries were found except one colony, where - contrary to our expectations - drones with larger fore wing shape asymmetry lived longer.
This study was supported by the Polish National Science Centre (NCN) grant number UMO-2013/10/E/NZ9/00682 and MSHE grant number DS-3500.

Lack of partner preference system for incest avoidance in the bumble bee *Bombus terrestris*

Laura Bortolotti[1], Gherardo Bogo[1,2], Natasha de Manincor[2,3], Alessandro Fisogni[2]and Marta Galloni[2]
[1]CREA-API, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Unità di apicoltura e bachicoltura, Bologna, Italy
[2]Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, Bologna, Italy
[3]UMR 8198 - Evo-Eco-Paleo, Université de Lille, CNRS, F-59000, Lille, France.
E-mail: laura.bortolotti@crea.gov.it

Inbreeding is caused by the mating of closely related individuals and produces a decrease in the offspring fitness and deleterious consequences for adults. In haplodiploid social Hymenoptera inbreeding has a further negative effect because of their particular mechanism of sex determination (sl-CSD), that causes the emergence, in half of the founded colonies, of unviable or sterile diploid males. When these males are able to develop until adult stage, as in bumble bees, they represent a huge cost for the colony. With respect to these high inbreeding consequences, a selection for mechanisms of inbreeding avoidance would be expected in bumble bees. Social recognition is one of the most common and efficient system to avoid inbred mating in social insect, but it is poorly studied in bumble bees. In this study we investigated the mating choice between siblings or non-siblings in queens and males of *Bombus terrestris* reared in laboratory. To investigate the role of mating behaviour in mating choice, the tests were performed both in cage and in tunnel. As opposed to what would be expected, we found that *B. terrestris* males and gynes do not show a mating preference for non-siblings compared to siblings (49.3% in non-siblings and 50.7% in siblings) and the mating latency was even shorter for sibling matings than for non-siblings ones.

The Influence of Dopamine on fighting behavior of Honey bee virgin queens

Sayed Ibrahim Farkhary[1, 3], Ken Sasaki [2] , Satoshi Koyama [3]and Toshiyuki Satoh[3]

1- Applied Veterinary Science, United Graduate School of Veterinary Science, Gifu University, Gifu , Japan
2- Department of Bioresource Science, Tamagawa University, Tokyo , Japan
3- Laboratory of Ethology, Department of Veterinary medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo , Japan

E-mail: tsatoh@cc.tuat.ac.jp

The biogenic amine dopamine (DA) has physiological and behavioral effects in honey bees. It is already knownthat DA increases the locomotoractivity of honey bee virgin queens, however the effects of DA on other behaviors are still unknown. In the present study we determined the effect of DA on fighting behavior of honey bee virgin queens. We injected two concentrations(10^{-2} M and 10^{-3} M) of DA-antagonist(ciz-flupenthixol) into the abdomen of honey bee virgin queens and measured their fight initiation and duration times, and observed the result of the fight(wining / losing) in a fighting arena. The effect ofDA- antagonist on fight initiation and duration time wasn't significant between 10^{-2} M vs saline and 10^{-3} M vs saline injected queens. All 10^{-3} M DA- antagonist vs saline injected virgin queens lost the fight; on the other hand,most of 10^{-2} M DA- antagonist vs saline injected queens won the fight. These results suggest DA-antagonist (ciz-flupenthixol)at low level decreases the fighting ability, however, at high level increases the fighting ability. In future, to get clearer results, we will analyze the effect of DA-agonist on fighting behavior and also we will measure the effect of fighting on brain DA-receptors in honey bee virgin queens.

Balling of honeybee queens after returning from mating flights

Dariusz Gerula, Beata Panasiuk, Paweł Wegrzynowicz and Małgorzata Bienkowska
Research Institute of Horticulture, Apiculture Division, Kazimierska 2, 24-100 Pulawy, Poland
E-mail: dariusz.gerula@inhort.pl

Actually, there is no logical explanation for why worker bees ball queens returning from mating flights although they previously were accepted in the colonies. these queens Very often die due to injuries and stings.
The aim of the study was to clarify the reasons for balling bee queens returning from mating flights, determine the scale of this phenomenon and determine its significance in queens rearing.
Total number of 269 queens of different subspecies were observed in spring and summer (Carniolan, Caucasian and Black bee). The queens of each race were introduced into mating colonies with workers belonging to the same race as the queen, or into mating colonies with bees belonging to other subspecies.
Bees balled more than 15% of queens. Losses of balled queens were at similar rate as losses caused by other factors. Taking into account all reasons, including those undefined, total losses reached 30.8% of introduced queens.
Worker bees were balling queens of the same race with similar frequency as the queens belonging to the other races. There was not differences in the frequency of damaging the queens by bees among observed races. The most frequent balling was found in case of Black bee queens, slightly less Caucasian and Carniolan. No correlation was found between the age and physiological status of workers in mating colonies and tendency to damages. Spring bee workers were observed to injury queens twice more frequent than mid-summer bees. Balling the queens followed both, returning after mating with drones and returning after orientation flights. However, significantly higher rate of copulation flights ended with balling comparing to observation flights of queens.

Floral resources for honey bees: the application for agro-ecosystems in Asia, particularly in Thailand

Rod-im Preecha, and Duangphakdee O.
King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand
E- mail: preecha.rod@kmutt.ac.th

The honeybees play a major role as a key pollinator in ecosystem. Recently, honey bee populations have significantly declined. These losses include direct economic effect to beekeeping industry as well as economic plants such as fruit, vegetable, forage seed, oil seed and biofuel crops. The poverty of nectar and pollen resources has been considered as one of major cause for honeybees losses. We review the potential approaches to provide and maintain floral resources for honey bees in cultivation area. The management of non-cropped farmlands which are fallow areas, field margins, and conservation buffer strips to benefit honey bee health and enhance beekeeping activities was also described. For plant species selection and farm practices, bee flora was categorized as major, medium and minor sources of pollen and/or nectar and/or honey dew. A total of 1,565 plant species were identified as main bee flora in the studied area of Iran, India, Nepal and Thailand. Two hundred thirty three species of bee flora were recorded in Thailand, among them, 36 species were horticultural plants, 42 species were crop plants, 45 species were ornamental plants and 114 species were wild plants. Study has shown that in Asian ecosystem is rich in bee flora and has great potential for beekeeping as many plant species are available and bloomed even in dearth periods.

Qualitative variation of bee pollen nutritional components during the seasons.

Roxana Spulber, Eliza Căuia, Adrian Siceanu, Nicoleta Ion and Teodora Colţa
Institute for Beekeeping Research and Development, Bucharest, 42 Ficusului Blvd, sector 1, code 013975, Romania.
E-mail: spulberroxana@yahoo.com

Pollen represents the only natural source of protein for honey bees. The optimal development of these social insects is highly dependent on the ingested protein quantity which depends on the pollen quality. The essential nutrients of bee pollen vary strongly among botanical origin of bee pollen. In addition, over the seasons, the floral resources of an area are representative and in many cases, available in a low diversity. In this conditions, the bee pollen also may fluctuate according to the seasons with a potential impact on bee health. We proposed to quantified the main quality parameters (sugars, proteins, lipids, ash) and mineral content of bee pollen to evaluate a possible variability in bee pollen quality during the active season. We examined a number of nine samples of bee pollen collected in spring, summer and autumn. The results obtained had an average of 43.04 % sugar, 21.73% crude proteins, 4.98% lipids and 2.11% ash during the spring, while in summer, the levels of quality parameters investigated had an average of 30.13%, 14.53%, 2.03% and respectively 1.46%. High levels of elements such K, Fe, Ca, Zn and Mg were detected in pollen samples harvested in spring. The nutritional components in the examined samples through the season indicated highest values for all parameters in spring and lowest values in summer. The study was carried out in the frame of national research program ADER 2015-2020, the contract 9.1.1./2015.

Analysis of crude protein and lipids in honeybees in the optimal reproduction period of the year.

Teodora Colta, Eliza Căuia, Adrian Siceanu and Roxana Spulber.
Institute for Beekeeping Research and Development, Bucharest, 42 Ficusului Blvd, sector 1, code 013975, Romania.
E-mail:colta_teodora@yahoo.com

Pollen is an essential source of protein and lipid for normal physiological development of bees. Reproduction, development and productivity of bee colonies depend on the quality of pollen used in their nutrition. At the level of bee colonies, the different nutritional value of pollen is reflected in the production capacity of royal jelly by nurse bees. Also, the variability of the quality of beepollen can influence resistance to various diseases. The aim of this study was to analyze the content of proteins and lipids from nurse and worker bees belonging to different size bee colonies, during April, May and June as indicator of the strength and performance of bee colonies. Our results allowed the evaluation of these parameters and their importance in the reproductive period of bees to identify nutritional requirements of bee colonies according to their vital processes.The study was carried out in the frame of national research program ADER 2015-2020, the contract 9.1.1./2015.

Hygenic behaviour in honeybee: a composition of two assays

Elena Facchini, Emanuela Arrigoni, Flavia Pizzi, Francesca Dell'Orco, Michele Mortarino, Giulietta Minozzi, Giulio Pagnacco and Rita Rizzi
Address of first author: Dipartimento di Medicina Veterinaria - Università degli Studi di Milano - via G. Celoria 10 – 20133 - Milano.
E- mail: elena.facchini@unimi.it

The Western honeybee represents a relevant productive livestock due to both hive products and to its role as commercial pollinator of many agricultural crops. In the honeybee, hygienic behaviour (HB) is a heritable phenotype that confers to the colony resistance to foulbrood, chalkbrood, and to the parasitic mite *Varroa destructor*. Nurse bees manifesting HB are able to detect, uncap, and remove infested and/or parasitized pupae from the colony. The genetic and

biochemical factors that drive the manifestation of this behaviour are under investigation. Therefore, the selection of such trait still relies on field assays. Heretofore, there are two main tests to measure HB: Pin Killed Brood (PKB) test and Freeze Killed Brood (FKB) test. Concerning FKB test, a comparison between the standard FKB test and a new variant (FKB*) of this method has been performed in order to optimize the methodology in terms of time, costs, feasibility and safety for the operator and to choose the best option for large scale phenotypic characterization. The tests were carried out at regular intervals on a cohort of unselected and unrelated colonies in the apiary of the University of Milano located in Lodi, Northern Italy. Despite the limited number of observation, the analysis shows a good correlation (r = 0.65) between FKB and FKB* and an encouraging repeatability (75%) of FKB* test. These preliminary results suggest the opportunity of further investigation even for a better standardization of FKB* methodology.

Effect of chelatic mineral feed additives on bee colony development

Oleksiy M. Losyev [1] and Robert Chlebo [2]

1 National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony st., 15, Kyiv - 03041, Ukraine

2 Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

E-mail: robert.chlebo@uniag.sk

Positive impact of biogenic metals on bees in feed was experimentally demonstrated, depending not only on the amount of micronutrients in the diet, but also on the chemical structure of compounds from which these minerals forms complexes. Bees were fed by sugar candy with mineral additives in chelate and sulphate forms. Bees in most cases prefer mineral supplements in the form of chelates. The use of additives with the highest mineral content (cobalt - 0.10 mg; zinc - 50.0 mg; copper - 21.4 mg; iron - 97.5 mg and manganese - 40.0 mg per 1 kg of feed) animates the activity of bees to collect food sources. The use of mineral supplements in the form of chelate compounds extends the life of bees to 12-16 % compared to the method of application in sulphate form.

Assessment of heavy metal pollution in Córdoba (Spain) by biomonitoring foraging honeybee.

Miriam Gutiérrez, Rafael Molero, Miquel Gaju, Josef van der Steen, Claudio Porrini and José Antonio Ruiz.
Font del Riego, 38. 14009 Córdoba.
E-mail: jaruiz65@gmail.com

Due to features that make them outstanding environmental bioindicator, colonies of *Apis mellifera* are being used to study environmental pollution. The primary objective of this research was to use honeybee colonies to identify heavy metals and determine their utility for environmental management. Five stations each with two *A. mellifera* hives were strategically located in urban, industrial, agricultural and forested areas within the municipality of Córdoba (Spain), and foraging bees were collected from April to December in 2007, 2009 and 2010 to analyse spatial and temporal variation in Pb, Cr, Ni and Cd pollution. Metal concentrations, in milligram per kilogram of honeybee, were determined by inductively coupled plasma-atomic emission spectrometry and graphite furnace atomic absorption spectrophotometry. Significant differences in concentrations were found among the various locations and periods. The highest number of values exceeding the upper reference thresholds proposed for this study (Pb, 0.7 mg/kg; Cr, 0.12 mg/kg; Ni, 0.3 mg/kg; and Cd, 0.1 mg/kg) was observed for Pb and Cr (6.25 % respectively), station S4 (13.22 %), year 2007 (20.83 %) and in months of May and July (11.90 % each). Regarding the Cd, which was analysed only in 2010, the highest number of values exceeding the upper reference thresholds was 40 %. Biomonitoring with colonies of *A. mellifera* could contribute to improved surveillance and control systems for atmospheric pollution by integrating qualitative and quantitative assessments, thus facilitating prevention and readiness in the event of environmental crises.

The difference in the morphometric parameters of some honeybee (*Apis mellifera*) breeding lines from Serbia and Montenegro

Rašić Slađan, Mladenović Mića, Hatjina Fani, Bouga Maria, Đedović Radica, Charistos Leonidas and Stanisavljević Ljubiša
Educons University - Faculty of Ecological Agriculture, Vojvode Putnika 87, 21208 Sremska Kamenica, Serbia
E-mail: rasic.sladjan@gmail.com

The main scientific objective of this study was to investigate the morphological differences between geographically distant, selected honeybee lines from Serbia and Montenegro.
These lines are taken from the Centre for the of selection queen bees in Vrsac, and Vranje (Serbia) and Bijelo Polje and Sutomore (Montenegro). Lines from Bijelo Polje and Vranje are grown in mountainous conditions. The line from Sutomore has been selected in terms of coastal climate, a line from Vrsac belonged flatland region. Per colony was taken 15 worker bees (150 per line) and performed analyses by classical morphometry (12 measures on the left forewing and hindwing, and left leg) and geometric morphometry on the right wings in two repetitions.
Testing differences between the lines were performed by analysis of variance (ANOVA).
ANOVA has established the significance of differences between individual measured parameters on the wings, leg and language bees. Comparing all, covered by measuring the parameters were significant differences between the studied years on the following morphometric parameters:

- highly significant differences ($p < 0.01$) were: the width of the forewings, the length of the tongue, basitarsus width, length of the tibia, length and width of the hindwing.
- no significant difference in the length of the forewings, basitarsus length, the length of the femur and the cubital index.

The effect of bee food supplementation with selected bacterial isolates and commercial probiotic product for development and condition of honeybee workers

Beata Panasiuk, Małgorzata Bieńkowska, Dariusz Gerula, Paweł Węgrzynowicz, Urszula Smolińska and Michał Oskiera
Research Institute of Horticulture, Apiculture Division, Department of Bee Breeding, Kazimierska 2, 24-100 Puławy, Poland
E-mail: beata.panasiuk@inhort.pl

Observed bee colony losses or their weakening during the beekeeping season may be caused by periodic food shortage, diseases, parasites or pesticide poisoning. These factors, influencing individual bees, disrupt the balance in their bodies and whole colonies. Beekeepers, trying to prevent adverse environmental factors, supplement bee food with plant extracts or probiotic preparations.
The aim of the study was to evaluate the effect of food supplementation with isolates of bacteria selected from the bee bread and the effect of addition of commercial probiotic preparation on food consumption, development of hypopharyngeal glands and body fat in bees in laboratory tests.
The study was performed three times during the summer 2015. 1-day old worker bees were introduced into cages and kept in incubator (temperature 31°C; relative humidity about 70%) and fed with honey-pollen-sugar or pure honey-sugar candy and sugar syrup supplemented with: *Bacillus* sp., *Lactobacillus* sp., commercial probiotic product or pure sugar syrup.
It was stated that, regardless of the feeding group, the smallest food consumption was in the first period of the study. At the same time the highest intake of both, sugar syrup and pollen-sugar candy, was recorded in the groups where the bees received a commercial product. Preliminary analysis of the hypopharyngeal glands development in bees showed the weakest growth in the group where bees did not receive pollen in their food. The greatest wax production was observed in the first period of the study.

The regulation of circadian rhythms in honeybee (*apis mellifera*) and bumblebee (*Bombus terrestris*) nurses: a possible mechanism for improved brood care and social immunity

Moshe Nagari and Guy Bloch
The Department of Evolution, Ecology and Behavior
The A. Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel
E-mail: mukinagari@gmail.com

Division of labor between workers is crucial for the success of social pollinators such as honeybees and bumblebees. Two key tasks are foraging for pollen and nectar from flowering plants, and brood caring ("nursing") inside the dark and thermoregulated nest. In honeybees, there is evidence that some nurses are also involved in hygienic behavior which is important for social immunity. Nurses and foragers differ in many behavioral and physiological traits, including their activity rhythms. Nurses care for the brood around the clock with no circadian rhythms whereas foragers have strong circadian rhythms that are needed for sun-compass navigation and timing visits to flowers. This form of behavioral plasticity is influenced by the circadian (daily) clock, and is modulated by direct contact with larvae. We tested the hypothesis that around-the-clock activity in nurses is also induced by older brood stages that do not require feeding (pupae and pre-pupae). We developed protocols for studying brood-tending in isolated nurses. We found that isolated honeybee nurses tended, and manipulated, the wax sealing of post-feeding brood stages. Isolated honeybee nurses showed typical brood-cell capping behavior, and uncapping of sealed brood cells which is typical of hygienic behavior. The isolated bumblebee nurses tended pupae, and showed increased wax-pot building compared to broodless controls. In both species, post-feeding brood induced around-the-clock activity with attenuated circadian rhythms. In bumblebees this was also associated with reduced sleep duration. Evidence from both species supports the involvement of brood pheromones in affecting the nurses' sleep and circadian rhythms. Our findings show that around-the-clock activity can be induced by factors other than the need to feed larvae. It may serve additional functions, such as better thermoregulation, hygienic behavior, parasite resistance, and thus may contribute to both improved brood rearing and enhanced social immunity.

Population Genetics

Genetic structure of Iberian Populations of *Bombus lucorum* (Hymenoptera: Apidae) using mitochondrial COI sequences and microsatellite loci

Nuria Blasco, D. Cejas, C. Ornosa and P. de la Rúa.
Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, 30100 Murcia, Spain.
E-mail: nuria.blasco@um.es

Bombus lucorum (Linnaeus, 1761) is a palearctic species that reaches from the south of Europe to the coasts of the Barents sea to the north. Its great similarity to *Bombusmagnus* (Vogt, 1911) and *Bombus cryptarum* (Fabricius, 1775) leads to it being included in the LCM complex to refer to all three species simultaneously. Molecular studies conducted to facilitate identification of specimens to the species level have revealed a remarkable mitochondrial cytochrome oxidase subunit 1 fragment haplotype diversity in *B. lucorum* compared to that shown by other species of *Bombus*. Despite these findings, subspecies *B. lucorum congruens* (Krüger 1951), found in Iberia and southern France, has not yet been genetically characterized. This paper had the aim of characterizing the Iberian *B. lucorum* populations through the use of mitochondrial cytochrome oxidase subunit 1 fragment and microsatellite markers. The results obtained with the mitochondrial COI suggest a separation between central Iberian populations and those from the north of Iberia and the rest of Europe. However, the analysis with 11 microsatellite markers pointed towards genetic homogeneity for all the Iberian populations encompassed by this study. Therefore, although there could have been events of population differentiation in the past, it seems that there is currently genetic exchange underway between them.

Geometric morphometrics of wings of drones and workers supporta process of secondary contact inthe Iberian honey bee (*Apis mellifera iberiensis*)

Julio Chávez-Galarza, Juliana S. Galaschi-Teixeira,Cátia Neves, Dora Henriques, Tiago M. Francoy and M. Alice Pinto
Mountain Research Centre(CIMO), Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.
E-mail:jchavez@ipb.pt

A recent survey of the Iberian honeybee genetic patterns usingconcurrently mtDNA and nuclear (SNPs) markers revealed the presence of a concordant southwestern-northeastern cline in Iberia,supportinga post-glacial secondary contact process. Here, we followed up those findings with geometric morphometrics of wings. The aim was two-fold:(1) evaluating the effectiveness of this approachin capturing the clinal pattern and (2) comparing the effectiveness of drones and workers in detecting the clinal pattern. To that end, we useda fine-scale sampleof 711 colonies taken across three Iberian north-south transects. For each colony, we recorded the geographical coordinates and collected the right forewing of fiveworkers and fivedrones. We plotted19 landmarks in the forewing venation of over 7100 individuals, and after the Procrustes alignment, the distances between landmarks were calculated. The distance matrix was used to infer population structure by applying a spatial multivariate analysis based on principal component analysis and Moran's autocorrelation. A correlation analysis was performed between the first spatial component of drones and workers witha SNP dataset. Interestingly, the spatial patterns inferred from the wings of both workers and drones, which were greatlyconcordant, displayed the presence of two clusterswith a spatial distribution overlapping with mtDNA and SNP data. Our findings suggest that geometric morphometrics is able to detect the signature of complex evolutionary processes. CN is funded through the 2013-2014 BiodivERsA/FACCE-JPI Joint call for research proposals, with the national funders FCT (Portugal), "CNRS" (France), and"MEC" (Spain).

Do morphology patterns correlate genetic ones within the insular honeybee populations *Apis melllifera* ssp. from the South Western Indian Ocean islands?

Julien Galataud, Hélène Delatte, Maéva Techer, Pascal Rousse, Anaïs Damour, Lakshmi Sujeen and Johanna Clémencet
UMR PVBMT – Reunion University – CIRAD, Université de La Réunion , 15 Av. R. Cassin - CS 92003 97744 SAINT-DENIS CEDEX 9, Réunion – FRANCE
E mail: : johanna.clemencet@univ-reunion.fr

Honeybees are present on all islands of the South Western Indian Ocean (SWIO), including Madagascar and three surrounding archipelagos (the Comoros, Seychelles and Mascarenes). Recent genetic studies using mitochondrial DNA described those insular populations as belonging to one African sub-group private to the SWIO. Results also revealed recent human importation of European subspecies in the Mascarene Islands (Mauritius, Rodrigues, La Réunion) for beekeeping. Further studies based on nuclear DNA highlighted significant genetic differentiations among insular populations from this African sub-group, suggesting ancient colonization events.
In order to better understand the processes of population divergence assessed by molecular studies, we investigated potential divergences of morphological traits. To do so, a large dataset including 1724 workers (genetically characterized) coming from Madagascar, the three archipelagos and some continental outgroups from Europe and Africa has been used. Morphometric analyses based on forewing size and geometric shapes were carried out to discriminate populations by using between-group PCA and LDA. We also tested whether the genetic diversity and structure of those insular populations were correlated with wing shape differences.
Results suggest that wing shape patterns corroborate genetic ones. They also highlighted current hybridization in Mauritius where European subspecies have been recently imported. Given the potential major role of *A. melllifera* ssp. as pollinator in a context of high biodiversity, results are discussed in evolutionary terms considering the relative parts of drift, selection and local adaption to explain those patterns of divergence.

Validation of microsatellite amplification in different species of bumblebee (genus *Bombus* Latreille, 1802).

D. Cejas, C. Ornosa, P. De la Rúa and I. Muñoz.
Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, 30100 Murcia, Spain.
E-mail: diegomanuel.cejas@um.es

Microsatellites or short tandem repeats (STR) are very useful to determine different parameters of evolutionary and ecological interest, like migration rates, bottlenecks, or population isolation coefficients. In the case of bumblebees (genus *Bombus*Latreille, 1802) STRs are used to analyze their biodiversity in order to conserve their populations. In this study, eleven loci of STR designed originally from the genome of *Bombus terrestris* (Linnaeus, 1758) (B10, B100, B11, B124, B126, B96, B118, B119, B121, B131 and B132), amplified in two multiple reactions, were evaluated in other ten species of the genus *Bombus*. The parameters analyzed were the quality of each locus (Q), the variation range of the allele size (R), the number of detected alleles (Na), the existence of null alleles and the presence of privative alleles in each species. Eight loci amplified in every species with acceptable values of Q, R and Na; however, six of them did not show allelic variability in some species. Amplification of loci B10, B96 and B118 was negative, or with a low value of Q, in the majority of species. A homocigosis excess was found in two loci for *B. humilis* (Illiger, 1806), five loci for *B. lucorum (*Linnaeus, 1761*)*, six loci for *B. lapidarius* (Linnaeus, 1758), and in seven loci for *B ruderarius* (Müller, 1776) which might be due to the existence of null alleles. Ten loci presented privative alleles, which might be useful to differentiate the species studied. These results are discussed in the context of conservation of the bumblebee species.

Novel flow cytometry assay for evaluation of sperm viability in honey bees.

Filipp Savvulidi, Karina Vargova and Dalibor Titera.
Charles University in Prague, First Faculty of Medicine, Institute of Pathological Physiology, Flow cytometry and Image analysis laboratory, U Nemocnice 5, 12853, Prague, Czech Republic.
E-mail: immunophi@gmail.com

Assessment of semen quality (sperm viability) is an essential for the efficient honeybee semen storage. The motility of spermatozoa is not the ultimate parameter of sperm viability. Instead, the viability of spermatozoa is assessed by testing their structural or functional plasma membrane integrity. These assays are based primarily on the technique of microscopy evaluation of either fluorescent DNA dyes or hypo-osmotic "tail-coiling" reaction of spermatozoa. However, the technique of microscopy evaluation is time-consuming, subjective and often is not accurate. On the other hand, the technique of flow cytometry is rapid, objective and precise. Nowadays, this technique is affordable for many institutions, as the price of "benchtop" flow cytometer is relatively low. To best of our knowledge, there is currently no flow cytometry assay available for evaluation of functional integrity of honeybee spermatozoa. Here we report the establishing of novel flow cytometry assay for simultaneous evaluation of structural and functional plasma membrane integrity. DRAQ7, the new fluorescent DNA dye was used to test the structural integrity of plasma membranes. The hypo-osmotic "tail-coiling" reaction was used for flow cytometric assessment of functional integrity of spermatozoa plasma membranes. The combination of both is a valuable tool for rapid and precise evaluation of sperm viability in honey bees.

Morphometric and genetic variability of two Algerian subspecies of honey bee, *Apis mellifera intermissa* and *Apis mellifera sahariensis*

Halima Hami[1,2] and Marina Meixner[1]
[1)] LLH Bieneninstitut Kirchhain, Erlenstr. 9, 35274 Kirchhain, Germany
[2)] National Superior School Agronomy, ENSA Algiers, Algeria
E- mail: hamihalima@gmail.com

Several subspecies of *Apis mellifera* occur in North Africa (*A. m. lamarckii*, *A. m. intermissa*, *A. m. sahariensis* and *A. m. major*), but their distribution across the region is not yet completely understood and may still be considered controversial. In Algeria, two subspecies, *A. m. intermissa* and *A. m. sahariensis* are reported. *A. m. intermissa* (the Tellian bee) is distributed in the northern and central region of the country, and *A. m. sahariensis* (the Saharan bee) is restricted to the oases of southern Algeria. However, this distribution is becoming increasingly distorted due to frequent transhumance and commercial migration of colonies between the north and south, resulting in increasing hybridization of the Saharan bee. The purpose of this study is to provide a reliable characterization of the morphometric and genetic variability of the two Algerian subspecies to provide data for an effective strategy for conservation of the genetic heritage.

A total of 81 colonies were sampled from 34 localities throughout Algeria, including oasis locations in the south which have not been part of any previous collections. The samples are currently being subjected to standard morphometric analysis and analysis of mtDNA variation using using amplification of the tRNA ^{leu}-COX-2 fragment followed by digestion with *Dra*I. The morphometric data will be analyzed together with published reference data of adjacent subspecies and contribute to a comprehensive analysis of morphometric honey bee variation in North Africa. Microsatellite analysis of the samples will also be included and contribute to the estimation of introgression between the two subspecies in Algeria.

The analysis of diversity and variability of phenotypic and behavioural traits of Apis mellifera mellifera bees in Poland

Małgorzata Bieńkowska, Beata Panasiuk, Dariusz Gerula, Paweł Węgrzynowicz, Ewa Skwarek and Tomasz Białek
Research Institute of Horticulture, Apiculture Division, Department of Bee Breeding, Kazimierska 2, 24-100 Puławy, Poland
E - mail: malgorzata.bienkowska@inhort.pl

The subjects of the study were the colonies of natural population Apis mellifera mellifera bees kept in the conservation breeding areas in Poland. The study was conducted in 2015 in 29 apiaries with M Augustowska colonies and in 6 apiaries with M Kampinoska colonies. In total, 679 colonies were tested- 550 with M Augustowska bees and 129 with M Kampinoska bees. It was assumed that the typical Apis mellifera mellifera bees' coloration should be uniformly dark, but brownish sternits, tergits and legs and brighter hair are tolerated. It was found that in the studied populations' coloration of queens, workers and drones is compatible with the standard for the race, and the percentage of individuals uniformly dark is very high. The results show that the bees kept in the Augustow and Kampinos Forests permanently keep the features typical for the race. This is additionally confirmed by the high percentage of uniformly dark drones in both lines (99.1 and 93.8%, respectively). This ensures that populations bred in these regions maintain their original form. The behavior of bees on combs and the way of honey stores capping in studied populations is typical for Apis mellifera mellifera bees. M Augustowska bees move fast on combs and during colony inspections form small clusters at frame edges and store honey with dry cappings. M Kampinoska bees move fast on combs also and run off the combs during colony inspections, but do not form clusters. The bees cap stores with semi-wet cappings.

Unraveling the basis for honeybee functional genetic diversity using functional studies in Drosophila.

Martin R. Schmid, Andreas Wallberg and Matthew T. Webster
Department of Medical Biochemistry and Microbiology (IMBIM), Uppsala University, Uppsala, Sweden
E- mail: martin.schmid@imbim.uu.se

To expose the genetic basis for adaptation amongst worldwide honeybee (A. mellifera) populations, we have sampled and sequenced the genomes of individual worker bees collected in Africa and Europe. We identified several hundred single-nucleotide polymorphism (SNPs) that are highly differentiated between populations on these continents and therefore indicative of local adaptations. One of the SNPs predicted to cause the most drastic change on the amino acid level is located within the honeybee gene GB52073 coding for citrate synthase. Being the pacemaker in the first step of the citric acid cycle, this enzyme is highly conserved in evolution. The D. melanogaster homologue is known as knockdown (kdn), and flies carrying loss-of-function alleles display paralysis and seizure like activity after mechanical or electrical stimuli. In line with the idea that these fly mutants are energy impoverished, we showed that they are temperature sensitive, less active in an arena assay and have lower overall metabolic rates measured as CO2 production per weight and time. Despite being separated by 300 million years of evolution with the help of the UAS/GAL4 system it was possible to rescue all phenotypes of kdn null flies by making them express either the African or the European version of the bee gene coding for citrate synthase. Preliminary data also indicates that the behavior of these flies may differ depending on which of these constructs is expressed. If confirmed, this may help to explain why the gene carries a particular SNP allele in some bee populations but not in others.

New honey bee (apis mellifera l.) genotype in western Black Sea of Turkey

[1,2]Meral. Kekeçoğlu
[1]Düzce Üniversity, Faculty of Science, Department of Biology, 81620 Konuralp-Düzce, Turkey
[2]Düzce University Beekeeping Research Development and Application Centre 81620 Konuralp-Düzce, Turkey
E- mail: meralkekecoglu@duzce.edu.tr

Yığılca honeybee is different from other bee subspecies in the aspect of the length of their wings and legs, being resistant to bad weather conditions and also having the capacity of honey production three times more than other bee subspecies. A population of honey bees (Apis mellifera L.) with an annual colony brood cycle adapted to a locally abundant floral source in the Yıgılca district of western Black Sea of Turkey is the subject of genetic conservation efforts.

Present study used morphology, mitochondrial DNA and microsatellites to characterize Yigilca honey bee population and suggests further genetic conservation strategies. These methods yielded different degrees of discrimination of native and imported colonies and provided a powerful suite of tools for local resource managers. Colonies from the Yigilca could be differentiated from the out of the Yigilca populations using morphometric analysis, and from reference populations using mtDNA and microsatellites. Four morphological characters were identified by discriminant analysis as informative for delineating the Yigilca ecotype from other A. mellifera populations. Mitochondrial haplotypes for the population were characterized and One microsatellite loci were found to be informative in characterizing the Yigilca population.

Acknowledgements: This work, Project No. BAP 2010.05.01.03., was supported by Duzce University Scientific Research Projects Commission. The authors are grateful for support given by Duzce University Scientific Research Projects Commission.

Assessing species boundaries in Mesoamerican *Scaptotrigona* species (Apidae: Meliponini) using Bayesian multilocus species delimitation

Miguel Hurtado–Burillo, William de Jesús May–Itzá, José Javier G. Quezada–Eúan, Pilar De la Rúa and Carlos Ruiz
Departamento de Zoología y Antropología Física, Facultad de Veterinaria, Campus de Espinardo, Universidad de Murcia, Murcia, 30100, Spain
E-mail: pdelarua@um.es

An accurate and objective method of species delimitation is critically important to establish appropriateconservation strategies given the current biodiversity crisis,particularly in biodiversity hotspots as Mesoamerica. Previous morphological and molecular studies on three managed stingless bee species of the genus *Scaptotrigona*distributed in Mexico (*S. mexicana*, *S. pectoralis* and *S. hellwegeri*) suggested the existence of cryptic species complexes in both *S. mexicana*and *S. hellwegeri*. Using a Bayesian multilocuscoalescent-based approach, we test these putative cryptic species hypotheses by analysing sequence information of five markers (two mitochondrial: cox1 and 16S, and three nuclear: ITS1, EF1-α andArgK). Two different hypotheses were obtained using a Generalized Mixed Yule Coalescent (GMYC) model: four (cox1) and six (16S) species. After the species validation step with the Bayesian species delimitation analysis (BPP), we proposed a four species scenario for the genus in Mexico. We confirmed that *S. mexicana*is a complex of two species with different distribution (along the Pacific and the Atlantic coasts respectively). To avoid the colony exchange between geographic regions is recommended in order to conserve the genetic integrity of both taxa.

Characterization of the Slovak bee population using the mtDNA COI–COII intergenic region

Róbert Chlebo and Radoslav Židek
Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
E- mail: robert.chlebo@uniag.sk

Studies on the the COI-COII intergenic region was used to analyze population of Slovak bees. All 32 samples of mtDNA clearly demonstrate affiliation to haplotype C, which is typical for the population of Central and Eastern Europe. C2C haplotypes are dominant for the Slovak population of Apis mellifera carnica (40%), followed by C1a haplotype (34%). Less frequent are haplotypes C2E (6%), C2y (6%), and C2D (3%). Study supported by VEGA grant 1/0511/15.

Use of larvae transferring method for queen rearing in condition of Mongolia

Selenge Dooshin and Khaliunaa Tsevegmid
P.O.B 1091, Central Post Office, Ulaanbaatar, Mongolia
E-mail:khaliun2008@gmail.com

We have used several queen rearing methods for intensive developing needs of beekeeping Mongolia. Among them larvae transferring method was most interesting in condition of the country which has very short hot summer and long winter. The result showed that the method was effective and the weight both of queen and its ovaries were increased. Successful larval transfer, traits for survival queens, surrounding natural and environmental condition, queen productivity (laid eggs) and many other analyses were provided within the study

Behavior of the microsporidium *Nosema ceranae* in experimental infections: the effect of low temperature in the viability of different spore populations

Soledad Sagastume De Andrés[1], Raquel Martín Hernández [1,2], Nuno Henriques-Gil[3] and Mariano Higes Pascual[1]

[1] 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, Guadalajara, España.

[2] Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FEDER), Fundación Parque Científico y Tecnológico de Albacete, Albacete, Spain

[3] Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad CEU San Pablo, Campus de Montepríncipe, 28668 Madrid, Spain.

E-mail: solsagastume@gmail.com

The nosemosis type C is a disease closely related with the phenomenon of massive colony losses of honeybees worldwide, and it is produced by the microsporidium *Nosema ceranae*. Since its first detection in *A. mellifera*, a number of genetic studies were carried out focused in phylogenies, with incongruent results due to an unexpected high intraspecific variability and recombination. Recently, two genome projects were performed using new generation sequencing techniques (NGS), but both of them were not totally assembled. Genetic studies based in specific markers suggest that *N. ceranae* may undergo sexual reproduction, which would explain the high genetic variability, recombination events and the unclear phylogenies. Sex in *N. ceranae* would not only imply that genetically different cells may be formed in each cycle, but also supports the hypothesis that heterosis (outbreeding enhancement) contributes to the higher pathogeny of the microsporidium.

In this work we demonstrate the existence of genetically different spores that evolve differently in experimental infections. We used freezing acts to produce bottlenecks in *N. ceranae*. Interestingly, the variability increases when the infections occur at room temperature, and heterozygosis decline after freezing steps. These results shed light about why the infections are more virulent in warm regions than mainly cold ones. On the other hand, the existence of different cells within each isolate of *N. ceranae* explains recurrent difficulties for genome assembly and phylogenetic inferences after analyses based in isolates of millions of spores. Therefore a number of experimental designs based in genetic sequences should be carefully reconsidered.

Acknowledgements: We would like to thank the team of the Laboratorio de Patología Apícola del CIAPA-IRIAF. This work was supported by (INIA-FEDER): RTA2012-0076-C02-01 y RTA2013-00042-C10-06.

Slovenia – the Land of Quality Carniolan Honeybee Queens

Maja Ivana Smodiš Škerl, Janez Prešern, Eva Cukjati and Peter Podgoršek
Agricultural institute of Slovenia, Hacquetova ulica 17, 1001 Ljubljana
E- mail: maja.smodis.skerl@kis.si

Carniolan bee was firstly described and named in 1879 by August Pollman. The subspecies *Apis mellifera carnica* was sent to him from Carniola, the Central geographical region of Slovenia. Carnica is the second most spread honeybee subspecies in the world and the only one reared in our country. Agricultural institute of Slovenia established the Service for Carniolan Bee Selection Program in 1984 and is registered as the Second Breeding Organization for Carniolan Bee. The breeding activities include the basic selection in each apiary including regular examination of the colonies by the beekeepers, the selection in queen breeding stations according to Carniolan Bee Selection Program, which is under supervision of the Institute. Each year the potential breeding colonies are tested on racial characteristics, swarming tendency, gentleness, honey production and *Varroa* tolerance and round 150 samples of workers from colonies are examined on *Nosema* spores and the Cubital Index in measured. The sample of reared queens from each breeding station (round 700/year) undergoes the performance test. Round 30 beekeepers test the queens and evaluate the breeding values for the main characters. The professional service at the Institute helps the breeders to rank the potential breeder colonies in their selection apiaries for breeding material in the next year. Performance of effective selection and quality of the queens is reached through the queen rearing program and over 37,000 queens are yearly recorded in the Herd Book.

Present day characters of the honeybee Apis mellifera ruttneri, observations and implications

Sammut S, Zammit-Mangion M, Meixner M. and Mifsud D
University of Malta, Department Physiology and biochemistry
E-mail: mzam1@um.edu.mt

The honeybee Apis mellifera ruttneri is an endemic sub-species of the Maltese Islands (Sheppard et al., 1997), closely resembling its counterparts from Sicily (A.m. siciliana) and North Africa (A.m. intermissa). As with other island populations, A.m. ruttneri is threatened by the anthropogenic expropriation of its natural habitats and unregulated importations of honeybees. This paper investigates the morphological and genetic characters of local managed colonies in Malta in an attempt to determine whether A. m ruttneri is still extant. A tot al of 332 bees from 35 colonies were subjected to morphometric analysis and examined for 33 of the original 42 parameters described by Ruttner et al. in 1978. Concurrently, mitochondrial DNA from the abdomen and legs of specimens from 52 colonies were also extracted and the region between the tRNAleu gene and the second subunit of the cytochrome oxidase (COX-2) gene were amplified. The calculated means and standard deviations for each character were compared and compared using Discriminant Analysis (DA) with data for honeybees from the closest neighbouring countries. DA confirmed that the present-day characters of A.m. ruttneri, which are described in the paper, share features with A.m. siciliana and A.m. intermissa but, more importantly, retain the features described by Sheppard et al. in 1997. The investigation of the mitochondrial DNA shows that there were six haplotypes (A4, A8, A9, C1, C2 and M7). The implications of these results are then discussed.

Pollinator ecology

The effects of climate-induced phenological shift of apple trees on pollinators and apple yield

Ádám Kőrösi, Viktor Markó, Anikó Kovács-Hostyánszki, László Somay, Ákos Varga, Zoltán Elek, András Báldi, Virginie Boireux and Alexandra-Maria Klein
MTA-ELTE-MTM Ecology Research Group, Hungary
E-mail: korozott@gmail.com

One of the main consequences of climate change on ecological systems might be the potential mismatch between the phenology of plants and life-cycle of insects, delivering important ecosystem services. Our study aimed to investigate the potential effects of climate change on pollinator insects and the effectiveness of pollination in apple orchards. We manipulated flowering time of young apple trees by keeping them in either a greenhouse (earlier flowering) or a cooling house (delayed flowering). Trees were placed into apple orchards one week before flowering in five consecutive steps resulting in five treatments (2 greenhouse, 2 cooling treatments and one control). We sampled wild bees, honeybees and hoverflies on each tree twice. Compared to the controls trees kept in greenhouse advanced flowering with 6-9 days, while flowering of cool stored trees delayed 16-38 days. The earliest flowering trees were mostly visited by wild bees, while abundance of honeybees and hoverflies showed a clear increase during the season. Species composition of wild bee assemblages profoundly changed during the ~7 weeks. We found equal pollination-maturation success on advanced as on control trees, however total biomass of fruit was much higher on control trees. We suggest that high diversity of wild bees can ensure phenological synchrony due to complementarity among bee species' activity periods and to differential responses to warming climate. Honeybees may supersede wild bees in the case of a delayed flowering scenario. Wild bees, however, can have an outstanding importance in apple tree pollination in the case of advanced flowering.

Survey of short-rotation tillage willow biomass-plantation's expedience in bee farming

Balázs Kiss
Institute for Apiculture and Bee Biology, Research Centre for Farm Animal Gene Conservation (HáGK) H-2100 Gödöllő, Isaszegi út 200, Hungary
E-mail: kiss.balazs@hagk.hu

Pollen is the major source of *protein* for honey *bees*. The willow pussy pollen is one of the first pollen and nectar source for honey bees after the winter, which pollen is very important in the development of the colony. Short rotation willow plantations are able to supply high quality of honey and healthy bee populations.

It would be profitable at an economic and ecological optimum to notice the bees' demand in forestry, agriculture and urban development – plant species, varieties, technology, distribution and rotation of production area. Reconsideration is also a require in bee farming.

The aim of the survey was to promote the spread of ecological-reconsidered territory usage and rejuvenation of willow habitats. In procession of ecological-reconsidered territory usage short-rotation tillage willow biomass-plantations will spread at disadvantageous areas. Plough-lands which are less good for food production could be integrated to production at a diversified level by tecnology change. Sections of land in agricultural plots are unsuited for intensive grain production. Sections which were habitat of willow in the past - before the so called „green revolution" and area aid – could renew as bee pasture early in spring, instrument of rain water conservation, biomass plantation as renewable energy source, instrument of soil conservation, instrument of climate conservation.

A case of acute intoxication with carbofuran in honeybees

Cristina Ţoca and Daniela Nica
Institute For Diagnosis and Animal Health, Bucharest, Romania
E-mail: toca.cristina@idah.ro

Honeybees are vulnerable to many pesticides used to control damaging pest species by fruit, vegetable, seed growers.
At the begining of 2016 a beekeeper of an individual farm observed a high mortality of his colonies. He found an excesive number of dead bees in all the hives. To establish the cause of the death he called the veterinarian who decided to sent immediately bees samples for toxicological investigations.
Due to the rapid death of the bees chemical analysis were performed to identify pesticides (organophosphorates, neonicotinoides and piretroids) using a LC-MS screening method. The results were negative for the compounds mentioned above and positive for carbofuran.
The detection of carbofuran at low levels was achieved using an accurate mass confirmation on high resolution exactive benchtop LC-MS Orbitrap mass spectrometer.
This method is suited for routine analysis for the determination of pesticide residues in cases of suspected honeybee poisoning incidents.

Foraging behaviour of honey bees in different sunflower varieties

Mariano Higes Pascual[1], Amelia-Virginia González Porto[2], Carlos García-Villarubia Bernabé[3], Antonio López Pérez[3,4], Cristina Pardo Martín[2] and Raquel Martín-Hernández[1,4]

[1] Laboratorio de Patología Apícola. Centro Apícola/Centro Agrario de Marchamalo, IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Guadalajara, Spain[2] Laboratorio de Productos de la colmena. Centro Apícola/Centro Agrario de Marchamalo, IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Guadalajara, Spain.

[3] Departamento de Experimentación Agraria. Centro Apícola/Centro Agrario de Marchamalo, IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Guadalajara, Spain

1 Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FEDER), Fundación Parque Científico y Tecnológico de Castilla La Mancha, Albacete, Spain

E-mail: avgonzalez@externas.jccm.es

Sunflower is an interesting crop for beekeepers because its flowering ensures honey harvest in summertime. However, in the last decade, it has been speculated about its negative effect on bees and other pollinators due to seed coating with neonicotinoids.Previous observationscarried out by our working team, raised an alternative to the problems of hive decline/depopulation and a loss of honey yield when beehives are in sunflower fields reported by beekeepers. Such problems may be related to changes on foraging behavior influenced by sunflower varieties.In order to sustain this hypothesis, 8 plots were seeded with different sunflower varieties (Transol, PR65A40, Mowgli, LG5543CL), whose seeds were untreated.A representative number of beehives were placed close to each variety. Foraging behaviour along the ten blossom days in the different varieties was evaluated. Results showed differences between varieties and discontinuity on the foraging, mainly affected by climatic variations related to the average of daily relative humidity andspeed wind. LG5543CL and PR65A40 sunflowers were the most visited varieties when humidity and wind flow were lower, while Mowgli was the most visited with high humidity and wind flow. Transol variety wasn't visited by honey bees, despite weather conditions.The results obtained proved that sunflower considered by beekeepers as a refuge when scarcity of wild bee pastures, gives a short term resources due to its short flowering period as well as foraging is affected by climatological

conditions. These factors limit the honey and pollen yield obtained, which affect negatively the development of bee colonies

Acknowledgements

We thank to all the CAR staff and the RTA2013-00042-C10-06(INIA-FEDER) project for funding this research.

Ecological risk of pollination of loosestrife (Lysimachia vulgaris) by oligolectic bee species Macropis eupopaea

Khvir Victor
4, Nezhaleznasti av., 220030 Minsk (Belarus)
E-mail: khvirv@gmail.com

Lysimachia vulgaris is the ornamental and medicinal species and good honey plant which recommended for industrial and domestic use by the Ministry of Environmental Protection of Republic of Belarus. The loosestrife flowers visited by two species of solitary bees – *Macropis fulvipes* and *M. europaea* in Europe. We study the pollinator's community of loosestrife was in the vicinity of the ecological reserve "Grove" (Belarus, Minsk) in 2014–2015. Determination of insects conducted under a key provided by D. Michez and S. Patiny. We caught 73 specimens of pollinators belonging to a single species – M. europaea. This bee species was not discovered by us on other flowering plants growing in the vicinity of the venue of research. However, in the composition of the pollen cargo of 75% specimens we have found from 5.7% to 40% of pollen of other plants species primary Umbelliferae (Apiaceae). Thus, *Lysimachia vulgaris* pollinated by only bee species which, however, has alternative food sources. It reveals the vulnerability of pollination of this plant because if of M. europaea disappear, or else his final move to other plants, possibly complete cessation of cross-pollination between flowers of loosestrife. It will certainly have a negative effect down to full disappearance of loosestrife in a given locality.

Asymmetry, size and unusual venation in honey bees (*Apis mellifera*)

Sylwia Łopuch and Adam Tofilski
Department of Pomology and Apiculture, Agricultural University, 29 Listopada 54, 31-425 Krakow, Poland
E-mail: slopuch@ogr.ur.krakow.pl

Symmetry is common in nature, however, it is rarely perfect. It appears that asymmetry should increase along with deviation from the average phenotype. Therefore, the aim of this study was to compare the level of asymmetry between normal individuals and phenodeviants categorized as minor or major on the basis of abnormalities present in forewing venation of queens, workers and drones of honey bees. Asymmetry was assessed using both geometric and traditional morphometrics analyses. The proportion of phenodeviants in drones was higher than in workers and queens. In drones, shape fluctuating asymmetry (FA) was lower in normal individuals and minor phenodeviants compared with major phenodeviants, and the former two categories were comparable. In workers and queens, there were not significant differences in FA shape between categories. FA size was significantly lower in normal individuals compared with major phenodeviant drones and higher than in minor phenodeviant workers, whereas there were no significant differences between categories in queens. The correlation between FA shape and FA size was significantly positive in drones, whereas it was insignificant in workers and queens. Significant directional asymmetry (DA) was found in all castes. Moreover, normal individuals were significantly smaller than minor phenodeviant queens and drones, and they were comparable to major phenodeviants in all castes. Smaller individuals were more asymmetrical as the correlation between wing size and wing asymmetry was negative. Thus, the results of this study showed that minor phenodeviants were not always intermediate as it might have been expected.

This study was funded by the National Science Centre under grant UMO-2013/10/E/NZ9/00682.

Honeybees (*Apismellifera*) as bioindicators of heavy metal pollution

Nenad Zarić, Konstantin Ilijević, Ljubiša Stanisavljević and Ivan Gržetić
Inovation center Faculty of Tehnology & Metallurgy, Karnegijeva 4, 11120 Belgarde, Serbia
E-mail:nzaric@tmf.bg.ac.rs

Metals in the environment can be of natural or anthropogenic origin. Anthropogenic sources of pollution can be different: industry, traffic, urban development, intense agriculture, etc. One of the results of these processes is increasing metal concentrations in soil, water, air and other elements of the environment. Honeybees (*Apis mellifera*) are increasingly being used to determine metal concetrations in the environment. They are a good biological indicator because they can be used to monitor the level of soil, water, plant, and air pollution in areas of several square kimoleters.

The aim of this study was to compare metal concentrations between regions characterized by different environmental impacts. The studied metals were Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr and Zn. The sampling regions were city of Pančevo, which is characterized with heavy industry; city of Belgrade, an urban area; the vicinity of two coal fueledThermal Power Plants (TPP);city of Vršac which has no industrial facilities; and village Mesić that is surrounded with land used for agriculture.Samples were mineralized using microwave digestion. Quantitative analysis was done using ICP-OES.

The results show that statistically significant differences can be seen for Al, Ba, Cu, Fe and Ni between at least two of the locations. Al and Fe had statistically higher concentrations in TPP region. Ba and Ni had higher concentrations in Mesić.Cu had higher concentrations in urban area of Belgrade. This is in accordance with pollution sources in these regions.

Comparison of Iberian honey bee colony variables continuously monitored with thermo-hygro-buttons and electronic scales set up in two latitudinal extremes of Portugal

Cátia Neves, Pedro J. Rodrigues, Fernando Pérez-Rodríguez, Miguel Vilas-Boas, Paulo Ventura, Julio Chávez-Galarza, Lionel Garnery, David G. Biron and M. Alice Pinto
Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.
E-mail: catia.jose7@gmail.com

Honey bee colony data collected continuously together with climate data are of great importance because they provide the opportunity to understand colony phenology. Continuous monitoring of honey bee colonies initiated long time ago with Gates (1914) and Hambleton (1925), when they assessed weather effects on hive weight using mechanical scales. Currently, the study of colony dynamics has been intensified with development of new technologies such as electronic scales, hygro-buttons, thermo-buttons, and computer-assisted digital image analysis of brood combs. Studies of colony dynamics are of great interest in Portugal because of large climatic (and flora) differences between the two latitudinal extremes and because of distinct genetic backgrounds of the native subspecies, *Apis mellifera iberiensis* (Pinto et al. 2013). In this study we will compare the temporal dynamics of colony weight and nest temperature and humidity of 12 colonies, which have been continuously monitored since July of 2015 with electronic scales and thermo-hygro-buttons, set up in apiaries located in two latitudinal extremes of Portugal. These colony variables will be correlated with climatic data (temperature, humidity, wind speed, and rain) obtained from automatic weather stations installed in the two apiaries.

This research is funded through the 2013-2014 BiodivERsA/FACCE-JPI Joint call for research proposals, with the national funders FCT (Portugal), CNRS (France), and MEC (Spain).

Nectar and pollen value at *Trifolium* genus and its importance for honeybees

Nicoleta Ion, Roxana Spulber, Eliza Cauia and Adrian Siceanu
Institute for Beekeeping Research and Development, Bucharest, 42 Ficusului Blvd, sector 1, code 013975, Romania.
E-mail: nicoleta.ion@icda.ro

Trifolium genus consists of about 300 species of plants, widely used for pasture. In some European countries, the presence or absence of *Trifolium* species from the floristic composition of the pastures characterize the honey harvest. Trifolium species exhibits a wide amount of variability in morphological characters with high impact in plant-animal interaction. However, these have not been studied in detail despite its importance.

In order to redress this imbalance, we sought, in the present paper, to analyze the level of nectar production of flowers (in mg) and pollen value of this botanical source. In addition, some morphological aspects of the flowers were tracked under magnifying glass to characterize Trifolium species from point of view of the variation of floral tube length and their appreciation in relation to agriculture. Nectar production was investigated using capillary method, while pollen loads quality was evaluated using selected parameters (proteins and lipids). The study was carried out in the frame of national research program ADER 2015-2020, the contract 9.1.1./2015. Our analysis have revealed that *Trifolium* genus present a large variation regarding nectar level and nectaries architecture in comparison with other genus from Leguminosae family.

Remotely-sensed vegetation indices as predictors of bee diversity in an urban landscape

Andrzej Oleksa and Ewelina Motyka
Kazimierz Wielki University, Chodkiewicza 30, 85-064, Bydgoszcz, Poland
E-mail:olek@ukw.edu.pl

Advances in remote sensing techniques allow detecting vegetation diversity between different land use types, but it is not clear how this translates to other communities in the ecosystem. Here, we examined if vegetation indices could be used as proxy for wild bee species richness across a human-altered urban landscape in Bydgoszcz, a city in the northern Poland (human population ~360K). Our dataset covered ~4K records of bees from 187 species from the years 2000-2015. Remote-sensing vegetation indices were estimated using Landsat 8 imagery (averaged data for imagery obtained in the growing seasons 2013-2015). Variability of vegetation indices within the city was grouped into five classes (zones) using K-means clustering. We found that normalised differential vegetation index (NDVI) was highly significant predictor of species richness of wild bees. Rarefied species richness of bees for vegetation zones showed a strong correlation with the average NDVI for the zone ($r = 0.99$, $p < 0.001$). The rate of decline of species richness with decreasing NDVI (≈increasing urbanization) was lower in cavity-nesters compared to ground-nesters, and in social compared to solitary bees. This evidence supports the importance of urban green areas in protection of pollinator diversity, and diversified response of bees with different ecological specialisation to urbanization pressure. Our results also highlight the potential to utilize remote sensing data to make predictions for components of biodiversity that have tight vegetation associations, such as pollinators.

Floral resources for honey bees: the application for agro-ecosystems in Thailand

Preecha Rod-im and Orawan Duangphakdee
King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok, Thailand
E-mail: preecha.rod@kmutt.ac.th

The honeybees play a major role as a key pollinator in ecosystem. Recently, honey bee populations have significantly declined. These losses include direct economic effect to beekeeping industry as well as economic plants such as fruit, vegetable, forage seed, oil seed and biofuel crops. The poverty of nectar and pollen resources has been considered as one of major cause for honeybees losses. We review the potential approaches to provide and maintain floral resources for honey bees in cultivation area of Thailand. The management of non-cropped farmlands which are fallow areas, field margins, and conservation buffer strips to benefit honey bee health and enhance beekeeping activities was also described. The species of bee flora was identified and categorized as major, medium and minor sources of pollen and/or nectar and/or honey dew. Total two hundred thirty three species of bee flora were recorded and studied, among them, 36 species were horticultural plants, 42 species were crop plants, 45 species were ornamental plants and 114 species were wild plants. Study has shown that in agro-ecosystems is rich in bee flora and has great potential for small scale beekeeping as many plant species are available and bloomed even in dearth periods.

Geometric contrast feature for automatic visual counting of honeybee brood capped cells

Pedro João Rodrigues, Cátia Neves and M. Alice Pinto
School of Technology and Management, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal
E-mail:pjsr@ipb.pt

Assessment of honey bee colony strength by measuring adults or broodis often required for ecological studies. The brood has typicallybeen estimatedthrough a subjective mode (Lieberfeldermethod), although it can also be objectively determined by counting (manually or automatically)the brood cells (capped or uncapped)from digital images.The manual counting of capped cellsis highlyprone to errors and a time-consuming and tedious task. An automatic way to accomplish that task allows reducing those drawbacks.The main challenge for developing an automatic method is, however, the presence of intraclasscolor variation; it is not possible to make a reliable detection based just on the pixel color presented by the capped cells. While several researchers are using the Hough transform to solve that problem, at certainlight, noise, and surface conditions the automatic detection fails. After carefully observing capped cell regions of several combs, we identified a set of geometrical relations that couldbe used to build a consistent contrast feature. That feature is the key to detect the capped cells with a high accuracy in our work. Afunctional optimizer is performing a searching on the imagelooking for the locations that maximize the contrast on that feature. Ourexperimental results are showing a good detection rate (over 96%), despite thewide intraclasscolor variation.

This research is funded through the 2013-2014 BiodivERsA/FACCE-JPI Joint call for research proposals, with the national funders FCT (Portugal), CNRS(France), andMEC (Spain).

European Orchard Bee *Osmia cornuta* as commercial pollinator of apple and pear orchards in Serbia

Ljubiša Stanisavljević, Slađan Rašić and Jelena Stanisavljević
University of Belgrade - Faculty of Biology, Studentski trg 16, 11000 Belgrade (Serbia)
E-mail: ljstanis@bio.bg.ac.rs

The orchard bee *Osmia cornuta* (Latr.) has been used as an excellent pollinator of almond, apricot, pear, apple and several other rosaceous fruit plants in Europe. In the last decade, there has been increased interest in Serbia in growing apple and pear in the modern type of orchards, primarily because the fruits are widely used and have an excellent price at the global market level. The present study was carried out in an apple orchard in central Serbia and a pear orchard in the north-east Serbia with the aim of considering two aspects: 1) to optimize the *O. cornuta* pollinating potential and 2) to reveal and enhance its field augmentation. Cocoons of *O. cornuta* were kept under special temperature conditions during the winter in order to synchronize the activity of females with the later flowering period of apple and pear. Furthermore, a hedge of *Prunus spinosa* L. was used as a complementary food source in order to anticipate the release of pollinator cocoons in the apple and pear orchards, which allowed females to initiate the nesting/foraging activity before the target plants start blooming. In the first week of April 2014 and 2015, cocoons were placed in the apple orchard on an area of 14 ha, while the other in the last week of March 2014 and 2015 in the pear orchard on an area of seven ha. Analysis of pollen from the *O. cornuta* nests showed an average of 85% Malus pollen and nearly 15% *Prunus* pollen, and 65% *Pyrus* pollen, 17% Malus pollen and nearly 18% *Prunus* pollen in the apple and pear orchards, respectively. The data showed that the augmentation of *O. cornuta* in apple orchards was near twofold, in the pear orchards near 1.4 fold, in relation to the number of cocoons taken outdoors. In addition, the amount and quality of apple and pear fruits was increased by about 15% in the both orchards.

Pollen Identification Software for Capacity Building in Palynology - West Indian Ocean (Madagascar – Reunion Island) Bee-foraging Plant Pollens

T M Rasoloarijao, G Lebreton, H Santacreu, P Grard, J Clémencet, B Reynaud and H Delatte
7, ch de l'IRAT, 97410 Saint Pierre La Réunion
E- mail: delatte@cirad.fr

Despite the rich biodiversity of indigenous plants in the West Indian Ocean islands (Madagascar and the surrounding archipelagos), pollen reference collections are still scarce for this part of the world. The present study focus on describing pollen morphology of melliferous plants of tropical rainforest zones in Madagascar and Réunion island.

During 2013 to 2015, pollens were collected from honeybee foraging resources. Pollen grains were extracted, reference slides mounted (store for collection), photography and measurements of pollen grains in light microscopic made. The samples were processed employing the standard acetolysis method. Data are being integrated into pollen recognition software, IDAO developed by CIRAD, a user friendly pollen taxa identification method developed to make easier the study of Pollen grains. Vectored drawings of a composite picture and of about 150 character states of selected pollen taxa have been prepared. Information on the pollen is organized in a database accessed by the identification system. This platform has already been initiated by collaboration between the UMR PVBMT and the Department of Plant Biology and Ecology at the University of Antananarivo. The bank already contains 192 taxa of Madagascar and Reunion with 74 families and 149 genera of melliferous plants of the tropical rainforests zone of the West Indian Ocean islands.

The most common and the most relevant composition of toxic pesticide puzzle in dead honeybees

Tomasz Kiljanek, Alicja Niewiadowska, Stanisław Semeniuk, Marta Gaweł, Milena Borzęcka and Andrzej Posyniak
National Veterinary Research Institute, 57 Partyzantow Avenue, 24-100 Pulawy, Poland
E- mail: tomasz.kiljanek@piwet.pulawy.pl

During last years the problem of honeybee acute poisoning more or less converts but do not disappeard. 'Modern' sources of honeybee poisoning resulted from the application of systemic pesticides showed to be the same important as 'traditional' sources related to spray application.

In order to investigate suspected incidents of honeybee poisoning the sensitive and reliable multi-residue method has been developed, validated and implemented [1]. Each sample was analysed for a most actual range of 200 pesticides and their metabolites, which in 98% are approved to use within European Union.

The monitoring of honeybee poisonings carried out in the years 2014-2015 showed spectrum of 57 pesticides and their metabolites in dead bee samples. Determination of fungicides were almost as often as insecticides. Fungicides, particularly systemic ones, as well as insecticides could play a synergistic role in poisoning. Results from the poisoning investigations confirms the significant positive relationship between the probability of a colony showing disorders and the total number of fungicides [2]. This presentations shows the most common and the most relevant composition of toxic pesticide puzzle in poisoned honeybees, with particular attention to systemic insecticides and systemic fungicides.

Colony Traits of Native *Bombus terrestris dalmatinus* (Hymenoptera: Apidae) from the Black Sea Region of Turkey: Comparison with Commercial Colonies*

Ayhan Gosterit and Ahmet Cicek
Department of Animal Science, Faculty of Agriculture, Süleyman Demirel University, 32260, Isparta, Turkey
Email: ayhangosterit@sdu.edu.tr

Knowledge of the life history of native bee populations is vital to secure pollination services and to improve conservation strategies. The present experiment was conducted to determine the developmental characters of the native bumblebee *Bombus terrestris dalmatinus* that occurs in the Western Black Sea region of Turkey, where commercial colonies have not yet been used as pollination agents. The colony traits of this native population were compared with the traits of commercial colonies of *B. terrestris* that originated in other regions. A total of 200 queens, 100 naturally diapaused ('native' population) and 100 artificially diapaused ('commercial' population), were allowed to found colonies. There were no differences in egg laying and colony founding success between the native and commercial queens. Queens collected from the field commenced egg-laying earlier than commercial queens, and produced less egg cells and workers in the first brood. Colonies founded by commercial queens produced more gynes (82.11 ± 9.32) than colonies founded by native queens (32.85 ± 3.97). Native and commercial colonies also differed in their patterns of production of males and gynes.

***** This work was supported by the Scientific and Technical Research Council of Turkey (TUBİTAK- TOVAG; grant number: 214O576). This study is also Master Thesis at Süleyman Demirel University Graduate School of Natural Sciences.

The assessment of pollinating insects biodiversity on oilseed rape plantations in Poland (preliminary study)

Zbigniew Koltowski, Mikolaj Boranski
Research Institute of Horticulture, Apiculture Division, Plant Pollination Laboratory, Kazimierska Str. 2, 24-100 Pulawy, Poland
E-mail: zbigniew.koltowski@inhort.pl

Oilseed rape (*Brassica napus* ssp. *napus*) is the most important oil crop in Poland. The acreage of this crop in Poland oscillate about 1 mln hectares, where winter cv. represent about 98%. The yield of seeds of this crop is about 30% higher after cross pollination of flowers by insects. The most important pollinators are bees (Apoidea).

The aim of the study was to assessment the species composition and abundance of foraging insects on winter oilseed rape plantation in vicinity of Pulawy, central east part of Poland.

The study was performed on three localizations Policzna, Bartodzieje and Osiny with the area of plantations 40, 10 and 80 hectares, respectively. Observations of pollinating insects were done at the beginning, during full and at the end of blooming. They were conducted during nice weather, with temperature at least 21°C, from 9 am to 1 pm, when the activity of all groups of pollinators was the highest. Density of bees was performed by transect method and pollinating insects were registered during walking along the observation plot in size 200 m x 1 m during 20 minutes. Observations were done at margin and central parts of plantations.

It was stated that, among of Apoidea noted on winter oilseed rape in Pulawy vicinity, honey bees (*Apis mellifera*) were dominant (98% of all insects). Solitary bees (*Andrena* sp. mainly) and bumblebees were rarely observed. Among bumblebees *Bombus terrestris*, *B. lapidarius*, *B. lucorum* and *B. pascuorum* were noted. Density of bees on tested plantations varied between 0.7 and 1.4 per 1 m^2.

Biologic activity of bee products

Total phenols, flavonoids and antioxidant activity of honey with propolis

S.M. Osés, A. Pascual-Maté, D. de la Fuente, A. de Pablo, M.A., Fernández-Muiño and M.T. Sancho
Department of Biotechnology and Food Science, University of Burgos, Pla. Misael Bañuelos s/n, 09001, Burgos, Spain.
E-mail:smoses@ubu.es

Propolis is a bee product with high amount of phenols and flavonoids and with a known antioxidant activity. However, this product is not good accepted by consumers due to its bitter and astringent flavour. The purpose of this work was to obtain an acceptable food product (honey with propolis) with higher antioxidant activity than the honeys used. First, 0.1%, 0.3% and 0.5% from each soft propolis (A, B, C and D) were added to each honey sample (H1, H2, H3 and H4) and sensorial evaluation by sixty-five untrained adult panellist, using a 9-point hedonic scale was done. As we were interested in making an edible product with honey and the highest amount of soft propolis extract, 16 mixtures were selected from all the samples evaluated.In order to remove all sugars and other polar compounds a phenolic extraction was performed through a column with Amberlite XAD-2 resin, eluting the phenol compounds with methanol. After extraction, total phenolics were determined by Folin-Ciocalteu assay, flavonoids content was determined by flavonols (type quercetin) and flavan-3-ols (type catechin) spectrophotometric methods based on the formation of aluminium-flavonoid complexes and antioxidant activity was evaluated by two methods: ABTS scavenging activity test and radical-scavenging effect on hydroxyl radicals. Total phenols, flavonoids and antioxidant activity were higher in honeys with propolis than in honeys. Therefore, soft propolis extract added to honey, even at a concentration as low as 0.1%, is able to increase the antioxidant activity of the base honey, enhancing honey's bioactive properties.

Novel rapid method for the determination of honey physicochemical parameters by vibrational spectroscopies and chemometrics to assess honey quality

Rosa M. Alonso-Salces, Juan A. Fernández-Pierna, Ouissam Abbas, Inés Aubone, Marisa Amadei Enghelmayer, Valeria S. Rosso, Vincent Baeten and Sandra R. Fuselli
Grupo de Investigación Microbiología Aplicada (GIMA), Centro de Investigación en Abejas Sociales (CIAS), Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Pcia. Buenos Aires, Argentina; Comisión de Investigaciones Científicas, La Plata, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.
E-mail: sfuselli@gmail.com

Nowadays, according to the *Codex Alimentarius*, honey quality is evaluated by determining a number of physicochemical parameters, such as moisture, electrical conductivity, pH, free acidity, colour, sugar content and hydroxymethylfurfural content, among others, using official methods (AOAC, International Honey Commission). The compulsory use of several of these methods for assessing honey quality makes the analytical procedure for honey quality control costly, long and tedious; besides considerable amounts of solvents and reagents are consumed. To overcome these drawbacks, the use of vibrational spectroscopies combined with chemometrics is proposed in order to quantitatively determine such physicochemical parameters; allowing to obtain rapid on-line non destructive information without performing any special sample preparation, and to determine various physicochemical parameters in a single run. The objective of the present work is to develop a novel rapid method based on NIR, MIR and/or Raman spectroscopies and chemometrics for the determination of the honey physicochemical parameters. The physicochemical data, obtained by official methods, and vibrational spectral data of 454 Argentinean honeys from 3 harvests (2013-2015) were analysed by multivariate data analysis. Validated Partial Least Squares regression models were achieved to determine with acceptable precisions, honey physicochemical parameters, i.e. the contents of fructose, glucose, reducing sugars, sacarose, turanose, maltose and erlose, fructose/glucose ratio, free acidity, color, moisture, and electrical conductivity, by just recording NIR, MIR and/or Raman spectra of the honeys.

Geographical characterization of Argentinean honeys by vibrational spectroscopies and pattern recognition techniques

Rosa M. Alonso-Salces, Juan A. Fernández-Pierna, Ouissam Abbas, Inés Aubone, Valeria Salazar, Vincent Baeten and Sandra R. Fuselli
Grupo de Investigación Microbiología Aplicada (GIMA), Centro de Investigación en Abejas Sociales (CIAS), Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Pcia. Buenos Aires, Argentina; Comisión de Investigaciones Científicas, La Plata, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.
E-mail: sfuselli@gmail.com

Argentina is a major producer of quality honey; 90% of the honey produced in Argentina is acquired by the United States and the European Union. The globalization of the world market makes the authentication and characterization of botanical and geographical origins of honey an important issue. Quality schemes, such as Protected Designation of Origin and Protected Geographical Indication, protect the identity and quality of a product from certain region, in order to prevent fraud and illicit practices, and give it an added-value. These schemes are linked to the characteristics of the production systems, geographical origin, and cultural and historical practices. In particular, for honeys, sensorial, pollen and physicochemical characteristics depend largely on the botanical and geographical origin, and reflect regional aspects of beekeeping management. Honey is traditionally characterized by performing the analysis of physicochemical parameters, melissopalynological and sensory analyses; which are tedious, time-consuming, use considerable amounts of reagents, and requires skilled personnel with extensive experience in pollen analysis and honey tastings. To overcome these drawbacks, vibrational spectroscopies are considered powerful alternatives due to their simplicity, speed, cost-effective and non-destructive character. MIR, NIR and/or Raman along with chemometrics are one of the strategies proposed for discrimination of the botanical and/or geographical origin of honeys. The objective of the present work was to develop analytical tools for the characterization of Argentinean honeys: Samples from three production regions from three harvests (2013-2015) were analysed by vibrational spectroscopies and pattern recognition techniques to develop classification models to distinguish honeys according to their geographical origin.

Pollen Identification Software for Capacity Building in Palynology - West Indian Ocean (Madagascar – Reunion Island) Bee-foraging Plant Pollens

T M Rasoloarijao, G Lebreton, H Santacreu, P Grard, J Clémencet, B Reynaud and H Delatte
Cirad Umr Pvbmt, Pôle de Protection des Plantes 7, chemin de l'IRAT Ligne Paradis 97410 SAINT-PIERRE, Réunion
E mail: delatte@cirad.fr

Despite the rich biodiversity of plants in the island of Madagascar and the archipelago or Mascarenes, pollen reference collections are still scarce for this part of the world. The present study was undertaken during 2013 to 2015, where pollens were collected from honeybee foraging resources in rainforests of Madagascar and Reunion Island.

Pollen grains were extracted, reference slides mounted (store for collection), photography and measurements of pollen grains in lightmicroscopic made. The samples were processed employing the standard acetolysismethod.

Data are being integrated into pollen recognition software, IDAO developed by CIRAD, a user friendly pollen taxa identification method developed to make easier the study of Pollen grains. Vectored drawings of a composite picture and of about 150 character states of selected pollen taxa have been prepared. Information on the pollen is organized in a database accessed by the identification system. This platform has already been initiated by collaboration between the UMR PVBMT and the Department of Plant Biology and Ecology at the University of Antananarivo.The bank already contains 192 taxa of Madagascar and Reunion with 74 families and 149 genera of melliferous plants of the tropical rainforests zone of theWest Indian Ocean islands.

Development of pre-processing and detection of bee venom in milks of dairy cattle

Sang Mi Han, In Phyo Hong, Soon Ok Woo, Se Gun Kim and Hye Ri Jang
Department of Agricultural Biology, National Academy of Agricultural Science, Wanju, 565-851, South Korea
E mail:sangmih@korea.kr

This research was performed in order to development a pre-processing and to investigate bee venom residues in the milks of cow treatment with bee venom which has a potential as a therapeutic agent for bovine mastitis. The pre-processing was consisted of the steps in freeze drying, dissolution with distilled water, ultrasonic, centrifuging and then filtering with 0.45 μm membrane filter. Melittin, as the main component of bee venom, in milks with pre-processing was detected by ultra-high performance liquid chromatography(UHPLC). Also, histamine and phospholipase A2 were detected using this pre-processing. We monitored bee venom reside in the milks for bee venom used for cow mastitis treatment. by UHPLC. Melittin, histamine and phospholipase A2 were not detected in the milks of cow for treatment with bee venom. These result that pre-processing showed good performance to be used for bee venom extracts of milk and bee venom didn't remain in the milk of cow treatment bee venom.

Isolation of abscisic acid from honey of acacia in Korea

Se Gun Kim, In Phyo Hong, Soon Ok Woo, Hye Ri Jang and Sang Mi Han
Department of Agricultural Biology, National Academy of Agricultural Science, Wanju, 565-851, South Korea
E mail:sangmih@korea.kr

Honey is nectar of flowers brought by the worker honeybees to their hives and modified during the process of storing and ripening. Actually it is a plant product. In this study, we carried out the extraction and identification of the botanical origin constituents from honey of Black Locustg (Robiniapseudoacacia) in Korea. To isolate characteristic constituent from the honey, sample was extracted with ethyl acetate and then the extract was subjected to column chromatography using silica gel and ODS resins. We isolated (+)-abscisic acid and its structure was identified by the basis of physicochemical method such as 1D-

NMR(1H, 13C, DEPT 90 and 135), 2D-NMR(HMQC and HMBC) and mass spectrometery. (+)-Abscisic acid was detected at UV 260 nm as characteristic peak by UPLC-PDA and was obtained for the first time from Black Locust honey. This insight might lead to important characterization features for differentiate honeys.

New UPLC method for quality assessment of propolis from Korea

Se Gun Kim, In Phyo Hong, Soon Ok Woo, Hye Ri Jang and Sang Mi Han
Department of Agricultural Biology, National Academy of Agricultural Science, Wanju, 565-851, South Korea
E-mail:sangmih@korea.kr

A new UPLC-DAD method for simultaneous determination of p-coumaric acid and trans-cinnamic acid in propolis was developed using Halo-C18 column and step gradient elution of MeCN and 0.1%H3PO4 in 8 min. The method was validated by specificity, linearity, limit of detection, limit of quantification, precision(intra- and inter-day variability) and recovery tests. The validated method was sufficiently applicable for quantitative analysis of propolis products as well as propolis collected from different regions in Korea. The established method is simple, appropriate and efficient, and can be used for quality assessment of Korean propolis.

Novel Lactic Acid Bacteria from the honey bee *Apismellifera*inhibiting bee pathogens

Sepideh S. Lamei[a,b],Anette Mårtensson[a], Alejandra Vásquez[a] and Eva Forsgren[b]
[a]Department of Laboratory Medicine Lund, Section of Medical Microbiology, Lund University, Building 404C, Medicon Village, ,SE-223 81 Lund, Sweden.
[b]Department of Ecology, Swedish University of Agricultural Sciences, PO Box 7044, 75007 Uppsala, Sweden.
E-mail:sepideh.lamei@med.lu.se

Honey bees, Apismellifera are one of the most important pollinating insect worldwide. Hence, apiculture has a great economic impact on insect pollinated crops throughout the world.Unfortunately, the health status of honey bees has become an important concern in many countries. In this study we focus on the role of the honey bee Lactic Acid Bacteria (LAB) symbionts in defense against thehoney bee *Apismellifera*pathogens *Paenibacillus larvae*, causing American foulbrood (AFB). In previous study has been shown thata large amount of different proteins are being produced when the LAB symbionts encounter honey bee pathogens[1]. We hypothesize that the LAB symbionts act as a natural barrier against pathogens in honey bees. Dual culture overlay with LAB symbionts was performed as previously described by Butler *et al.*[1]. In order to study the effect of cell free supernatants (CFS) from the LAB, well diffusion tests were conducted. LAB symbionts were cultured in de Man, Rogosa and Sharpe broth (MRS)(OXOID LTD, England) supplemented with 2% fructose and 0.1% L-cysteine. The individual LAB species showed different inhibition properties against *P. larvaein vitro*, whereas a combination of all thirteen LAB species resulted in a clear inhibition (no visible growth) of *P. larvae*. Species within LAB microbiota have different ability to produce bioactive compounds such as organic acids, free fatty acids, ethanol, benzoate, enzymes and hydrogen peroxide. However further studies have to be made to reveal the mechanisms of action behind the LAB symbionts properties to inhibit honey bee pathogens. Moreover the colony level interaction between the LAB microbiota and these honey bee pathogens will be investigated in the field, and a formula based on the honey bee LAB bacteria and their metabolites will be evaluated for long term impact on prevalence of these honey bee pathogens.

Physicochemical characteristics of *Robinia* honeys from Hungary

M. Bohus Hományiné, T. Kiss, M. Flórián, E. BükiRőzséné and E. Zajácz
Institute for Apiculture and Bee Biology, Research Centre for Farm Animal Gene Conservation (HáGK) H-2100Gödöllő, Isaszegiút 200, Hungary
E mail:bohus.magda@hagk.hu

One of the most important unifloral honeys in Hungary is the *Robinia* honey (*Robinia pseudoacacia*). The present studyaimed to establish a database and a characteristics profile of the *Robinia* honey from Hungary. Within the framework of a 3-year project (2014-2016) honey samples directly originated from different beekeepers were collected from 3 regions of Hungary (North, East and Southwest region; 10-12 honey samples/region/year). The honey samples are studied by sensory, physicochemical and melissopalinological analysis. The physicochemical studies included colour, moisture content, electrical conductivity, pH, acidity, diastase activity, invertase activity, HMF content determinations and sugar analysis. The most important parameters for *Robinia* honeys are fructose content (average value of 41,6 g/100 g in 2014) and the fructose/glucose ratio (average value of 1,65 in 2014).The investigated honeys showedlower colour values (8 mm,on the Pfund scale) and higher invertase activities (average value of 60,8 U/kg, in 2014)compared to other *Robinia* honeys from other parts ofEurope. It is noteworthy that, the quality of honeys was influenced by the climatic conditions, geographical origin and handling. As the differences among the unifloral honeys that have different geographical backgrounds can solely be described by conducting examinations in the same place in three consecutive years, the continuation of these studies is very important.

Evaluation of antibacterial activity of honey samples collected from *A.florea* combs in District Khairpur

Naheed[1] Rajper and Shakeel R. Farooqi [1, 2],

Department of Genetics[1], University of Karachi,
Biological Research Centre[2], University of Karachi, Karachi, Sind, Pakistan
E-mail: rajpernaheed28@gmail.com, naheedrajper@yahoo.com

Honey is a prospective competitor for fighting antimicrobial resistance in view of the fact that it contains an ample range of antibacterial compounds working at multiple sites. This work was directed to study the inhibitory effects of honey collected from different geographical regions of District Khairpur against certain pathogenic bacteria. It has been observed that the valuable use of honey in the management of bacterial infection is when it can be applied directly to the bacteria without dilution. There are few published reports on the physicochemical and antibacterial characteristics of honey from *A. florea*, the dwarf honeybee native to Pakistan. Current study explores the variation in physicochemical properties and the level of antimicrobial potential of honey samples collected from wild bee combs of *A. florea* showing potential genetic diversity from District Khairpur. The acacia honey found effective to stop growth of isolates except *Proteus* and *Shigella*. The antibacterial action of honey was attained in high concentrations of honey both in well diffusion as well as disc diffusion methods.

Antimicrobial and anti-inflammatory activities of honey with propolis

S.M. Osés, A. Pascual-Maté, D. de la Fuente, A. de Pablo, M.A., Fernández-Muiño and M.T. Sancho
Department of Biotechnology and Food Science, University of Burgos, Pla. Misael Bañuelos s/n, 09001, Burgos, Spain.
E-mail:smoses@ubu.es

Nowadays, propolis is being researched as a potential food ingredient, having already demonstrated to be able to improve the shelf-life of other foods, providing them with potentially interesting functional properties. However, propolis alters the sensory characteristics of foods combined with them,

transferring bitterness and astringency. The aim of this study was to make likeable products of honey with propolis and to research their antimicrobial and *in vitro*anti-inflammatory activities. 0.1%, 0.3% and 0.5% soft propolis extracts were added to four honey samples and then, all mixtures were sensory assessed using a 9-point hedonic scale. Antimicrobial activity was performed against six bacterial and six fungi species by agar well diffusion. Anti-inflammatory activity was evaluated by hyaluronidase inhibition assay based in the mechanism of the Morgan-Elson reaction.Results showed that when soft propolis extracts were added to honeys, both antifungal and antibacterial activities dramatically increased, in comparison with honeys' activities. In respect of dilutions, anti-inflammatory activity for samples diluted at 75% was higher than the activity found for honeys diluted at 50%. Additions of soft propolis extracts to honey samples diluted at 50% increased anti-inflammatory activity of honeys, while in samples diluted at 75% such rises were only observed in few samples. Therefore, a soft propolis extract, even at a concentration as low as 0.1%, proved to be able to increase both antimicrobial and anti-inflammatory activities of the base honeys. Synergic antimicrobial activity was observed between honeys and propolis.

Botanical, zoological and geographical identification of honeydew honey by quantitative analysis of multielement isotope ratios

Amine Simay Yaycioglu, Annette Schroeder and Susanne Voerkelius
Apicultural State Institute, University of Hohenheim, Augustvon- Hartmann-Strasse 13, 70599 Stuttgart, Germany
E-mail: simay.yaycioglu@uni-hohenheim.de

Honeydew honey is among the most popular honeys in Germany and economically important. However there is no chemical analysis method available to distinguish the different variation of honeydew honeys according to their botanical, zoological or geographical origin.
Therefore, we will establish a data base that includes the characteristic multielement isotope ratios of honeydew honeys from locations with different climatic and geological conditions. As a first step, we had to reveal specific isotope parameters for honeydew honeys. A general problem for the classification of honeydew honeys is the fact that usually various aphid species (*Aphididae*)

occur in one region, which aggravates the clear identification of samples. For this reason we established a method to collect honeydew drops directly from individual aphids and to analyze their composition according to aphid and host plant species. One focus of this chemical analysis is the trisaccharide melezitose, which is considered to be responsible for the so called "cement honey". The proportion of melezitose in honeydew varies thereby among species and host plants. Feeding experiments with "artificial sugar liquids" will be realized to examine the crystallization behavior of honey in combs and to measure the threshold value of this melezitose induced "cement honey". We here present this new cooperation project together with preliminary results of the first research season.

Biological Properties of Honey Bee Venoms in Thailand

Wannapha Mookhploy, Jakkrawut Maitip, Sukum Eitssayeam and Panuwan Chantawannakul
Bee protection laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 50200
E--mail: panuwan@gmail.com

Honey bee venom has long been utilized for traditional medical application. In this study, we report biological properties of crude honey bee venoms collected from European honey bee and Asian honey bees in Thailand. The Minimum Inhibitory Concentration (MIC) technique was used to detect the antibacterial activities of bee venom samples against the tested microorganisms (*Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumonia*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhimurium*). In addition, crude venom extracts were also tested for antioxidant activities. The results showed that European honey bee venom exhibited the highest antibacterial and antioxidant activities.

Antimicrobial activity of different bee pollen methanolic extracts and correlation with their polyphenolic content

Rodica Mărgăoan, Liviu Al. Mărghitaş, Daniel S. Dezmirean, Otilia Bobiş, Corina Cătană, Flore Chirilă, Claudia Pașca and Adriana Urcan
University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Romania
E-mail: rodica.margaoan@usamvcluj.ro

In vitro antimicrobial activity of studied bee pollen samples, was carried out by disk diffusion technique. Bee pollen samples used were extracted into 70% methanol. The concentration of bee pollen extract was 15%. To test the antibacterial activity against S. aureus, B. cereus, B. laterosporus, E. coli, P. aeruginosa, S. enteritidis and S. typhi strains, Mueller-Hinton agar was used, meanwhile for testing P. larvae P. alvei. and C. albicans, glucose agar substrate was used. Streptomycin was tested as positive control and as negative control, methanol 70% (extraction solvent). The predominant bee pollen (>45%) in the analyzed samples was: BP1: Malus domestica (Fam. Rosaceae), BP2: Prunus spp. (Fam. Rosaceae), BP3: Calendula officinalis (Asteraceae), BP4: Taraxacum officinale (Asteraceae), BP5: Calluna vulgaris (Ericaceae) and BP6: Salix spp (Salicaceae). The total phenolic content was estimated according to Folin-Ciocalteu spectophotometric method using gallic acid as reference standard. The absorbance was read at 760 nm with Synergy HT MultiDetection Microplate Reader with 96 well plates. Total phenols content was expressed as mg gallic acid equivalents/g dry matter samples. Total flavonoid content was assayed following the method of Tămaş with ZrOCl2 2,5 % in methanol adapted for this matrix. The absorbance was read at 425 nm after 10 min with Synergy HT Multi-Detection Microplate Reader with 96 well plates and quercetin was used as a standard and the results expressed as mg quercetin/g dry matter samples.

Extraction characteristics of propolis with ethanol

Woo Soon Ok, Inpyo Hong and Sangmi Han
National Academy of Agricultural Science, RDA, Republic of Korea
E-mail: wooso1@korea.kr

Propolis is a sticky material made from plant growth point protection secretion and resin which are collected by bees, then mixed with bee saliva enzyme, it is used to keep bee colony safe by applying inside of bee hive, and it is consisted of about 50% of resin and aromatic, 25% of beeswax, 10% of essential oil, pollen and mineral.

In this study, it was necessary to extract the active ingredient in crude to use of propolis, since the use of a health food and extracted with ethanol. We reported a summary of the main results of the extraction of propolis by ethanol. Propolis extract yield and total flavonoid content tends to increase the higher the ethanol concentration. Total phenolic content exhibited the highest value in the 50-60% EtOH, the EtOH concentration showed a tendency to decrease further enhanced.

The extraction yield and DPPH free radical scavenging effects of liquor ratio from Korean propolis

Woo Soon Ok, Inpyo Hong, and Sangmi Han
National Academy of Agricultural Science, RDA, Republic of Korea
E-mail: wooso1@korea.kr

Propolis is a sticky material made from bee collected growth point protection materials or resin of plants mixed with bee saliva enzyme. It is used to keep bee colony safety by applying inside of bee hive with various color including dark brown and yellowish brown.

Propolis is extracted with ethanol, so as to take advantage of edible material, this experiment was carried out in order to investigate the relationship between liquor ratio and extraction yield and antioxidant effects.

Although differences in the extraction yield according to the collected area, when one solution ratio 1:10 showed the highest extraction yield.

The results confirm the DPPH free radical scavenge ratio (%) as antioxidant effects, the greater the amount of extraction solution showed higher antioxidant

Improvement storage stability of the fresh-cut vegetables with propolis

Woo Soon Ok, Sangmi Han and Inpyo Hong
National Institute of Agricultural Science, RDA, Republic of Korea
E-mail: wooso1@korea.kr

Propolis is a health food, known that high antioxidant and antimicrobial effects, fresh cut vegetables that rapidly increasing consumption has recently faced the problem storability fall down after washing. To improve storability of fresh cut vegetables are being carried out various studies. In this study, using the characteristics of propolis we were performed to improve the storability of fresh cut vegetables. There was prepared in 18% solution of propolis extracts, by using this solution, propolis solution prepared diluting 0.001 to 1%, were dipped in fresh vegetables(cabbage lettuce, perilla leaf, and lettuce). Vegetables were measured the sensory evaluation and hardness after each treatment by placing in a certain period of time at room temperature and refrigerator. The results showed that the storage stability is excellent compared to non-treated as if diluted to 0.1-0.01% propolis solution is to improve the shelf life of fresh cut vegetables.

The effect of some alcoholic extracts of bee pollen and propolis against *E.coli*

Claudia Pașca[1], Liviu Al. Mărghitaș[1], Daniel S. Dezmirean[1], Otilia Bobiș[1], Victorița Bonta[1], Adriana Urcan[1], Rodica Mărgăoan[1], Flore Chirilă[2] and Nicodim Fiț[2]
[1]Department of Apiculture and Sericiculture: University of Agricultural Sciences and Veterinary Medicine, România
[2]Department of Microbiology (Veterinary Medicine): University of Agricultural Sciences and Veterinary Medicine, România
E-mail: claudia pasca@usamvcluj.ro

The honeybee (*Apis mellifera*) makes various bee products from plants, flower nectar and flower pollen. Propolis and bee pollen are natural bee products with wide range of biological and medicinal properties. The study investigated antimicrobial activity of 6 poplar propolis ethanolic extracts and 16 pollen samples of the following families: Rosaceae, Salicaceae, Fabaceae, Tiliaceae, Asteraceae, Brassicaceae and two polifloral pollen collected from Transylvania, using antibiogram method.
The results show a highest sensitivity 12.50±1.62 mm for propolis extracts and the low antibacterial activity 9.75±1.35 mm for bee pollen extracts.
In conclusion, the results of the present investigation revealed that propolis and bee pollen extracts could be useful in the prevention of diseases in which *E.coli* are implicated.

Effect of the storage temperature on volatile organic compounds and aroma profile of *Robiniapseudoacacia* honey

Sara Panseri, Federica Borgonovo, Marcella Guarino, Lucia Piana, Elena Facchini, Michele Mortarino, Luca Chiesa and Rita Rizzi
Department ofHealth, Animal Science and Food Safety – Via Celoria 10 – 20133 Milan, Italy
E-mail:sara.panseri@unimi.it

Honey retains its quality for a long time if it is properly processed and stored. The storage temperature has an impact on the physical and chemical properties of honey.

In this study we evaluated the trend of volatile organic compounds and aroma profiles of *Robiniapseudoacacia* honey samples during storage extended for 18 months at three different temperatures (15, 25 and 35° C)by using Solid-Phase Microextraction coupled with Gas-Chromatography Mass-Spectrometry (SPME-GC/MS) and Electronic Nose.The 103 volatile compounds identified belonged to different major chemical classes: aldehydes, alcohols, sulphur compounds, free fatty acids, furans, hydrocarbons, ketones and terpenes. The aromatic profile analyses were also evaluated using an electronic nose and the sensor array was composed of 10 different sensors. A stepwise discriminant analysis was used to determine the best combinations of variables that would separate the three storage temperatures. A mixed model was fitted to investigate the effect of temperature and time on the volatile compoundsand thearomatic profile. The discriminant analysis resulted in fourselected volatile compounds (furans, sulphur compounds, aldehydes, and alcohols) and three selected sensors reactive to aromatic, hydrogen and methane compounds. A consistent increase of furans was observed in honey samples after 4 months and after 14 months for samples stored at 35 and 25° C, respectively. No variations for furans were observed for honey at 15° C. A difference among the three storage temperature was detected for the sensor reactive to hydrogen compounds after 6 months.

Identification of volatile organic compounds,using SPME / GC-MS technique, in honey collected in apiaries from Transilvania area

Maria Pojar-Fenesan, Ana Balea, Irina Ciotlaus and Mihaela Vlassa
Babes-Bolyai University – Raluca Ripan Institute for Research in Chemistry
30 Fantanele street, 400294 Cluj-Napoca, Romania
E-mail: maria.fenesan@ubbcluj.ro

The aim of this study is to identify the profile of volatiles of honey with different geographical and botanical origins from Transylvania area, using SPME/GC/MS technique. Volatiles organic compounds VOCs from honey could besemiochemicals–pheromones and kairomones, involved inchemical communication of *Apismellifera* species.

The types of honey analyzed are from the 2015 year and were: multifloral honey harvested in Mures plain, Rodna Mountains, Lapus Subcarpathians and

surroundings of Cluj-Napoca city.The analysed unifloral honey were: acacia honey, sunflower honey, rape honey and linden honey; honeydew: mountain and hill. The varieties of honey from different areas: mountain, plain, hill and urban area showed interesting differences regarding the VOC content.

Antimicrobial effect of *Hedysarum coronarium* "Sulla" honeys

Messaouda Belaid, Salima Kebbouche-Gana and Farida Benzina
Département de Biologie, Faculté des Sciences, Université M'Hamed Bougara de Boumerdes. Avenue de l'Indépendance-35000-Algérie.
E-mail: belaidfo@yahoo.fr

Honey is produced by honeybees (*Apis mellifera intermissa*) from nectar of blossoms or from the honeydew and its antimicrobial activity depending greatly on the floral origin.

In this study, we have investigated the antimicrobial activity of *Hedysarum coronarium* "Sulla" honeys against *Staphylococcus aureus, Pseudomonas aeroginosae, Aspergillus niger* and *Candida albicans.* The agar well diffusion method was employed.

The results showed that those strains that appear to be very sensitive to the "Sulla" honey tested are *Staphylococcus aureus* and *Pseudomonas aeroginosae. Aspergillus niger* is more sensitive to honey studied compared to *Candida albicans.*

Investigation of the free amino acid profile and carbohydrates content of bee bread

Adriana Urcan, Liviu Mărghitaș, Daniel Dezmirean, Otilia Bobiș, Victorița Bonta, Claudia Pașca and Rodica Mărgăoan
University of Agricultural Sciences and Veterinary Medicine Fculty of Animal Science and Biotehnologies, Departament of Beekeeping and Sericiculture, Cluj Napoca, Romania
E-mail: adriana.urcan@usamvcluj.ro

Bee bread undergoes a lactic acid fermentation of pollen, stored in the combs and mixed with bee secretions. One of the contributions to their high nutritional value is the presence of significant amounts of proteins and carbohydrates. The aim of this study was the identification and quantification of free amino acids and carbohydrates from bee bread. Samples from different geographical origins were analyzed using liquid chromatography coupled with mass detection (LC-MS), and HPLC methods. Different content of free amino acids have been detected in the analyzed samples, which is assumed to be caused by Maillard reaction between amino acids and carbohydrates.
The results show that the predominant amino acid in bee bread is proline in an amount of 1060,01mg aa/100 g. Eight essential amino acids were detected in beebread, excepting histidine. Lisine was found in the highest amount (86.59 mg aa/100 g). Concerning the reducing sugars content, the most relevant are glucose and fructose with 18.50 and 11.57% from the total content of carbohydrates.

The potential application of bee bread as food supplement depends on the chemical composition, and the protein and aminoacid content are the most important components.

Is possible propolis composition to fix?

Meral Kekeçoğlu[1] and Sevgi Kolayli[2]
[1]Duzce University, Faculty of Arts and Sciences, Department of Biology, Konuralp, 81620, Duzce, Turkey
[2]Karadeniz Technical University Faculty of Science Department of Chemistry 61080 Trabzon / Turkey
E- mail: meralkekecoglu@duzce.edu.tr

Propolis is very valuable pharmaceutical mixture, recently, is used many purposes. There are many different drugs, solutions, pills, and creams produced from propolis. There is any standardization in the propolis mixtures since either productions or manufactures methods are variable. We have investigated bee ecotypes effects on propolis phenolic contents and composition as fixed other conditions in this study. Five different ecotypes (Muğla ecotype of Anatolia, Caucasica, Carniva, Yığılca ecotype of Anatolia, and Syriaca) of *Apis mellifera* were used and the propolis were collected from Ankara region of Turkey at the same time. Total phenolic content (TPC), total flavonoid content (TFC) and total tannin (TT) and phenolic profiles were also compared in the ethanolic extracts. Total phenolic content, total flavonoids and total tannins also reflects bioactive features of propolis samples.Although these propolis extracts were showed different phenolic profiles, and phenolic compositions, TPC were ranged from 16 to 26 mg gallic acid/ g extracts. The results of the study shows that different honeybee species or ecotypes have different quality propolis collecting capacity. In addition, the best way to determine propolis quality is to measure the amount of total phenolic and flavonoid contents. So bee differences can be used to advantage by beekeepers, depending on what traits interest them.

Antigenicity of honeybeevenom in Guinea Pigs

Sang Mi Han, In Phyo Hong, Soon Ok Woo, Se Gun Kim and Hye Ri Jang
Department of Agricultural Biology, National Academy of Agricultural Science, Wanju, 565-851, South Korea
E-mail:sangmih@korea.kr

This study was performed to examine the skin phototoxicity of purified bee venom (Apismellifera L.) collected using bee venom collector. To confirm whether the gel containing purified bee venom (BV gel) causes phototototoxicity when used for the skin medicinal products, phototototoxicity testing was conducted using guinea pig models. The BV gel (0.1 mL / site) was administered transdermally to guinea pigs. 8-MOP was used to introduce positive control response. After administration, the guinea pigs were irradiated with UVA (15 J/cm2) with doses based on standard phototoxicity study guidelines. In the weight measurement and clinical observation, BV gel groups didn't show any significant changes compared with control group. BV gel groups didn't show any symptoms such as erythema and edema formation of skin. This study demonstrated that BV gel has promising potential external treatment for topical uses that do not induce significant levels of skin phototoxicity.

Screening bioactivity and bioactive constituents of Nordic unifloral honeys

Anneli Salonen, Virpi Virjamo and Riitta Julkunen-Tiitto
Natural Product Research Laboratories, Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 111, 80110 Joensuu, Finland
E-mail: salonen.anneli222@gmail.com

Bioactivity of honey is a complex phenomenon originating from nectar, honeybees and physical properties of honey. The objectives of this study were to screen the bioactivity of Nordic unifloral honeys. Forty-four honey samples from Finland, Sweden, Norway and Denmark were collected. Their moisture, electrical conductivity, pH, colour, pollen and carbohydrate content and amounts of phenolic compounds, hydrogen peroxide and methylglyoxal (MGO) were analysed. Antioxidant activity was evaluated with DPPH radical scavenging assay and antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus with microdilution assay. The unifloral honey samples game from fifteen botanical

origins: buckwheat, caraway, clover, dandelion, fireweed, heather, honeydew, lime tree, lingonberry, mire, rape, raspberry, sweet clover, willow and polyfloral. Moisture varied between 14.0 and 19.9%, electrical conductivity 136 and 1663 µS cm-1 and pH 3.5 and 5. The colour L* value ranged between 50.2 and 96.6 indicating that all Nordic unifloral honey samples were rather light. Fructose was the main sugar and total amount of sugars was the highest in raspberry honey. Generally, the amount of MGO in honeys was low or zero. However, the honeys collected from mire or forest areas contained 94-166 mg/kg MGO. One fourth of the studied honeys (11 samples) had high antioxidant activity. With 15% honey dilution only three unifloral honeys had over 85% inhibition against the growth of *P. aeruginosa* where as ten honey samples had high inhibition against of S. aureus. Most bioactive honeys were buckwheat, raspberry and honeydew honeys, but bioactivity did not correlate with phenolics or MGO.

In vitro **efficacy of honeydew honey against** ***Staphylococcus*** **coagulase positive and negative strains of canine origin**

Mihaela Niculae[1] and Laura Stan[2]

[1] Department of Infectious Diseases (Veterinary Medicine): University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, România

[2] Department of Food Science: University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, România

E-mail: laurastan@usamvcluj.ro

Previous studies underlined the beneficial therapeutic properties of different types of natural honeys; among these, antimicrobial and wound-healing activities are of great interest, although some variations are noticed among research results. The aim of the present study was to evaluate the inhibitory properties of honeydew honey against antimicrobial resistant *Staphyloccocus spp* of canine origin and correlate these results with chemical profile of the samples. Authenticity and quality control of honeydew honey samples from different Transylvanian geographical locations was evaluated for compositional criteria proposed by European legislation: pH, acidity, moisture, ash, electrical conductivity, diastase, hydroxymethylfurfural and total polyphenols (Folin–Ciocalteau method). The

values were in the range of authentic and high quality honeydew honey. The honeydew honey samples were investigated *in vitro* for their antibacterial properties based on the results of two diffusion assays against coagulase positive (n=5) and coagulase negative (n=5) *Staphylococcus* strains isolated from dogs presenting external otitis and pyoderma. Both screening tests pointed out an important inhibitory effect when compared to the artificial honey and amoxicillin and clavulanic acid. The inhibition zones varied between 8-20 mm, with the lowest values recorded in case of *Staphyloccoccus pseudintermedius* and *Staphyloccocus schleiferi*, thus suggesting that the antibacterial activity of honeydew honey may be strain-dependent. The honeys were inhibitory at dilutions of 10% (v/v). These results indicate that natural products such as honeydew honey should be considered as valuable alternative in canine dermatology and further *in vitro* and *in vivo* studies are intended to formulate therapeutic protocols.

Changing dynamics of fatty acids concentration in bee pollen during the polliniferous season

Irina G. Kalinina and Rudenko Є.V.
Institute of Animal NAAS of Ukraine, Kharkiv, Ukraine
E-mail: it.lab12@ukr.net

Much attention has been paid to the presence of lipid fatty acids - Omega 3 family, omega-6, omega-9. These fatty acids have the highest biological value, indispensable for a complete regeneration of cells. The dominant family of acid Omega - 3 is α - linolenic acid, omega -6 - linoleic, omega -9 - oleic acid. As the season for bees bees bring pollen from different plants, of course, the content of substances in the feed is different. This entails the need to further study the dynamics of fatty acids.

The aim was to investigate the quality of bee pollen indicators for fatty acid in various pylkonosiv bloom from April to August for 2008 - 2010 years, and the average rate set needs oleic, linoleic and linolenic fatty acids for bdzholorodyn forest steppe zone for decades.

To accomplish the task, polliniferous for season 2008 - 2010 years, bee pollen samples were taken Polyphlore saw in the Kharkiv region, which is typical of the plants and feed crop agriculture and industrial cultural eastern Ukraine. The dynamics of changing concentrations of oleic C18: 1, linoleic C18: 2, C18 linolenic

acid in bee pollen season for polliniferous changed in different ways at different times. Summarizing data Decade for three years of research, was built polynomial trend line (approximation and smoothing), which allowed the correct concentration of oleic, linoleic, linolenic acids for normal development bdzholorodyn spring and summer. There were obtained using regression equations, which were possible to calculate the required concentration of oleic, linoleic, linolenic acid Feeding bees in the spring and summer.

Analyzing the quality of pollen brought by bees, it should be noted that during the most intensive growth of bee colonies coincides with the period of harvesting pollen from high zhyryh acids.

Phatology

Pollen nutrition influences bee survival and viral infection level

Branchiccela B., Corona M., Invernizzi C., Posadas F., Santos E., Zunino P. and Antúnez K
Microbiology Department, Institute for Biological Research Clemente Estable. Montevideo, Uruguay.
E-mail: belenbranchiccela@gmail.com

In recent years, large scale honey bee colony losses have been reported in different countries. It has been proposed that the malnutrition of the bees due to the increase in monoculture areas, linked with pathogen infection are involved in these losses. The aim of this study was to analyze the effects of different monofloral pollen diets on: i) bee survival, ii) expression level of genes related with its nutritional status, its physiology and immune response, and iii) natural infection level of Deformed wing virus (DWV). Bees emerged under laboratory conditions were divided in three groups and fed with: syrup, syrup and pollen from *Eucalyptus grandis* or syrup and pollen from *Baccaris trimera*. Bees fed with pollen survived more than those with protein deprivation showing that protein rich diets are important for bee survival. These proteins rich bees showed similar nutritional status according to the analyzed marker (*mrjp1*). However, bees fed with pollen of *B. trimera* survived more than the ones fed with pollen of *E. grandis* (supported also by the expression level of *vitellogenin* and *methyl farnesoate epoxidase* genes). By the other side, natural infection level of DWV was significantly higher 14 days post emergence in bees fed with pollen of *E. grandis*, which expression level of *pre-phenol oxidase* gene was down-regulated in comparison with the bees fed with pollen of *B. trimera*. These results show evidence that monofloral pollens diets affects differentially the bee physiology and immunity with consequences in their response to pathogen infection.

Improving the quality of laboratory diagnosis for American foulbrood through a proficiency assessment program

Gabriela Chioveanu
Institute for Diagnosis and Animal Health, Bucharest, Romania
E-mail: gabriela.chioveanu@idah.ro

To evaluate the microbiology laboratory performance in Romania over a 5-year period of participation in a quality assessment program and to assess the impact of quality improvement strategies.

Methodology of isolation and identification of *Paenibacillus larvae* bacteria from brood simulated samples, necessary to diagnose American Foolbrood.

Romanian veterinary medical laboratories, authorized by the National Sanitary Veterinary and Safety Food Authority have been subjected to mandatory testing by the NRL for Honey Bee Diseases Proficiency Testing Program of the official laboratories network. This PT Program is used as quality improvement strategies.

Veterinary laboratories were subjected annually to external quality assessment challenges. Performance was assessed against consensus reference values. Single survey and cumulative profiles were reviewed by NRL for acceptable or unacceptable performance. Specific interventions are used to improve collective and individual laboratory performance.

The number of participant laboratories was relatively constant. Since 2011, 90% of the laboratories have scored at or above 95% for isolation and identification, but 10% have scored at or below 50% on susceptibility testing. Poor susceptibility testing performance is due to inappropriate agent selection, not testing errors.

The emphasis of the Laboratory Proficiency Testing Program is on quality improvement, not punishment. Performance has improved, but poor performers have the same characteristics as always. Identification to species is common owing to the use of commercial systems. Automated susceptibility testing has increased to 55% of participants.

Real time PCR coupled to High Resolution Melting Analysis for detection and quantitation of *Nosema ceranae* in honey bees

Francesca Dell'Orco, Monica Loiacono, Francesca Albonico, Sergio Zanzani, Antonella Cersini, Giovanni Formato, Elena Facchini, Mario Colombo and Michele Mortarino
Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy
E- mail:francesca.dellorco@unimi.it

Nosemosis is a gut disease of honey bee, *Apismellifera,*caused by the microsporidia *Nosemaapis*and *N. ceranae*. These two species are morphologically similar but differ in epidemiological pattern and virulence (Fries 2010). Proper control strategies of the infection rely on correct species differentiation and quantitation of parasite load. The discrimination between the two species using the classical methods can be difficult since *N. apis*and*N. ceranae*spores are not easily distinguishable under microscopic examination. Correct species identification thus requires molecular analysis (Fries 2013). Over the last years, several molecular methods with higher sensitivity and specificity compared to classical microscopy were designed. Nevertheless, a need for simple and fast molecular protocols to discriminate between *N. apis* and *N. ceranae* still remains. High resolution melting analysis coupled to real-time PCR (qPCR-HRMA). is a widely used quantitative technique to target sequence polymorphisms in different species without the need to perform DNA sequencing or to use species-specific probes (Reed 2007). The present work allowed to setup a qualitative and quantitative assay for the detection of *N. ceranae*in honey beesand discrimination from*N. apis*infection, based on qPCR-HRMA. The qPCR-HRMA protocolis simpler to use than most other DNA-based methods and provides comparable discrimination between the two sibling species.The results obtained with the real time PCR-HRMA method showed correlation with the results obtained on the same DNA samples using the two reference methodsfor *N. ceranae*: spore microscopical counting and molecular quantitation with the MITOC218 primer pair (http://www.coloss.org/beebook/II/nosema/2/2/2/3).

Beekeeping and recent colony losses in turkey

İbrahim Çakmak

Uludag University, Beekeeping Development-Application and Research Center, Bursa, Turkey,
E-mail: icakmak@uludag.edu.tr

Turkey having at least five subspecies of *Apis mellifera* is a bridging country connecting Europe, Asia and Africa by Middle East and gene center of Western Honey Bee *Apis mellifera*.

Anatolia also has three out of 37 phytogeography rich areas in the world and there are about 10,000 plant species and 3506 of them are endemic to this country. There are recently about 7.709.636 colonies, more than 150.000 families in beekeeping business, 56.000 professional beekeepers, 79 Beekeeping Unions in each province as parts of Central Beekeeping Union of Turkey representing 56.000 professional beekeepers and 107.665 tons of honey production annually in Turkey.

There are a number of factors affecting colony losses in Turkey including Varroosis, Nosemiosis, Foulbrood diseases, Queen Failure, Colony Management, Long Distance Migratory Beekeeping and Pesticides as general and recently new generation of pesticides as Neonicotinoids.

Even though high colony losses are recorded in some regions of Turkey recently colony number in the country is surprisingly increasing almost half a million yearly. Finally, Turkey still has great potential of genetic reservoir of European honey bee, *Apis mellifera* and may provide vital solutions for a number of beekeeping problems in the world facing today.

Honeybee health in South America

Matías Maggi[1], Karina Antúnez[2]*, Ciro Invernizzi[3], Patricia Aldea[4]*, Marisol Vargas[5], Pedro Negri[1], Constanza Brasesco[1], David De Jong[6], Dejair Message[7], Erica Weinstein Teixeira[8], Judith Principal[9], Carlos Barrios[9], Sergio Ruffinengo[10], Rafael Rodríguez Da Silva[4] and Martín Eguaras[1]

[1]- Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CONICET, Mar del Plata, Argentina.

[2]- Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.

[3]- Facultad de Ciencias. Universidad de la República. Montevideo, Uruguay.

[4]- Facultad Silvoagropecuaria de la Universidad Mayor. Santiago, Chile.

[5]- Universidad de Concepción. Chillán, Chile.

[6]- Faculdade de Medicina, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

[7]- Universida de Federal do Semiárido, Mossoro, RN, Brazil.

E- mail: kantunez03@gmail.com; patricia.aldea@mayor.cl

Over the last decade, high rates of managed honey bee colony losses have been reported worldwide. There is no single explanation for those losses. Instead, are the results of synergistic interactions between different stressors, including pest and pathogens, agricultural intensification and use of pesticides. South America is not the exception; several cases of colony losses were reported by beekeepers throughout the continent, although, no accurate, comparable data, or systematic nation-wide surveys have been conducted.

Therefore, this study attempts to analyze the situation of honey bee health in South America, specifically in Argentina, Chile, Uruguay, Brazil, and Venezuela. A mean of 30% of colony losses was estimated per year, at least in temperate climates. The impact of the main parasites and pathogens affecting honey bee colonies, including *Varroa destructor*, RNA virus, *Nosema* spp., *Paenibacillus larvae*, *Melissococcus plutonius* and *Acarapis woodi*, and their role in colony losses in different countries is discussed. We also contemplate the main challenges that each nation must confront with regards to honey bee health.

This study is intended to serve as a comparison with future colony losses studies as well as to provide guidance for future hypothesis-driven research on the causes of colony losses.

Nosema ceranae goes on killing colonies in Spain

Miguel Llorens-Picher, Aránzazu Meana, Cristina Rodríguez, José Bernal, José Luis Bernal, María Dolores Hernando, Raquel Martín-Hernández and Mariano Higes
Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid
E-mail:migllore@ucm.es

A beekeeper reported low efficacy of the treatment against *Varroa destructor*, high levels of phoretic mites and high colony mortality rates. Fifty-four colonies with the same symptomatology were sampled to determine the underlying reason. Visual inspection allowed determining colony strength and other disease signs. The test for mite resistance to pyrethroids and coumaphos designed by Dr. J. Pettis (USDA-ARS Bee Research Laboratoy, Beltsville, Maryland) was performed to detectpossible mites resistantto previous applied treatment against *Varroa* (Checkmite® MA coumaphos). Adult bees and stored pollen samples were randomly takento performa broad pathogen analysis and detection of neonicotinoid insecticidespresence. All analyses were performed using previously published methods.

Inspection confirmed a precarious health status: twelvecolonies (22.22%) were found dead and most of them(46.29%) had less than 20% of the frames covered by bees. Most common findings were chalkbrood mummies (38.88%), phoretic mites (37.03%), and bees with deformed wings (14.81%). Mean *Varroa* phoretic levels were 9.4% (sd 2.4) and no resistances to coumaphoswere detected.Bee samples analized by PCR showed presence of *N. ceranae*, BQCV, DWV and trypanosomatidsin all sampleswhile were negative to *N. apis* and neogregarines.Percentages of *N. ceranae*were far higher than 40% of inner bees with spores, a parameter previously related with colony deaths. Pesticide residue test did not detect any of the following: acetamiprid, clothianidin, fipronil, imidacloprid, thiacloprid and thiamethoxam.All the data from the analysis supported the implication of *N. ceranae*playing a leading role in the deaths observed.

Work founded by INIA-FEDER (RTA2013-00042-C10-03-06).

Metabolization of a neonicotinoid and interaction with the chronic bee paralysis virus in honeybees

Marianne Coulon, F. Schurr, A.-C. Martel, N. Cougoule, A. Bégaud, P. Mangoni, A. Dalmon, C. Alaux, Y. Le Conte, R. Thiéry, M. Ribière-Chabert and E. Dubois
Anses Sophia Antipolis, 105 route des Chappes, CS 20111, F-06902 Sophia Antipolis cedex, France
E mail: marianne.coulon@anses.fr

Multiple factors are suspected of being the cause of the recent honeybee decline, either by themselves or in combination with others. Amongst these factors, and based on field observations, we have chosen to study the effects of a co-exposure to thiamethoxam, a neonicotinoid insecticide, and the chronic bee paralysis virus (CBPV).

Thiamethoxam is known to be rapidly converted to clothianidin in both plants and insects. We first studied the kinetics of this metabolization in honeybees fed with supplemented syrup at a thiamethoxam concentration of 0.28 ng/bee/day (mean consumption measured), over 18 days. We found by LC-MS/MS that the metabolization rate of thiamethoxam increased over time, leading to the insecticide being present only as traces after 12 days, while the bees were still exposed to it. Clothianidin seemed to accumulate in honeybees. The survival rates of bees fed with thiamethoxam were not significantly different from control bees.

We subsequently exposed honeybees to various doses of thiamethoxam (from 0.28 to 5ng/bee/day) and to infection by CBPV via exposure to infected honeybees, thus reproducing the natural route of infection. Mortality and CBPV loads were recorded. No significant effects were observed on CBPV viral loads in bees co-exposed to the different doses of thiamethoxam. However a synergistic effect was observed on mortality at the highest thiamethoxam dose, compared to the sum of the observed effects in honeybees exposed to thiamethoxam or CBPV alone.

Further studies investigating potential changes at the immune and detoxification levels will help to better understand such interactions.

Distribution of pathogens in honey bee colonies from Azores and Madeira archipelagos.

Raquel Martín-Hernández, Mariano Higes, Carmen Rogerio, Cátia Neves, Dora Henriques, Julio Chávez-Galarza, Irene Muñoz, João C. Azevedo, Pilar De la Rúa and M. Alice Pinto.
Laboratorio de Patología Apícola. CIAPA, IRIAF, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha. INCRECYT, Parque Científico de Castilla- La Mancha, Marchamalo, Guadalajara, Spain
E mail: rmhernandez@jccm.es

Honey bees are recognized as one of the most important pollinators. Nowadays they are facing a substantial impact in their populations being pathogens amongst the greatest threats. Unwanted spread of pathogens can occur through the introduction of honey bee queens or movement of colonies, as illustrated in recent studies on the Canary Islands. In this context, we present the results of the study of the health status of honey bee colonies located in other archipelagos of the Macaronesian region (Azores and Madeira). These islands are particularly interesting because four out of the nine islands sampled have been recently colonized by *Varroa destructor* while other five are still Varroa-free. This allows to study the interactions among the different pathogens and parasites detected on each island.

Here we present the first results on the prevalence and geographical distribution patterns of the mite *V. destructor*, Microsporidia (*Nosema apis* and *N. ceranae*), Diptera (*Braula coeca*) and some viruses. A total of 576 colonies (three per apiary) from Madeira and the Azores archipelagos were sampled between July and August of 2014 and 2015. This comprehensive survey provides new insights into the influence of insularity and apicultural trade on pathogen dispersal in island honey bee populations.

We acknowledge the "Direção Geral de Agricultura dos Açores e Madeira" for their inestimable collaboration in the sampling. Funded by RTA2012-00076-C02-01 (INIA-FEDER), 19908/GERM/2015 (Fundación Séneca del Gobierno Regional de Murcia), and the 2013-2014 BiodivERsA/FACCE-JPI joint call for research proposals with the Portuguese Foundation for Science and Technology (FCT).

First record of *Megaselia scalaris* (Diptera: Phoridae) parasitizing *Apis mellifera* (Hymenoptera: Apidae).

Menail Ahmed Hichem, Piot Niels, Meeus Ivan, Smagghe Guy and Ayad-Loucif Wahida.
Laboratory of Applied Animal Biology, Faculty of Science, Badji Mokhtar University, 23000-Annaba, Algeria.
E mail: menail.hichem@yahoo.fr

The honey bee *Apis mellifera* was so far the host of two Phorid fly species; *Apocephalus borealis* and *Megaselia rufipes*. During laboratory experiments on *Apis mellifera intermissa*, we noticed a parasitism of honey bees by a fly, leaving the former ones as empty exoskeletons. DNA and RNA extraction was done on samples of adult flies and larvae. A PCR was performed on DNA to identify the fly species. The infected honey bees came from a hive infected by Deformed Wing Virus located in Annaba, Algeria. So, a standard PCR to detect DWV was performed on adults and larvae phorid fly RNA, followed by a negative strand detection of the virus as proof of a true infection which was done by a multiplex ligation-dependent probe amplification technique (MLPA). PCR revealed that the Phorid flies belong to *Megaselia scalaris* species. Both fly stages were positive for DWV but the negative strand of the virus was detected only in the larvae. To our knowledge, this is the first time this virus is detected in *M. scalaris.* These discoveries shed light on the new threat *M. scalaris* can be for honey bees and on its possible role as a new vector of DWV.

First molecular detection of *Crithidia mellificae*, *Apicystis bombi* and *Paenibacillus larvae* in the Algerian honey bee; *Apis mellifera intermissa.*

Menail Ahmed Hichem, Piot Niels, Meeus Ivan, Smagghe Guy and Ayad-Loucif Wahida.

Laboratory of Applied Animal Biology, Faculty of Science, Badji Mokhtar University, 23000-Annaba, Algeria.
E-mail: menail.hichem@yahoo.fr

In the northern hemisphere, global warming is triggering the migration of biotopes northwards. This situation might lead to the emergence of southern honey bee subspecies north of their usual distribution. Studying these subspecies like *Apis*

mellifera intermissa, native to North Africa (Algeria, Morocco and Tunisia) is very important as it is adapted to high temperatures. Since the nineties, honey bees are facing a lot of threats like pesticides, ecosystems degradation and increasing number of pathogens. To understand pathogen spreading in the context of globalization and climate change, we performed a pathogen screening in *Apis mellifera intermissa*. Two surveys were done during 2013 and 2014. Asymptomatic hives of 18 apiaries from 12 geographical locations were sampled in the region of Annaba (36°54'0 N and 7°46'0 E), the extreme North-East of Algeria. An average of 30 honey bees and 10 larvae were sampled from each hive and stored in 70% ethanol. PCRs were performed for parasites, fungi and bacteria detection. Results confirmed the presence of *Nosema ceranae* and *Paenibacillus larvae* in Algeria and revealed for the first time the occurrence of *Apicystis bombi* and *Crithidia mellificae*. *A. bombi* detection reflects its arrival in Africa. Neither *Melissococcus plutonius* nor *Acarapis woodi* were detected during our screening. Our study confirms the global spreading of honey bee pathogens. However, because sampled hives didn't display typical symptoms triggered by detected pathogens i.e, American foulbrood, nosemosis, *A. mellifera intermissa* known for its cleaning behaviour seems to be well adapted to the worldwide sanitary situation and global warming.

Diversity of pathogens of Honeybee (*Apis mellifera unicolor*) in the South-West Indian Ocean area

Olivier Esnault, Deodass Meenowa, Preaduth Sookar, Jimmy Mélanie, Maria Tirant, Marc Naken, Marcellin Biarmann, Henriette Rasolofoarivao, Marie-Pierre Chauzat and Hélène Delatte
GDS Réunion & CIRAD UMR PVBMT, **GDS Réunion** 1, rue du Père Hauck, 97418 La Plaine Des Cafres, Réunion
E-mail: olivier.esnault@gds974.re

In the South West Indian Ocean, apiculture is a high value activity, important in Madagascar, Mauritius and Rodrigues, but lesser in the Seychelles. Sanitary status and pathogen diversity of the endemic honeybee subspecies (*Apis mellifera unicolor*) populations on the different island of the area were poorly known and studied.

The survey was conducted on 60 colonies randomly sampled in September 2014 for Mauritius, 50 colonies in October 2014 for Rodrigues, 43 colonies in April 2015 for the 3 main islands of Seychelles and 43 colonies in May 2015 for Madagascar. For each colony, 50 honeybee forager workers were sampled. Incidence and severity of several pathogens and parasites were investigated: 3 ectoparasites (*Varroa destructor*, *Tropilaelaps sp*. and *Braula sp.*), 2 bacteria (*Melissococcus plutonius* and *Paenibacillus larvae*), 2 nosema species (*Nosema apis* and *N. ceranae*) and 6 viruses (ABPV, BQCV, CBPV, DWV, KBV and SBV). During this survey mortalities and abnormal clinical signs were also investigated.

Different pathogen patterns were observed within the different islands (prevalences and diversity). *Nosema ceranae*, BQCV, CBPV and DWV were detected in each country, while *Varroa destructor* was detected in only two countries: Madagascar and Mauritius. No *M. plutonius* nor *P. larvae* were detected in the SWIO area.

All these results are discussed in the light of a tropical bee sanitary context with or without *V. destructor* and indigenous or exotic *Apis mellifera* subspecies.

Microsporidia prevalence in bumblebee species from Spain

María Benito, Concepción Ornosa, Mariano Higes, Raquel Martín-Hernández and Pilar De la Rúa
BeePathologyLaboratory, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal. Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha. Marchamalo, 19180 Guadalajara, Spain
E-mail:pdelarua@um.es

Spread of parasites are among the main causes of worldwide pollinator decline. One example are Microsporidia of the genus *Nosema*Nägeli, 1857. These obligate intracellular parasites have significant negative effect on individual bees, colony fitness and development, and show a high prevalence in temperate countries as Spain where it has been related with extensive honeybee colony losses. These parasites have also been observed in bumblebees: the species *Nosemabombi* is

highly prevalent in North America, while *Nosemaceranae* has been found inbumblebeesin South America, Europe and Asia.

Herein we present the results of a comprehensive sampling of 1013 bumblebees collected during 2013, 2014 and 2015, in National Parks from northern, central and southern Spain (Pyrenees, Sierra de Guadarrama and Sierra Nevada respectively), and other complementary points where these pollinators display high diversity. We conducted *Nosema* detection at both genus and species levels with molecular techniques (PCR amplification of a fragment of the ribosomal gene and sequencing). We aim to test the hypothesis stating that the populations of bumblebees owe their decline to the high prevalence of these pathogens. A low prevalence of Microsporidia (2.7%) was detected. We found *Nosemabombi* in 20 bumblebees and *Nosemaceranae* in one, whereas six bumblebees were infected by new *Nosema* genetic variants. Further studies are needed since other driversmay be implicated in the decline of bumblebees in this territory.

New primers for VDV-1 detection

Katrine Hansen Lemming and Per Kryger
Aarhus University, Department for Agroecology, Forsøgsvej 1, 4200 Slagelse, Denmark
E-mail:katrine.lemming@gmail.com

The colony losses have increased especially in USA and on a smaller scale in Europe. In 2014 a Kenyan beekeeper announced that also he had problems with big losses of his colonies, spread across South-Western Kenya. He discovered Varroa mites (*Varroa destructor*) in his hives. Varroosis is thought to be one of the main reasons the colonies are collapsing, due to virus transmission by the mite, in particular Deformed Wing Virus (DWV). Severe levels of DWV are responsible for deformed wings and a high mortality rate in the honey bees.
Samples from the Kenyan beekeeper were sent to Denmark to be screened for several viruses: acute bee paralysis virus complex, sac brood virus, black queen cell virus and DWV, but none of these found. Similar to previous studies from Kenya and South Africa however, the Varroa destructor Virus (VDV-1) was detected in 3 of 20 brood samples. Analysis of beta-Actin confirmed that all samples had arrived in Denmark with intact RNA.
Mutations can arise over time and space and the primers published in 2008 might not fit perfectly current variants of the virus in Africa. Hence, new VDV-1qPCR primers were developed based on sequences from the three positive samples. The new primers only detected one additional case with VDV-1, in this case in Varroa mites taken from the brood of a fourth colony. Danish bees with clear symptoms of deformed wings have since tested positive with the new primers too.

Honey bee viruses in Lithuania

Laima Blažytė-Čereškienė, Vesta Skrodenytė-Arbačiauskienė, Sandra Radžiutė, Violeta Apšegaitė and Vincas Būda
Nature Research Centre, Akademijos St. 2, LT-08412 Vilnius, Lithuania
E-mail: blazyte@ekoi.lt

Within the Lithuanian honey bee population, six honey bee viruses: Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), Deformed wing virus (DWV), Sacbrood virus (SBV), and Black queen cell virus (BQCV) were studied by application of molecular methods. A three-year study (2012-2014) revealed the dramatic increase of virus-infected colonies from 66.2 % in 2012 to 90.6 % in 2014. BQCV and SBV were the most widespread viruses with higher prevalence in summer, compared to autumn. The prevalence of DWV was lower compared to other European countries, whereas ABPV and IAPV were not detected, thus estimated much less spread compared to western EU countries. A Single colony was infected by KBV. No differences in prevalence of any virus within the country were detected.

The decrease of single virus infections and increase of double and triple infections during the three-year period were revealed. BQCV and SBV were involved in multiple-infections most often.

A phylogenetic analysis revealed that the partial polyprotein gene sequences of the viruses detected in Lithuanian honey bee colonies, were closely related to those of viruses recorded in European countries, e. g. Hungary, UK, Belgium, and Germany. Two European and Asian geographically separated genotypes of both SBV and DWV in honey bee populations were revealed.

Field trials on trial - Evaluation of the information of honeybee field test for pesticide risk assessment

Simon-Delso N, Aupinel P, Brandt A, Colli M, Fourrier J, Giffard H, Gregorc A, Hatjina F, Hernández-López J, Laurino D, Malagnini V, Manino A, Medrzycki P, Porporato M, Renzi T, Sgolastra F, Tosi S and Williams G
Place Croix du Sud 4 te L7.7.9, 1348 Louvain la Neuve Belgium
E- mail: simon@cari.be

Field trials (FT) have been traditionally employed as tests used to ultimately determine the risk of a pesticide for honey bees in the risk assessment scheme, the so called highest tier. Methodological guidelines exist for their development (e.g. EPPO 170, CEB 230); however, either they are not systematically used when developing tests for regulatory purposes or they remain imprecise. As a result, risk assessors have access to variable information for the authorisation of pesticides in Europe. The objective of this study is to evaluate the quality of FTs performed to date for regulatory purposes. Test summaries publicly available in the Draft Assessment Reports of different active ingredients (aa.ii., n=6) were randomly allocated and evaluated by bee ecotoxicologists (n=17), each one receiving 2 summaries. Reviewers did not know either the a.i. nor the authors/company/etc developing the study allocated to them. The purpose of the exercise was to verify and score the quality of the information included in these summaries according to the criteria proposed by the EFSA guidance document for the risk assessment of pesticides on bees (2013) related to exposure and toxicology assessment, statistical requirements, design of the FT and duration and frequency of assessments. The results will be discussed in the view of improving the quality of information available for risk assessment purposes and public availability of data.

Evaluation of propolis of stingless bee for the control of Nosemosis in *Apis dorsata*

Guntima Suwannapong[1]* and Mark Eric Benbow[2]
[1*]Department of Biology, Faculty of Science, Burapha University, Chon Buri 20131, Thailand
[2]Departments of Entomology and of Osteopathic Medical Specialtie, Michigan State University, East Lansing, MI 48824, USA
E-mail:guntima@buu.ac.th

The potential of natural product, propolis of stingless bee, *Trigona apicalis* was evaluated for the control of *Nosema ceranae* infected giant honeybee, *Apis dorsata*. Newly emerge of *Nosema* free bees were individually fed with 2 µl of 50% sucrose solution (w/w) containing 250, 000 spores per bee and then fed with 50 and 70% propolis extract (v/v) of stingless bees compared to those of controls. All propolis treated bees showed significantly higher survival rate than those of untreated bees. Interestingly, propolis extract can increase protein content of hypopharyngeal glands, while it reduced the trehalose level in bee haemolymph and protease activity compared to those of infected bee without propolis treatments. The result suggests that probably propolis extract might have direct negative effect on *N. ceranae* spores.

Effectiveness of treatments with thymol in controlling varroa destructor parasite of the honey bee in Algeria

Noureddine Adjlane , Ounesse Tarek and Nizar Haddad
M'hamed Bougara University,Department of Biology, Boumerdes, 35 000 Algéria
Laboratoire de Biologie et de physiologie animale, ENS kouba Algérie
E-mail: adjlanenoureddine@hotmail.com

Varroa disease is a parasitic disease of adult bees and brood, due to a blood-sucking external parasitic mite, Varroa destructor. This is one of the most dangerous diseases in Algeria. The purpose of this study is to evaluate the effectiveness of two treatments approved based thymol (Thymovar® and Apiguard®) in the fight against varroa in Algerian conditions and to determine the side effects of these treatments on bee colonies local Apis mellifera intermissa. The

test was performed on 80 colonies with two different formulations for each treatment. As the results show in our test, a wide variation in the effectiveness between colonies is recorded for both treatments. The average efficiency for the group of colonies ½ Thymovar strap in two applications stood at 84.43%. This efficiency is lower for the group ½ straps in one application (77.23%). For Apiguard, the average efficiency is 81% for formulation 2 x 25g spaced two weeks, against a 79% rate for the formulation of 2X25 spaced one week. During our tests, with a few dead bees and nymphs found on nappies, we never recorded unusually high bee mortality. Further tests are needed to determine the optimal dose and the best time for treatment.

Evaluation of the resistance of the mite *Varroa destructor* to the Amitraz in colonies of honey bees (*Apis mellifera*) in Algeria

Noureddine Adjlane and Nizar Haddad
M'hamed Bougara University,Department of Biology, Boumerdes, 35 000 Algéria
Laboratoire de Biologie et de physiologie animale, ENS kouba Algérie
E-mail : adjlanenoureddine@hotmail.com

Varroa mite has become a major concern of beekeepers in Algeria since the discovery of the first cases of infestation in the year 1982. Amitraz is the predominant compound used in Algeria to control *V. destructor*, its constant application has caused the appearance of resistant mite populations to this product in several parts of the world. This study was conducted to detect the possible existence of populations of resistant mites to Amitrea in Algeria. To determine the mites mortality percentage to the Amitraz, they were exposed to a trips of 2.5 x 1.0 cm. Varroa mortality in apiaries treated with Amitraz was 39.23%, lower than the 87.40% mortality obtained in apiaries that only received an alternative treatment. A significant difference ($P > 0.05$) was found between two mortality of Varroa. These results show for the first time the existence of *Varroa destructor* populations resistant to Amitraz in Algeria.

Isolation and Identification of bacterial microflora from heamolymph of emerging worker honeybee (*Apis mellifera intermissa*) parasitized by *Varroa destructor*

Messaouda Belaid, Farida Benzina and Fatma Acheuk
Département de Biologie, Faculté des Sciences, Université M'Hamed Bougara de Boumerdes. Avenue de l'Indépendance-35000-Algérie.
E-mail: belaidfo@yahoo.fr

Varroa destructor is an obligatory ectoparasite of the honeybee (*Apis mellifera* L). The mites use their piercing monthparts to suck out heamolymph from immature and adult bees caused direct damage (morphological, physiological abnormalities) and indirect damage due to microbial pathogens. The aim of this work is to research the bacterial microflora in heamolymph of emerging worker honeybee parasitized by *Varroa destructor.* The results show that the isolates were *Bacillus sp, Pseudomonas sp, Serratia sp* and *Aeromonas sp.*

Influence of two different Varroa summer treatments on honey bee hemolymph proteome

Cabbri R., Ferlizza E.,Isani G., Galuppi R., Tampieri M.P. and Nanetti A.
Department of Veterinary Medical Sciences, Alma Mater Studiorum – *University of Bologna*
E-mail:rcabbri@gmx.com

In summer, when Varroa infestationis important, a highly effective acaricidal treatment is needed to obtain successful wintering of the colonies.
Mediterranean climates are particularly demanding due to the long reproductive season of the mite, so the use of hybrid approaches (mechanical plus chemical) are gaining ground among beekeepers.
The two main techniques, brood interruption and brood removal, rely both on obtaining a broodless period to treat on phoretic mites only.
The aim of this research is to explore the effect of the abovementioned techniquesat the proteomic level, beyond the mere acaricidal efficacy.
Specifically, we focused on vitellogenin, the main hemolymph protein, already proposed as a colony vitality parameter in other studies.

This glicolipoprotein, component of the egg yolk in all oviparous, assumes other roles in honeybees: trophic, antioxidant, hormonal and immunological.
Two groups of five colonies each were arranged: one underwent brood interruption and the other brood removal according to the COLOSS Varroa Task-force protocols.
A pool of hemolymph from 30 bees was sampled for each colony in four critical moments: pre manipulation, after manipulation, autumn(wintering phase) and winter.
All the samples were subjected to SDS-PAGE and vitellogenin was isolated, identified and quantified.
As predicted the techniques did have a different impact on colonies with a significant lower vitellogenin abundance, after the manipulation, in brood removal group.
This is not surprising since brood removal is a much more radical technique, involving the split of the colony in a moment of natural population contraction.

The effect of subliminal applications of formic acid on the reproduction and population dynamic of Varroa destructor in honey bee (Apis mellifera) colonies

Peter Rosenkranz, Sebastian Nicodemus, Jasmin Fidyka and Bettina Ziegelmann
Apicultural State Institute, University of Hohenheim, August-von-Hartmann-Str. 13, 70599 Stuttgart, Germany
E-mail: peter.rosenkranz@uni-hohenheim.de

In beekeeping practice Varroa treatments usually start at the end of the season after honey flow. However, at that time the damage threshold has often been exceeded due to the exponential growth of the Varroa population. A reduction of the mite´s reproductive success during the breeding period could prevent such damages in honey bee colonies. We here show how mite reproduction within the honey bee brood cell can be disturbed by subliminal applications of formic acid (FA). FA 60% was evaporated in two-story hives at 10 – 15 ml per day. After a treatment period of 7 days, combs were examined for living and dead mite stages and the numbers of spermatozoa in adult daughter mites were counted. It could be shown that even low evaporation rates of FA lead to a higher mortality of the sensitive nymph stages, whereas honey bee brood and adult mites were hardly

affected. However, in brood cells with living males and daughter mites more than 90% of these daughters did not have spermatozoa at all. Furthermore, we performed a field test in full sized colonies where this subliminal FA was applied twice during the season. At the end of the season, the treated colonies revealed a significant lower mite infestation compared to untreated control colonies. This confirms that a subliminal FA treatment – that didn´t cause visible harm to the developing bees - can disturb the reproducing Varroa females within the brood cells leading to a sustainable effect on the growth of the mite population

Application of next generation sequencing (NGS) technologies to identify microbial factors important to the survival of a Swedish Varroa resistant honey bee population

Srinivas Thaduri, Barbara Locke, Eva Forsgren and Joachim de Miranda
Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
E-mail: srinivas.thaduri@slu.se srinivas.thaduri2801@gmail.com

The parasitic mite Varroa destructor in combination with viruses it transmits is main cause for honeybee colony losses worldwide. However, there are several feral honeybee populations world-wide that manage to survive long-term without Varroa control. The best studied of these is on Gotland. Central to colony survival, particularly during winter or other long brood-less periods, is the health and longevity of its adult bee population. Recent studies have shown major differences between Gotland's Varroa-surviving 'Bond' bees and non-resistant bees in the levels of certain pathogens, which manifests itself increasingly towards autumn. The objective of this study was to confirm these findings, and to identify possible other metagenomics and transcriptomic differences between 'Bond' and control bees relevant to survival, using next generation sequencing (NGS) technologies.

The original analyses identified four of the most common honeybee viruses (Deformed wing virus (DWV), Sac brood virus (SBV), Black queen cell virus (BQCV) and Kashmir bee virus (KBV)) with real time PCR assay. The NGS analysis results further justified our real time PCR results where control colonies have higher SBV and BQCV titters then Bond colonies in autumn season. In

addition to this, with NGS analysis we also discovered few more viruses like Varroa destructor virus-1, Lake Sinai virus, VDV-1/DWV recombinant 4.etc. Follow-up studies will be performed with PCR to further characterize the newly identified virus levels between Bond and Control colonies.

Effect of oxalic acid on mite Varroa destructor and its host honey bee (Apis mellifera, L.)

Ivana Papežíková, Miroslava Palíková, Michael Fronc, Radka Heumannová and Stanislav Navrátil
University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1/1946, Brno, 612 42, Czech Republic
E-mail: papezikovai@email.cz

This work was aimed at evaluation of oxalic acid effect on mite Varroa destructor and its host western honey bee (Apis mellifera, L.). First, toxic effect of oxalic acid was studied on isolated Varroa mites. Further, oxalic acid effect was studied on Varroa mites parasitizing caged bees, which were treated per os or topically (by trickling or by sublimation). Effect of oxalic acid on individual bees was also studied, with focus on their lifespan, midgut morphology and function and morphology of Malpighian tubules. It was found that all modes of oxalic application (trickling, sublimation and per os) exerted strong acaricidal effect, which was mediated by direct contact, but also by oxalic acid-containing hemolymph of treated bees. Concerning health of treated bees, topically applied oxalic acid in doses used by practical beekeepers for routine treatments did not cause epithelial destruction in midgut and Malpighian tubules, or loss of digestive tract function. However, trickling with oxalic acid significantly decreased lifespan of treated bees. Thus, sublimation should be always preferred to trickling during field treatments, despite the higher expenses and labour input associated with this mode of application.

Conduct of studies to obtain authorisation of new veterinary medicine products for honey bees in EU

Gabriele Braun, Beate Lohr, Claudia Schneider and Klaus Hellmann
Gabriele Braun, Klifovet AG, Geyerspergerstrasse 27 80689 München, Germany
E-mail:gabi.braun@klifovet.com

The number of veterinary medicinal products (VMP) authorised for honey bees in EU is relatively small. Currently ~160 products in 29 EU countriesare on the market, with the vast majority of products licenced for the control of varroatosis.

For authorisation,beyond the quality a dossier with data on safety (target animal, user, consumer, environment)and efficacy (dose finding, dose confirmation, field efficacy) is required.

In Europe Directives 2004/28/EC and 2009/9/EC amending 2001/82/EC require clinical studies to obtain a marketing authorization for a VMP. Species and product specific European guidelines (e.g. Guideline on veterinary medicinal products controlling *varroa destructor*parasitosis in bees (EMA/CVMP/EWP/459883/2008)) give additional guidance to conduct valid studies.

Field studies should represent different beekeeping practices and geographical and climatic regions. A test permit and (preliminary) withdrawal period for honey are necessary. Studies need to follow a defined quality standard (Good Clinical Practice, VICH GL9). Study procedures, statistical hypothesis and method,as well as sample size should be pre-defined. Studies should be randomised, blinded and negative-controlled,and the VMP handled under surveillance of a veterinarian.

For a field study evaluating a VMP controlling varroatosis, relevant parameters need to be evaluated:

General aspects: details on apiaries, hive types, weather records.

Safety: colony strength determination (bees, brood), dead bee counts, queen viability, flight activity, overcoming winter, development in spring, honey production.

Efficacy: based on % mite reduction= (T1x100)/(T1+T2), where:T1 is no. mites killed by treatment under investigation, and T2is no. mites killed bylicensed follow-up treatment.

Detection of deformed wing virus by PCR in honey bees colonies Apis *mellifera intermissa* in southern Algeria

Noureddine Adjlane * and Nizar Haddad
* M'hamedBougara University of Boumerdes, Department of Biology
Laboraoire de Biologie et de Phyiologie Animale, ENS de Kouba, Alger, Algérie
E-mail: adjlanenoureddine@hotmail.com

Honey bees are threatened by many pathogens including honeybee viruses Among these viruses, deformed wings virus (DWV), it is considered as one of the most prevalent viruses in honeybee colonies and it is directly related to weakening and losses of infected colonies.

The aim of this study was to determine the prevalence of this virus in some apiaries in southern Algeria and to highlight the relationship between mortalities of these colonies and the presence of the virus. Sampling was carried out in Djelfa, El Bayadh, Laghouat, and Ain seffraBechar on bee colonies of *Apis mellifera intermissa* race. We collected 45 samples of adult honeybees , each samples represents one apiary with mortality rate is higher than 10%. . For the detection of the virus RNA is extracted using the NucleoSpin® RNA II kit (ACHEREY-NAGEL). Reverse transcription of RNA and DNA amplification is performed using a continuous process by the RT-PCR method with the RT-PCR Kit (Qiagen) according to manufacturer's recommendations. The results show a variation in the prevalence of the virus between apiaries and study areas. Apiaries the Bechar region recorded the highest rate of infection (45%). The least contaminated zone is that Djelfa with a rate of 25%. No correlation was detected between recorded deaths and prevalence of this virus. Other causes may be the source of the colonies marked losses in these regions.

Effects of pollen traps on the replication of chronic bee paralysis virus (CBPV) in honey bee (*Apismellifera*) colonies

Eric Dubois, Caroline Reis, Frank Schurr, Nicolas Cougoule and Magali Ribière-Chabert
Anses, European Union Reference Laboratory for HoneyBee Health, 105 route des Chappes, CS 20111, F-06902 Sophia Antipolis cedex, France
E mail: eric.dubois@anses.fr

Confinement of honey bees inside hive sincreases contact between individuals and promote the horizontal transmission of the chronic bee paralysis virus (CBPV). This can lead to clinical episodes mainly in strong colonies. In the present study, we aimed to assess whether the pollen traps would be a factor for development of chronic paralysis and for increasing of viral loads in honey bee colony population.

After a recent episode of chronic paralysis, five asymptomatic hives were separated into two groups: one group with pollen traps (three hives) and one group without traps (two hives). Bees were regularly sampled inside the hives and at the hive entrance, and analysed by real-time PCR for CBPV quantification.

Before the setup of pollen traps, viral loads higher than 10^{10} (10 $\log_{10}$) copies of viral genome per bee were only found in few dead bees and in the foragers collected at the entrance. Nurse bees and larvae had low viral loads (<4 $\log_{10}$). During the experiment, the viral loads decreased in bees from hives without pollen traps while they remained high (>10 $\log_{10}$) in alarge amount of symptomatic bees and in hundreds of dead bees in front of hives with pollen traps. In addition to reducing the hive entrance, these traps have impeded the elimination of dead bees resulting in their accumulation inside the hives. Clinical signs of the disease persisted during three weeks. They disappeared with the withdrawal of traps which was also concomitant with a decrease of viral loads in foragers. Results of this study indicate that it could be preferable to reduce the time of trapping period in order to limit chronic paralysis impact on colonies.

Virome composition of *Apis mellifera* colonies infested with *Varroa destructor*

Sofia Levin[1], Noa Sela[2] and Nor Chejanovsky[1]
[1]Entomology Department and [2]Department of Plant Pathology and Weed Research, Institute of Plant Protection, Agricultural Research Organization, Israel
E-mail: ninar@volcani.agri.gov.il

Varroa destructor infestation of *Apis mellifera* colonies carries and/or promotes replication of honey-bee viruses. Some of them like Deformed wing virus, Varroa destructor virus-1, Acute bee paralysis virus, Israeli acute bee paralysis virus and Kashmir virus have been well described and characterized, but others viruses associated with Varroa remained unknown. To learn about the viral population carried and exchanged between *V. destructor* and *Apis mellifera* in untreated Varroa-parasitized colonies we performed deep sequencing (RNA-seq), a technique that enables characterization of the virome richness, including less abundant viral components. Contig-assembly and blast analysis enabled identification of known viruses like DWV, ABPV and IAPV as well as insect and plant viruses unknown in this host-parasite system. After establishing a criteria to estimate the relevance of our findings we validated the presence of relevant new viruses in Varroa-infested honey bee colonies. Our data enabled characterization of new viruses that replicate in Varroa-infested colonies.

Accuracy of the real-time PCR for the quantification of the chronic bee paralysis virus (CBPV) evaluated throughan inter-laboratory study

F. Schurr, M. Ribière-Chabert and E. Dubois
Anses, European Union Reference Laboratory for HoneyBee Health, 105 route des Chappes, CS 20111, F-06902 Sophia Antipolis cedex, France
E mail: eric.dubois@anses.fr

The chronic bee paralysis virus (CBPV) causes massive mortalities in front of the colonies and disorders in individual bees. Some of the clinical signs frequently reported, such as trembling bees,may be confused with intoxication syndromes. Therefore, laboratory diagnosis is performed by quantifying CBPV loads by real-time PCR. Clinical signs of the chronic paralysis are usually associated with viral loads higher than 10^8 copies of the CBPV genome per bee (8

log_{10} cp./bee). This threshold is used by the European Union Reference Laboratory for Honey Bee Health for the diagnosis of the disease.

The accuracy of measurements at two levels of CBPV load(8 and 9 log_{10} cp./bee)was assessed throughan inter-laboratory study,in 2015.Twenty-one participants, including 16 European National Reference Laboratories, received 12 homogenates of CBPV-infected bees adjusted at both levels. The participants were requested to use their method employed for routine diagnosis (or the official method). Quantification resultsof the test (n=312) were analysed according to the international standard, ISO 13528. The standard deviation of measurement reproducibility (S_R) was 1.06 and 1.16 at the viral loads8 and 9 log_{10} cp./bee, respectively. The uncertainty of measurement ($U = 1.96\ S_R$) at the diagnosis threshold (8 log_{10} cp./bee)was about 2.08log_{10}cp./bee.

These results highlight the need to take into account the uncertainty of measurements in epidemiological studies using results from different laboratories. Considering this uncertainty, viral loads over6 log_{10} cp./bee could be considered as indicating probable cases of chronic paralysis.

Potential for virus transfer between European and Asian honey bees in mixed-species apiaries

Orlando Yañez[1,2], Huo-Qing Zheng[3], Xiao-Ling Su[4], Fu-Liang Hu[3], Peter Neumann[1,2,6] and Vincent Dietemann[2,5]
1 Institute of Bee Health, University of Bern, Bern, Switzerland
2 Agroscope, Swiss Bee Research Center, Bern, Switzerland
3 College of AnimalSciences, Zhejiang University, Hangzhou, China
4 Jinhua Academy of Agricultural Sciences, Jinhua, China
5 Social Insect Research Group,Zoology and Entomology Department, University of Pretoria, Pretoria, South Africa;
6 Bee Protection Laboratory, Department of Biology, Facultyof Science, Chiang Mai University, Chiang Mai, Thailand
Institute of Bee Health, Vetsuisse Faculty, University of Bern, Switzerland
Schwarzenburgstr. 161, CH-3003 Bern, Phone: +41 (0)31 631 57 66
E mail:orlando.yanez@vetsuisse.unibe.ch

Honeybee viruses have a much broader host spectrum than previously thought, suggesting occurrences of host shifts. This is of considerable concern as

emerging viral diseases are often the product of host shifts. The introduction of the European honeybee (*Apis mellifera*)into Asia exposed endemic *Apis* species to new viruses or viral strains and *vice versa*. To assess the potential of interspecific virus transferbetween the Eastern (*Apiscerana*) and Western honeybee, we took advantage offivemixed-speciesapiaries, consisting of three neighboring *A. mellifera* and *A. cerana*colonies each.Samples of 30-50 workers were collected from each colony tomonitor virus prevalence and obtain their sequences for phylogeneticalanalyses.*Deformed wing virus* (DWV), *Israeli acute paralysis virus* (IAPV) and *Sacbrood virus* (SBV) were found, but not black queen cell,varroa destructor, acute bee paralysis orchronic bee paralysis viruses.
DWV was not found in *A.cerana* without being detected in the respective *A. mellifera* colonies of the same apiary. The prevalence in *A. mellifera* was significantly higher than in *A. cerana*. IAPV was detected in a single colony of both species, but not in the same apiary. SBV was found in all colonies ofa single apiary. The sequence data provided evidence for interspecific transfer of DWV and IAPV but SBV strains appeared species specific. Prevalence and sequence results taken together indicate that interspecific transfers of viruses are rare and we found no evidence for recent host shift,even thoughthese honey bees species are commonly kept in close proximity.

Prevalence of *Paenibacillus larvae* agent of American foulbrood in some regions of Algeria

Mohamed Chahbar[1], N. Adidou-Chahbar, H. Tefiel, B. Doumandji-Mitiche and S.B.S. Gaouar
1: National *High School of Agriculture*, Department of agricultural and forest zoology, El-Harach, Algiers, Algeria.
E mail : chahbar_mohamed@yahoo.com

American foulbrood is a honey bee disease caused by the bacteria *Paenibacillus larvae*. Our study is to search and detect the disease in order to determine its prevalence in some regions of Algeria. The samples were collected in eleven regions. The isolation of *P. larvae* was done on the MYPGP-agar specific medium. We used microbiological, physiological and biochemical tests to identify the bacteria. The present study shows that all regions are infected by this bacteria

with different degrees. The region of Boumerdes is the most affected with a rate of 88,89 %, followed by region of Tipaza, with a rate of 83,33%. The regions of Tlemcen, Medea and Algiers represent 46,67 %, 33,33 % and 33,33 % of rate respectively. A rate low to 20 % was noted for the others regions. This high prevalence of pathology may explain the mortality observed in the last years among beekeepers.

Characterization of microorganisms associated with Algerian honeybee: *Apis mellifera intermissa* and *Apis mellifera sahariensis.*

Nora Chahbar-Adidou [1] and Amel Lamara Mahamed[1]

[1] Department of biology, Faculty of science, University of M'hamed Bougarra, Boumerdes, Boumerdes 35000, Algeria.

E-mail: chahbar_nora@yahoo.fr

The microorganisms associated to the two Algerian subspecies honeybees *A.m.intermissa* and *A.m.sahariensis* were characterized. The identification of the microflora associated with the digestive tract and pollen was made two seasons during the year 2012, namely in January -February (winter test) and May (spring test). The research of this microflora was realized by the digestive tract, stored pollen (bee bread) and pollen baskets. The Isolated bacteria belong to the genera *Enterobacter, Pantoae, Pseudomonas,* and *Lactococcus* and to genera neighbors, *Lactobacillus* and *Streptococcus*. All yeast strains are likely to belong to the Saccharomyces genera and the molds are identified genera *Penicillium, Aspergillus, Cladosporium, Alternaria* and *Mucor*. According to the biochemical and physiological characters that are found, certain bacteria may be involved in the degradation of the components of the two extern layers of pollen, be ingested by the bees with food and shared with other individuals of the hive by the phenomenon of trophallaxis. The presence of yeasts and molds can be explained by the close relationship as maintenance honeybee with its environment, soil and flowers.

Supplementation of colonies with a probiotic based on Lactobacillus kunkeei strains

Daniela Arredondo*, Juan Campá, Loreley Castelli, Jorge Harriet, Lucía Leyton, Pablo Zunino and Karina Antúnez
Department of Microbiology, Instituto de Investigaciones Biológicas Clemente Estable, Uruguay
E-mail: danielarpapiol@gmail.com

The administration of probiotics as food additives is a widely used approach to improve the health of the host. In previous studies our research group obtained a probiotic product composed of 4 strains of *Lactobacillus kunkeei*, isolated from the intestinal microbiota of bees. Preliminary 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 objective of this study was to evaluate the effect of the administration of this probiotic on commercial colonies including the possible protection against *N. ceranae* and RNA viruses, as well as their effect on colony strength.

The probiotic was lyophilized, suspended into sugar syrup (10^7 ufc/ml) and applied in autumn and spring by aspersion over the brood combs. Oxalic acid (6%) was simultaneously applied. Control groups included colonies treated with oxalic acid, amitraz (4%) and no acaricide treatment, 15-20 colonies per group were used.

One and three months after treatments colony strength was estimated and infection level of pathogens (*N. ceranae, Lotmaria passim*, RNA viruses and *Varroa destructor*) was evaluated.

The administration of the probiotic on colonies was safe since colony strength including population of larvae and adults was not affected. However, it did not significantly affect the infection level of pathogens, neither in autumn or spring, indicating that the tested concentration was not effective. However, considering the promising results of previous assays, new studies are being carried out to evaluate higher concentrations of probiotic and the use of other vehicles.

Efficacy of Lactobacillus spp. in controlling European foulbrood in Central Italy

Pietropaoli Marco[1], Jorge Rivera Gomis[1], Milito Marcella[1], Palazzetti Massimo[2], Giuseppe Micarelli1, Viviana Belardo[1], Francesco Scholl[1] and Formato Giovanni[1]
[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Apiculture Unit,
Via Appia Nuova 1411, 00178 Rome, Italy
[2]Veterinary Services, ASLVT, Via Alighieri 100, 01027 Montefiascone (Viterbo), Italy
E-mail : giovanni.formato@izslt.it

American Foulbrood (AFB) and European Foulbrood (EFB) are the most widespread and damaging honeybee brood diseases.
In our study we evaluated the efficacy of *Lactobacillus spp.* to prevent AFB and EFB.

On March 2014 in Viterbo (Central Italy) we treated 488 colonies with a water suspension of *Lactobacillus spp.* trickling it (5ml per inter---comb space) once a week for one month, while we left untreated 491 colonies (control group). After the treatment, we monitored AFB and EFB cases incurred in the total (979) of the honey bee colonies, until the end of September 2014.

Colonies treated with *L. plantarum* did not show a significant reduction of AFB prevalence respect the control group: the final AFB prevalence in the treated group was of 5,3%, while in the control group was 5,1%.

On the contrary, the colonies treated with *L. plantarum* at the end of the experiment showed an EFB lower prevalence (2,5%), compared to the untreated colonies (4,5%). Kaplan---Meier estimate showed a significant difference of colony survival after 34 days from the end of treatments. The use of Lactobacillus spp. against EFB could be a possible alternative to antibiotics, avoiding antibiotic---resistance and risk of residues in bee products.

The examination of powdered sugar to detect Paenibacillus larvae infections in honeybee colonies

Giulio Loglio and Stefano Bassi
Agenzia di tutela della Salute di Bergamo -Dipartimento di Prevenzione Veterinario - Via Mazzini 13, 24069 Trescore B.rio (BG) Italy
E-mail: stefano.bassi@izsler.it

The common strategy to control the American foulbrood is based on the application of correct preventive measures and early clinical diagnosis. Nevertheless, some honeybee colonies can be infected by Paenibacillus larvae spores, even in very large number, without showing clinical symptoms of American foulbrood. The detection of such colonies is crucial for the application of effective preventive measures.

In this work was evaluated the use of the powdered sugar to identify colonies with symptomatic or asymptomatic infections by Paenibacillus larvae. Twenty-eight honeybee colonies were included in the trial: seven colonies with American foulbrood symptoms (Group A), seven colonies without symptoms of disease but belonging to apiaries in which were present diseased colonies (Group B) and 14 colonies coming from an apiary where no cases of American foulbrood were reported in the last years (Group C).

The sugar was dusted on the top bars of brood combs, brushed between the frames and collected on a sheet of paper placed in the hive bottom. The sugar samples were examined with culture method.

All the sugar samples from Group A were positive with a very high load of Paenibacillus larvae spores, comprised between 40,400 and 2,000,000 UFC/g. In Group B, Paenibacillus larvae spores were found in six colonies out of seven with values between 140 and 8,100 UFC/g. All the samples taken from Group C were negative.

Based on the results of this preliminary trial the examination of the powdered sugar seems to be a good indicator of Paenibacillus larvae infection.

Optimal concentration of organic solvents to be used in the broth microdilution method to determine the antimicrobial activity of natural products against *Paenibacillus larvae*

Noelia Cugnata, Elisa Guaspari, Pablo D. Giménez-Martínez, Sandra R. Fuselli and Rosa M. Alonso-Salces
Grupo de Investigación Microbiología Aplicada (GIMA), Centro de Investigación en Abejas Sociales (CIAS), Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Pcia. Buenos Aires, Argentina; Comisión de Investigaciones Científicas, La Plata, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina; FONCYT, Buenos Aires, Argentina.
E mail: sfuselli@gmail.com

American Foulbrood (AFB) is a bacterial disease, caused by *Paenibacillus larvae*, that affects honeybees (*Apis mellifera*). Alternative strategies to control AFB are based in the treatment of the beehives with antimicrobial natural substances such as extracts, essential oils and/or pure compounds from plants, honey by-products, bacteria and moulds. The broth microdilution method is currently one of the most widely used methods to determine the minimum inhibitory concentration (MIC) of a substance. In this regard, the fact that most natural products, due to their lipophilic nature, must be dissolved in organic solvents or their aqueous mixtures is an issue of major concern because the solvent becomes part of the dilution in the incubation medium, and therefore, can interfere with bacterial viability depending on its nature and concentration. A systematic study to determine, by the broth microdilution method, the MIC and the maximum non inhibitory concentration (MNIC) against *P. larvae* of the most common organic solvents used to extract or dissolve natural products, i.e. ethanol, methanol, acetonitrile, n-butanol, dimethyl sulfoxide, and acidified hydromethanolic solutions, was carried out. From the MIC and MNIC for each organic solvent, recommended maximum concentrations in contact with *P. larvae* were established: 5% (v/v) of DMSO, 7.5% (v/v) of acetonitrile, 7.5% (v/v) of ethanol, 12% (v/v) of methanol, 1% (v/v) of n-butanol, and methanol-water-acetic acid (1.25:98.71:0.04, v/v/v).

Essential oils for the control of American Foulbrood: Antimicrobial activity and mode of action against *Paenibacillus larvae* on bee larvae reared *in vitro*

María Celeste Pellegrini, Rosa M. Alonso-Salces, Alejandra G. Ponce and Sandra R. Fuselli
Grupo de Investigación Microbiología Aplicada (GIMA), Centro de Investigación en Abejas Sociales (CIAS), Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Pcia. Buenos Aires, Argentina; Comisión de Investigaciones Científicas, La Plata, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.
E mail: sfuselli@gmail.com

Paenibacillus larvae, a gram-positive spore-forming bacterium, causes American foulbrood (AFB), which is the most destructive brood disease of honey bees (*Apis mellifera*). The antibiotic oxitetracycline, although being the traditional method of choice to control AFB, has led to the appearance of resistant bacterial strains and residues in beehive products. Therefore the development of alternative methods to control the disease is crucial. Among them, the use of natural products such as essential oils (EOs) is proposed. In the present work, the antibacterial activity against *P. larvae* of EOs of native plants from Argentina such as *Solidago chilensis* and *Schinus molle* is determined by the broth microdilution method, as well as the disruption of EOs on membrane permeability of *P. larvae* by the crystal violet assay and the determination of the released UV-absorbing intracellular material. Moreover, the ability of EOs to control AFB was tested on bee larvae reared *in vitro*, which were infected with 600 *P. larvae* spores/µL. *S. molle* and *S. chilensis* EOs showed antimicrobial activity against *P. larvae*, the minimal inhibitory concentrations (MIC) being 31.25 and 25 µg/mL respectively. The membrane disruption capacity of these EOs on *P. larvae* was higher than 50% when using concentrations of 4xMIC (100% of disruption was obtained when using EDTA as a positive control). Preliminary results showed that both EOs at concentrations of 40xMIC did not control the AFB disease, although this concentration was non toxic to the larvae reared *in vitro*.

Foulbrood, number of frames of bees and so-called winter colony losses

İbrahim Çakmak[1], Ebru Borum[2] and Mehmet Özden[3]
Uludag University, Beekeeping Development-Application and Research Center, Bursa, Turkey,
Balıkesir University, Veterinary Medicine, Department of Microbiology, Balıkesir, Turkey
Bornova Veterinary Institute, İzmir, Turkey
E-mail: icakmak@uludag.edu.tr

Colony losses have been great concern in recent years and a number of factors were considered in different countries. Even though and new generation of pesticides as neonicotinoids were considered as major factors in recent years, foulbrood should also be considered particularly in countries such as Turkey with common practice of long distance migratory beekeeping.

There are a number of different bacteria causing foulbrood, not only American foulbrood and European foulbrood but also a number of others to slow down brood development and cause colony losses in winter or early spring. Therefore, reduced number of frames of bees due to foulbrood in colonies before winter and during winter speed up colony losses during winter or early spring.

Bacterial analysis of samples from colonies suggests that not only American and European foulbrood agents but also others and mixed infections cause so-called winter colony losses. In conclusion *Varroa destructor*, *Nosema ceranea* and foulbrood should be followed closely and treated effectively to reduce colony losses.

Inter-laboratory comparison of molecular methods for the identification of *Paenibacillus larvae*, the aetiological agent of the American foulbrood

Marie-Pierre Rivière, Nicolas Gaïani and Magali Ribière Chabert
Anses, Honey Bee Patholgy Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes – CS 20111, 06902 Sophia Antipolis, France.
Email: marie-pierre.riviere@anses.fr

The American foulbrood (AFB), one of the most deleterious diseases of honey bee brood is caused by the spore-forming, gram positive bacterium, *Paenibacillus larvae*. AFB can lead to colony losses and is a notifiable disease in the European Union. In order to control the disease, the development and use of reliable diagnosis methods are of utmost importance.

In 2014, the European Union Reference Laboratory (EURL) for Honey Bee Health organised a proficiency test by inter-laboratory comparison aiming at assessing the reliability of molecular methods used within the network of European National Reference Laboratories (NRLs).

Twenty-six laboratories participated in the test (including 24 European NRLs). The participants received a panel of 17 DNA samples and were requested to identify *P. larvae* using the molecular method they routinely used for AFB diagnosis. The panel included samples containing DNA from *P. larvae* (including the four known genotypes) or from other bacteria infecting honey bees, in order to assess the method specificity and sensitivity. Twenty-one (81 %) participants provided results in accordance with the expected results.
The test was also the opportunity to gather information on the molecular methods used by the NRLs for AFB diagnosis. The analysis of an online questionnaire filled in by the participants revealed differences in the gene targeted, the primers and the technique used (e.g. conventional or real-time PCR).

This inter-laboratory comparison highlighted the diversity of molecular methods used for AFB diagnosis within the European NRL network as well as the need to harmonize and to improve their reliability.

Honey bee antimicrobial peptides jelleine I and jelleine II do not inhibit the growth of *Paenibacillus larvae*

Silvie Dostálková, Jiří Danihlík and Marek Petřivalský
Department of Biochemistry, Palacký University Olomouc, Czech Reoublic
Email: jiri.danihlik@upol.cz

Nonspecific and fast reactions of humoral immunity are integral part of insect immune system. Lectins, lysozymes, phenoloxidase and antimicrobial peptides belong to humoral immunity and play essential role in immune system. The representatives of honey bee (*Apis mellifera*) antimicrobial peptides include peptides apidaecins, abaecin, defensins and jelleines.

In this work an optimized protocol was developed for ascertaining the antimicrobial activity of peptides. The antimicrobial effect of peptides was monitored by measuring the growth curves of the bacteria in liquid medium. The protocol was optimized with antimicrobial peptide apidaecin Ia which was tested against bacterial strains of *Escherichia coli*. Antimicrobial effect of apidaecin Ia was determined, the minimal inhibitory concentration was 25 μg/ml.

The method was applied to test antimicrobial activity of peptides jelleine I and jelleine II which were previously detected in royal jelly of honey bee. Jelleines I and II were tested against 9 bacterial strains of *Paenibacillus larvae*, a causative agent of American foulbrood. Antimicrobial effects of jelleine I and jelleine II have been previously shown in diffusion assays; however no antimicrobial effect against *P. larvae* strains was monitored by our growth curve assay. This discrepancy of results can be caused by the interactions either of the peptide and media used for cultivation, or the peptide and tested bacteria. Hence, it is necessary to analyze in detail the effect of peptide on bacteria during incubation to accurately determine the kinetics of their antimicrobial activity.

The examination of bees, honey and hive debris as a tool to foresee the onset of American foulbrood

Stefano Bassi and Emanuele Carpana
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – Sezione di Modena – via Emilio Diena 16, 41100 Modena (Italy)
E- mail: stefano.bassi@izsler.it

Bees, honey and hive debris were collected in winter from 125 hives belonging to 10 apiaries and examined with culture method for the detection of *Paenibacillus larvae* spores. In the following spring the colonies were inspected for American foulbrood symptoms from the beginning of March to the end of May at 3 weeks intervals.

The aims were to compare the efficiency of bees, honey and debris in detecting the infected colonies and evaluate the relationship between the wintry contamination by *Paenibacillus larvae* in these three materials and the development of the American foulbrood in the same colonies in the spring. The examination of the bees has identified a greater number of infected colonies (117/125) compared to the examination of honey (87/125) and debris (62/125). Regarding the relationship between presence of *Paenibacillus larvae* and onset of American foulbrood 16 out of 62 (25.8%) colonies in which the debris were positive for *Paenibacillus larvae* showed disease symptoms in spring. The disease occurred in 13 out of 70 (18.6%) and 16 out of 117 (13.7%) colonies in which honey and bees were positive for *Paenibacillus larvae* respectively. None of the colonies with bees and debris negative for *Paenibacillus larvae* developed the disease while three colonies where the honey was negative have fallen ill in the spring.

The onset of the disease in spring is related to the level of wintry contamination by *Paenibacillus larvae*: for all materials the higher the number of spores detected the greater the probability that the colony becomes ill.

Detection of *Paenibacillus larvae* from beehive debris: a culture method based on watery extraction of spores

Stefano Bassi and Giorgio Galletti
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – Sezione di Modena – via Emilio Diena 16, 41100 Modena (Italy)
E- mail: stefano.bassi@izsler.it

The detection of Paenibacillus larvae spores in debris collected on the hive bottom is a non-invasive tool for the identification of infected colonies. We developed a quick and simple culture method for the detection of Paenibacillus larvae in beehive debris based on the extraction of the spores in water (Water method - WM).

Briefly, 1 g of debris was placed in a 15 ml test tube with a sealing cap containing 9 ml of sterile distilled water. The suspension was shaken for 30 seconds and then heated in a water bath at 85-90 °C for 15 min. After the heat treatment, the suspension was poured into a Stomacher bag with a lateral filter and the filtered liquid was transferred with a disposable pipette in another test tube. The sample was plated onto 5 plates (100 µl/plate) of MYPGP agar supplemented with nalidixic acid. The plates were incubated at 37 °C in an atmosphere with 10% CO2 and examined after 8 days.

We examined 50 samples of beehive debris with this method and compared the results with the same samples using the Tween Method (TM), which currently is the most effective culture method for the detection of Paenibacillus larvae in beehive debris.

WM has an excellent agreement with TM.

The use of WM compared to TM presents practical advantages: it is less expensive, it is easier to perform and the sample preparation time is shorter (approximately 25 - 30 min with WM vs. approximately 4 - 5 hrs with TM).

Comparison between the examination of powdered sugar and adult bees for the detection of honeybee colonies infected by Paenibacillus larvae

Stefano Bassi, Giulio Loglio
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – Sezione di Modena – via Emilio Diena 16, 41100 Modena (Italy)
E-mail: stefano.bassi@izsler.it

The detection of Paenibacillus larvae infected colonies is imperative for an effective prevention and control of American foulbrood.

How already reported in a previous communication the bacteriological examination of powdered sugar can be a useful tool for the identification of these colonies.

From 101 beehives, belonging to apiaries with different situations regarding the presence of American foulbrood, we collected samples of both powdered sugar and adult bees.

The powdered sugar was dusted over the frames and collected on a sheet of paper placed on the tray in the hive bottom. The bees were collected in the brood nest. Both materials were examined with bacteriological method for the detection and enumeration of Paenibacillus larvae spores. The results were expressed as CFU/g (powdered sugar) or CFU/bee (bees).

Concordant results between powdered sugar and bees analysis was observed in a total of 82/101 (81.2%) beehives: in 51 colonies both materials were positive and in 31 both were negative.

Discordance was observed in 19/101 beehives (18.8%): in 17 colonies the powdered sugar was positive and the bees were negative, whereas in two colonies the bees were positive and the sugar negative.

In the colonies (n. 51) where bees and powdered sugar were both positive, the number of spores was higher in the powdered sugar compared to bees in 44 colonies, conversely in seven colonies bees showed higher spores number. These preliminary results show that powdered sugar could be more effective than adult bees in detecting colonies with Paenibacillus larvae infection.

Epidemiology of *Paenibacillus larvae* in Germany, using MALDI-TOF mass spectrometry

Marc O. Schäfer, Noreen Formella, Barbara Bettin and Axel Karger
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald– InselRiems, Germany
E mail:marc.schaefer@fli.bund.de

Infection with *Paenibacillus larvae* is lethal for honey bee larvae and may lead to loss of the entire colony. Of the known ERIC genotypes (ERIC I – IV) of *P. larvae*, only ERIC I and II,which differ significantly in virulence and the course of the disease, are frequently found in the field. To test and improve the matrix-assisted laser desorption/ionisation time-of-flight (MALDI – TOF) mass spectrometry (MS) for the discrimination of *P. larvae* genotypes ERIC I and II, an epidemiological study was conducted in Germany on more than1500*P. larvae* samples, taken from colonies of more than900 German apiaries from August 2013 till August 2015. All *P. larvae*samples were genotyped by rep-PCR using ERIC primers and cultivated on Columbia sheep blood agar at 37°C and 5% CO_2 for 48 h till single colonies were harvested by suspension in300 µl of HPLC grade water. Proteins were extracted as previously described and MALDI – TOF MS was performed for each sample and the results were compared to the rep-PCR genotyping results. We showed that the already good reliability of genotype discrimination of German field isolates with MALDI Biotyper software was improved by adding more reference spectra to the database. Furthermore, it was shown that within the studied samples, which were spatially distributed over the whole country, both genotypes were present in equal numbers.

The interaction between *Nosema ceranae* and organophophorates on honeybees of Spain

Almudena Urbieta-Magro, Mariano Higes, Aránzazu Meana and Raquel Martín-Hernández
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, 19180 Marchamalo, Spain.

E mail: aurbieta@jccm.es

Honeybees are constantly exposed to a wide variety of environmental stressors such as parasites and pesticides. Among them, in Spain, the microporidium *Nosema ceranae* is one of the pathogens with the higher prevalence and clorphenvinphos and clorpiriphos are the organophosphorate pesticides that shows the higher prevalence in wax and stored pollen. These stressors might act in combination and cause maintained stress over time to honey bee colonies. In this work we studied the survival of honey bees (*Apis mellifera iberiensis*) exposed to *N.ceranae*, clorphenvinphos, clorpiriphos and the combination of all of them. We also investigated the immune response of bees exposed to these stressors.
Twenty new born bees were caged in groups (8 treatments with 6 replicates) and fed with different syrups according to the average concentrations of pesticide residues found in field. At 5 days of age, 4 groups of bees were infected individually with 40.000 fresh spores of *N.ceranae*. Syrups were replenished daily until the collapse of the controls on the 24th day after capping and the consumption and cumulative mortality were measured per bee and treatment daily. The days 0, 5, 7, 9, 11, 13, 15 and 18 post infection, 5 bees per treatment were sampled to analyze the comparative level of expression of 8 genes (quantitative RT-PCR) involved in the humoral immune response of bees to stressors, pathogens and pesticides. The food consumption increased after the fungal infection. Mortality was higher in treatments with pesticides until day 5 p.i. From day 9 p.i. the mortality increased dramatically in the 4 treatments with *N.ceranae* and from day 12 p.i. exceeded, in all cases, to uninfected controls. Infected and treated with both pesticides bees had the lowest survival while most survival was recorded in the control group fed with 50% syrup. The results on immune response are also discussed.

Our results confirmed that *N.ceranae* increases honeybee mortality and that *N.ceranae*-pesticides combination might act as a synergistic factor contributing to the colony collapse.

Funded by: INIA-CCAA RTA2012-00076-C02-01 and RTA2013-00042-C10-06 (INIA, FEDER).

Isolation and identification of fungal diseases in the local honeybee *Apis mellifera intermissa*

Nora Chahbar-Adidou [1], M. Chahbar[2], H. Delimi[1] and H. Lounici[1]

[1] Department of biology, Faculty of science, University of M'hamed Bougarra, Boumerdes, Boumerdes 35000, Algeria.

[2,] Département de Zoologie Agricole et Forestière, Ecole Nationale Supérieure Agronomique

El-Harrach, Alger, Algérie

E-mail: chahbar_nora@yahoo.fr

The local honeybee *Apis mellifera intermissa* suffers from many diseases which are currently a major concern for beekeepers worldwide. These diseases affect the bee as adults and brood. This study focused on the isolation and identification of fungi that attack honeybees. To do this, sampling is performed in the wilaya of Blida (Boufarik, and Sidi hamaed), the wilaya of Algiers (Bouchaoui) and the wilaya of Boumerdes (Hamadi) from the embossed wax, larvae, bees and mummified larvae). The identification is made on the macroscopic and microscopic characters. After purification of the different strains, 07 fungal species were identified namely: *Aspergillus niger*, Ascosphaera apis, *Alternaria sp*, *Penicillium sp* and Rhizopus sp. These fungi are implicated in the pathology of the honeybee.

Sporicice action in different substances against *Nosema ceranae* spores

Cristina Rodríguez-García, Riccardo Cabbri, Mariano Higes, Aránzazu Meana, Antonio Nanetti, Luca Lazzeri and Raquel Martín-Hernández
Bee Pathology Laboratory, Centro de Investigación Apícola y Medioambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal. Consejería de Agricultura, Junta de Comunidades de Castilla- La Mancha. Marchamalo, 19180 Guadalajara, Spain.
E mail: cristinarodriguez.crg@gmail.com

Nosemosis type C in *Apis mellifera* is caused by *Nosema ceranae*. In some countries, like Italy and in Spain, this pathogen is highly prevalent and causes important losses to the beekeepers. Since few treatments are available to control this disease, cleaning and disinfection of beekeeping equipment could be useful tool for lowering the number of infective spores decreasing the risk of infection.

In this work the effectiveness of different products against *N. ceranae* spores has beenevaluated based on International European Pharmacopoeia and UNE guidelines. Purified *N. ceranae* spores were obtained from naturally infected honey bees. Stainless steel surfaces were contaminated with a known spore concentration. The surfaces were exposed for 5 and 15 minutes with the following products: Sodium Hypochlorite 40g / L, and 0.4g / L, Inouko® (1%, CEVA), Mycoplasma-OFFTM (Minerva Biolabs), acetic acid (99%), ethanol 96º, ammonia 50%, Biocidal ZFTM (Wak-Chemie GmbH), Povidone-iodine (Betadine®, 1%), oxalic acid (Ecoxal), water (negative control) and autoclaved (mortality control). Additionally, the sporicidal effect of flours milled from *Brassica nigra* was tested.
To do this, water was added to the milled flour to determine the release of the corresponding isothiocyanate in a hermetic jar with the spore contaminated surfaces. The spore were exposed to the volatile compounds for 3, 6 and 24 hours. After exposition, the surfaces were washed and the spores recovered. Viability was analyzed by flow cytometry.
All tested products showed a certain degree of sporicidal activity, although ammonia, sodium hypochlorite, acetic acid and Mycoplasma-OFFTM showed sufficient effect to be used in equipment disinfection. Funded by RTA2012-00076-C02-01 (INIA-FEDER)

Tissue tropism of *Nosema apis* and *Nosema ceranae* in *Apis mellifera*

García-Palencia Pilar, Martín-Hernández Raquel, Meana Aranzazu and Higes Mariano
Department of Animal Health. Faculty of Veterinary. Complutense University of Madrid. Spain
E mail: mhiges@jccm.es

The aim of this study is to describe and compare the development in different host tissues of N*osema apis* and *Nosema ceranae,* both honeybee pathogens, with standard histopathological techniques for light microscopy (OM) and transmission electron microscopy (EM) observations.

Four replicates groups of newly born honeybees seven days after birth were individually oral inoculated with different spores doses (50000 to 100000) of *N. ceranae* or *N. apis*. Each replicate was checked daily and any dead bees were counted and removed. Surviving bees were collected at day 7, 10, 11 and 15 postinoculation (p.i.) to be dissected and processed for OM and EM observation.

Cephalic tissues, brain (nerve tissues), hypopharyngeal glands and salivary glands as well as foregut, midgut, Malpighian tubules and fat body of bees infected with either *Nosema* species were studied by the same experienced technician.

Tissue tropism was thoroughly studied in all infected bees and microsporidian spores or vegetative forms were not observed in any tissues other than the midgut tissues either in *N.ceranae* as *N.apis*.

Our results confirmed that *N. ceranae* only develops in midgut tissues and has identical tissue tropism with *N. apis.* Differences in specie distribution along the midgut as well as related to the intensity of parasitism among different groups were observed.

Work founded by INIA-FEDER (RTA2013-00042-C10-03-06).

Detection of *Lotmaria passim* in Spain

Mariano Higes, Raquel Martín-Hernández, Cristina Rodríguez, María Benito, María Buendía, Pilar de la Rúa, Tamara Gómez-Moracho, Xulio Maside and Carolina Bartolomé
Bee Pathology Laboratory, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF). Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha. Marchamalo, 19180 Guadalajara, Spain.
E mail: mhiges@jccm.es

The first genetic description of a trypanosomatid other than *Crithidia mellificae* in *Apis mellifera* took place in 2014, where a lineage – later named *Lotmaria passim*– was detected in apiaries located in different regions from Spain. The comparison of cloned sequences of two loci (*GAPDH* and *18S rDNA*), obtained from DNA of naturally infected worker bees and the *C. mellificae* reference strain *ATC30254*, respectively, revealed that both species are distantly related and that most, if not all, the sequences previously deposited in GenBank were wrongly attributed to *C. mellificae*; this finding put into question whether the presumed negative effects of *C. mellificae* on winter mortality, alone or in combination with other pathogens, are actually caused by *L. passim*. It is thus necessary to investigate the prevalence of each of these parasites and their potential effect on colony losses. The accurate identification of the infecting species is usually performed through the sequencing of PCR products, although this method is relatively expensive and time consuming. An alternative strategy is the use of specific primers, but this approach also presents caveats like low specificity and/or poor repeatability (the latter being highly dependent on the length of the amplicons and the quality of the DNA used). Here we make a comparison of several protocols and discuss their utility in the context of investigating the presence of trypanosomatids in Spanish honey bee colonies.

Acknowledgments: Financial support for this work was provided by INIA-FEDER (RTA2013-00042-C10-05-06 and E-RTA2014-00003-C03).

Survival of honey bees *(Apis mellifera*) infected with *Crithidia mellificae* (Langridge and McGhee: ATCC® 30254™) in the presence of *Nosema ceranae.*

Mariano Higes, Cristina Rodriguez-García, Tamara Gómez-Moracho, Aránzazu Meana, Carolina Bartolomé, Xulio Maside, Laura Barrios and Raquel Martín-Hernández

Bee Pathology Laboratory, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF). Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha. Marchamalo, 19180 Guadalajara, Spain.

E mail: mhiges@jccm.es

Crithidia mellificae is a trypanosomatid parasite of *Apis mellifera* that was first described in 1967. It was deposited in 1974 as microbiological culture (ATCC 30254). . However, this agent was not considered important until the infection with this protozoan was proposed to be related with the serious worldwide honey bee colony losses produced in the last decade, either alone or in association with *Nosema ceranae* infection. Since this association contradicts the results of the experimental infections originally performed by Langridge and McGhee almost 40 years ago, we have studied the effect of infecting *A. mellifera* worker bees with the *C. mell*ificae ATCC30254 reference strain under laboratory conditions in the presence or absence of *N. ceranae*. Our results showed that the highest mortality under these conditions was evident in bees infected with *N. ceranae*, irrespective of the presence of *C. mellificae*, which did not appear to affect the survival of the bees.

Acknowledgments: The authors wish to thank Prof. de Graaf and Dr Ravoet for supplying the ATCC30254 reference strain and for sharing their knowledge about *C. mellificae*. Financial support for this work was provided by INIA-FEDER (RTA2013-00042-C10-06 and E-RTA2014-00003-C03).

Improving the DNA extraction method for pathogen (*Nosema* spp) detection in honeybees

Laura Jara, Carmen Rogerio, Pilar De la Rúa, Mariano Higes and Raquel Martín-Hernández
Departamento de Zoología y Antropología Física, Facultad de Veterinaria, Campus de Espinardo, Universidad de Murcia, Murcia, 30100, Spain
E mail: rmhernandez@jccm.es

The microsporidium *Nosema ceranae* is currently considered a predominant pathogen in bees related to honey bee declines in Mediterranean countries. Studies about its prevalence are needed to evaluate its role in the worldwide pollinators decline. However, the reliability of its molecular detection can be highly influenced by the efficiency of the DNA extraction step. In this work we evaluate the effectiveness of different methods of sample processing and DNA extraction for the detection of *N. ceranae*. Individual honey bees (20 per colony, 5 colonies) were macerated directly (one by one) in Milli-Q water (N=100) and then aliquots incubated either with proteinase K (56ºC, overnight) or in AL buffer (Qiagen). DNA of both groups was extracted in a BioSprint station with the BioSprint 96 DNA Blood kit (Qiagen). PCR analyses for *N. ceranae* detection were subsequently performed. Higher levels of infection were detected in the samples macerated directly in AL buffer so this method appears to be more efficient for the detection of this microsporidium. Furthermore, to establish the best methodology for processing honey bee samples, 11 composite honey bee samples (120 bees each) were macerated in AL buffer and different phases were subsequently analyzed by PCR: 1) either taken directly after the maceration process; 2) from the sediment after centrifugation (800g, 10 min) or 3) from supernatant. Although the direct analysis of the samples did not show any difference in the detection, the sensibility of the reaction was influenced by the phase.

Funded by RTA2012-00076-C02-01 and RTA2013-00042-C10-05 (INIA-FEDER).

Identification of Characterization of *Nosema bombi* in *Bombus ignitus* and Diagnosis using ultrafast PCR

Kyu-Won Kwak, Hyung Joo Yoon, Myung-Sae Han, Kwan-Ho Park, Ji-Young Choi, Wontae Kim and Sung-Hee Nam*
Industrial Insect Division, Department of Agricultural Biology, National Institute of Agricultural Science, Wanju, Korea
E-mail: nawoon23@korea.kr, kyuwon97@hanmail.net

Microsporidia, especially, *Nosema bombi* is the critical pathogenic species to *Bombus ignitus*. We have identified the morphology of the microsporidia by light and electron microscopy, and found it to have fairly small oval spores, as has been described previously in many other articles. For the specific and sensitive diagnosis of the microsporidian parasite *N. bombi* in *B. ignitus*, we have developed an improved method of the Polymerase Chain Reaction (PCR) for expeditious diagnosis. Two pairs of primers are tested on *N. bombi* and the related microsporidia *Nosema apis* and *Nosema* sp., both of which infect *B. ignitus* and *B. hypocrita sapporoensis.* Furthermore, we have verified and analyzed the 16SrRNA sequence data of *N. bombi* by using the Basic Local Alignment Search Tool (BLAST) server at the National Center for Biotechnology Information. On the other hand, while conventional PCR takes several days to identify the microsporida, the portable ultra-fast PCR machine can be identified *N. bombi* from infected tissues. Therefore, we used a one-stop method using specific buffer that is possible genomic DNA from *N. bombi* infected *B. ignitus* samples which are extracted at the farm, and identified *N. bombi* within approximately 12 minutes as compared 90 min. using conventional PCR. In addition, relative quantification of PCR product using the ultrafast PCR is also possible, which will enable estimation of disease prognosis.

The influence of European foulbrood on the mortality of adult honeybees

Andrea Cecchini, Pascal Herren, Vincent Dietemann, Jean-Daniel Charrière, and Daniela Grossar
Jean-Daniel Charrière, Agroscope, Swiss Bee Research Centre, CH-3003 Bern, Switzerland
E -mail:jean-daniel.charriere@agroscope.admin.ch

Since 1999 the disease European foulbrood (EFB) gains in prevalencein Switzerland.The gram-positive bacterium*Melissococcus plutonius*, is the causal agent of this disease and is known to have a pathogenic effect on the young honeybee larvae. Its effect on adult workers remains unclearsince it is poorly documented. The goal of this study was to measure the influence of two Swiss strains of *M. plutonius* onthe mortality rate and food consumptionof adult workersunder laboratory conditions. Despite the oral exposure of treated workers with a high quantity of bacteria, no significant difference in mortality and in food consumption have been observedcompared to control groups not exposed to the bacteria. Our results suggest that *M. plutonius* is not adapted to infect adult workers or that the latter possess effective defencemechanismsagainst this pathogen. The fact that adult workers are healthy carriers of *M. plutonius* should be considered in the control strategies against this bacterial disease.

Presence of *Lotmaria passim* is in Uruguay, Argentina and Chile, and its association with other bee pathogens

Loreley Castelli1, Belén Branchiccela1, Ciro Invernizzi2, Ivanna Tomasco2, Marina Basualdo3, Marta Rodriguez4, Pablo Zunino1 and Karina Antúnez1*
1Instituto de Investigaciones Biológicas Clemente Estable.Montevideo, Uruguay.
2Facultad de Ciencias. UdelaR. Montevideo, Uruguay.
3Facultad de Ciencias Veterinarias, UNCEN, Tandil, Argentina.
4Biobichos Ltda. Chillán, Chile.
E-mail: kantunez03@gmail.com

The presence of different pathogens is one of the main threats for honey bee health worldwide. However, trypanosomatids affecting honey bees, including *Crithidia mellificae*and *Lotmaria passim,*have been poorly studied. Both pathogens have acquired attentionduring last years due to their potential association with colony losses. In the present study we evaluated their presence in honey bee

samples from South America, specifically in samples from Uruguay, Argentina and Chile collected between 1990 and 2011. Honey bee samples were processed, DNA was extracted and the presence of both pathogens was evaluated through PCR and sequencing. The association of those pathogens with *Varroa destructor*, *Nosema ceranae*and RNA viruses was evaluated. The obtained results confirmed that L. passim is widely spread in Uruguay, Argentina and Chile, while *C. mellificae*was not detected. In Uruguay, older positive samples were collected in 2007, and the prevalence for 2011 was calculated in 16%. *L. passim*infected samples showed significantly higher level of parasitism with *V. destructor*than the negative samples. Evenmore, the presence of *L. passim*increased 3 times the risk to develop high level of parasitism with the mite. No association with other pathogen was detected. Future studies are needed to evaluate the impact of this pathogen in honey bee colonies.

An Inter-laboratory Comparison of molecular methods, from DNA extraction to PCR Assays, for Identification of *Nosema* species in Honey Bee Samples

Véronique Duquesne, Aurélie Delcont, Nicolas Cougoule and Magali Ribière-Chabert

[1]Anses, Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes – CS 20111, 06902 Sophia Antipolis, France.

E-mail: veronique.duquesne@anses.fr

Two microsporidia could be involved in honey bee colony losses: *Nosema apis* (Zander) and *Nosema ceranae*. Several PCR-based methods (conventional and real-time PCRs) have been developed and applied to identify these *Nosema* species.

In order to evaluate the possibility to identify the *Nosema* species using the different molecular methods used by the European national reference laboratories (NRLs), an inter-laboratory comparison was organized by the European Union Reference Laboratory (EURL) for Honey Bee Health in 2015.

Twenty European and one non-European NRLs for honey bee disease diagnosis participated in this trial. The specificity and the sensitivity of the methods were tested on *Nosema apis*, *Nosema ceranae* and *Nosema bombi*. The sample panels distributed to each participating laboratory contained 17 crushed abdomen suspensions from naturally and artificially infected honey bees or bumble

bees. Data on the methods used by the participants were collected in an online questionnaire covering all the steps of the protocols. The analysis showed that a large panel of protocols was used. Twenty one protocols were recorded with variation from the DNA extraction step to the PCR step. For this comparison test, 47% of the participating laboratories gave the expected results. Considering the 21 different methods, 57 % of the results were satisfactory for the sensitivity and 72 % were satisfactory for the specificity.

The results of this inter-laboratory comparison clearly highlight the need of improvement and of harmonization of the methods used for identification of *Nosema* species.

Characterizations of *Ascosphaera apis* from *Apis mellifera* larvae infected by Chalkbrood Disease in Korea

Sung-Hee Nam*, Ji-Young Choi, Kwan-Ho Park and Kyu-Won Kwak
Industrial Insect Division, Department of Agricultural Biology, National Institute of Agricultural Science, Wanju, Korea
E-mail: creative716@korea.kr

The purpose of this study was to accurately identify the certain symptoms of each stage of chalkbrood disease. Chalkbrood-infected larvae of *Apis mellifera* were obtained from 8 apiaries in Korea. Among the 548 samples, 306 black mummies were identified *Ascosphaera apis* (Maasen ex Claussen) Olive & Spiltoir. The symptoms were observed during whole stage of *Ascosphaera apis*. In the early stage of chalkbrood diseases, the white/grey mass was formed on the surface of larvae. The larvae were soon shrunken the whole body and getting form hard mummies. In the late stage of chalkbrood diseases, the mycelium grew densely and covered the larvae to the extent that it filled the whole cell. When the larvae were infected with one sexual type of *A. apis*, they became white mummies while the grey-black mummies were developed by the invasion of both sexual types. *A. apis* (Aaj623) was heterothallic and mostly grown 57.6 ㎜ in diameter in 7 days on potato dextrose agar (PDA). Spore cysts were globose, nearly smooth on outer surface and evenly verrucate on inner surface, 45~9㎛ in diameter (average 74㎛ diameter). Spore balls were globose, 9~17 ㎛ in diameter (average 12.9 ㎛ in diameter) and lacking a conspicuous granular coating. Ascospores were hyaline, ellipsoid and smooth, 1 cell, 2.3~3.0 × 1.0~1.5㎛ (average 2.49 × 1.35 ㎛ in diameter).

Culture conditions of honeybee trypanosomatids

T. Gómez-Moracho, Mª D. Jiménez-Antón, MªJ. Corral, A.I. Olías, R. Martín-Hernández, Jª Alunda and M. Higes
Bee Pathology Laboratory, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF). Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha, Marchamalo, 19180 Guadalajara, Spain.
E mail: tamaragomezmoracho@gmail.com

Trypanosomatids (Kinetoplastea: Trypanosomatidae) are a group of unicellular parasites with a single flagellum and the kinetoplast, an organelle which contains the mitochondrial DNA. Two species of trypanosomatids have been described in the western honeybee *Apis mellifera*: *Crithidia mellificae* and *Lotmaria passim*. In recent years, the prevalence of the later specie has increased in honeybee colonies. However, there is no knowledge neither on its putative pathogenicity in *A. mellifera*, nor its infective form; a cell stage which should be characterized prior to infection experiments. Therefore, the optimization of cell culture media to determine the growth dynamics and the cellular phases of *L. passim* is urgently needed.

C. mellificae (ATCC 30254) and *L. passim* (ATCC PRA403) cell lines were grown in several basal liquid cell culture media including RPMI-1640, Medium 199, Brain Heart Infusion, Schneider's Insect Medium and PYFNH Medium. Media formulations were supplemented with peptides, amino acids, hemin or serum at different concentrations. Cells were incubated in culture flasks at 27 ºC, temperature commonly used for trypanosomatids culture maintenance, and 33ºC, the average temperature found in honeybee colonies and suitable for other honeybee pathogens (e.g. *Nosema ceranae*). Adaptation of trypanosomatids to culture media was assessed thorough monitoring, cell morphologies examined by staining and adequate culture conditions selected. Growth curves from both trypanosomatid species have been established by cell counting every 2h in the first 12 hours, and every 24h up to day 5. Results obtained are discussed.

Financial support for this work was provided by INIA-FEDER, project E-RTA2014-00003-C03.

Infection levels of *Apis mellifera iberiensis* pupae by *Nosema ceranae* under field conditions in Spain

Almudena Urbieta-Magro, Mariano Higes, Aránzazu Meana and Raquel Martín-Hernández
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, 19180 Marchamalo, Spain.
E mail: aurbieta@jccm.es

The nosemosis type C, produced by *Nosema ceranae*, has been considered a disease of adult bees capable of infecting European bees (*A. mellifera*), Asian honeybees and some species of the genus *Bombus*. Recently, *N. ceranae* infection has also been reported in pre-pupae of *A. mellifera* under laboratory conditions, demonstrating the infectivity of this microsporidium in bee breeding.

Since this fact has important epidemiological consequences, we aim to determine the risk of exposition of bee brood under natural field conditions by evaluation the levels of infection in naturally infected honey bee colonies. To do this, five experimental colonies, that had been monitored to determine monthly microsporidia prevalence, were selected to investigate the levels of infection in brood. One frame per colony was taken in October 2015 and in January 2016. Up to 85 capped cells per colony were analyzed in each colony (when this amount of brood cells was available). Brood samples were aseptically collected one by one and they covered all phases of development from larva to pre-pupae. Molecular detection of *Nosema sp*. was carried out by triplex PCR previously developed in our laboratory. Additionally, PTP3 gene expression (qPCR), which encoding a protein of *N. ceranae* during the infective stage, was analyzed in PCR positive samples to rule out external contaminations. Only two samples of pre-pupae (7 to 9 days post capping) were positive in the PCR and confirmed by PTP3 expression. We studied the correlation between adult and brood prevalence of the parasite in the adult bee population. Our work describes the first natural infection of *N. ceranae* in *Apis mellifera iberiensis* brood in Spain, although in very low level (0.45%) only found in autumn. According with our results, the natural risk of propagate the infection to brood is low. However, it is necessary to determine the temporal evolution and the effect this may have on the epidemiology of the disease and the final collapse of honeybee colonies.

Funded by: INIA-CCAA RTA2012-00076-C02-01 y RTA2013-00042-C10-06 (INIA, FEDER)

The German bee monitoring (DeBiMo): Report 2014/ 2015

Annette Schroeder, Werner von der Ohe, Elke Genersch, Marina Meixner**,** Christoph Otten, Stefan Berg, Marc Schäfer and Dieter Martens
Apicultural State Institute, University of Hohenheim, 70599 Stuttgart, Germany
Email: annette.schroeder@uni-hohenheim.de

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 2014/ 2015, we will present and discuss prevalence of pathogens (i.e. *Varroa destructor*, *Nosema* spp., *Paenibacillus larvae* and four honey bee viruses), residues of 402 different pesticides and winter mortality based on data from 1,036 bee colonies and 104 apiaries and compare the data with results from previous years.

15.1% of the monitored colonies (N=1,036) and 15.6% of all wintered colonies of the involved apiaries (N=5,822) died during winter 2014/2015. The prevalence of acute bee paralysis virus (ABPV) was 6.9%, of sacbrood virus (SBV) 1.4%, of deformed wing virus (DWV) 38.6 % and of chronic bee paralysis virus (CBPV) 26.1% (N=575). 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 but not the infections with *Nosema* spp..

We identified 83 different pesticides in bee bread (N=193), most of them in traces. Only 23 samples (11.9%) were free of measureable residues. The most frequent pesticides originate from applications in flowering oil seed rape. We found up to 22 different substances in one sample with a mean of 5 different substances per sample. However, no differences in overwintering were observed between apiaries with high or low number of pesticides.

SEA: an epidemiological surveillance network dedicated to bee health and pathogen diversity monitoring on Reunion Island using different tools.

Olivier Esnault, Jérémy Hascoat, Bernard Xavier, Vincent Porphyre, Marie-Pierre Chauzat and Hélène Delatte
GDS Réunion & CIRAD UMR PVBMT, 1, rue du Père Hauck, 97418 LA PLAINE DES CAFRES, Réunion
E -mail: olivier.esnault@gds974.re

Beekeeping is a major economic activity on Reunion Island with an estimated number of beekeepers reaching 500 for 24 000 colonies. Free of *Varroa destructor*,*Aethinatumida* and American foulbrood, issues for Reunion beekeeping are to stay free of these main biological threats. Since the beginning of 2016, a surveillance network ("SEA", French acronym for "epidemiological monitoring of honeybee diseases") is efficient.

The aims of this epidemiological surveillance network are to detect any early introduction of exotic pathogen and to follow the dynamics of several honeybee pathogens, such as *Nosemaceranae* and CBPV in different places. Active surveillance is coordinated by the technical team of the GDS in close collaboration with the French Veterinary Services (DAAF). A web of sentinel nucelli in each township of the island (24 townships) is used for *V. destructor* and *Paenibacillus larvae* surveillance. Each month, 50 forager bees are sampled in the same 5 sentinel colonies located in 4 different apiaries. CBPV and *N. ceranae* are quantified by qPCR. in addition of this network, an application called SAVA® developed for Android and iOS allows beekeepers to geolocate their apiary and to report all sanitary event in their colonies.

First occurrence of *Varroa destructor* in the Republic of Mauritius: sanitary survey and co-infestation with the bee louse *Braula pretoriensis*

Olivier Esnault, Deodass Meenowa, Preaduth Sookar, Marie-Pierre Chauzat and Hélène Delatte
CIRAD UMR PVBMT, Pôle de Protection des Plantes 7, chemin de l'IRAT**,** Ligne Paradis
97410 SAINT-PIERRE, Réunion
E-mail: olivier.esnault@gds974.re

Honeybees are under numerous threats in different parts of the world. Among them, the ectoparasite*Varroa destructor* is of major concern. *V. destructor* has been observed since 2010 in Madagascar, an island located in the South West of the Indian Ocean. At the end of July 2014, the introduction of *V. destructor* was suspected in Mauritius, an island located east of Madagascar (900 km). Two surveys were conducted in September and October 2014 in Mauritius and Rodrigues islands. In each survey, honeybees were sampled from different sites and apiaries covering the all territory of the islands. The ectoparasites (*V. destructor* and *Braulapretoriensis*) infestation rates were measured for each colony and apiary. *V. destructor* presence was confirmed and observed in the Central and Northern part of Mauritius, and was not detected in Rodrigues. *B. pretoriensis* was observed on both islands. The *B. pretoriensis* infestation rates were significantly higher in Mauritius when the colonies hosted *V. destructor*. The sequence analysis of *V. destructor* partial mitochondrial gene (COXI) showed that the Mauritius strain was different from the one from Madagascar, thus excluding an introduction from this country. Several sanitary measures were proposed, among which some are currently being applied to try to restrain the spread of *V. destructor* across the island.

Honey bee colony losses in Uruguay during 2013-2014

Karina Antúnez[1]*, Ciro Invernizzi[2], Yamandú Mendoza[3], Dennis vanEngelsdorp[4] and Pablo Zunino[1]

[1] Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay. *kantunez03@gmail.com

[2] Facultad de Ciencias, Universidad de la República. Montevideo, Uruguay.

[3] Instituto Nacional de Investigación Agropecuaria. Colonia, Uruguay.

[4] Department of Entomology, University of Maryland, College Park, Maryland, USA

E-mail:kantunez03@gmail.com

Over the last decade, high rates of managed honey bees colony losses have been reported worldwide. These reports have almost exclusively included countries in the north hemisphere including North America and several European countries. By contrast, information about honey bee colony losses in south hemisphere countries is scarce. The aim of the present study was to quantify colony losses in Uruguay in the period 2013-2014, and to identify potential causes of these losses. An estimated 2.6% of beekeepers, managing 5% of national managed honey bee population responded the survey. They reported similar rates of total summer and winter losses [summer: 19.0% (95% CI: 13.26- 24.77%), winter: 20.2% (95% CI: 14.98-25.39)], as average losses [summer: 19.8% (95% CI: 14.01-25.52); winter: 18.3% (95% CI 13.15- 23.56%)]. The total annual loss was 28.5% (95% CI: 22.42-34.51%), with each beekeeper losing 28.6% (95% CI: 22.52-34.61%) colonies.

Loss rates were similar across operations regardless of operation size. Queen failure, diseases and parasites, and pesticides were the leading self-reported causes of colony losses. This study is the first to document honey bee colony losses in Uruguay, establishing a baseline for future long term monitoring.

Varroa destructor infestation rate of bees and overwintering success in the Austrian surveillance programme of the project "ZukunftBiene"

Hemma Köglberger, Linde Morawetz, Irmgard Derakhshifar, Josef Mayr and Rudolf Moosbeckhofer
AGES GmbH, Spargelfeldstraße 191, 1220 Wien
E-mail: hemma.koeglberger@ages.at

In recent years winter losses of honey bee colonies were high, culminating in winter 2014/15 with losses of 28.4 % of Austrian bee colonies. The Austrian surveillance programme aims at clarifying the reasons of winter losses. In summer (July/August 2015), fall (September/October 2015) and spring (March/April 2016) 190 apiaries all over the country, covering the full range of geographical distribution and size of beekeeping operations,were inspected by trained bee inspectors. A representative number of colonies was examined visually for clinical symptoms of bee diseases,and bee samples (300 bees) were taken from up to ten colonies per apiaryin order to analyse varroa infestation ratesduring the visits of summer and autumn 2015. The bee samples were checked for varroamites employing the washing method and the infestation rate was calculated as the percentage of the number of detected varroa mites per number of bees.During the third inspection in March/April 2016 the colony losses wererecorded.

In summer the infestation rate of the bee samples ranged from 0 % to 40.4 %(median 0.3 %). In autumn the infestation rate ranged from 0 % to 137.9 %(median 0.6 %). In 48 % of the summer-samples and in 31 % of the autumn-samples no varroa mite was found (0 % infestation rate).

Bee samples with varroa mites were detected in92 % of the apiaries in summer and 97 % of the apiaries in autumn. On apiary level the varroa infestation rate was often low in summer (0 % median varroa infestation rate in 44 % of the apiaries) and increased in autumn (0 % median varroa infestation rate in 2 % of the apiaries). However, a small proportion of apiaries was highly infested with varroa: the median of varroa infestation levels exceeded 5 % infestation rate in 2 % (summer) and in 9 % (autumn) of the apiaries, respectively.

Winter colony losses will be evaluated for correlations with varroa infestation rates of bees in summer and autumn.

The study is part of the project "ZukunftBiene" (www.zukunft-biene.at), funded by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management, BieneÖsterreich and the Austrian federal provinces (DaFNEProj. 100972, www.dafne.at).

Prevalence of the main pathogens of Honey bees determined by passive laboratory surveillance

Raquel Martín-Hernández and Mariano Higes
Bee Pathology Laboratory, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF). Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha. Marchamalo, 19180 Guadalajara, Spain. INCRECYT.
E mail: rmhernandez@jccm.es

The health status of honey bees is receiving much attention due to the recent decline of these populations. To detect potential disease outbreaks or changes in honey bee health, a passive pathogen surveillance system has been developed at the pathology laboratory of the CIAPA. A total of 210 (in 2014) and 200 (in 2015) adult honey bee samples were received in our laboratory for detection of the main pathogens of bees. The number of pathogens detected in those samples coming from apiaries with pathological problems in both years was higher than those detected in samples submitted for routine analysis. The most prevalent pathogen was *N. ceranae* in both types of samples, significantly higher in those reporting pathological problems (around 90% both years) than in samples from routine analysis (<3%). Trypanosomatids were found in 17,4% (2014) and 21,4% (2015) in samples from apiaries with pathological problems and in 5,1% 6,1% of samples from routine analysis. In all cases, the Trypanosomatids-positive samples were also positive for *N. ceranae.* Also, *Varroa destructor* was detected in around the 22 % of samples with pathological problems and in less than 6% in routine ones. All positive samples to *V. destructor* in group with pathological problems were also positives to *N. ceranae*. Therefore, the synergistic action of both pathogens may be lethal to the bee colony. The high prevalence of these pathogens requires continuous monitoring measures in time in order to obtain the epidemiological data and to harmonize the necessary control actions for each case.
Work funded by the Gobierno de Castilla-La Mancha (Consejería de Agricultura), INIA-FEDER (RTA2013-00042-C10-06).

Epidemiological surveillance in Spain: pathogens as risk factors for honey bee colony collapse.

Almudena Cepero, Raquel Martín-Hernández, Carolina Bartolome, Tamara Gómez-Moracho, Laura Barrios, José Bernal, María Jesús del Nozal, José Luis Bernal, Aránzazu Meana and Mariano Higes
Bee Pathology Laboratory, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF). Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha. Marchamalo, 19180 Guadalajara, Spain.
E -mail: rmhernandez@jccm.es

Many European and North American countries have reported losses in honey bee populations over recent years. Although multiple factors appear to be involved in this decline, the influence of pathogens may be particularly important. In the present study, we analyzed the presence of the major honey bee pathogens and neonicotinoid insecticides in ten professional apiaries (five affected by high honey bee colony mortality and five asymptomatic apiaries). Our results confirm that *Nosema ceranae* and trypanosomatids were very prevalent in the apiaries affected by depopulation and high colony losses. By contrast, , neonicotinoid insecticides were not found so they did not play an important role in colony mortality in these apiaries. This kind of passive surveillance system is important to collect information about pathogen incidence, particularly when supplemented with a strong system of active disease surveillance that enables emergent diseases to be detected.

Acknowledgments: Financial support for this work was provided by the Gobierno de Castilla-La Mancha (Consejería de Agricultura), and INIA-FEDER (RTA2013-00042-C10-03-06).

Discrepancy between acute and chronic toxicity induced by Thiamethoxam in *Apis mellifera intermissa* and *Apis mellifera sahariensis*

Nora Chahbar[1], Mohamed Chahbar[2] and Salaheddine Doumandji[3]
1Département de Biologie, Faculté des Sciences, Université M'Hamed Bougara, Boumerdes, Algérie
2,3Département de Zoologie Agricole et Forestière, Ecole Nationale Supérieure Agronomique
El-Harrach, Alger, Algérie
Email: chahbar_nora@yahoo.fr

Thiamethoxam [3-(2-chlorine-1, 3-thiazole-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro) amine], a systemic insecticide of the group of neonicotinoids with a large spectrum of action at low concentrations. It is used in the control of sucking insects and some chewing species, because of its excellent absorption and translocation in plants. Oral acute and chronic toxicity of thiamethoxam were investigated in two *Apis mellifera* subspecies, *Apis mellifera intermissa* and *Apis mellifera sahariensis.* Acute intoxication by thiamethoxam resulted in the rapid appearance of neurotoxicity symptoms, such as hyperresponsiveness, hyperactivity, and trembling and led to hyporesponsiveness and hypoactivity. For acute toxicity tests, bees were treated with doses of toxic compounds ranging from 1 to 90 ng/bee. The LD50 values of thiamethoxam at 24h were about 12,3 ng/bee for *A. m. intermissa* and 13,3 ng/bee for *A. m. sahariensis.* The dose–mortality relation revealed directly proportional relationship between the administered dose of thiamethoxam and mortality observed. The mortality is reached maximum at 24 hours after treatment with doses above 50 ng / bee after oral application. Response kinetics showed classic kinetics. The higher the dose of thiamethoxam and faster is high mortality appears. To test chronic toxicity, worker honeybees were fed sucrose solutions containing 0,01µg, 0,1µg, 1µg et 10 µg/L of thiamethoxam for 10 days. For the high dose, it is apparent that 50% average mortality are obtained after 7 days of exposure to toxic to *A. m. intermissa* and after 8 days for *A. m. sahariensis.*

A case of acute intoxication with carbofuran in honeybees

Cristina Țoca and Daniela Nica
Institute For Diagnosis and Animal Health, Bucharest, Romania
E-mail: toca.cristina@idah.ro

Honeybees are vulnerable to many pesticides used to control damaging pest species by fruit, vegetable, seed growers.

At the begining of 2016 a beekeeper of an individual farm observed a high mortality of his colonies. He found an excesive number of dead bees in all the hives. To establish the cause of the death he called the veterinarian who decided to sent immediately bees samples for toxicological investigations.

Due to the rapid death of the bees chemical analysis were performed to identify pesticides (organophosphorates, neonicotinoides and piretroids) using a LC-MS screening method. The results were negative for the compounds mentioned above and positive for carbofuran.

The detection of carbofuran at low levels was achieved using an accurate mass confirmation on high resolution exactive benchtop LC-MS Orbitrap mass spectrometer.

This method is suited for routine analysis for the determination of pesticide residues in cases of suspected honeybee poisoning incidents.

Toxic nectar is responsible of River disease syndrome in Uruguay

Daniela Arredondo, Pablo Juri, Enrique Nogueira, Estela Santos, Belén Branchiccela, Karina Antúnez and Ciro Invernizzi
Department of Microbiology, Instituto de Investigaciones Biológicas Clemente Estable, Uruguay
E-mail: cirobee@gmail.com

"River disease" (RD) is a syndrome that affects honey bee colonies located close to the rivers with abundant riparian vegetation in Uruguay, at early summer. This syndrome is characterized by the massive death of young larvae (1- 2 days old), which progressively lead to the colony loss. Colonies are recovered when they are relocated and pollen and nectar stores are removed. Previous studies suggested that nectar from RD colonies is toxic for larvae. According to palynological analysis, this nectar contains honeydew; and field observation confirmed the

presence of forager bees on the leaves of *Sebastaiana schottiana*, collecting honeydew of a planthopper (Hemiptera, Flatidae).
In the present study we aimed to confirm that nectar collected from RD colonies, as well as the honeydew produced by the planthopper are toxic for larvae. We modified the model of larvae rearing to include nectar in the food, and evaluated the survival of larvae fed with nectar collected from healthy colonies, nectar from healthy colonies supplemented with planthopper honeydew (30%), or nectar from RD colonies. Experiments were carried out by triplicates with n=24 per group.
Statistical analysis of survival curves showed that larvae fed on nectar from RD colonies, as those that received honeydew, showed significantly higher mortality than control groups, confirming their toxicity (Gehan Beslow test, $p < 0.001$, multiple comparison $p < 0.001$ in both cases). Studies are being carried out to identify the chemical component of nectar and honeydew to confirm their role in larvae mortality.

A rapid and sensitive LC-MS/MS method for determination of neonicotinoids in beebread

Anne-Claire Martel, Romain Ducros, Patrick Mangoni and Adrien Bégaud
ANSES, Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes - CS 20111, 06902 Sophia Antipolis, France
E-mail: anne-claire.martel@anses.fr

Neonicotinoids are insecticides highly toxic to honey bees. Until 2013, they were widely used on different crops as foliar sprays or seed treatments. In December 2013, the European Union had suspended the use of imidacloprid, clothianidin and thiamethoxam on four field crops (maize, colza, sunflower and cotton) for two years. Since Januray 2016, new evaluations of these three pesticides were requested by the European Commission.
When these systemic compounds are applied in the environment, low concentrations are subsequently found in the nectar and pollen of the crops, which are then collected, stored in hives and consumed by the colony.
A procedure for the determination of thiamethoxam, imidacloprid, clothianidin, acetamiprid and thiacloprid in beebread (pollen and honey mixture) is described.

The method involves the extraction of pesticides using acetonitrile and liquid partitioning with *n*-hexane. One clean up is then performed on a Bond Elut Plexa cartridge (200 mg, 6 ml) and the extract is analyzed by LC-ESI-MS/MS in SRM mode. The recoveries were obtained by spiking beebread samples free of pesticides at two concentration levels of the various neonicotinoids. The recoveries were in the range between 89.6 and 108.0% with relative standard deviation (RSD) less than 20%. Detector linearity covers the range of 0 to 40 µg/l for thiamethoxam and thiacloprid, of 0 to 20 µg/l for clothianidin and imidacloprid and of 0 to 8 µg/l for acetamiprid. The limits of quantification (LOQ) were 0.5 µg/kg for thiamethoxam and thiacloprid, 1 µg/kg for acetamiprid and 2.5 µg/kg for imidacloprid and clothianidin.

Gene expression in worker honeybees (*Apis mellifera carnica*) exposed to neonicotinoid thiametoxam and Varroa mites (*Varroa destructor*)

Ivanka Cizelj, Tanja Tesovnik, Janko Božič, Gordana Glavan and Mojca Narat
University of Ljubljana, Biotechnicalfaculty, DepartmentofAnimalSciences, Chairforgenetics, animalbiotechnologyandimmunology
E mail: tanja.tesovnik@bf.uni-lj.si

Intensive agriculture depends heavily on the use of pesticides.There is a limited information available about the effectsof pesticides on infected bees. The ingestion of certain chemicals significantly alters the gut microflora and could affect bee development, several metabolic pathways, weakens the bee's immune defense mechanisms.In spite of laws and regulations, Slovenia has lost 100.000 bee families in the period of 2007-2009. Europe and America also face the disturbing phenomenon of disappearance of bee families termed Colony collapse disorder (CCD), the cause of which is presumed to be the suppression of their immune system as a consequence of prolonged exposure to chemicals.In this study, the effects of neonicotinoid thiametoxamon honeybees *Apis mellifera carnica* previously infested by *Varroa destructor* miteswereanalyzed at the molecular level. One week before the experiments were undertaken four honeybee colonies without a queen were established. Just before the start of the experiments unsealed honeybee broods originating from the same honeybee colony to reduce genotypic variability were introduced. Two groups of *Varroa* mite infested brood were

exposed to mites for 10 days. Afterwards allcolonies were fed with 100 g protein cake, provided daily for the bees. One infested and one non infestedcolonyreceived protein cake with thiametoxam (10µg/kg). After 10 days exposure to thiametoxam, samples of adult worker honeybees were collected. Total RNA was extracted from 12 bees/group and complementary DNA (cDNA) was synthesized. Expression of 8detoxification, 2 developmental, 17 immune related and 5 apoptosis related genes were analyzed by real-time PCR.

Primary cell lines isolated from honeybees *Apis mellifera carnica*

Manuela Čitar, Irena Oven, Ivanka Cizelj, Gordana Glavan and Mojca Narat
University of Ljubljana, Biotechnical faculty, Department of Animal Sciences, Chair for genetics, animal biotechnology and immunology
E mail: manuela.citar@bf.uni-lj.si

Majority of studies regarding the impact of different chemicals and pathogens were performed on bees/ bee families. The studies are carried out under unstandardized conditions therefore the results obtained are difficult to compare. We aim to establish a permanent honeybee cell lines for standardized *in vitro* testing of the effects of xenobiotics and pathogens, which would enable learning and understanding the mechanisms present *in vivo*. The first step was the preparation of primary cell lines. Here we describe methods that are effective for maintaining various honeybee cells in *in vitro* conditions. The preparation of cells from different honeybee *Apis mellifera carnica* developmental stages and the optimization of growth conditions and media for different cell types, including supplements were standardized. Multiple bee cell cultures were produced using bee eggs, larvae, pupae, queen bee ovaries, brains and hypopharyngeal glands as a starting material. Only cells from eggs and pupae were used for further tests. Supplemented L-15 media was found to support long-term growth of eggs while modified WH2 media was established for long-term cultivation of pupae cells. The cells were successfully cultured for up to 6 months and the bee origin of the cells was confirmed using PCR specific primers. Multiple cell phenotypes were observed including free-floating small suspension cells, neuron-like monolayers and cells with multiple nuclei. Cells obtained from pupae were successfully cryopreserved. After thawing their viability was estimated and was always higher

than 85 %. For this purpose trypan blue and *propidium iodide were used, the latter being more* appropriate.

Pathogen spillover of Crithidia mellificae from honey bees to solitary bees (Osmia cornuta)?

Verena Strobl, Matthias Albrecht, Orlando Yañez and Peter Neumann
Schwarzenburgstrasse 161, 3003 Bern, Switzerland
E-mail: verena.strobl@vetsuisse.unibe.ch

Pathogen spillover from managed honey bees, Apis mellifera, has been suggested to contribute to the decline of wild bees. However, the detection of honey bee pathogens in wild bees does not necessarily imply that the latter can actually serve as novel hosts. Moreover, there might be differeces in susceptibility between haploid males and diploid females as predicted by the haploid susceptibility hypothesis. Here, we test if the trypanosome Crithidia mellificae, an endoparasite of honey bee, can successfully infect the solitary bee Osmia cornuta and whether males of this bee are more susceptible. Male and female Osmia cornuta (Megachilidae), were mass fed with C. mellificae for 48 h using contaminated sucrose solution ad libitum (106 cells / ml). Bee gut dissections were conducted every 4-6 days up to 15 days in males (N=30) and 24 days in females (N=45) post infection and C. mellificae cells counted using microscopy and Neubauer chambers. Our results clearly show that C. mellificae cell numbers increased in both male and female O. cornuta 2.5 times between days 6 and 15 post infection. A significant positive correlation was observed between C. mellificae cell replication and days post infection (Spearman $|r| = 0.6976$, P 0.05). Our results clearly show that the solitary bee O. cornuta is susceptible to infections with C. mellificae, thereby supporting a considerable potential for pathogen spillover between managed and wild bees.

Diagnosis of the small hive beetle (*Aethinatumida* Murray, 1867): optimization and standardization of two identification methods based on morphological examination and real-time PCR

Stéphanie Franco[1], Aurélie Delcont, Nicolas Cougoule, Magali Ribière-Chabert and Véronique Duquesne
[1]Anses, Honey Bee Pathology Unit, European Union Reference Laboratory for Honey Bee Health, 105 route des Chappes – CS 20111, 06902 Sophia Antipolis, France.
E-mail: stephanie.franco@anses.fr

The small hive beetle (SHB), *Aethinatumida*Murray 1867(Coleoptera: Nitidulidae), is an invasive pest of bee hives, originally from sub-Saharan Africa.It was introduced in different countries throughout the world severely damaging honeybee colonies.Previously absent in Europe, SHB was detectedin Reggio Calabria, South West Italy, on 5$^{th.}$ September 2014; several dozens of cases were subsequently confirmed in 2014 and 2015 in this region.The SHB infestation is regulatedin the European Unionand belongs to the OIE list of diseases.
Considering these sanitary issues, in case of detection, rapididentification is crucialto confirm the suspicion and to implementsanitary measures to avoid spreading.Twomethods have been optimized by the European Union Reference Laboratory for HoneyBee Health to ensure the quality of analytical results. Morphological identification is performed for primary diagnosis. Specific criteria have been selected to differentiate SHB from other Nitidulidaecoleoptera found in honey bee hives and from wax moths larvae. In a second stage, molecular identification is carried out for confirmation, particularly on larvae or when specimensare damaged.This method adapted from a previously published assay is based on a real-time PCR. The complete method has been characterized and validated in accordance with international standards. Several reference materials have been produced to control the parameters of the PCR step and of the complete method.
These two methods have been disseminated to European national reference laboratories in order to standardize and ensure reliablediagnosis of SHB at the European level.

Investigation and molecular characterization of important honey bee (*Apis mellifera* L.) viruses in Hakkari province (Turkey) by using RT-PCR

Mustafa Rustemoglu and Hikmet Murat Sipahioglu
[2]Department of Plant Protection, Faculty of Agriculture, Sirnak University, Sirnak/İdil – TURKEY
Email: mustafa.rustemoglu@gmail.com

The present study investigates the existence and prevalence of the infections of *Deformed wing virus* (DWV), *Black queen cell virus* (BQCV), *Israeli acut paralysis virus* (IAPV), *Sacbrood virus* (SBV), *Kashmir bee virus* (KBV) and *Chronic bee paralysis virus* (CBPV) by RT-PCR method in Hakkari province of Turkey. Specific primers were designed for the genome of each virus in order to use for the molecular detection of these viruses. The viruses were investigated in 90 apiaries in total. BQCV was detected in 32,2% of apiaries, DWV in 23,3%, SBV in 12,2%, CBPV in 8,8% and ABPV in 2,2%. The identification tests showed that many apiaries were infected with one or more viruses: 35.5% of apiaries were positive for a single infection, 20% a double infection and 1.1% a triple infection. KBV and IAPV were not be detected in any apiaries. In Hakkari province, this is the first report for the four viruses. The presence of SBV is the first record in Turkey. For each identified virus species, a virus isolate was selected randomly for molecular characterization. For BQCV and SBV partial coat protein (CP) genes and for DWV and CBPV partial RdRp genes were cloned in a proper cloning vector and sequenced. Blast analysis in NCBI database, the sequenced Hakkari isolates of BQCV, DWV, SBV, and CBPV have been showed nucleic acid similarity 90%, 99%, 90%, and 86% as compared with other world isolates, respectively.

SAVA: a mobile app to declare disorders and disease outbreaks in bees

Porphyre Vincent.[1], Esnault O.[2], Tendero A.[3] and Armand J-.M.[4]
(1) Cirad, Umr112 Selmet, Station Ligne Paradis, Saint-Pierre (La Reunion); (2) Gds974, Le Tampon (La Reunion); (3) Cirad, Umr Aida, Station Ligne Paradis, Saint-Pierre (La Reunion) ; (4) Inra Cr Bordeaux-Aquitaine, Umr 1065 Save, Villenave D'ornon (France).
E-mail: vincent.porphyre@cirad.fr

In the South-West Indian Ocean, beekeepers produce high value and specific honeys thanks to local bees and indigenous or cultivated exotic melliferous plants. Sanitary hazards are numerous: Reunion and Rodrigues Islands have a remarkable sanitary situation with no *Varroa destructor*, which was recently reported in Madagascar and Mauritius. Poor sanitary information is available for Madagascar, Comoros and Seychelles.
SAVA (French acronym for "Alert and Surveillance System for Bee keeping") is a smartphone app to help beekeepers from Reunion and Indian Ocean islands to send sanitary alerts to the regional surveillance network for bee diseases. Targeted users are beekeepers, technicians and other professionals of the bee industry.
The 2016-version of SAVA app allows users (1) to identify a possible bee disease or disorder thanks to a decision tree and images, and (2) to better manage any suspected outbreaks in apiaries by sending georeferenced and standardized information about observed disorders. The observed disorders or macroscopic elements to declare are parasites, small hive beetle, acute mortalities, brood disease and bee abnormalities. The SAVA web interface allows the network managers to map reported cases, and to better organize the field operations to confirm or/and control outbreaks.
SAVA app allows also to declare the position of hives and inform the Official Health Services that are in charge of mosquitoes control campaigns, and to declare robberies of hives and inform the beekeepers community.
SAVA was recently implemented in the field in La Reunion Island and will soon be used in South East region of Madagascar.

Acknowledgements
This work is part of theQualiREG network and supported by the Regional Cooperation Program of the French Regional Council of La Reunion(French overseas territory), the European Regional development Funds (ERDF) and the French Government. The QualiREG network is dedicated to scientific and technical cooperation in agriculture and agribusiness to improve food safety and quality of agri-products, by increasing information sharing, improving research

capacities, and supporting economic initiatives in Indian Ocean. QualiREG gathers 60 institutions from Madagascar, Comoros, Mauritius and Rodrigues, Reunion, Seychelles, and South Africa. **More details:**www.qualireg.org
The authors thank gratefully the INRA Ephytia team for their technical support. **More details:** http://ephytia.inra.fr

More information

To download the SAVA app (Android only)

Notes:

Author index

SPONSORS

Primăria
Municipiului
Cluj Napoca