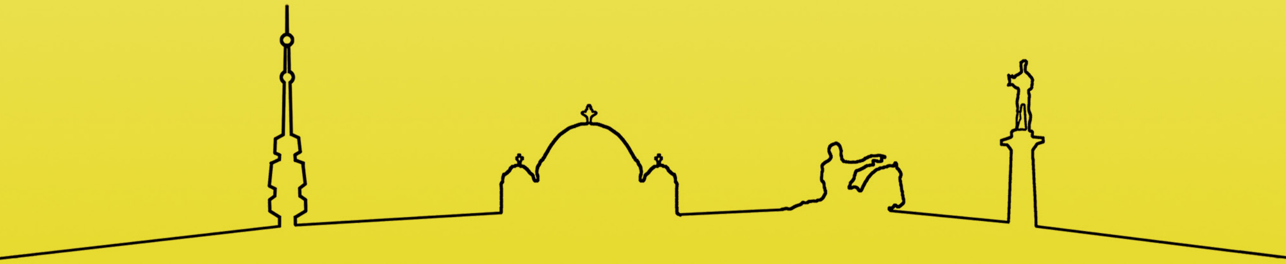
EurBee 9
9th European Congress of Apidology

20-22 September 2022
Belgrade, Serbia

Abstract Book

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WELCOME

On behalf of the European Association for Bee Research (EurBee) I have the great honour and pleasure to invite you to attend the 9th European Congress of Apidology (EurBee 9) convened under the motto: "Save the bees for our future".

The conference intends to promote knowledge exchange and international cooperation and to enable the further exchange of up-to-date scientific information on different aspects of managed and wild bees among researchers, teachers, students, experts in extension. EurBee has become the main European event for bee scientists studying how bees respond to climatic changes to discuss problems with bee health, conservation, ecology, diversity, pollination, beekeeping and colony losses.

The six world-renowned experts in bee's science are invited to give keynote lectures to present the latest developments in their research fields. Each morning and afternoon will commence with a plenary lecture, followed by three parallel symposia. Scientists from Europe and far beyond are welcome to Belgrade to exchange recent advancements and ideas that emerge from basic and applied research.

The conference serves as a connection platform for top European research in Apidology and hosts the pan-European research projects and networks. EurBee 9 congress should contribute to identifying new approaches, tools, and techniques to meet future needs and challenges facing bee science.

We will do our best to make the EurBee 9 successful and enjoyable as possible and look forward with great anticipation to seeing you in Belgrade, Serbia, September 20-22, 2022.

Sincerely Yours,
Ljubiša Stanisavljević,
Host Committee President

COMMITTEES

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PRACTICAL ISSUES

Badge

Your personal name badge is your entrance ticket to all sessions and other activities of the EurBee 9 congress. Please remember to wear this badge at all times during the meeting and the social activities. At the back of your badge is a number that you can call in case of emergencies.

Languages

The meeting language will be English. No simultaneous interpretation in other languages will be offered.

Dress code

The dress code will be ‘casual’ throughout the meeting.

Time zone

The time in Serbia is Central European Time (CET), which is GMT+1, plus 1 hour in summer.

Emergency numbers

In Serbia - to contact the police dial 192 - to contact the fire brigade dial 193 - to contact the ambulance service dial 194

Electrical supply

Electricity is supplied at 220 volts, 50 Hz. Plugs and sockets are the same as in the Continental countries of the European Union. British, Irish and non-European participants need plug adaptors for electrical appliances.

Driving license

International Driving Licenses are recognized throughout Serbia. Car rental companies ask for a valid driving license. For your requests please contact Congress secretariat.

Currency

Serbian official currency is the dinar (RSD). One dinar consists of 100 para. Currency exchange is done in all banks and exchange offices. The current exchange rate may be checked on the website of the National Bank of Serbia, but rates at different exchange offices might differ slightly.

Insurance

The Meeting organizers cannot accept liability for personal injuries sustained, for loss of, or damage to, property belonging to meeting participants (or their accompanying persons), either during or as a result of the meeting. Please check the validity of your own travel insurance.

Liability

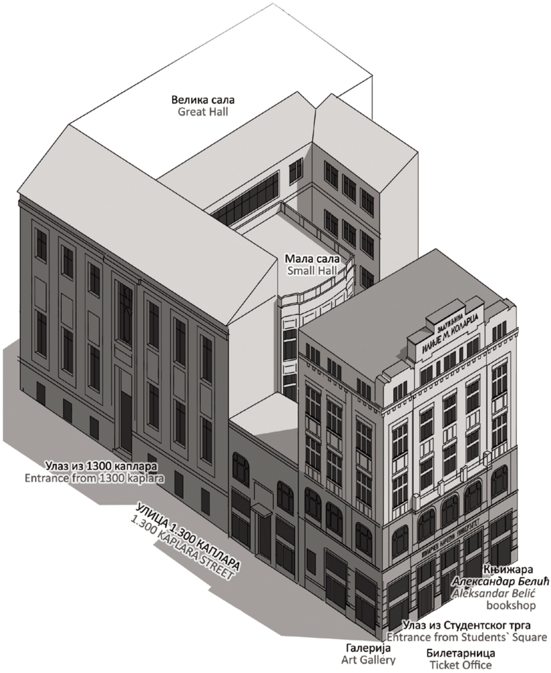
In the case of Government intervention or regulation, military activity, strikes or any other circumstances that make it impossible or inadvisable for the meeting to take place at the time and place provided, the participant shall waive any claim for damages or compensation except the amount paid for registration after deduction of actual expenses incurred in connection with the congress and there shall be no future liability on the part of either party.

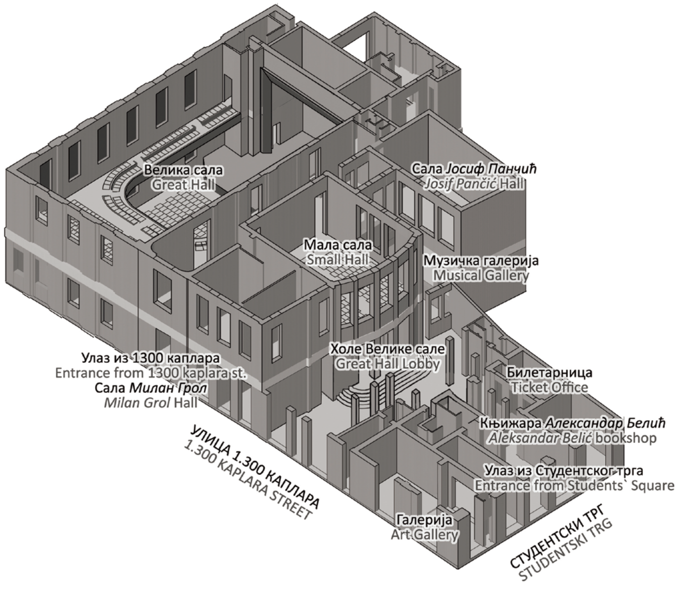
Coffee/tea will be served at the “Entrance Hall” of Ilija M. Kolarac Endowment.

Lunch will be served daily at the hotel Palace. Address: Topličin Venac 23, 11000 Belgrade

VENUE MAP

ILIJA M. KOLARAC ENDOWMENT





PROGRAM OVERVIEW

MONDAY 19 SEPTEMBER

12:00-19:00	**Registration**	*Ilija M. Kolarac Endowment*
19:00-21:30	**Opening Ceremony and Welcome Reception**	*Great Hall*

Legend:

Symposia

S01	Genetics and genomics
S02	Ecology and biodiversity
S03	Pathology
S04	Immunity
S05	Nutrition
S06	Microbiota
S08	Ecotoxicology, pesticides
S09	Pollination and bee flora
S10	Pan-European research networks
S11	Quantitative genetics, reproduction and bee breeding
S12	Communication and chemical ecology
S13	Monitoring bees
S14	Open

O– Oral contribution
P – Poster contribution
* – *Silver Sponsor*
** – *Faculty of Biology*

TUESDAY 20 SEPTEMBER

08:00-12:00	**Registration**	*Ilija M. Kolarac Endowment*
08:15-08:30	**Welcome Address**	*Great Hall*
08:30-09:30	**Plenary Session 1** **Juliet Osborne**, *Bee Ecology*	*Great Hall*
09:30-11:00	**Oral Contributions**	
09:30-11:00	**S01** Genetics and genomics (O1-O6)	*Room "Josif Pančić"*
09:30-11:00	**S02** Ecology and biodiversity (O1-O6)	*Music Gallery*
09:30-11:00	**S03** Pathology (O1-6)	*Great Hall*
09:30-11:00	**S10** Pan-European research networks (O1-O6)	*Alveis Room**
11:00-12:00	**Poster session 1 & Coffee Break**	*Entrance Hall*
	S01 Genetics and genomics (P1-P7)	
	S03-1 Pathology (P1-P18)	
	S05 Nutrition (P1-P7)	
	S8 Ecotoxicology, pesticides (P1-P22)	
12:00-13:00	**Plenary Session 2** **Sara Diana Leonhardt**, *Nutrition*	*Great Hall*
13:00-15:00	Lunch	
15:00-16:00	**Oral Contributions**	
15:00-16:00	**S01** Genetics and genomics (O7-O10)	*Room "Josif Pančić"*
15:00-16:00	**S10** Pan-European research networks (O7-O10)	*Alveis Room**
15:00-16:15	**S02** Ecology and biodiversity (O7-O11)	*Music Gallery*
16:00-16:30	**S03** Pathology (O7-O8)	*Alveis Room**
16:30-17:30	**Poster session 1 & Coffee Break**	*Entrance Hall*
	S01 Genetics and genomics (P1-P7)	
	S03-1 Pathology (P1-P18)	
	S05 Nutrition (P1-P7)	
	S08 Ecotoxicology, pesticides (P1-P22)	
17:30-18:45	**Oral Contributions**	
17:30-18:15	**S04** Immunity (O1-O3)	*Music Gallery*
17:30-18:30	**S03** Pathology (O9-O12)	*Alveis Room**
17:30-18:45	**S06** Microbiota (O1-O5)	*Room "Josif Pančić"*
18:45-19:30	**Members Meeting**	*Alveis Room*

WEDNESDAY 21 SEPTEMBER

08:00-12:00	**Registration**	*Ilija M. Kolarac Endowment*
08:15-08:30	**Report from Members meeting**	*Great Hall*
08:30-09:30	**Plenary Session 3** **Maj Rundlöf**, *Pesticides*	*Great Hall*
09:30-11:00	**Oral Contributions**	
09:30-11:00	**S03** Pathology (O13-O18)	*Alveis Room**
09:30-11:00	**S08** Ecotoxicology, pesticides (O1-O6)	*Great Hall*
09:30-11:00	**S09** Pollination and bee flora (O1-O6)	*Room "Josif Pančić"*
09:30-11:00	**S13** Monitoring bees (O1-O6)	*Music Gallery*
11:00-12:00	**Poster session 2 & Coffee Break**	*Entrance Hall*
	S02 Ecology and biodiversity (P1-P13)	
	S03-2 Pathology (P1-P19)	
	S04 Immunity (P1-P2)	
	S06 Microbiota (P1-P5)	
	S09 Pollination and bee flora (P1-P10)	
12:00-13:00	**Plenary Session 4** **Martin Beye**, *Genetics, CRISPR-Cas*	*Great Hall*
13:00-15:00	Lunch	
15:00-16:00	**Social Event** Visiting the "Naš med" (Our honey) factory of the Association of Beekeeping Organizations of Serbia. Visiting Topola a town and municipality located in the Šumadija District of central Serbia.	
16:00-23:45	Dinner	

THURSDAY 22 SEPTEMBER

08:00-12:00	**Registration**	*Ilija M. Kolarac Endowment*
08:15-08:30	**Eva Crane Trust**	*Great Hall*
08:30-09:30	**Plenary Session 5** **Quinn McFrederick**, *Pathogen spill-over*	*Great Hall*
09:30-11:00	**Oral Contributions**	
09:30-11:00	**S03** Pathology (O19-O24)	*Alveis Room**
09:30-11:00	**S09** Pollination and bee flora (O7-O12)	*Room "Josif Pančić"*
09:30-11:00	**S011** Quantitative genetics, reproduction and bee breeding (O1-7)	*Music Gallery*
09:30-11:00	**S13** Monitoring bees (O1-O6)	*Great Hall*
11:00-12:00	**Poster session 3 & Coffee Break**	*Entrance Hall*
	S10 Pan-European research networks (P1-P6)	
	S11 Quantitative genetics, reproduction and bee breeding (P1-P8)	
	S13 Monitoring bees (P1-P20)	
	S14 Open (P1-P15)	
12:00-13:00	**Plenary Session 6** **Jane Stout**, *Pollination*	*Great Hall*
13:00-15:00	Lunch	
15:00-16:00	**Oral Contributions**	
15:00-16:30	**S05** Nutrition (O1-O6)	*Room No. 11 ***
15:00-16:30	**S08** Ecotoxicology, pesticides (O7-O12)	*Music Gallery*
15:00-16:15	**S09** Pollination and bee flora (O13-O17)	*Room "Josif Pančić"*
15:00-16:45	**S13** Monitoring bees (O13-O19)	*Alveis Room**
16:30-17:30	**Poster session 3 & Coffee Break**	*Entrance Hall*
	S10 Pan-European research networks (P1-P6)	
	S11 Quantitative genetics, reproduction and bee breeding (P1-P8)	
	S13 Monitoring bees (P1-P20)	
	S14 Open (P1-P15)	
17:30-19:00	**Oral Contributions**	
17:30-18:15	**S05** Nutrition (O7-O9)	*Room No. 11 ***
17:30-18:00	**S08** Ecotoxicology, pesticides (O13-O14)	*Music Gallery*
17:30-19:15	**S12** Communication and chemical ecology (O1-O7)	*Alveis Room*
	S14 Open (O1-O5)	*Room "Josif Pančić"*
20:15-24:00	**Awards and Closing Ceremony**	***Restaurant on boat***
20:15-24:00	Banquet	*Restaurant on boat*

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PLENARIES

Juliet Osborne
Environment & Sustainability Institute, University of Exeter, Cornwall, UK

Bee Ecology

Professor Juliet Osborne is a the Director of the Environment and Sustainability Institute (ESI) at the University of Exeter and she leads a research team studying bee movement, ecology and pollination in agro-ecosystems. The team uses a combination of novel technology, experiments and models to predict the effects of changing threats on bee survival, and subsequent impacts on pollination. In particular Juliet is best known for her work tracking individual flying bees and butterflies with harmonic radar, and most recently tracking asian hornets to their nests with radio telemetry. Juliet's research team has also developed and validated individual based systems models of bee colonies and populations: the **BEEHAVE models** which can be used to predict the impact of multiple stressors on bee colonies, in realistic landscapes, over large spatial scales and long time frames.

MIND THE GAP: TRANSLATING BEE ECOLOGY RESEARCH INTO REAL WORLD CONSERVATION

Bee ecology research is thriving with hundreds of papers published each year. Many of these have a practical message relating to the application of that research to help conserve bee communities. But how many of these practical actions are implemented and actually make a difference to bee populations? The field of bee ecology has transformed over the last 30 years: I will share some of our studies uncovering the hidden flight patterns of bees and hornets, and modelling bee populations, and I will reflect on whether this work has had the practical impact we had hoped to support the conservation of bees. In short, has 30 years of research saved many bees?! To make future progress: we need much better understanding of the socio-ecological systems in which we are aiming to conserve pollinators, working directly with communities and businesses to close the gap between scientific knowledge and the delivery of conservation action at scale.

Keywords: Ecology, Conservation, Bumblebees

Sara Diana Leonhardt
Technische Universität München, Germany
Nutrition

Sara Leonhardt is a professor at the Technical University of Munich in Germany. She is interested in the chemistry underlying plant-insect interactions – with particular focus on the proximate and ultimate mechanisms underlying the exploitation and use of chemically and functionally diverse plant resources by bees. Projects in her group aim at unravelling the link between resource quality & diversity and the sensory ecology, behavior, organization & fitness of both social and solitary bees.

ARE YOU WHAT YOU EAT? HOW BEES MASTER THE CHALLENGE OF GETTING GOOD FOOD

Co-authors: Johannes Spaethe, Fabian Rüdenauer, Carmen Nebauer, Marielle Schleifer, Alejandra Parreno, Susanne Butschkau, Alexander Keller, Michael Kuhlmann et al.

Bees are unique in obtaining all required micro- and macro-nutrients from pollen and nectar. Nectar provides mainly sugar, while pollen is the main source of protein, lipids and various micro-nutrients, such as phytosterols or vitamins. Pollen is thus pivotal for offspring production and development, but also supports adult health. However, while nectar is a reward specifically produced for pollinators, pollen is only occasionally a reward. It primarily serves the plants' own reproductive interests carrying the male gametes. Moreover, pollen is highly variable in its chemistry with regard to both nutrients and plant secondary metabolites. On top, chemical variation occurs within and between plant species. Bees consequently need to constantly assess pollen chemistry, i.e. quality, to ensure allocation of appropriate pollen diets. As most pollen is not consumed directly by adult foragers, but transported to nests for offspring provisioning, pollen assessment should take place prior to consumption.

Allocating appropriate pollen diets clearly represents a challenging task for female bees, even more so in today's landscapes, where floral abundance and diversity has dramatically decreased due to intensive land-use by humans. Recent studies indicate that pollen (and nectar) quality are additionally affected by climatic changes, e.g. drought, and the use of agrochemicals.

Together with our collaborators, we aim to better understand: (i) the role of different nutrients for bee performance, (ii) how bees allocate appropriate pollen diets, and (iii) how changes in floral diversity and composition affect nutritional niches, health and fitness of (wild) bees. To address our questions, we combine behavioral experiments, DNA metabarcoding, analytical chemistry and field observations. Our findings point to species-specific nutritional niches and nutrient-foraging strategies and thus strong differences between species in their sensitivity towards alterations of floral communities.

Maj Rundlöf
Department of Biology, Lund University, Lund, Sweden
Pesticides

Dr Maj Rundlöf is an ecologist and environmental scientist at Lund University in Sweden, interested in understanding the multitude of factors influencing biodiversity and the functions organisms provide in ecosystems and to humans. She is particularly interested in how land use change and management of agricultural landscapes affect pollinators and the pollination services they provide to both crops and wild plants. In the past few years, she has also been developing the field of landscape ecotoxicology, to understand how bees are exposed to and effected by pesticide use in the landscape.

BEES AND PESTICIDES – LANDSCAPE ECOTOXICOLOGICAL PERSPECTIVES ON EXPOSURE, EFFECTS, AND MITIGATION

A bee is a bee is a bee – or? Well, this may in general be true from a toxicological perspective, even if there are exceptions, removed from their (non-)social and ecological context and into a laboratory exposed to some quantity of a pesticide. However, with the diversity of bee species and related life-history traits, both exposure and resulting effects will vary. Contemporary agricultural pesticide uses result in bee exposure to a mixture of pesticides, but we know less about how this exposure varies among species and landscape contexts, what the results are for bee health in agricultural landscapes or how to mitigate potential effects. We suggest a trait-based framework to predict pesticide mixture-related environmental risk among bee species and landscape context, where extensive foragers, which form large colonies and use advanced forage resource communication, would experience the highest risk and least landscape context sensitivity compared to more limited solitary foragers. We use pesticide residues in multi-species bee collected pollen and nectar along agricultural land gradients from the MixToxBee project in southern Sweden to challenge these predictions. We find that the pesticide mixture-related environmental risk estimated based on pollen collected by the extensive forager Apis mellifera can be extrapolated precautiously to two other bee species and nectar. The consequences for bee health of this pesticide mixture exposure are not known, even if agricultural use of some pesticides, specifically neonicotinoid insecticides, has been associated with negative consequences for wild bee reproduction and distribution. We use a large feeding experiment and a European-wide dataset from the PoshBee project to explore this. Finally, we draw on work from northern California to suggest that flower plantings can be used to support bee reproduction and for some species they may also mitigate pesticide exposure effects. The increased awareness of the context dependency of bees' pesticide exposure and resulting effects bring the use of single model species, field, and pesticide product-focus in the environmental risk assessment into question. Fortunately, this awareness also forms the basis for developing new roads towards more holistic and realistic environmental risk assessment of pesticides – called for by both scientists and authorities.

Keywords: wild bees, pesticide risk, landscape ecotoxicology

Martin Beye
Heinrich Heine University Düsseldorf, Germany
Genetics, CRISPR-Cas

I am the head of the Evolutionary Genetics Institute at the (HHU) University of Duesseldorf (Germany). The goal of our research is to understand how genes can specify the fascinating developmental and behavioral features of honey bees (*Apis mellifera*). The exact topic will be announced.

THE MOLECULAR BASIS OF SEX DETERMINATION IN HONEYBEES

In 1845 Dzierzon uncovered that parthenogenesis in honeybees (*Apis mellifera*) is a mechanism to determine male and female sexes. This was the first rigorous report of a sex determination mechanism that also explained the strong female sex ratio in honeybee colonies in which most colony members are sterile females (workers). More than 170 years later we have uncovered that the genotype of *complementary sex determiner* (*csd*) gene controls the sexual fate in honeybees. Heterozygosity at *csd* determines femaleness, while homo- or hemizygosity induces maleness. In local populations, more than 15 alleles segregate and at the amino acid level, alleles in heterozygous individuals differ from 10 to 25%. However, how the amino acids differences regulate a reliable binary sex determination signal is unknown. I will present our results on the molecular decision process which we gained from our evolutionary, genetic and biochemical studies.

Quinn McFrederick
Department of Entomology, University of California, Riverside, United States
Pathogen spill-over

Quinn McFrederick is an professor and entomologist at the University of California, Riverside. The McFrederick lab's goal is to improve wild bee conservation and managed bee health via the study of symbiosis. We study microbial parasites, commensals, and mutualists with the goal of leveraging our knowledge of how bee-associated microbes interact with environmental stressors and parasites to sustain bee populations for the future.

THE WEIRD WORLD OF PATHOGENS, MICROBES, AND MEAT-EATING BEES

Flowers are more than just a source of food for bees; they can also act as hubs of microbial transmission. Some of these microbes can spillover from social bees into solitary species and move through plant-pollinator networks, while others have more restricted host ranges. We use a combination of fieldwork, laboratory assays, molecular ecology, and genomics to understand the evolution and ecology of these microbes. In this talk I will discuss how plant-pollinator networks can help us understand relationships between bee hosts and pathogens and other microbes. I will then explore the evolution of pathogenicity in the fungal genus *Ascosphaera*. While *Ascosphaera* is best known as the causative agent of chalkbrood disease, the genus is ancestrally commensal and pathogenicity has evolved independently several times. I will finish by discussing the microbiomes of bees that have reverted to a carnivorous lifestyle – the so-called "vulture bees". Our ultimate goal is to leverage these symbionts to improve bee health, and we are just beginning to understand many of these weird and wonderful relationships.

Keywords: Pathogen spillover, bee microbiome, diet

Sponsored by the Eva Crane Trust

Jane Stout
School of Natural Sciences, Trinity College Dublin, Dublin, Ireland
Pollination

Jane Stout is a Professor in Botany in the School of Natural Sciences and Vice President for Biodiversity and Climate Action, at Trinity College Dublin. She is an ecologist who seeks to understand the processes and consequences of human activity on natural and managed ecosystems. Her research focuses on pollination ecology, exploring how interactions shape ecological processes at a range of scales and the value of these interactions for human society and well-being. Her work addresses global challenges associated with climate change, food security, biodiversity conservation and human health, which are national and international research priorities, and crucial for achieving the Sustainable Development Goals. She is co-founder of the All-Ireland Pollinator Plan and co-founder and former Director of Natural Capital Ireland.

POLLINATOR CONSERVATION: FROM BEES TO BOARD ROOMS

One of the justifications for the conservation of bees is because of the fundamental role they play in plant pollination. Given that the vast majority of plant species on earth are animal pollinated, and bees are primary pollinators for many species, they play an essential role in our ecosystems. They also are important for maintaining yields of food, timber and fiber crops, and for maintaining populations of other plant species of value to humans. These may be non-cultivated species that are collected from the wild (e.g. plants that produce edible berries or seeds, or have medicinal properties), or those that help regulate climate, water and nutrient cycles. Or they may be plants that contribute to landscape aesthetics, culture or spirituality.

The multiple values of pollination to human society and well being, although not well quantified, have stimulated widespread concern about pollinator decline, and action at a range of scales, from individuals to businesses and governments, often cooperating at regional and even global levels. To implement effective conservation of pollinators, a transdisciplinary approach is required. Of course, we need to understand the ecology of pollinators to determine what, where and when to implement conservation action. But we also need to understand the socio-economic and political contexts in which conservation is being undertaken, and the psychology of behaviour change.

Thus scientists who specialize in pollination ecology need to work across disciplines and with a multitude of stakeholders to achieve conservation goals. This has been successfully achieved in various places, including in Ireland, through the All-Ireland Pollinator Plan. This plan has engaged citizens from across the island of Ireland, from a range of sectors, and has resulted in a widespread change in the way public and private lands are managed to promote pollinators.

However, challenges remain to integrate actions in the context of broader environmental, social and economic goals at local, regional and global scales.

Keywords: Values of pollination, Pollinator conservation, Transdisciplinary research

GENETICS AND GENOMICS

ORAL PRESENTATIONS

CHAIRPERSONS
Alice Pinto
Martin Hasselmann

RECONSIDERING THE CRITERIA FOR FUNCTIONAL HETEROZYGOSITY OF THE *APIS MELLIFERA* COMPLEMENTARY SEX DETERMINING GENE

Mroczek, Robert, Laboratory of Molecular and Cellular Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland
Laszkiewicz, Agnieszka, Laboratory of Molecular and Cellular Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland
Blazej, Pawel, Department of Bioinformatics and Genomics, Faculty of Biotechnology, Wroclaw University, F. Joliot-Curie 14a, 50-383 Wroclaw, Poland
Adamczyk-Weglarzy, Kinga, Laboratory of Molecular and Cellular Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland
Niedbalska-Tarnowska, Joanna, Laboratory of Molecular and Cellular Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland
Cebrat, Malgorzata, Laboratory of Molecular and Cellular Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland

The complementary sex determiner (*csd*) gene is responsible for controlling the sex-determination molecular switch in western honey bees. Individuals that are heterozygous for *csd* develop into females, whereas those that are hemizygous or homozygous develop into males. The diploid males that are homozygous for csd are destroyed at an early stage of their development. The *csd* gene is present in numerous variants in the bee population but not all alleles are expected to form functional pairs with each other because of insufficient sequence difference. It has been proposed that the minimal number of amino acid differences (including both changes in the length of the hypervariable region (HVR) of the protein and other changes in the potential specifying domain) between two *csd* alleles needed to fully determine femaleness is five. It has also been proposed that smaller differences may result in forming an evolutionary intermediate that is not fully capable of female determination, but has increased fitness compared to the homozygous genotype. By analysing numerous (118) diploid genotypes present in worker bee larvae we have identified fully functional *csd* pairs differing in the *csd*-PSD by only a single amino acid in the length of HVR. We show that the larvae carrying this genotype express only female-specific transcripts of the *feminizer* (*fem*) and *double-sex* (*dsx*) genes. We conclude that criteria for a functional heterozygosity of the csd gene are highly dependent on the sequence context and position of the change, and show that the generation of a new csd specificity may occur during a single mutation step with no need for evolutionary intermediates accumulating further mutations.

Keywords: sex determination, functional heterozygosity, genotype

CONSERVATION STATUS OF THE ENDEMIC EUROPEAN DARK HONEY BEE (*APIS MELLIFERA MELLIFERA*) IN BELGIUM & THE NETHERLANDS

Elen, Dylan, School of Natural Sciences, Bangor University, Bangor, United Kingdom
Malhotra, Anita, School of Natural Sciences, Bangor University, Bangor, United Kingdom
Henriques, Dora, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Herremans, Mathijs, Taskforce Research, ZwarteBij.org vzw, Gavere, Belgium
Pinto, M. Alice Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Cross, Paul, School of Natural Sciences, Bangor University, Bangor, United Kingdom

Due to past and present imports of *Apis mellifera ligustica* (Italian bees), *Apis mellifera carnica* (Carniolan bees) and the Buckfast bee (a hybrid strain) across its entire natural range, the European Dark Honey bee (*Apis mellifera mellifera*) is currently threatened with extinction by genetic pollution through hybridization. In the past, genomic research has shown the European Dark Honey bee was still present in the Belgian Chimay area and on the Dutch North Sea Isle of Texel. However, as the risk of beekeepers importing non-native honey bees into the area remains, which could rapidly threaten the genetic integrity of the endemic honey bee population, monitoring hybridization is a key element in conservation and breeding. Within the framework of the Flemish Beekeeping Programme, 246 honey bee colonies originating from the Belgian population of Chimay (108) and the Dutch North Sea island populations of Texel (6) and Terschelling (132) were sampled in autumn 2021 to identify pure European Dark Honey bee colonies with which to support the Flemish *A. m. mellifera* breeding activities. The population of Terschelling, which has not been studied before, was included because local beekeepers claimed their colonies looked and behaved like European Dark Honey bees. Of each sampled honey bee colony 10 worker bees were used for this study, including measuring wing morphometrics (based on cubital index, discoidal shift angle and hantal index) and estimating the extent of C-lineage introgression in the ncDNA by applying a custom-tailored SNP genotype assay. Q-values for most sampled honey bee colonies were above 90% for M-lineage: 85 out of 108 colonies for Chimay; 106 out of 132 colonies for Terschelling and all 6 colonies for Texel. Both the populations of Chimay and Texel seem to be spared from intense hybridization over the past decades, whilst a novel remnant population of the European Dark Honey bee was found on the Isle of Terschelling. The correlation between wing morphometrics and molecular data was significant but very weak (Spearman r = 0.20, p = 0.002), indicating the SNP genotype assay is a more valuable tool for assisting in breeding and conservation activities.

Keywords: *Apis mellifera mellifera*, Wing Morphometrics, SNP, honey bee conservation

CHROMATIN ACCESSIBILITY-BASED CHARACTERIZATION OF THE GENE REGULATORY NETWORKS UNDERPINNING PHENOTYPIC PLASTICITY IN THE HONEY BEE

Robert Lowe,[1,3,5] Marek Wojciechowski,[2,4,5] Nancy Ellis,[2] and Paul J. Hurd[2]

1 RER Consultants, 28 Worbeck Road, London, SE20 7SW, UK.
2 School of Biological and Behavioural Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, UK.
3 Current address: Altos Labs, Cambridge, UK.
4 Current address: International Institute of Molecular and Cell Biology in Warsaw, 4 Ks. Trojdena Street, 01-109 Warsaw, Poland.

5 These authors contributed equally to this work.

The honey bee genome has the capacity to produce three phenotypically distinct organisms (two diploid female castes: queen and worker, and a haploid male drone). Previous studies have implicated metabolic flux acting via epigenetic regulation in directing nutrition-driven phenotypic plasticity in the honey bee. However, the cis-acting DNA regulatory elements that establish contrasting epigenomes and determine phenotype-specific gene expression remain unclear. Using a high resolution multiomic approach including assay for transposase- accessible chromatin by sequencing (ATAC-seq), RNA-seq and ChIP-seq, we produce the first genome-wide maps of the regulatory landscape across all three adult honey bee phenotypes identifying more than 5000 regulatory regions in queen, 7500 in worker and 6500 in drone, with the vast majority of these sites located within intronic regions. These regions are defined by positive enrichment of H3K27ac and depletion of H3K4me3 and show a positive correlation with gene expression. Using ATAC-seq footprinting we identify queen, worker and drone -specific transcription factor occupancy and uncover novel phenotype-specific regulatory networks targeting two key nuclear receptors involved in caste determination; the ecdysone receptor and ultraspiracle. Collectively, this study provides novel insights into key gene regulatory networks that underpin phenotypic plasticity in the honey bee.

A POPULATION GENOMICS AND IMMUNOGENOMICS STUDY OF IRISH BUFF-TAILED BUMBLEBEES (*BOMBUS TERRESTRIS AUDAX*)

Larragy, Sarah, Biology Dept, Maynooth University, Maynooth, Ireland
Colgan, Thomas, Institute of Organismic and Molecular Evolution, Johannes Gutenberg University Mainz, Mainz, Germany
Stout, Jane, School of Natural Sciences, Trinity College Dublin, Dublin, Ireland
Carolan, James, Biology Dept, Maynooth University, Maynooth, Ireland

Bombus terrestris (L.) is naturally distributed across Europe and Northern Africa and includes numerous subpopulations that often have distinctive behaviour, morphology or genetics. In Ireland, there is a native population of *B. terrestris* (spp. *audax*) in addition to imported *B. terrestris* colonies, used for supplemental crop pollination. To protect bumblebees from potential hybridisation with commercial varieties, it is essential that the distinctness of Irish *B. terrestris* is evaluated. Furthermore, we can investigate patterns of selection in groups of important genes such as those involved in defence against pathogens.

Whole genome sequencing (WGS) is a powerful tool with extensive applications. We used WGS to gain insight into population structure, selection patterns and genes important in fundamental physiological processes (e.g. immunity). We sequenced genomes of 33 wild *B. terrestris* males across the island of Ireland. We then performed variant calling on filtered, aligned genome sequences to assess single nucleotide polymorphisms (SNPs). We assessed the population structure by carrying out a principal component analysis (PCA) and a cluster analysis with both our Irish and non-Irish (including commercial) *B. terrestris* genomes. To examine patterns of selection acting on this wild population, we used PopGenome to calculate genetic diversity and performed an extended haplotype homozygosity (EHH) analysis to identify genes undergoing positive selection. We then applied these analyses to Bombus canonical immune genes to take a deeper look at how selection acts on a highly important physiological system.

We found that the Irish *B. terrestris audax* population shows genetic distinction from non-Irish and commercial populations. Interestingly, male bumblebees collected from the Eastern counties in Ireland show potential signs of admixture with non-Irish populations. We find general signatures of positive selection in genes associated with neural development and larval morphogenesis and, in terms of immune genes, we find significant selection signatures in pathogen recognition proteins.

The findings of this study will have implications for future policy development and the management of commercial bumblebee imports in Ireland to ensure protection of this locally adapted population as a genetic resource. It also provides a framework to investigate patterns of selection in genes associated with essential processes.

Keywords: Genomics, selection, bumblebees

IN SEARCH OF THE HONEY BEE CENTROMERES: REGIONS WITH TANDEM REPEATS, HIGH AT-CONTENT AND LOW RECOMBINATION RATES

Vignal, Alain, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Eynard, Sonia, Labogena DNA / GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Klopp, Christophe, UMR MIAT, INRAE, 31326, Castanet Tolosan, France
Servin, Bertrand, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Canale-Tabet, Kamila, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Labarthe, Emmanuelle, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Marande, William, CNRGV, INRAE, 31326, Castanet Tolosan, France
Vandecasteele, Céline, INRA, US 1426, GeT-PlaGe, Genotoul, 31326, Castanet-Tolosan, France
Roques, Céline, US 1426 GeT-PlaGe, INRAE, Genotoul, 31326, Castanet-Tolosan, France
Donnadieu, Cécile, US 1426 GeT-PlaGe, INRAE, Genotoul, 31326, Castanet-Tolosan, France

Several high-quality long-read assemblies of the *Apis mellifera* honey bee genome are now available. One of their important characteristics is that they are based on single haploid drones, making the assembly of complex repeat elements easier and more accurate. As an example of such complex repeats, centromeres are frequently formed on long arrays of satellite DNAs and/or transposable elements and AT-rich sequence. However, despite the improved genome assemblies and of several cytogenetics studies, the position of the centromeres in the honey bee chromosomes remain unclear. In order to locate them, we searched in the *Apis mellifera mellifera* AMelMel1.1 assembly for tandem repeats with a structure similar to alpha satellites, such as observed in other species. Surprisingly, although we successfully detected several repeat families, none of them clustered into one specific position per chromosome, as expected for conventional monocentric chromosomes. In *Apis mellifera mellifera*, we identified at least one repeat family occurring predominantly. It is a 371 bp sequence repeated up to 50 times in tandem, thus forming clusters covering up to 20 kb, that were detected in several locations up to several Mb apart on most chromosomes in the assembly. Its consensus sequence is 28.5 % GC, which is only slightly lower than the genome average of 33 %. Furthermore, it does not appear to be located in AT-rich regions or in regions having specifically low recombination rates and nucleotide diversity as one could expect. These results raise questions on the structure of the honey bee genome, especially the possibility that it could be composed of polycentric or holocentric chromosomes. Additional cytogenetic studies could help answer this question.

GENOMIC AND PHYSIOLOGICAL ADAPTATIONS TO CLIMATE IN WILD AND MANAGED BEE SPECIES

Cejas, Diego. Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium
Webster, Matthew T. Department of Medical Biochemistry and Microbiology, 75237 Uppsala, Sweden.
Maebe, Kevin. Laboratory of agricultural entomology, acarology and nematology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.
Hart, Alex. Laboratory of agricultural entomology, acarology and nematology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.
Wood, Thomas. Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium.
Smagghe, Guy. Laboratory of agricultural entomology, acarology and nematology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.
Vereecken, Nicolas J.. Agroecology Lab, Université libre de Bruxelles (ULB), Avenue FD Roosevelt 50, B-1050 Brussels, Belgium.
Vandamme, Peter. Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium.
Michez, Denis. Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium.

As we improve our knowledge of bee population trends, we have uncovered that climate change and its synergies with other drivers can affect species differently depending on their traits and distribution. In recent years, the honeybee (*Apis mellifera* Linnaeus, 1758) and the buff-tailed bumblebee (*Bombus terrestris* Linnaeus, 1758), both of strong economic value, have been used as model species to understand bee decline. However, their ecology, life cycle and overall characteristics may be too different from most other wild bees to accurately draw comparisons for conservation. In contrast to other bee species, *A. mellifera* and *B. terrestris* are social species with broad diets and positive population trends, while the adaptive potential of other wild bees remains unknown.

The aim of this work is to investigate species variation in sensitivity to global warming using genomic and physiological data collected from three common European bee species, *Andrena dorsata* (Kirby, 1802), *A. flavipes* Panzer 1799 and *Osmia cornuta* (Latreille, 1809) and the two managed species *A. mellifera* and *B. terrestris*. Genomic DNA from one individual of each wild bee species was sequenced using PacBio technology, with RNA sequencing implemented for their annotation. Moreover, 15 female individuals of each species were sampled to estimate their time before heat stupor (THS), a proxy for heat stress sensitivity.

FREE-LIVING VS. MANAGED HONEY BEES – A POPULATION GENETIC APPROACH

Davidović, Slobodan, Department of Genetics of populations and ecogenotoxicology
Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia
Patenković, Aleksandra, Department of Genetics of populations and ecogenotoxicology
Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia
Erić, Katarina, Department of Genetics of populations and ecogenotoxicology
Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia
Erić, Pavle, Department of Genetics of populations and ecogenotoxicology
Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia
Stanisavljević, Ljubiša, Center for Bee Research, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Tanasković, Marija, Department of Genetics of populations and ecogenotoxicology
Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

The honey bee is one of the most economically and ecologically important species currently facing serious challenges. Its history is marked by strong anthropogenic influence and its local populations are rapidly changing while its diversity is constantly manipulated by beekeepers through the import of foreign queens, selection, and migratory beekeeping. The practice of queen importation caused one of the most disastrous events in the history of beekeeping when in the mid-20th century *Varroa* mite was accidentally imported to Europe. This infestation, combined with the degradation of natural habitats, was believed to have caused the disappearance of free-living populations throughout European forests. Recent studies challenge this view and demonstrate that honey bees are able to survive in nature without human interference.

To assess the genetic diversity of different honey bee colonies, we analyzed mitochondrial DNA and 14 microsatellite loci in samples collected from 102 apiaries (with 542 hives) and 55 free-living colonies (FC) from the urban and natural environments throughout Serbia.

Standard parameters of genetic diversity for both genetic markers showed that FC exhibit higher values compared to managed colonies (MC). Values of pairwise F ST genetic distances were higher when FC were compared to MC from different parts of Serbia while MC exhibited somewhat lower values when compared among themselves. Relatedness estimates demonstrated that feral colonies are more related among themselves than they are to other MC and vice versa, implying the existence of a viable and stable free-living population of honey bees. A significant change in the distribution and diversity of mtDNA lineages among MC compared to the historical data for Serbia was detected while genetic analysis based on microsatellite data demonstrated a high genetic uniformity.

Our data confirmed the existence of a genetically diverse and distinctive free-living population of honey bees in Serbia worthy of protection as well as a significant change in the genetic diversity of managed honey bees that calls for immediate change in the current beekeeping practices so that genetic diversity of locally adapted populations could be preserved.

Keywords: genetic diversity, *Apis mellifera*, feral honey bees

DETECTION OF DIPLOID MALES IN *BOMBUS TERRESTRIS* FROM HIGH-THROUGHPUT SEQUENCES

Shah, Abhijeet; & Hasselmann, Martin; Department of Lifestock Genomics, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Diploid males in the buff-tailed bumble bee, *Bombus terrestris,* with a haplodiploidy in single-locus complementary sex determination system, represent significant costs in terms of colony growth and reproduction. Differentiating between diploid and haploid males requires morphological (sperm count and testis area), morphometric (wings) and genetic (microsatellite) analysis, and therefore specialized expertise. The presence of diploid males in a bumble bee colony presents a risk of inbreeding depression due to shared sex alleles, monogyry, and monandry. As *B. terrestris* is mass bred for commercial purposes, this poses a threat to commercial viability of breeding lines with high number of diploid males. With the help of high-throughput sequencing, we can assess ploidy levels in several datasets and studies to determine the prevalence of diploid males in natural populations and in commercial mass breeding lines. Here, we investigate the presence of diploid males in natural populations and a highly inbred colony using high-throughput short-read sequencing followed by alignment-based and alignment-free methods to estimate allele frequencies and k-mer frequencies. Alignment based methods such as NGSploidy rely on estimating the allele frequencies at many loci. The distribution and counts of these allele frequencies at a specific heterozygosity provides us with evidence of the ploidy level. K-mer based approaches such as KMC/GenomeScope2 rely on k-mer coverage and distribution modelling to make reliable estimations of ploidy levels. However, most previous studies have not considered these approaches to answer this question crucial to the buff-tailed bumblebee. We show that post-hoc high-throughput sequencing methods may also yield compelling evidence to identify ploidy levels in the buff-tailed bumblebee.

Keywords: *Bombus terrestris*, ploidy, genomics

GENOME WIDE ASSOCIATION STUDY ON *VARROA* RESISTANCE TRAITS IN FRENCH HONEYBEE POPULATIONS

Eynard, Sonia, Labogena DNA / Genphyse INRAE, Castanet-Tolosan, France
Vignal, Alain, GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France
Basso, Benjamin, INRAE Abeilles et Environnement, Avignon, France
Bouchez, Olivier, INRAE, GeT-PlaGe, Genotoul, Castanet-Tolosan, France
Bulach, Tabatha, INRAE, GeT-PlaGe, Genotoul, Castanet-Tolosan, France
Canale-Tabet, Kamila, GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France
Le Conte, Yves, INRAE Abeilles et Environnement, Avignon, France
Dainat, Benjamin, Agroscope, Bern, Switzerland
Decourtye, Axel, ITSAP, Avignon, France
Genestout, Lucie, Labogena DNA, Jouy-en-Josas, France
Guichard, Matthieu, Agroscope, Bern, Switzerland
Guillaume, François, Innoval, Noyal-sur-vilaine, France
Labarthe, Emmanuelle, GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France
Mahla, Rachid, Labogena DNA, Jouy-en-Josas, France
Mondet, Fanny, INRAE Abeilles et Environnement, Avignon, France
Neuditschko, Markus, Agroscope, Posieux, Switzerland
Phocas, Florence, Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France
Poquet, Yannick, Labogena DNA, Jouy-en-Josas, France
Sann, Christina, Labogena DNA, Jouy-en-Josas, France
Serre, Rémy-Félix, INRAE, GeT-PlaGe, Genotoul, Castanet-Tolosan, France
Servin, Bertrand, GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France

Infestation by *Varroa destructor* is a major concern worldwide for the survival of the honeybee *Apis mellifera* colonies. Breeding for resistance or tolerance may be an approach to reduce or cease chemical treatments. However, although genetic components to such a resistance have been suggested by numerous studies, their exact determinants are still poorly known, making selection for resistant honeybees a challenge. In France, this problem is complexified by a context of hybrid populations in which a mosaic of genetic background is used, going from pure *A. m. carnica*, *A. m.ligustica* or *A. m.mellifera* lines to highly hybrid colonies including also some A. m. caucasia genetic background. In this context, we performed a genome wide association study (GWAS) on over 1,500 *A. mellifera* colonies, grouped into three distinct populations, based on their genetic backgrounds: two pure and one hybrid. We focused our study on three traits linked to infestation by *Varroa destructor*: its infestation in the different compartments of the hive (on adult bees, in the brood and overall) and resistance to such infestation by measuring Suppressed Mite Reproduction and recapping capacity of the colony. The GWAS were performed separately for each trait in each population and were subsequently combined into a meta-analysis using Bayesian methods. Such meta-analysis was possible thanks to the tremendous sampling effort providing us with one of the largest dataset available. We identified several chromosome regions showing significant effects for these polygenic traits, which were associated with relevant genes. We reported heritability values and standard errors for most traits in most genetic background groups. This study allows us to improve our knowledge on the genetic determinism underlying traits of resistance to varroa, highlighting differences between the honeybee genetic backgrounds. These results will pave the way towards the selection for such crucial traits, especially in the context of a landscape of highly hybrid honeybee populations, such as observed in France.

Keywords: Resistance, GWAS, pool-sequence

A MOLECULAR TOOL TO DETECT GENETIC INTROGRESSION FROM SPERMATHECA CONTENT

Henriques, Dora, CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Smith, Stephen, National University of Ireland, Galway, Ireland
Lopes, Ana R., CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Wegener, Jakob, Institute for Bee Research Hohen Neuendorf, Germany
Musin, Eduard, Institute for Bee Research Hohen Neuendorf, Germany
Hoppe, Andreas, Institute for Bee Research Hohen Neuendorf, Germany
Parejo, Melanie, University of the Basque Country, Leioa, Spain
McCormack, Grace, National University of Ireland, Galway, Ireland
Pinto, M. Alice, CIMO, Instituto Politécnico de Bragança, Bragança, Portugal

The genetic integrity of *Apis mellifera mellifera* is threatened by introgression in many places of its native distribution, after recurrent importations of commercial queens typically belonging to the divergent C-lineage. A growing interest in keeping and protecting *A. m. mellifera* has motivated the development of conservation programs in many places of Europe. As part of the conservation efforts, isolated mating stations are set to avoid unwanted crosses, but these are not always effective as matings with unwanted drones are frequently reported. An interesting method to monitor the degree of isolation of mating stations could be through genetic analysis of the queen spermatheca contents. While this method implies that queens selected for monitoring are sacrificed, it can be a powerful way of assessing the effectiveness of mating stations because it would allow easy detection of unwanted alleles. Here, we developed an SNP-based tool suited to the analysis of DNA extracted from spermatheca or from pooled DNA of varying sources. To that end, we first designed an SNP panel from whole-genome sequence data generated from 228 drones, of which 148 belonged to the M-lineage (117 *A. m. iberiensis* and 31 *A. m. mellifera*) and 80 to the C-lineage (46 *A. m. carnica* and 34 *A. m. ligustica*). A total of 5,007 highly differentiated SNPs was found. Based on different criteria, 130 SNPs were selected to be included in the genotyping tool. This tool is based on the NEBNext Direct Genotyping Solution that allows high-throughput, sequence-based target genotyping of single-individual or pooled DNA. To assess the tool's sensitivity and accuracy, 142 samples (DNA extracted from spermatheca and tissue, as well as known DNA mixtures) were genotyped. After removing the problematic SNPs, 81 were retained and these were able to provide an estimate of the pool introgression level with great accuracy. This tool represents a significant advance in the genetic analysis of honey bee colonies with a variety of applications, including breeding and conservation of *A. m. mellifera*.

Keywords: SNPs, Pools, Spermatheca content

GENETICS AND GENOMICS

POSTERS

NON-LETHAL SNP DETECTION IN BUMBLEBEES – A PILOT STUDY FOR LOW DNA SAMPLES

Sickel, Wiebke, Thuenen Institute of Biodiversity, Braunschweig, Germany
Rees, Jana, Ruhr University Bochum and University Duisburg-Essen, Germany
Krüger, Lasse, Thuenen Institute of Biodiversity, Braunschweig, Germany
Sommerlandt, Frank, Thuenen Institute of Biodiversity, Braunschweig, Germany
Dieker, Petra, Thuenen Institute of Biodiversity, Braunschweig, Germany

The fine-scale analysis of intraspecific genetic diversity and population genetics is typically done via the detection and analysis of single nucleotide polymorphisms (SNPs). For this, restriction site associated DNA sequencing is commonly applied, which requires high quantities of DNA. In the case of bumble bees, this results in lethal sampling, as DNA is extracted from whole individuals to achieve sufficient yields. Genotyping by Random Amplicon Sequencing-Direct (GRAS-Di), which relies on a random set of PCR primers rather than restriction enzymes, can theoretically be applied to low amounts of DNA. We tested this approach for two bumble bee species (*Bombus pascuorum* (BP) and *Bombus lapidarius* (BL)) by using small amounts of DNA extracted from a single leg per individual.

Specimens of the two bumble bee species were caught (BP = 9, BL = 12) in agricultural landscapes in Saxony-Anhalt, Germany. One middle leg was removed and used for DNA extraction. DNA was sequenced using the GRAS-Di workflow and SNPs were detected using the STACKS pipeline. The number of sub-populations was assessed using STRUCTURE. We detected 8256 (BP) and 8231 SNPs (BL). Nucleotide diversity was $\pi = 0.4$ in both species, but mean observed heterozygosity was $H_e = 0.3$ (BP) and $H_e = 0.2$ (BL). The mean inbreeding coefficients were $F_{IS} = 0.1$ (BP) and $F_{IS} = 0.4$ (BL). F_{ST} values between individuals of different sampling sites ranged from 0.21 – 0.35 (BP) and 0.20 – 0.59 (BL). Using STRUCTURE, we identified five clusters in BP and three clusters in BL, indicating the existence of some sub-populations.

In conclusion, the GRAS-Di workflow was successfully applied to both bumblebee species with the same set of primers. Despite low sample sizes and DNA yields (6-10 ng), SNPs were detected and differences between the two species were shown. Therefore, GRAS-Di is a suitable method for the assessment of genetic diversity of bumble bees whilst allowing for non-lethal sampling. It can potentially also be applied to other wild bee species. The workflow holds great potential for the application in genetic diversity monitoring.

Keywords: Genetic diversity, Monitoring, Population genomics

MEDIBEES PROJECT: DISENTANGLING DIVERSITY PATTERNS AND PROCESSES OF MEDITERRANEAN HONEY BEE SUBSPECIES BY WHOLE-GENOME SEQUENCING

Yadró, Carlos A., Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Henriques, Dora, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Martín-Hernández, Raquel, Centro de Investigación Apícola y Agroambiental de Marchamalo, Marchamalo, Spain
Nanetti, Antonio, Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy
Haddad, Nizar, National Agricultural Research Center, Jordan
Necati, Mustafa, University of Namik Kemal, Faculty of Veterinary Medicine, Turkey
Hosri, Chadi, Lebanese University, Faculty of Agriculture, Lebanon
Zammit, Marion, University of Malta, Faculty of Medicine and Surgery, Malta
Adjlane, Noureddine, M'Hamed Bougara University of Boumerdès, Faculty of Science, Algeria
MEDIBEES CONSORTIUM
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal

Apis mellifera is represented by 31 presently acknowledged subspecies that are native to Asia, Africa, and Europe. Approximately 40% of that richness evolved in the Mediterranean region, as the result of adaptation to diverse environmental conditions. Understanding and preserving such diversity will help to promote the honey bee's resilience to climate change and biotic stressors, which is the main goal of the MEDIBEES project. This goal will be addressed by the following objectives: a) elucidate the genetic background of the Mediterranean subspecies, b) uncover the genetic basis of their adaptation to the local conditions, c) evaluate the respective conservation status, and d) assess their resilience to climate change. To that end, over 1300 geo-referenced colonies representing 9 subspecies (*A. m. ligustica, A. m. siciliana, A. m. ruttneri, A. m. sahariensis, A. m. intermissa, A. m. syriaca, A. m. anatoliaca, A. m. caucasia* and *A. m. meda*) are being sampled in 6 Mediterranean countries (Algeria, Italia, Jordan, Lebanon, Malta and Turkey). The samples will be whole-genome sequenced using Illumina technology. These newly generated genome datasets will be combined with those previously obtained for *A. m. iberiensis* from Spain and Portugal, and the data will be subjected to a battery of analytical tools. Here, we describe how the massive whole-genome dataset will be scrutinized to fully address the MEDIBEES objectives. This project promises to generate unprecedented knowledge on the differential vulnerability and resilience of the Mediterranean honey bee subspecies to the main environmental stressors typical of this region.

Keywords: honey bee genomics, whole-genome sequencing, genetic basis of adaptation, genotype x environment associations

ANTHROPOGENIC SELECTIVE PRESSURES HAVE A DISCERNIBLE EFFECT ON THE GENOMES OF WILD EUROPEAN BUMBLEBEES

Hart, Alex, Laboratory of Agricultural Entomology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
Verbeeck, Jaro, Laboratory of Agricultural Entomology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
Ariza, Daniel, Laboratory of Agricultural Entomology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
Cejas, Diego, Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Belgium
Ghisbain, Guillaume, Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Belgium
Marshall, Leon, Laboratory of Agroecology, Université Libre de Bruxelles, Belgium
Honchar, Hannah, Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine, Ukraine
Radchenko, Vladimir, Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine, Ukraine
Straka, Jakub, Department of Zoology, Division of Biology, Faculty of Science, Charles University, Czechia
Ljubomirov, Toshko, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria
Lecocq, Thomas, Unité de recherches Animal Et Fonctionnalités des Produits Animaux, University of Lorainne, France
Ferreira, Juliana, Department of Ecology, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, Sweden
Flaminio, Simone, Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment, Italy
Bortolotti, Laura, Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment, Italy
Karise, Reet, Institute of Agricultural and Environmental Sciences, Estonia University of Life Sciences, Estonia
Meeus, Ivan, Laboratory of Agricultural Entomology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
Smagghe, Guy, Laboratory of Agricultural Entomology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
Vereecken, Nicolas, Laboratory of Agroecology, Université Libre de Bruxelles, Belgium
Vandamme, Peter, Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Belgium
Michez, Denis, Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Belgium
Maebe, Kevin, Laboratory of Agricultural Entomology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium

Anthropogenic drivers of bumblebee population decline are well-established, particularly in Europe and North America. Agriculturalizsation is associated with a loss of nesting sites, habitat fragmentation, agrochemical toxicity and nutritional stress; climate change is affecting population distribution, phenology, and voltinism, among other traits.

This study aimed to find genetic evidence of these anthropogenic selective pressures in the genomes of European bumblebees. We sampled 97 sites across the continent for workers of two common species, *Bombus pascuorum* and *B. lapidarius*, and processed the specimens for Restriction-site Associated DNA Sequencing (RADSeq) to generate thousands of loci across the genome. After performing sample QC, data cleaning, and read alignment steps, 97 *B. pascuorum* specimens and 73 *B. lapidarius* were retained. Sampling sites were categorised using CORINE land cover data for agricultural analysis, and WorldClim 2.1 data for climate-based analysis.

Using two Bayesian approaches, and one Latent Factor Mixed Model (LFMM) approach, we uncovered significant correlations between the RADSeq data and both agricultural and climate variables, in both species. 191 unique loci were associated with agricultural land cover gradients in *B. pascuorum*, and 260 in *B. lapidarius*. 757 unique loci were highlighted in relation to four climate variables (related to temperature and precipitation) in *B. pascuorum*, and 550 in *B. lapidarius*.

Further investigation into the genomic regions highlighted revealed evidence of possible non-synonymous mutations in a wide range of genes related to transcriptional regulation and neurodevelopment, in both species and across both climate and agricultural variables, thought to be involved in responses to changing environments or optimising local habits. Of special interest are also loci found in genes related to muscle structure and spermatogenesis in *B. pascuorum*, such as *titin* and *VPS54* respectively; in *B. lapidarius*, a locus was found in *TipE*, known for its role in heat stress response and pyrethroid resistance, and several genes related to wing and limb morphogenesis.

These results call attention to anthropogenic stressors as a source of evolutionary pressure, and with further study, may help inform conservation efforts. Furthermore, the highlighted genes are strong evidence for the considerable impact of the Aanthropocene, shaping populations' evolutionary trajectory.

Keywords: Bombus, Bioinformatics, Anthropocene

GENETIC CHARACTERIZATION OF ALGERIAN HONEY BEES REVEALS A GEOGRAPHIC PATTERN

Salvatore, Giovanna, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France,
Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100, Campobasso, Italy
Canale-Tabet, Kamila, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Fridi, Riad, Department of Biotechnology, Faculty of SNV, University of Oran1 Ahmed Benbella, Oran, Algeria
Nacera, Tabet Aoul, Department of Biotechnology, Faculty of SNV, University of Oran1 Ahmed Benbella, Oran, Algeria
Labarthe, Emmanuelle, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Faux, Pierre, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
D'Andrea Mariasilvia, Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100, Campobasso, Italy
Vignal, Alain, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France

The original geographical distribution of the honey bee, *Apis mellifera*, is Africa, Europe, and Western Asia, from where their export throughout the world, mediated by human beekeeping activities. In recent years, many studies have been carried out in order to better understand *Apis mellifera* genetic diversity. However, the genetic composition of some subspecies located in the African continent is still poorly described. The Algerian honey bee populations are currently regarded as being members of two African honey bee subspecies: *Apis mellifera intermissa* and *Apis mellifera sahariensis*. Introduction of foreign queens and migratory beekeeping practices are factors that can affect the genetic diversity of Algerian honey bees. To finely reconstruct the genetic structure of Algerian population and their phylogenetic relationships with other honey bee subspecies, we sequenced a population of haploid drones, each from one colony and we included, as reference populations, subspecies of honey bees from various European locations. Our genetic analysis demonstrates a clear clustering of the samples from Algeria, which are geographically isolated from the other subspecies in our dataset. Within the Algerian population, our analysis also suggests the presence of a genetic pattern characterized by continuous east-to-west and north-south gradients. The east-west gradient along the north coast and mountains demonstrates the persistence of a genetic structure within the *Apis mellifera intermissa* population, despite potentially high levels of gene flow that could happen following the exchange of queens for breeding purposes and transhumance. Honey bee samples collected in more southern parts of the country showed higher genetic

divergence from the other and likely correspond to *Apis mellifera sahariensis* colonies. The outcomes of our study should be considered in local honey bee biodiversity improvement and conservation initiatives.

Keywords: genetic diversity, sequencing, *Apis mellifera*

EPIGENETIC DETERMINATION OF PHENOTYPIC PLASTICITY IN THE BUFF-TAILED BUMBLE BEE, *BOMBUS TERRESTRIS*

Nancy Ellis[1] and Paul J Hurd[1]

1 School of Biological and Behavioural Sciences, Queen Mary University of London, London E1 4NS, United Kingdom.

Polyphenism in the buff-tailed bumble bee, *Bombus terrestris*, is a profound example of developmental plasticity. One genome produces three phenotypically and behaviourally distinct organisms (two diploid female castes, the reproductive queen and workers, and haploid male drones). Reproductive division of labour is central to eusociality, queens are the only female in the colony that mate and behavioural division of labour between the three adult phenotypes (with specialised tasks to support the colony) persists throughout life. The queen/worker phenotype in the bumble bee is thought to be determined by the amount of diet and the pheromone environment during post-embryonic development. Studies in eusocial honey bees, where differential diet directs polyphenism, have demonstrated that metabolic flux acting via epigenetic regulation is a key determinant of distinct patterns of gene expression that drive caste-specific phenotypic and behavioural differences. We produce the first genome-wide maps of chromatin structure in all three bumble bee phenotypes (queen, worker and drone) at the end point of post embryonic development. We find genome-wide differences in H3K4me1, H3K4me3, H3K27ac and H3K27me3 between newly emerged queen, worker and drone bumble bees that correlate with caste-specific transcription. This study provides the first genome-wide map of chromatin features in all three adult phenotypes of *Bombus terrestris*. As a more primitively eusocial insect, these findings in the bumble bee provide novel data on environmentally driven phenotypic plasticity, caste-specific behaviours and the molecular basis of eusociality.

Key words

Epigenetics, Bumble bee, Eusociality

MOLECULAR DIVERSITY AND SELECTIVE SWEEPS IN IBERIAN HONEY BEE

Muñoz, Irene, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain.
Henriques, Dora, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal.
González-Veiga, Manuel, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain.
Chávez-Galarza, Julio, Departamento Académico de Ciencias Básicas y Afines, Universidad Nacional de Barranca, Barranca, Perú.
Pinto, M. Alice, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal.

Deciphering the genetic basis of the process of adaptation of organisms has been and remains one of the fundamental goals of evolutionary biology. Genomes contain information related to the history of natural populations. The Iberian honey bee (*Apis mellifera iberiensis* Engel 1999) exhibits a complex genetic diversity pattern of clinal variation shaped not only by evolutionarily neutral processes but also by selection. Unravelling the variation of subgenomic regions in this subspecies not only allows us to better understand the complexity of clinal patterning, but also the identification of genetic variation involved in local adaptation.

Several regions related to vision, xenobiotic detoxification, and immune response have shown signals of selection in *A. m. iberiensis.* In this study, analyses of sequence variation around candidate subgenomic regions (~100 kpb) have been carried out. The aims of this work are to provide further evidence of positive selection in the Iberian honey bee, to localize at a much finer scale the direct points of such selection, and to find the underlying source of the beneficial alleles or variants.

Keywords: Genomic conservation, Introgression, *Apis mellifera iberiensis*

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AN INTEGRATED RESOURCE OF GENOTYPE–PHENOTYPE ASSOCIATIONS IN THE HONEY BEE

Zorc, Minja, Department of Animal Science, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
Ristanić, Marko, Department of Biology, Faculty of Veterinary Medicine, Belgrade, Serbia
Glavinić, Uroš, Department of Biology, Faculty of Veterinary Medicine, Belgrade, Serbia
Jovanović, Nemanja, Department of Parasitology, Faculty of Veterinary Medicine, Belgrade, Serbia
Stevanović, Jevrosima, Department of Biology, Faculty of Veterinary Medicine, Belgrade, Serbia
Dovč, Peter, Department of Animal Science, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
Stanimirović, Zoran, Department of Biology, Faculty of Veterinary Medicine, Belgrade, Serbia

Honey bee genomics is an expanding field of research with a large number of publications on biology, health, and diseases. Linkage and association mapping have been used to link genetic polymorphisms to phenotypic variation observed in individuals, colonies, and populations of honey bees. Quantitative trait loci (QTL) mapping has proven valuable for studying the genetic architecture of various traits in honey bees and determining genomic positions associated with causal polymorphisms and their relative effect sizes. However, given the decreasing cost of genome sequencing, genome-wide studies (GWAS) are expected to improve the resolution of genotype-phenotype mapping. Information on identified genomic regions associated with various phenotypic traits is scattered in the scientific literature. The results come from studies exploiting different methodological approaches and experimental populations, and the traits studied have also been defined in different ways. Here, we collected QTL in honey bees associated with a variety of traits (e.g., fertility, pathogen resistance, behavioral traits) from the available literature. Data were manually curated and mapped to the current honey bee genome assembly (Amel_HAv3.1). The BeeQTLdb database created by our team is freely available at https://www.beeqtldb.org. The database provides comprehensive information on QTL (e.g., marker system used, experimental population, reference) and allows combining results from multiple studies. Users can confirm or narrow down the candidate genomic regions from overlapping QTL results. BeeQTLdb was developed to facilitate interpretation of genotype-phenotype correlations and prioritization of candidate genes in the honey bee genome.

Keywords: database, honey bee, QTL

ECOLOGY AND BIODIVERSITY

ORAL PRESENTATIONS

CHAIRPERSONS
Maria Bouga
Pilar de la Rúa

A NEW METHOD TO EVALUATE HONEY BEE COLONY STATUS: MATCHING VISUAL COLONY ASSESSMENTS WITH MULTI THERMAL IN-HIVE SENSORS

Albertazzi, Sergio, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Capano, Vittorio, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Guerra, Irene, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Carpana, Emanuele, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Medrzycki, Piotr, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Generally visual assessments are used to evaluate the strength or status of a honey bee colony. The principle of the modified Liebefeld method according to Accorti, is based on the assessment of the surface covered by brood and bees on each frame and on the hive's structure. Also honey and pollen stores are taken into account. This way of evaluation is time-consuming, requests specialized technicians and foresees good weather conditions to allow to open the hive. For these reasons, the assessment is often scheduled in occasion of other operations (e.g. samplings), and in any case not possible on a frequent basis. Moreover, visual assessment, being based on human&conditions-depending interpretation, produces rough estimates rather than exact numeric data. During the last decades technological improvement has produced "high-tech-hive" which allows continuous assessment of some colony parameters, like hive's weight, in hive and environmental temperature, sound, etc. A single-point temperature sensor, usually included in the standard high-tech hive, allows only to roughly evaluate the colony status (alive, dead, very small, very big).

In the context of the project "BeeNet – monitoring the environment through bees and biodiversity" , based on hundreds of apiaries and thousands of colonies, we designed and tested a new instrument to evaluate brood and bee extension on frames. The tool counts 5 thermal sensors mounted over transversal support which is placed over the top of the frames. The sensors go down between the frames for 15 cm deep, covering the nearly whole hive space (inter-frames 2,4,6,8,10). In this way they should discriminate the presence of brood, bees or empty inter-frames for each sensor. The 5 inter-frame records should roughly show the tridimensionality extension of brood and bees.

In this work we report the comparison between the visual colony assessments and data provided by the high-tech hives installed in BeeNet apiaries in the central Italy.

FOREIGN HONEY BEE INTRODUCTION IN A MEDITERRANEAN BIODIVERSITY HOTSPOT: THE CASE OF THE BALEARIC ISLANDS

De la Rúa, Pilar, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain
Yaniz, Jesús, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.
Angel-Beamonte, Ernesto, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.
Santolaria, Pilar, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.
Espejo, Pablo, Grup Balear de Cria i Selecció Dotze Reines, Mallorca, Spain
Muñoz, Irene, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain

The spread of honey bee subspecies has been identified as one of the current threats to honey bee biodiversity. This endangers the conservation of local populations, not only by the joint spread of pathogens and parasites, but also by introgressive hybridisation leading to the loss of locally adapted populations. This is especially true when dealing with Mediterranean islands such as the Balearic Islands, as they are biodiversity hotspots where the design of local fauna conservation policies is an urgent issue.

In this work, the evolutionary lineage of 123 honeybee colony samples collected during 2021 in Formentera (4), Ibiza (36), Menorca (18) and Mallorca (65) has been determined by analysing mitochondrial haplotypes. Their genetic integrity has been inferred by analysis of the genetic structure through SNPs and population differentiation by geometric morphometry analysis. For the first time, foreign haplotypes of subspecies from other European evolutionary lineages have been found, which usually corresponds to introductions of honey bee queens of the subspecies *Apis mellifera ligustica* and *carnica* and the hybrid strain Buckfast. The results of the SNPs analysis corroborate this, as the presence of hybrid individuals has been observed especially in the samples from Mallorca, suggesting that genetic introgression is taking place. This hybridisation seems to be recent, so there is still time to reverse it by eliminating those colonies in which honey bees of foreign lineages or with a hybridisation percentage higher than 10% are detected. Such conservation practice is particularly important as the geometric morphometry of honey bee samples from the Balearic Islands has revealed their differentiation from other Mediterranean honey bee populations, which may well be due to the effect of insularity. Although further studies are needed to corroborate the presence of this potential ecotype of *Apis mellifera iberiensis*, the results of this study can be applied by local beekeepers for the conservation of the genetic resources of the honey bee population in the Balearic Islands.

Keywords: Biodiversity, Conservation, Introgression

EXPLORING THE WILD BEE (HYMENOPTERA:ANTHOPHILA) DIVERSITY IN THE CANARY ISLANDS THROUGH DNA BARCODING

Lugo, David[1], De la Rúa, Pilar[2], Ruiz, Carlos[1].

1Department of Animal Biology, Edaphology and Geology, University of La Laguna, San Cristóbal de La Laguna, Spain.
2Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain

The Canary Islands are a known hotspot of bee endemism that is threatened by habitat loss, the introduction of invasive species and climate change. Although the bee fauna has received considerable taxonomic attention during the last decades, recent molecular and morphological studies have revealed several cases of overlooked cryptic species. Therefore, a correct characterisation of the biodiversity is urgently needed in order to establish adequate conservation measures. To this end, 900 specimens were collected in this study from 160 localities on the seven main islands. A DNA barcode library of Canary Islands bees was constructed, including 351 sequences from 71 species and 28 genera. This dataset represents the 55% of the bee diversity and 59% of the described endemic species of the archipelago. In order to understand patterns of diversification and the possible detection of cryptic species, three delimitation methods (ASAP, GMYC and BPTP) were carried out.

Species delimitation results suggest general agreement with traditional taxonomy (70% of coincidence between species and MOTUs); however, they reveal a high percentage of potential cryptic diversity (15%). These data also suggest the existence of three new, previously undetected exotic species. These results confirm the usefulness of DNA barcoding as a first step for characterize the fauna in a hotspot, as well as to detect unnoticed exotic species, and to infer their source of origin and route of entry to the archipelago.

Keywords: Wild bees, Barcoding, Cryptic species

A SURVEY OF BEE DIVERSITY PATTERNS AND BEE-PLANT INTERACTIONS IN AGRICULTURE-DOMINATED LANDSCAPES OF THE SOUTH BANAT REGION, SERBIA

Raičević, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Bila Dubaić, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Ćetković, Aleksandar, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Plećaš, Milan, Faculty of Biology, University of Belgrade, Belgrade, Serbia

Within the framework of EcoStack project, we explored the possibilities to improve the provisioning and stability of pollination services to oilseed rape in southeastern Pannonian part of Serbia, focussing on wild bees. The study region (South Banat) is largely dominated by intensive agriculture, with variable and unevenly distributed fragments of natural or semi-natural habitats. Since oilseed rape is the mid-spring mass-flowering crop managed under annual rotation, maintenance of pollinators is possibly affected by the availability of landscape scale non-crop floral resources which bloom in late-spring/early-summer. Since diversity of wild bees and their trophic interactions with local plant communities were poorly studied in this region, we initiated the baseline study of bee diversity patterns in relation to landscape scale agricultural intensity. We selected 10 sampling sites within landscape sectors (1 km radius) differing in share of annual crops and we grouped them in two broad landscape classes: High share of Agriculture (>90%; HA) and Moderate-to-Lower share of Agriculture (<85%; MLA). Bees were sampled twice per site in the period June – early July 2020, by timed hand-netting on each plant taxon found at the surveyed site visited by at least some bees.

We recorded 2,084 bees from 25 bee genera, representing about 46% of the potential regional diversity. Assessment was conducted at 23 plant genera, which were unevenly recorded among landscape classes (9 in HA, 17 in MLA, only 3 found in both). Nevertheless, the bee generic richness was similar among classes (19 in HA, 21 in MLA, 15 found in both; 9.6–10.7 per average site), and Shannon diversity (*H'*-value) was almost identical. Ten most abundant bee genera comprised >97% of all recorded bees (range: 1.2–34.3%), while remaining 15 were represented with less than 0.5% each. The average bee abundance (per site) was somewhat higher in MLA than in HA, but more variable, with 7 out of 10 most abundant genera being markedly more abundant in MLA; this was likely affected by the unbalanced sampling intensity due to local differences in plant availability. Further in-depth and species-level analysis (ongoing) should enable a more insightful, interaction-based pollinator management.

Keywords: agricultural landscapes, pollinator floral resources, EcoStack project

INFLUENCE OF LANDSCAPE MANAGEMENT ON WILD BEES COMMUNITIES

Zavatta Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Ranalli Rosa, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Flaminio Simone, CREA Research Centre for Agriculture and Environment, Bologna, Italy; Laboratory of Zoology, University of Mons, Avenue du Champs de Mars 6, 7000 Mons, Belgium
D'Agostino Marco, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Giovanetti Manuela, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Quaranta Marino, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Intensive practices in agriculture can lead to a biodiversity reduction and consequentially simplification of pollinator communities. The multiple and frequent maintenance actions required for agriculture can cause a severe disturbance to forage and nesting activities necessary for wild bees' survival. In the context of the project "BeeNet – monitoring the environment through bees and biodiversity" in-depth information investigates whether different landscape management can lead to different wild bee community compositions. Sampling sites include both intensive and seminatural agroecosystems in the north (Piedmont), north-central (Emilia-Romagna), and south Italy (Campania). The definition of intensive and seminatural sites was based on CORINE land cover (CLC) categories. We explored the composition of wild bee communities through main ecological traits such as home distance foraging classes, sociality, and diet typology. We calculated CLC categories percentage contained in a 2km ray buffer for each site. Our initial hypothesis was that higher heterogeneity in landscape composition could lead to different wild bees communities, rewarding most sensitive categories such as oligolectic or primitively social species. Moreover, the presence of specific CLC categories (heterogeneous agricultural areas, mixed with forests and natural habitats) can encourage the "long traveler pollinators", bees with larger intertegular distances, and potentially capable of more distant flights from the nest sites. Our conclusions lead us to confirm that seminatural sites, which turned out to have a higher level of both species' richness and abundance, offer a better environment for oligolectic and primitively social species compared with intensive ones, as well as being more likely to host species that potentially come from greater distances.

Keywords: Wild bees, CORINE land cover inventory, ecological traits

LANDSCAPE COMPOSITION MODULATES PLANT-POLLINATOR COEXTINCTIONS

Proesmans, Willem[1]
Laurent, Emilien[1]
Felten, Emeline[1]
Biju-Duval, Luc[1]
Cyrille, Nathan[1]
Vanbergen, Adam[1],

1 Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Dijon, France

Extinction of plant or pollinator species may initiate a coextinction cascade, resulting in further species losses via interspecific links in mutualistic networks. Land-use can modify plant-pollinator network structure (e.g. connectance, nestedness, modularity) in ways that modulate community robustness, coextinction risks and species persistence.

We used a Stochastic Coextinction Model (SCM) to model plant-pollinator coextinction cascades in 12 intensively-sampled agricultural, rural or urban landscapes. We correlated landscape composition with network structure and coextinction risk (e.g. number of extinction episodes, number and variance of species lost). We explicitly corrected for network size to separate effects of species richness and network structures on extinction risk. We also accounted for intrinsic dependence on mutualism among the plant and pollinator species that affected extinction risk, for example, obligate outcrossing wild plants c.f. cultivated plants.

Significant differences in network architecture between the different landscapes were driven by both urbanization and agricultural intensification. Urban networks were more asymmetric, less modular and had a lower connectance. Intensively managed agricultural landscapes were the most nested. Additionally, network architecture changed throughout the season. Network metrics had a very strong effect on coextinction cascade degree, variability, proportion and number of extinct species. Our results suggest that landscape structure and seasonality drive network architecture, which in turn predicts network vulnerability to coextinction cascades. In addition, landscapes modulate coextinction risk through intrinsic differences in pollinator dependence of plants present in each landscape type.

Our study sheds new light on how plants and pollinators may not only go extinct due to direct disturbances, but also indirectly through differential losses of mutualistic partners in anthropogenic landscapes.

Keywords: Mutualistic Networks, Stochastic Coextinction Model, Landscape

COMPOSITION AND FUNCTIONALITY OF AUSTRIAN WILD BEE COMMUNITIES IN THE CONTEXT OF CLIMATE WARMING AND LANDSCAPE TRANSFORMATION

Scharnhorst, Victor Sebastian, Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
Ockermüller, Esther, Biology Centre of the Upper Austrian State Museum, Linz, Austria
Neumayer, Johann, free lancing entomologist, Elixhausen, Austria
Formayer, Herbert, Institute of Meteorology and Climatology, BOKU, Vienna, Austria
König, Barbara, Institute of Meteorology and Climatology, BOKU, Vienna, Austria
Becsi, Benedikt, Institute of Meteorology and Climatology, BOKU, Vienna, Austria
Christa Hainz-Renetzeder, Institute of Landscape Development Recreation and Conservation Planning, BOKU, Vienna, Austria
Christina Bauer, Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
Biella, Paolo, Dipartimento di Biotecnologie e Bioscienze, University of Milano-Bicocca, Milano, Italy
Dötterl, Stefan, Pflanzenökologie & Botanischer Garten, Paris-Lodron-University, Salzburg, Austria
Meimberg, Harald, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria
Pachinger, Bärbel, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria

Wild bees are keystone species of many ecosystems worldwide, pollinating wild plants and crops, which in turn is essential for human well-being. However, their populations are declining and many species are at risk, with habitat loss, stress from pesticides and parasites, climate change being the most important drivers. In this ongoing study, we are investigating the effects of climate change on wild bee communities and the distribution of functional traits, and how these effects interact with changes in landscape heterogeneity. The faunal dataset is provided by the Upper Austrian State Museum and contains several thousand records from 1910 to 2021 from eight different sampling sites around the city of Linz. Historical aerial photographs and recent orthophotos were analyzed to investigate changes in landscape heterogeneity. Climate reconstructions were developed using the HISTALP and SPARTCAUS temperature and precipitation dataset. Preliminary results show a strong effect of sampling period on wild bee community composition and ecological functional traits that correlate with higher annual or seasonal temperatures and changes in the prevalence of certain landscape features. Some effects appear to depend on individual sampling sites, and some species and specific functional traits, such as the prevalence of parasitic species, are more affected than others. Our study demonstrates the potential of exploring large historical biodiversity datasets and their importance for understanding the ecological impacts of climate and landscape change, which in turn is essential for prospective conservation strategies.

Keywords: Antophila, historical data, Austria

MULTISCALE EFFECT OF *APIS MELLIFERA* ON THE WILD BEE COMMUNITY OF TEIDE NATIONAL PARK (CANARY ISLANDS)

Ruiz, Carlos[1]; Pérez, David[2]; González Castro, Aarón[1]; Pérez, José Antonio[1].

1 Department of Animal Biology, Edaphology and Geology, University of La Laguna, San Cristóbal de La Laguna, Spain.
2 Área de Medio Ambiente. Gestión y Planeamiento Territorial y Ambiental (GesPlan S.A.), Las Palmas de Gran Canaria, Spain

Intensive beekeeping can have a variety of negative effects on wild plants and pollinators. These impacts can be exacerbated both in conserved areas and oceanic islands due to the uniqueness and vulnerability of their biotas. In this study we have assessed the impacts of *Apis mellifera* competition on the wild bee community in the high mountain scrub of the Teide National Park (Canary Islands, Spain). The effects of competition along a gradient of beekeeping intensity were evaluated at different levels: wild bee community composition, the structure of pollination networks, and the reproductive success and lipid composition of two wild species (*Osmia submicans* and *Hylaeus canariensis*). The results of this study showed no significant impacts of intensive beekeeping on wild bee abundance and richness, which is mainly determined by the availability of floral resources. However, several direct and indirect impacts of Apis competition were observed at both community and species level. Differences in pollination structure networks were observed along the beekeeping gradient. In addition, several negative impacts on reproductive fitness and lipid content were detected for both wild bee species studied.

Keywords: *Apis mellifera*, competition, wild bee, intensive beekeeping

SMALLER, MORE DIVERSE, AND ON THE WAY UP: RAPID CHANGES IN MONTANE WILD BEE COMMUNITIES WITHIN AN EXCEPTIONALLY HOT DECADE

Fabienne Maihoff[1], Nicolas Friess[2], Bernhard Hoiss[3], Christian Schmid-Egger[4], Janika Kerner[1], Johann Neumayer[5], Sebastian Hopfenmüller[6], Claus Bässler[7,8], Jörg Müller[1,8], Alice Classen[1]

1 Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
2 Faculty of Geography, University of Marburg, Deutschhausstrasse 12, 35037 Marburg, Germany
3 Bayerische Akademie für Naturschutz und Landschaftspflege, Seethalerstraße 6, 83410 Laufen
4 Independent Researcher, Fischerstr. 1, 10317 Berlin, Germany
5 Independent Researcher, 5161 Elixhausen, Austria
6 Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany
7 Department of Conservation Biology, Institute for Ecology, Evolution and Diversity, University of Frankfurt, Max-von-Laue-Str.13, 60438 Frankfurt am Main, Germany
8 National Park Bavarian Forest, Freyunger Str. 2, 94481 Grafenau, Germany

Global warming is assumed to restructure mountain insect communities in space and time. Theory and observations along climate gradients predict that insect abundance and richness, especially of small-bodied species, will increase with increasing temperature. However, the specific responses of single species to rising temperatures, such as spatial range shifts, may weaken such prediction, asking for intensive monitoring of real-world communities over time. Here, we examined the temporal and spatial change in wild bee communities and its drivers along two largely well-protected elevational gradients (alpine grassland vs. prealpine forest), each resampled within the last decade. Along both gradients, we detected clear upward shifts in bee communities, with cold-adapted bumblebee species reacting particularly sensitive, demonstrating the speed with which mobile organisms can respond to climatic changes. Mean annual temperature (MAT) was identified as the main driver of species richness in both regions. Accordingly, and in large overlap with expectations under climate warming, we detected an increase in bee richness and abundance, and an increase of small-bodied species in low- and mid-elevations along the grassland gradient. While changes in upward shifts, species richness, abundance and body size in the prealpine forest gradient were partly consistent with the alpine grassland system, they were generally weaker, which could be due to a much less severe warming trend than in the alpine grassland system or due to the variable and possibly confounding effect of canopy cover. Our study highlights the use of accurate assessed abundance data revealing rapid changes in bee communities over only one decade. We conclude that in well-protected temperate regions, small-bodied bees may initially profit from warming temperatures, by getting more abundant and diverse.

INTENSIVE AGRICULTURE VS. RURAL ENVIRONMENT – SEASONAL ANALYSIS OF POLLINATION NETWORKS

Szentgyörgyi, Hajnalka, Institute of Botany, Jagiellonian University, Kraków, Poland
Żmuda, Aleksandra, Institute of Botany, Jagiellonian University, Kraków, Poland
Nobis, Agnieszka, Institute of Botany, Jagiellonian University, Kraków, Poland
Kierat, Justyna, Kraków, Poland
Gajda, Anna, Department of Pathology and Veterinary Diagnostics, Warsaw University of Life Science, Warsaw, Poland
Albrecht, Matthias, Agroecology and Environment, Agroscope, Zürich, Switzerland
Neumann, Peter, Institute of Bee Health, University of Bern, Bern, Switzerland
Paxton, Robert J., Institute of Biology, Martin Luther University Halle-Wittenberg, Germany
Schweiger, Oliver, UFZ Helmholtz Center for Environmental Research, Halle (Saale), Germany
Settele, Josef, UFZ Helmholtz Center for Environmental Research, Leipzig, Germany
Vanbergen, Adam, INRAE National Research Institute for Agriculture, Food and Environment, France

Landuse patterns may affect bee diversity and bee numbers, however, some geographic areas like Central and Eastern Europe are still poorly represented. In 2021, we conducted three plant-pollinator samplings (May, June, July) on eight sites (circles = 0.5 km radius) in the vicinity of Kraków with different intensities of agriculture: four intensively managed large monocultures and four rural, small-scale agricultural areas. At each sampling, all bees foraging on a 1 km transect together with the visited plant species were collected. Next, the plant-pollinator data were used to construct pollination networks between sites and across the season. Sites were characterized using Corine Landscape Cover, grouping the habitats present on the sites into three major categories: i) intensive agricultural, ii) rural small scale agriculture and housing and iii) semi-natural habitats including extensively managed pastures. The four agricultural sites were covered by monocultures (corn, wheat and non-flowering oilseed rape, but not during flowering) by at least 69% to 100% of their total area, while the rural sites were covered by rural habitat from 44% - 100% while the remaining area of these sites were covered by semi-natural habitat in both types.

Rural habitats supported higher numbers of bee species (63 in total), plant species (76 in total) and greater numbers of individual bees (970 in total) over the season compared to intensively-managed agricultural sites (53, 68, 672, accordingly). Honey bees were constituting 17% to 71 % of all observed individuals per site and affecting the network properties, accordingly. Such high honey bee densities are characteristic of the Lesser Poland landscape. Networks revealed that *Taraxacum officinale* to be a key floral resource for pollinators in the early season in rural areas. Network properties tended to be better on the rural sites, however, not significantly. Seasonal differences on both rural and agricultural sites were more pronounced between May and June than between June and July. Vicinity of Krakow is characterized by rather diverse landscapes supporting also healthier intense agricultural sites on them.

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Keywords: pollination networks, large-scale monocultures, rural habitats

QUANTIFYING THE IMPACT OF AN INVASIVE HORNET UPON BUMBLE BEE COLONIES

O'Shea-Wheller, Thomas, Environment and Sustainability Institute, University of Exeter, Penryn, United Kingdom
Kennedy, Peter, Environment and Sustainability Institute, University of Exeter, Penryn, United Kingdom
Osborne, Juliet, Environment and Sustainability Institute, University of Exeter, Penryn, United Kingdom

The invasive Asian hornet (*Vespa velutina nigrithorax*) has spread rapidly across extensive areas of Europe and Asia over the last two decades, and is of considerable concern due to impacts upon beekeeping, agriculture, and invertebrate biodiversity. While a substantial body of research has examined its effects on honey bees (*Apis mellifera*), comparatively less is known about the potential threat that it poses to wild pollinators. Dietary analyses have indicated that the hornets prey upon a diversity of insects—including many important wild pollinators—however there is a paucity of data quantifying the direct and indirect consequences for pollinator health and survival in invaded areas. Eusocial bees are of particular concern, as the hornets will intensively predate upon honey bee foragers at colony entrances, indicating that other colonial species may be similarly at risk. Here, we quantify the impact of hornet predation and behaviour on colonies of a native European bumble bee (*Bombus terrestris*). Our experiment employs a combination of direct colony health monitoring, and automated behavioural tracking, to assess the health and survival of colonies across a range of hornet densities in the field, while examining inter-specific behaviours at the individual level. Results constitute the first comprehensive study of interactions between invasive hornets and bumble bee colonies at the landscape scale, and quantify concordant effects on survival and health across the lifespan of colonies.

Keywords: Invasive Species, *Vespa velutina, Bombus terrestris*

ECOLOGY AND BIODIVERSITY

POSTERS

WILD BEES OF THE REPUBLIC OF SRPSKA (B&H)

Nikolić, Petar
Institute of Genetic Resources, Faculty of Agriculture
University of Banja Luka, Banja Luka
Bosnia and Herzegovina

Wild bees are the most important pollinators of agricultural and native plants in the world. Recent research shows that successful pollination depend on functional and biological diversity of bees why most basic research for one country is to meet species present in the area.

In the Republic of Srpska melittology was pointed only on honeybee (*Apis mellifera* L.) research while other bees where not in scientific interest. From 2018. inventarisation of wild bee species in the territory of the Republic of Srpska started. This area cover three different biogeographical regions: alpine, continental and mediterranean why there was an assumption that wild bee diversity could be very high.

Bees were collected in the years 2018-2020 from early spring to autumn across the state. Two sampling method where used: transect walk with a net and pan traps of different colors (white, blue and yellow). Speciemens were pinned, marked and stored in entomological boxes. Most of the speciemens were sent in Belgium for identification as a part of the CLIPS project or identification was done at the Institute of genetic resources following entomological keys.

So far, 46 different species were confirmed among which most numerous are genuses Andrena, Lasioglossum and Bombus. Among these species, an invasive one was also found - sculptured resin bee (*Megachile sculpturalis*, Smith) as well as natural populations of european orchard bee (*Osmia cornuta*, Latreille) which can be reared and used as a pollinator in orchards. Paper also discuss effective ways for promotion of wild bee significance, importance of scientific collaboration and future of melottology in the Republic of Srpska.

Keywords: wild bees, diversity, Republic of Srpska

EFFECT OF SEMI-NATURAL HABITAT AND HABITAT FRAGMENTATION ON BEE SPECIES RICHNESS AT WILDFLOWER COMPENSATION AREAS IN SCHLESWIG HOLSTEIN, GERMANY

Bennett, David (Christian Albrechts Universitat zu Kiel, Germany)

Wildflower compensation areas (WCA) in agricultural ecosystems are becoming increasingly common methods to attempt to conserve wildlife in the landscape. However, little research has been done to determine what factors influence their effectiveness for insect conservation and ecosystem services.

In theory, the quantity of semi-natural habitat and the degree of habitat fragmentation may influence whether insects (including Syrphids) can colonise and utilise wildflower compensation areas. In summer 2021 we performed sampling at 37 WCAs across Schleswig Holstein, northern Germany with yellow pan traps. This was combined with high resolution spatial data on the quantity of semi natural habitats and modelling of habitat fragmentation (using the MESH and Connectance indexes).

Our 2021 results indicated a significant positive relationship between bee species richness and both semi-natural habitat percentage and the two fragmentation indices. We have not yet detected a significant relationship between semi-natural habitat or either habitat fragmentation metric for the richness or abundance of Syrphids (33 species detected across all WCAs).

We are repeating and expanding the study in summer 2022, and we will also implement an eDNA method for detecting insects visiting wildflower heads (following a promising prototype last year).

BEE-PLANT INTERACTIONS OF *MEGACHILE SCULPTURALIS*: EVIDENCE VS. MISCONCEPTIONS, BASIC PATTERNS, AND THE WAY FORWARD

Ćetković, Aleksandar, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Lanner, Julia, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria
Plećaš, Milan, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Raičević, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Stanisavljević, Ljubiša, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Bila Dubaić, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia

There is a growing concern about the current trends in alien bee introductions, regarding their potential to cause various environmental problems. Among other negative effects, non-native bees may alter the pollination interactions and reproduction of various plants, including the enhancement of invasive ones. More generally, it is important to understand trophic interactions of each introduced bee in a non-native range, in order to evaluate its potential to become a successful invader, and to establish an efficient monitoring. We studied the trophic affinities of *Megachile sculpturalis*, an alien bee native to East Asia which is now widespread in North America and Europe (since 1994 and 2008, respectively). This bee is commonly referred to as polylectic, but without clear evaluation of the documented patterns of visitation and pollen usage, or possible source of evidence bias. The genuine trophic preferences of this species are still insufficiently clear, to some extent even controversial or partly misinterpreted.

We compiled extensive evidence of *M. sculpturalis* plant visitations from various public sources and our unpublished data: on the worldwide basis until 2019, and at European scale for 2020–2021 (after initiating the targeted monitoring and citizen science engagement). We designed a semi-quantitative approach to evaluate various obscured or contentious patterns in food-plant preferences (relevance of plant phylogenetic relatedness vs. geographic origin, relevance of availability in spatial and phenological aspect, recording biases, etc.). With limited availability of direct analyses of pollen usage by bees, we indirectly quantified the relative merits of compiled interactions (counts of localities, specimen totals, female presences, pollen collecting).

Worldwide, *Megachile sculpturalis* was recorded on over 75 plant genera and at least 25 families. Majority of recorded visitations were made on species of E-Asian Fabaceae, with greatest share of the single genus *Styphnolobium*. Generally, the Fabaceae account for the highest share of female-based records, and largely dominate in pollen-based records. Arguably, a more suitable category for *M. sculpturalis* trophic patterns could be the mesolecty, or at most a polylecty with very strict and narrow preference. Our parallel extensive metabarcoding survey of bee-collected pollen (on-going) indicates a possibly more complex usage pattern.

Keywords: Exotic Asian bee, bee trophic preferences, Fabaceae

CHANGES IN POLLINATOR ABUNDANCE AND RICHNESS ALONG A LAND-USE GRADIENT

De La Paz, Carmen[1], Lugo, David[1], Macías, Nuria, Ruiz, Carlos[1].

1 Department of Animal Biology, Edaphology and Geology, University of La Laguna, San Cristóbal de La Laguna, Spain.

Oceanic islands represent excellent simplified and isolated models both with a high diversity of endemic pollinators and a high vulnerability to anthropogenic disturbances. However, the response to main drivers of decline such as land use change are poorly studied. In the Canary Islands the coastal euphorbia scrublands, is one of the most impacted ecosystems by land use degradation in the last decades due to mass tourism development. This work assesses the response of their pollinator's community to the land use change at three different scales (site, intra-island, inter-island). For this purpose, we sampled three families of flies and five families of wild bees in the two central islands (Tenerife and Gran Canaria). Representative five coastal scrubland areas comprising 45 sampling units were selected in a land use perturbation gradient. Land-use intensity and land-use change between 1990 and 2018 were evaluated using Corine Land Cover and SIOSE databases. Our data reveal an overall decrease in abundance and richness of pollinator with anthropogenic land-use. However, disparate response was observed among families and between islands. Land use change also generated a diverse response depending on the pollinator group studied, although there was a trend towards a decrease in abundance and diversity with the loss of natural vegetation.

Keywords: richness, abundance, wild bees, habitat loss, land-use intensity

CONSERVATION OF POLLINATORS IN THE CANARY ISLANDS

Diaz, Natalia[1]; Peña, Gustavo[2], Ruiz, Carlos[2]

1 Camino La Patita sn. 38430. Icod de los Vinos. La Abejera de Ecoalpispa
2 Department of Animal Biology, Edaphology and Geology, University of La Laguna, San Cristóbal de La Laguna, Spain.

Pollinators face global decline due to direct or indirect impacts of human activities, endangering a key ecosystem service for natural and agricultural ecosystems. Oceanic islands represent both ecosystems with a high diversity of endemic pollinators and a high vulnerability to anthropogenic disturbances. In this project we aim to promote the conservation of pollinators in the Canary Islands through three key axes. The first is to improve knowledge about the ecology and conservation status of the main pollinators in the archipelago. A large species database will be created using citizen science, literature review and photographs in social networks, with the help of citizen science. All the information gathered will be essential to establish appropriate conservation measures. The second axis is dedicated to promoting conservation actions, through the design of a protocol for the creation of spaces to attract pollinators, as well as the elaboration of guidelines on good agricultural and beekeeping practices. Finally, the third axis is to enhance the value of the Canary Islands' natural heritage and to strike a balance between the conservation of pollinators and sustainable development of primary sector and tourism, which are crucial sectors for the economy of Canary Islands. The measures developed in this project will be implemented in *La Abejera de Ecoalpispa*, on the island of Tenerife which aims to become an eco-agrotourism benchmark for the conservation of pollinators, honey bees and agro-ecological practices.

Keywords: Pollinator conservation, Oceanic island, Macaronesia

DETERMINING THE STATUS OF WILD POLLINATOR BEES (HYMENOPTERA: APOIDEA) IN ISTANBUL BY USING BEE HOTELS

Inci, Harun, Biology, Istanbul University, Istanbul, Turkey
Dikmen, Fatih, Biology, Istanbul University, Istanbul, Turkey
Dabak, Tunç, Biology, Istanbul University, Istanbul, Turkey

Pollinator bees are key to existence of flowering plants on earth. Almost 90% of pollinator bees are not social like honeybees and live solitary. In megacities such as Istanbul, existence of solitary bees is threatened via high urbanization. As a result of this the availability of nesting sites and food sources of solitary bees decreased. Also, wrong landscaping and planting of non-bee-friendly plants negatively affected the solitary bee populations.

To investigate such situation in Istanbul, bee hotels were designed to be placed at 34 different stations. These nests were controlled regularly throughout 2 years monthly among the active seasons for bees. Each year by the end of the summer period, the nests were removed from the area and taken to the laboratory. The nesting specimens are identified by morphological and molecular inspection. Molecular barcoding analysis were performed using the Cytochrome Oxidase Subunit 1 (CO1) gene region. In addition, the pollens collected and stored by the bees in the nests were analyzed and thus the preferred plants were determined. As a result of the study 4405 specimens were analyzed. Owing to results of morphological and genetic analyses we could identify the solitary bees that inhabit our nests are belong to the genus *Osmia* dominantly. According to our palynological analyses we found out that family Asteraceae is the most common food source among these bees in Istanbul. It was concluded that the nesting success of solitary bees were much higher at rural area compared to urban habitats. It was strikingly found that big parks in the city did not have high bee population and diversity. Lack of food sources, nesting sites and constantly mowing grass may affect solitary bees negatively.

Keywords: Solitary Bees, Bee Hotels, Urbanization

IMPACT OF LANDSCAPE STRUCTURE WITH DIFFERENT OILSEED RAPE COVERAGE ON POLLEN DIVERSITY AND PESTICIDE RESIDUES IN POLLEN COLLECTED BY THE RED MASON BEE *OSMIA BICORNIS*

Misiewicz Anna, Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland
Mikołajczyk Łukasz, Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland
Bednarska Agnieszka J., Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland

Transformation of land use into large-scale agricultural monocultures and pesticide application are one of the main threats to pollinating insects. Large monocultures of mass flowering crops attractive to pollinators, such as oilseed rape, may provide a high amount of floral resources, but on the other they are monofloral, short-term, unbalanced and may be potentially dangerous as they are treated with pesticides.

We studied how the landscape structure with different oilseed rape coverage (ORC, % land cover) around the *Osmia bicornis* nests affects floral diversity, contamination with pesticides and energetic value of pollen collected by females as a food for their offspring. Twelve nests with cocooned adults were located at sites representing 6% - 65% of ORC within no overlapping circles of 500m radius. The ORC, landscape diversity index, LDI (calculated as exp(H’) using 7 landscape characteristics functionally relevant for bees), and two variables - FA1 and FA2 (obtained from Factor Analysis used to reduce 15 different landscape characteristics into two main axis) were used to describe the sites. The bees were allowed to build their nests during the entire period of oilseed rape blooming and then pollen provisions were extracted and mixed to create a combined representative sample for the entire nest. Floral diversity was expressed as Pollen Effective Number of Species, PENS (calculated as exp(H’), where H’ is Shannon-Wiener diversity index), 11 residues of pesticides found in pollen were expressed in Toxic Unit and pollen energetic value was measured in kJ.

The bees collected pollen from 28 floral taxa and provisions were dominated by *Brassica napus* (6% - 54%), but no relationship between any of dependent variables and ORC was found. Surprisingly, PENS decreased with increasing LDI (p=0.011) and negative relationship of PENS with FA1 (p=0.007) was found, although its significane was driven by a single nest. Similarly, negative realtionship of pollen energetic value with FA1 was found indicating that energetic value of pollen decreased on sites with a large share of built-up areas and was greater in more natural areas. Additionally energetic value of pollen was positively related with LDI (p=0.003).

Research founded by NSC Poland (SONATA 2017/26/D/NZ8/00606).

Keywords: solitary bees, floral resource, pesticide

PERENNIAL ENERGY CROPS AS “ENVIRONMENTAL ISLANDS” IN HIGHLY MODIFIED AGRICULTURAL LAND – BIOENERGY PLANTATIONS AS A SOURCE OF BENEFITS FOR POLLINATING INSECTS

Piotrowska Natalia [1], Czachorowski Stanisław [1], Stolarski Mariusz [2];

1 Department of Ecology and Environmental Protection, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland;
2 Department of Plant Breeding and Seed Production, Faculty of Environmental Management and Agriculture, University of Warmia and Mazury in Olsztyn, Poland;

Declines in wild pollinator species in the last decades are well documented. They are driven primarily by habitat loss and a decrease in floral resources resulting from agricultural intensification. Large, monocultural crops, devoid of herbaceous plants don’t provide nectar and pollen throughout the all growing season. Negative ecosystem changes have sparked a debate on sustainable agriculture, the main assumption of which is to implement solutions that are beneficial both from an economic and environmental point of view. Landscape mosaicism increase is one of them. It can be achieved by incorporating extensively used plantations in industrial agriculture areas. Some of the most beneficial for this model are perennial energy plants. They can be grown on marginal land, of little use for other types of agricultural production, and also require a small amount of agrotechnical treatments. The most popular energy plants in Central Europe today include willow (*Salix* spp.), giant miscanthus (*Miscanthus×giganteus*), virginia fanpetals (*Sida hermaphrodita*) and cup plant (*Silphium perfoliatum*). Based on a literature review, they were assessed for suitability for pollinating insects. The following factors were taken into account: the use of pesticides and the number of agrotechnical treatments on the plantation, the presence of herbaceous plants, the usefulness of the plant itself as forage, and the potential for invasiveness. Conventional miscanthus plantations have been shown to be of little use for pollinating insects. The value of this plant, however, can be increased by intercropping with melilot (*Melilotus officinalis*). Flowering perennials – cup plant and virginia fanpetals were evaluated much better. However, they have a high invasive potential, which should be taken into account when introducing them into the environment. Additionally, they do not provide nectar in drought conditions. The greatest number of publications concerning willow, which was assessed positively as a source of forage. Additionally in the preliminary study, carried out in Leginy (north-eastern Poland) the cup plant and virginia mallow plantations have been observed during the flowering period. The abundant presence of bumblebees (*Bombus* spp.) and honey bee (*Apis mellifera*) have been indicated. Supplementary photographic documentation was prepared.

Keywords: perennial energy crops, bumblebees, wild pollinators

MORPHOLOGICAL VARIATIONS OF HONEY BEE (*APIS MELLIFERA CARNICA*) FROM DIFFERENT SITES OF SERBIA

Nedić, Nebojša, Institute for Zootehnics, Faculty of Agriculture University of Belgrade, Belgrade, Serbia

Different natural conditions in the Republic of Serbia contributed to breeding of the honey bee. Due to adaptation to different habitats and conditions, it is assumed that there are different ecotypes of bees in Serbia. In the border zones with neighboring countries, the influence of other subspecies of bees is possible, and there is also the anthropogenic influence, which affects the hybridization of domestic honey bees. The study examined 14 morphological characters on the front wing of worker bees collected from 6 locations in Serbia and one from Slovenia. Measurements were made with a stereo microscope Leica XTL-3400D and software package IM1000 in accordance with the standard method. Analysis of variance revealed very significant differences between the examined groups of bees. Based on the Duncan test, we determined that the bees from the area on the border of southern and central Serbia differed significantly ($P<0.01$) from the other groups in terms of angles E9 and N23. Bees from Banat region differed significantly ($P<0.01$) from the other groups in terms of angles K19 and G18. Bees from western Serbia had the smallest length and width of the front wing and differed significantly ($P<0.01$) from the other groups. In the analysis of the values of the angles A4, D7, L13, J16 and O26 and the cubital index of domestic bees with the values from the sample of bees from Slovenia, no statistically significant difference was found ($P>0.01$). In the case of angles B4, E9, J10, N23, K19 and G18, statistically significant differences between groups of bees from Serbia and Slovenia were at the individual groups level. Bees from Slovenia had the highest average wing length and width and it were very significantly different from bees from Serbia, except in the case of the group of bees from the area on the border of southern and central Serbia. The data and results obtained from this morphometric analysis contribute to the phenotypic characterization of the domestic carnica and the future strategy of its conservation.

Keywords: Honey bee, Morphometry, Forewing

VESPA VELUTINA DISTRIBUTION AT LANDSCAPE LEVEL – PORTUGUESE CASE STUDY

Nave, Anabela, UESAFSV, INIAV, Oeiras, Portugal; Godinho, Joana, UESAFSV, INIAV, Oeiras, Portugal; Fernandes, João, UESAFSV, INIAV, Oeiras, Portugal; Branco, Manuela, ISA, Lisboa, Portugal

The Asian hornet, *Vespa velutina* (Hym: Vespidae) is the most important predator of honeybees and other pollinators in the Mediterranean countries. Its expansion endangers the conservation of pollinators, as well as crop production. The main objective of this work was to analyse landscape variables that can explain the spatial distribution of *V. velutina* in the Portuguese territory. We also aimed at understanding the relationship between the density of the hornet nests with the abundance of beehives. We used data collected by the Portuguese Institute for the Conservation of Nature and Forests, ICNF, available on an online platform, with occurrences of *V. velutina* nests at the parish level during the last two years. For the Land-use and Land-cover, data we retrieved information from the Portuguese Land Cover database COS2018. Finally, we gather the number of apiaries and hives at the parish level.

We present information on the most relevant land cover associated with a higher density of nests and how the abundance of honeybee colonies affects its distribution.

Keywords: *Apis mellifera*, landscape, land-use, environmental factors

CONSTRUCTION OF A 16S MINI BARCODE LIBRARY FOR FRENCH WILD BEES

Kamila, Canale-Tabet; GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Emmanuelle, Labarthe, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Alain, Vignal, GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Nathalie, Escaravage 3; EDB, UMR 5174 Bâtiment 4R1 31062 cedex 9, 118 Rte de Narbonne, 31077 Toulouse, France
Géraldine, Pascal ; GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
Christophe, Klopp 4; UMR MIAT, INRAE, - 24 Chemin de Borde Rouge, 31320 Auzeville-Tolosane, France
André, Pornon, EDB, Université Paul Sabatier, UMR 5174, 118 route de Narbonne 31062 Toulouse, France
Rémi, Rudelle, Rudelide Expertise muséologie, 21 rue tour de ville, 12240 Rieupeyroux, France
Annie, Ouin, INP-ENSAT, Av. de l'Agrobiopole, 31326 Auzeville-Tolosane, France
Mélodie, Ollivier ; INP-ENSAT, Av. de l'Agrobiopole, 31326 Auzeville-Tolosan, France
Magalie, Pichon, UMR Dynafor, INRAE, 31326, Castanet-Tolosan, France

The use of molecular biology tools: barcoding/metabarcoding is particularly attractive and complementary to conventional methods for studying biodiversity. For wild bees, the development of DNA barcodes has been carried out in Canada and in various European countries (Germany, UK, Switzerland, etc.). In France, a national project CODABEILLES, has been initiated in 2021 to barcode the 968 species listed. Most barcoding and metabarcoding experiments on wild bees have been performed using 650 bps of the CO1 gene. Despite high amplification and sequencing success (about 70%) for most bee's families, some genera like *Andrena*, or old specimens from collections are difficult or even impossible to amplify with classical Folmer primers. In the present work, we have built a 16S (250 bp) mini DNA-barcode database. Data come from a regional collection of French wild bees that contains about 8000 individuals belonging to 174 species and 21 genera previously identified by taxonomists. The specimens had been sampled between 2013 and 2020 using pan traps or sweep nets and kept dried in the collection. DNA was extracted from front legs (one to three individuals per species) and sequenced using MiSeq and Sanger technologies. All mini-barcodes were then validated by distance tree inference. We demonstrated that the 16S mini barcode is well adapted to delineate wild bee species, particularly whenever CO1 is unsuccessful. Moreover, this mini barcode should be useful to perform low-cost metabarcoding and therefore opens opportunities for environmental DNA approaches by analysis of the traces left on foraged flowers.

Keywords: wild bees, 16s barcoding, sequencing.

MULTISPECIES GENETIC DIVERSITY INVESTIGATIONS ON *ANDRENA* USING SSR-GBAS MICROSATELLITE ANALYSIS

Rupprecht, Christina, Institute for Integrative Nature Conservation Research, University of natural resources and life sciences, Vienna (BOKU), Austria
Lanner, Julia, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria
Bila Dubaić, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Ćetković, Aleksandar, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Ockermüller, Esther, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria
Scharnhorst, Victor, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria
Pachinger, Bärbel, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria
Meimberg, Harald, Institute for Integrative Nature Conservation Research, BOKU, Vienna, Austria

With about 150 species in central Europe, the genus *Andrena* covers a variety of ecological groups, comprising widely distributed generalists as well as specialists. Phylogenetic and taxonomic analyses of some species were inconclusive because mitochondrial loci used for barcoding can comprise only a low number of variable characters. In particular, for species which diverged recently, multilocus markers like microsatellites can be informative for the determination of species concepts, reproductive isolation and potential hybridization.

In this study, we use genetic structure of multiple *Andrena* species to verify species concepts and investigate patterns of relationship. The main focus was on the cross-species functionality of the marker panel, which can be applied to differentiate on species and population level. Long-term, it could be used for cross-species comparisons of genetic diversity to investigate evolutionary ecology questions. We developed 46 microsatellite markers, 22 for the *Andrena cineraria* species complex and 24 for *Andrena flavipes.* The cross-species applicability was then tested on 37 selected *Andrena* species of different subgenera (555 samples). Genotyping was conducted using the SSR-GBAS approach (short sequence repeat-genotyping by amplicon sequencing), a high-throughput method using the Illumina technology.

The marker panel discriminated most of the analysed 37 species and successfully resolved the *A. cineraria* species complex. Not all species in the subgenera *Micrandrena* and *Notandrena* could be differentiated, indicating that with increasing phylogenetic distance to the source of markers, alleles show lower levels of divergence. However, we identified one individual which appears to be a first-generation hybrid between *A. danuvia* and *A. cineraria*. While the microsatellites confirm general divergence between these species, occasional hybridization exists.

Our investigation shows the suitability of genetic structure analysis to verify taxonomic

concepts, especially for species groups with ambiguous barcoding outcomes. Wild bees are particularly threatened by land use change, causing habitat fragmentation and loss of population connectivity. Resulting genetic erosion might also lead to widespread bee decline. Our multispecies approach allows to implement comparative investigations on genetic diversity. It could be further developed into a genetic monitoring approach which can serve as an early warning system for the detection of species at risk.

Keywords: biodiversity loss, *Andrena*, population genetics

CHANGES IN POLLINATOR DIVERSITY ALONG A LAND-USE GRADIENT

De La Paz, Carmen[1], Lugo, David[1], Macías, Nuria, Ruiz, Carlos[1].

1 Department of Animal Biology, Edaphology and Geology, University of La Laguna, San Cristóbal de La Laguna, Spain.

Oceanic islands represent excellent simplified and isolated models both with a high diversity of endemic pollinators and a high vulnerability to anthropogenic disturbances. However, the response to main drivers of decline such as land use change are poorly studied. In the Canary Islands the coastal euphorbia scrublands, is one of the most impacted ecosystems by land use degradation in the last decades due to mass tourism development. This work assesses the response of their pollinator's community to the land use change at three different scales (site, intra-island, inter-island). For this purpose, we sampled three families of flies and five families of wild bees in the two central islands (Tenerife and Gran Canaria). Representative five coastal scrubland areas comprising 45 sampling units were selected in a land use perturbation gradient. Land-use intensity and land-use change between 1990 and 2018 were evaluated using Corine Land Cover and SIOSE databases. Our data reveal an overall decrease in abundance and richness of pollinator with anthropogenic land-use. However, disparate response was observed among families and between islands. Land use change also generated a diverse response depending on the pollinator group studied, although there was a trend towards a decrease in abundance and diversity with the loss of natural vegetation.

Keywords: richness, abundance, wild bees, habitat loss, land-use intensity

PATHOLOGY

ORAL PRESENTATIONS

CHAIRPERSONS
Uroš Glavinić
Anne Dalmon
Joachim de Miranda

REPRODUCTION OF *VARROA DESTRUCTOR* AND ITS IMPACT ON VARROA SENSITIVE HYGIENE (VSH) AND RECAPPING BEHAVIOUR IN HONEY BEE COLONIES (*APIS MELLIFERA*)

Sprau, Lina, Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany
Hasselmann, Martin, Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany
Rosenkranz, Peter, Apicultural State Institute, University of Hohenheim, Stuttgart, Germany

A sustainable approach to the prime threat of honey bees, *Varroa destructor* is selective breeding. Worldwide selection programs have been established with the focus of mostly three different selection criteria: (i) Suppressed mite reproduction (SMR): the amount of non-reproductive mites in a colony is increased, (ii) varroa sensitive hygiene (VSH): the adult bee actively removes varroa infested brood and (iii) recapping (REC): adult bees remove the cell cap of varroa infested brood and reclose the cells again to disturb the varroa mite. Oftentimes a clear phenotypical distinction between SMR and VSH is not made because of the assumption that the reproduction of the mite acts as trigger for VSH behaviour by preferably removing the reproducing mites. Based on this assumption some breeding programs use the ratio of non-reproductive to reproductive mites to determine the VSH value. This study focused on the removal rate of the adult bee compared between reproductive and non-reproductive mites as an investigation of the potential trigger for VSH. The two groups were created by artificially infesting half of the mites into freshly capped cells (not longer than 6 hours capped; FCC) and the other half into cells which were capped for at least 24 hours (24HCC). Mites were placed manually inside of the cell by making a small incision into the cell cap. In FCC, mites reproduce normal with a reproduction success of about 75%. Mites which were placed into older cells do rarely start to reproduce (2% reproduction success) and therefore we were able to create two distinct groups. No preference between the two groups were observed with a removal rate after 8 days of approx. 40%. The REC behaviour of both groups also did not differ. This strongly indicate that the mite offspring is not a crucial trigger for VSH behaviour. The percentage of non-reproducing mites are therefore not an appropriate measure for the selection of colonies with VSH. The determination of VSH is advised to be solely done by artificially infesting combs with varroa mites.

Keywords: varroa resistance, varroa sensitive hygiene (VSH), recapping

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SOLITARY BEE FITNESS IS IMPAIRED BY A TRYPANOSOME PARASITE BUT NOT BY A BUTENOLIDE INSECTICIDE

Hellström, Sara, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Paxton, Robert, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Albrecht, Matthias, Agroecology and Environment, Agroscope, Zürich, Switzerland

The impact of honeybee-associated pathogens, and in particular their potential interactions with pesticide stressors, on solitary bees remain largely unexplored. In a series of laboratory and semi-field experiments using O*smia bicornis*, we investigated the lethal and sublethal impacts of the trypanosome parasite *Crithidia mellificae* on fitness-relevant life-history traits of *O.bicornis*. Across experiments, we individually inoculated bees with 10 or 20 thousand trypanosome cells derived from a cell culture, thereby achieving high infection rates. In a benign laboratory setting, we found no impact of the trypanosome on survival, but found a decrease in sucrose consumption of infected bees. In a second laboratory assay, a chronic, field-realistic exposure to the butenolide insecticide flupyradifurone together with *C. mellificae* infection did not significantly impact bee survival or infection intensity. To further investigate fitness-related consequences under field-realistic conditions, we performed a fully crossed semi-field cage experiment. Using individually marked bees, the per-female lifespan and reproductive output of *C. mellificae* infected and non-infected females were assessed with or without flupyradifurone spray treatment. Using a novel computer vision tool, individual females were automatically tracked at the nest through video recordings. We found a lower likelihood of successful establishment (i.e. initiated brood provisioning) among infected females upon release. Once established, preliminary results indicate a slight but significant reduction in survival of infected bees. There was no observable effect on brood cell production, brood survival, sex ratio or body size of offspring produced by infected versus uninfected females once established. No effects of flupyradifurone could be observed, including on foraging trip duration, reproductive output, brood survival or male offspring body weight. We conclude that infection with *C. mellificae* is achievable under controlled conditions, and that infection has slight negative effects on *O. bicornis,* but can be tolerated under benign conditions. We found no negative impacts of flupyradifurone alone, nor additive or synergistic interactions between the pathogen and the insecticide. These experiments clarify the pathology of trypanosomes in *O. bicornis* and provide evidence for the safe use of the agrochemical flupyradifurone in agricultural systems with regards to solitary bee health.

Keywords: solitary bee, trypanosome, combined stressors, flupyradifurone

EFFECTS OF EMERGING GUT PARASITES ON BUMBLEBEE AND HONEY BEE COGNITION

Gómez-Moracho, Tamara, Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier – Toulouse III, Toulouse, France; Department of Parasitology Biochemical and Molecular Parasitology Group CTS-183CTS-183, University of Granada, Granada, Spain
Higes, Mariano, Regional Institute for Agricultural food and Forestry Research and Development (IRIAF), Laboratory of Bee Pathology, Center for Beekeeping and Agro-environmental Research (CIAPA), Marchamalo, Spain
De Pablos, Luis Miguel, Department of Parasitology Biochemical and Molecular Parasitology Group CTS-183CTS-183, University of Granada, Granada, Spain
Lihoreau, Mathieu, Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier – Toulouse III, Toulouse, France

Social insects face numerous parasitic microorganisms that can affect their physiology and behaviour. In particular, the microsporidia Nosema ceranae and the trypanosomatid Lotmaria passim are emergent parasites of bees that have been reported in a high prevalence in domestic honey bee colonies worldwide. These two obligate parasites colonize the bee gut (N. ceranae is an intracellular parasite of the mid-gut, and L. passim attaches to the walls of the hind-gut) and reduce bee survival at high exposition doses. However, sublethal effects at ecologically relevant exposure doses are still poorly understood. Since these parasites have also been found in wild bumblebees, wasps or butterflies, understanding there effects on pollinator behaviour is an important issue. Here I will present a series of experiments about the effects of N. ceranae and L. passim infection on learning and memory in bees using standard cognitive tests in the lab. Bumblebees feeding N. ceranae spores had impaired olfactory learning and memory abilities. Honey bees exposed to L. passim also showed reduced learning, although this result as dependent on the parasite strain used. I will discuss the underlying mechanism by which gut parasites may alter cognitive abilities of their hosts, the consequences on social insects at the individual and colony level, and the possible interactions between these gut parasites.

Keywords: Cognition, parasite, learning and memory

DOES MIGRATORY BEEKEEPING IMPACT THE PREVALENCE OF PATHOGENS AND PARASITES IN MANAGED AND WILD BEES?

Martínez-López, Vicente[1,2]; Ruiz, Carlos[3]; De la Rúa, Pilar[2]

1 Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, UK
2 Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain
3 Department of Animal Biology, Edaphology and Geology, Faculty of Sciences, University of La Laguna, La Laguna, Spain

Human population is growing, forcing an increase in plant production. Many crops are insect-pollinated and their yields depend on healthy pollinator populations. Thereby, there is a growing demand for managed pollinators to ensure adequate pollination rates. Migratory beekeeping, also known as transhumance, is a practice in which honey bee hives are transported seasonally to different locations after crop flowering. These movements are pivotal for crop pollination, but they can have a negative impact on both domestic and wild bees due to numerous factors associated with migratory beekeeping and dispersal of pathogens between managed and wild pollinators. To understand the impact of migratory beekeeping on bee health, we conducted a systematic review to identify its main trends and provide a complete picture of existing knowledge on the subject. Our search yielded 185 studies, but only 52 studies actually analysed the impact of migratory beekeeping on honey bees in relation to pathogens. Some of these studies assessed the effect of transhumance on the genetic composition of honey bee stocks and colony losses. Of the 35 investigations related to pathogens and parasites associated with migratory beekeeping in honey bees, only 16 tested the effect of migratory practices on the prevalence and spread of pathogens and parasites. We found no studies on the effect of transhumance on wild bees. Overall, migratory beekeeping tends to increase the prevalence of pathogens and parasites in honey bee colonies. However, the results were very heterogeneous, probably due to several uncontrolled underlying factors, such as management, biological and geographical factors, and the interactions between them. Migratory beekeeping is expected to increase in the future due to the increasing demand of bees for crop pollination as well as for the climate change, and therefore, there is an urgent need for studies to assess its impact on bee health, as it may be perceived as an emerging threat for pollinators in the upcoming decades.

Keywords: Migratory beekeeping, Pathogens, Parasites

THE IMPACT OF HONEY BEE RNA VIRUSES ON THE STINGLESS BEE *MELIPONA BEECHEII* IN THE YUCATAN PENINSULA, MEXICO

Fleites-Ayil, Fernando A, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Quezada Euán, José Javier G, Departamento de Apicultura Tropical, Universidad Autónoma de Yucatán, Yucatán, México
Medina-Medina, Luis A, Departamento de Apicultura Tropical, Universidad Autónoma de Yucatán, Yucatán, México
Paxton, Robert, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

The subtropical Yucatan Peninsula of Mexico has 17 native species of eusocial stingless bee, including *Melipona beecheii*, a culturally, economically, and ecologically important species. It is also a worldwide renowned beekeeping region using Africanized honey bees (AHBs) that, like temperate honey bees, harbour many RNA viruses. We have sampled stingless bees and AHBs from Yucatan Peninsula, screened them by qPCR and detected deformed wing virus (DWV) genotypes A and B and black queen cell virus (BQCV). Moreover, we have evidence of viral replication of the same viruses through controlled infections by injection in *M. beecheii* pupae, which suggests a potential threat of virus spillover from AHBs to M. beecheii populations. These viruses might in part account for the decline of native stingless bees in this subtropical region. To evaluate this hypothesis, we performed controlled infections by feeding and then recorded their survival and the viral loads. Freshly eclosed worker bees were individually infected with 10 µl of a viral inoculum consisting of one µl of 10^8 or 10^6 viral genome equivalents of DWV genotype A, B, or BQCV in nine µl of sucrose solution 50% (w/v); control bees were fed with 10µl of sucrose solution 50% (w/v). Bees were then stored in groups of a maximum of 10 individuals per cages, separated by treatment, and then their survival was monitored every day until the last bee died. To quantify viral titers, treated bees were removed at three time points: two, four, and six days post-inoculation. We found that infected bees had reduced survival compared with the control treatment (χ^2= 12.2 p= 0.007) but were not different among viral treatments. On the other hand, we registered a decrease in viral load in the virus treatments across time. Our results demonstrate for the first time an impact of so-called honey bee viruses on the survival of infected *M. beecheii* adult bees as well as changes in viral titer over time under laboratory conditions. Our results suggest that viral spillover from AHBs could have potentially adverse effects on the population of *M. beecheii* in this subtropical region of Mexico.

Keywords: RNA virus, survival, *M. beecheii*

ENCLOSED IN THE WAX: HOW CAN THE BEE PUPA BREATHE?

Kubasek J[1], Svobodova K[1], Puta F[2] and Bruce-Krejci A[1]

1 University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
2 Charles university, Faculty of Science, Prague, Czech Republic

Wax is a common constituent of animal and plant cuticles but some social bee species also use it to build their nests. The construction of wax cells closed with wax cappings allows the bees to store food and ensure colony survival over periods of dearth, providing them with an evolutionary advantage over other bee species. However, during their evolution, these insects had to solve a practical question of how to use wax to satisfy two contrasting biological purposes. Whilst the hydrophobic properties and low permeability of wax are well suited to store honey, how could the bee brood breathe and develop properly when sealed in wax cells? Does the structure of brood cappings relate to bee pathology and host - parasite interactions?

In order to elucidate the selection pressure and its adaptations that lead to the diversification of wax functions, we measured the CO2 conductance of wax honey cappings and worker brood cappings of the honey bee (*Apis mellifera carnica*) and bumble bee (*Bombus terrestris*) and the respiration of pupae developing within the brood cells. We also compared the wax composition of the distinct wax cappings. We show that despite being built from wax the CO2 conductance of brood cappings resembles the CO2 conductance of living cells, organs or stomatous plant surfaces. The honey cappings have conductance on the other end of the biologically relevant spectrum, resembling the gas impermeable apical membrane of gut cells or the astomatous plant cuticles. Moreover, we identified unique pores present only in the brood cappings that permit the maintenance of CO2 concentrations in the brood cell within physiological limits.

Our data suggest efficient gas exchange as the most probable primary selective pressure in the evolution of social bees that drived their adaptation for using wax in two highly contrasting biological contexts. Pores in the brood cappings allow gas exchange to allow breathing of the pupae, but they are also important for the exchange of the pheromones and other volatiles present within the hive. Importantly, the pores could also be the points of entrance for chemical contaminants used by beekeepers to combat these pathogens. The porosity of the brood cappings and the level of pupa respiration determines the CO2 gradient established inside the wax cells and, consequently, this could affect the pupa development and its interactions with pathogens and parasites.

REDUCING WINTER MORTALITY OF HONEYBEE COLONIES (*APIS MELLIFERA*) BY APPLYING TREATMENTS AGAINST *VARROA DESTRUCTOR* PARASITE DURING SUMMER

Plamondon, Laurence, Département de Biologie, Université Laval, Québec City, Canada
Paillard, Marilène, Centre de recherche en sciences animales de Deschambault, Deschambault, Canada
Dubreuil, Pascal, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Canada
Giovenazzo, Pierre, Département de Biologie, Université Laval, Québec City, Canada

Varroa mite (*Varroa destructor*) infestation has been identified as one of the major causes of winter colony loss in Canada. Many beekeepers follow a varroa integrated pest management strategy (IPM) to keep fall infestation rates below the recommended threshold of 3% to avoid negative impact on winter colony health. Unfortunately, there are only few strategies that reduce varroa infestation rates below the 2% threshold (July and August) without risk of honey residues. The objective of this project was to measure the efficacy of adding a novel mid-summer varroa treatment within an existing IPM strategy to help reduce varroa summer infestations and verify its impact on health status and zootechnic performances of hives over a 10-month period. During summer 2021, 135 hives from three beekeepers (6 apiaries) in 3 different regions of Quebec, Canada, were used for the protocol. Treatments within each apiary were: Group 1: control, no mid-summer treatment; Group 2: mid-summer treatment with formic acid (FA, MAQS®); Group 3: mid-summer treatment with oxalic acid (27g)/glycerin (OA) impregnated towels. Dependent variables measured were: efficacy of treatment, colony performance (brood and bee population, cluster size and weight), winter mortality, spring brood and population buildup, varroa infestation level and six varroa related virus infestation levels (ABPV, BQCV, DWV-A and B, IAPV and KBV). The AF-MAQS® treatment showed the highest efficacy, reduced varroa infestation levels below the fall 3% threshold but was detected in honey compared to the control group. The OA-glycerin impregnated towels treatment leaves no residues in honey but does not reduce varroa infestation levels below the fall 3% threshold. The results show that control of varroa infestation by mid-summer treatments reduce winter mortality (Control: 53%, FA-MAQS®: 28%, OA-glycerin: 44% of mortality) without reducing colony performance. The prevalence of DWV-B is higher than DWV-A and winter mortality is associated to the presence of several viruses in the same colony. Virology dynamic data will be presented. This research shows the importance of adding a mid-summer treatment within a IPM strategy to control varroa populations, reduce varroa related virus infestation rates and reduce winter mortality.

Keywords: summer treatment, winter mortality, *Varroa destructor*

USE OF FUMAGILLIN AND *AGARICUS BLAZEI* MUSHROOM EXTRACT IN *NOSEMA CERANAE* CONTROL AND HEALTH IMPROVEMENT OF INFECTED HONEY BEES

Glavinic Uros, Niketic Mia, Ristanic Marko, Rajkovic Milan, Dominikovic Nina, Stevanovic Jevrosima, Stanimirovic Zoran

Department of Biology, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia

Nosemosis is a common disease that affects bees and it is known to cause great problems in their reproduction, productivity and vitality. Fumagillin is an antibiotic with proven effect used in the treatment of nosemosis. Lately, there have been problems with production and registration of this medication worldwide, therefore leaving all cases of the disease without proper treatment. Two important factors – significant presence of nosemosis within bee population and residues of fumagillin found after its application, motivated scientists around the world to search for another medication or supplement that could be used in controlling the disease. The growing popularity of alternative non-antibiotic treatments inspired us to carry out a cage experiment and investigate the effect of natural extract of mushroom *Agaricus blazei* and fumagillin on survival rates of *Nosema* infected bees, *Nosema* spore loads and expression levels of immune-related genes (abaecin, hymenoptaecin, defensin, apidaecin and vitellogenin) and oxidative stress (superoxide dismutase - SOD, catalase - CAT and glutathione S-transferase - GST activities and malondialdehyde - MDA concentration). The results unquestionably showed the anti-*Nosema* effect of fumagillin which is reflected in lower *Nosema* loads, better bee survival and improved immunity and oxidative status. However, it has been noted that preventive application of fumagillin caused certain drawbacks. On the other side, positive effects of A. blazei extract were expressed both in preventive and in curative application. The favorable effects of *A. blazei* extract revealed in this study indicate a necessity of a further research of the extract in *Nosema* control as an alternative for fumagillin treatment.

Keywords: Royal sun mushroom; immune stimulation; antioxidative protection

CAN TRANS-GENERATIONAL IMMUNE PRIMING BE USED TO PROTECT HONEY BEES AGAINST EUROPEAN FOULBROOD?

Ory Florine[1,2], Duchemin Vincent[1,2], Kilchenmann Verena[1], Charrière Jean-Daniel[1], Dainat Benjamin1, Dietemann Vincent[1,2]

1 Swiss Bee Research Centre, Agroscope, Bern, Switzerland
2 Department Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

European foulbrood (EFB) is a disease affecting honey bee brood, and is caused by the bacterium *Melissococcus plutonius*. *M. plutonius* is distributed globally and causes EFB with regionally varying prevalence. In many countries, treatment of EFB with antibiotics is prohibited. EFB outbreaks must be reported to veterinary authorities and require costly intervention, sanitation and control measures. In order to provide beekeepers with sustainable tools to prevent EFB and to reduce the costs associated with EFB outbreaks, we tested whether the honey bee's own immune priming system could be exploited to protect the colonies against *M. plutonius* infections. Trans-generational immune priming is a natural mechanism transferring the immunological experience of parents to their offspring, leading to the latter's protection against infections. Our aim was to test whether natural or experimental exposure of honey bee queens to *M. plutonius* reduced the susceptibility of their brood to infection by this pathogen. The survival rates of *in vitro* reared brood produced by previously exposed or non-exposed queens and inoculated with *M. plutonius* were compared in order to assess the degree of protection conferred by the previous exposure of the queens. We also determined whether this exposure resulted in individual or colonial fitness costs. Our results showed no fitness costs of queen exposure to the pathogen, but provided no evidence of trans-generational immune priming. Neither natural nor experimental exposure induced protection of honey bee brood against *M. plutonius* infection. Our choice of queen exposure by feeding, which could be easily performed by beekeepers for colony health management, may have prevented immunisation. Immunisation might be achieved under different priming procedure, which should be identified to achieve a biologically relevant effect, while ensuring that the costs to the health and fitness of the queen and colony remain minimal.

Keywords: Honey bee health, European foulbrood, Trans-generational immune priming

DWV-A PRESENCE IN WILD VS. MANAGED BEES FROM POLAND

Gajda, Anna, Laboratory of Bee Diseases, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland
Mazur, Ewa, Laboratory of Bee Diseases, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland
Szentgyörgyi, Hajnalka, Institute of Botany, Jagiellonian University, Kraków, Poland
Żmuda, Aleksandra, Institute of Botany, Jagiellonian University, Kraków, Poland
Albrecht, Matthias, Agroecology and Environment, Agroscope, Zürich, Switzerland
Neumann, Peter, Institute of Bee Health, University of Bern, Bern, Switzerland
Paxton, Robert J., Institute of Biology, Martin Luther University Halle-Wittenberg, Germany
Schweiger, Oliver, UFZ Helmholtz Center for Environmental Research, Halle (Saale), Germany
Settele, Josef, UFZ Helmholtz Center for Environmental Research, Leipzig, Germany
Vanbergen, Adam, INRAE-National Research Institute for Agriculture, Food and Environment, France

Insect pollinators face multiple threats, with diseases a potentially leading cause of mortality. One of the most common disease risks comes from Deformed Wing Virus. Most studies focus on Western honey bees, *Apis mellifera*, as the most common and abundant managed pollinator, but recent studies show that wild bee populations also harbor viral infections and exhibit disease symptoms associated with the same pathogens. The most likely route of transmission between these managed and wild pollinators is via shared flower use.

In this study, we examined 288 honey bees and 152 wild bees (of different species) collected simultaneously from transects in agricultural and urban landscapes directly from flowers in the Lesser Poland region. We checked for the presence of DWV-A, as the most common genotype of DWV in Poland, and for infection intensity with common molecular biology methods, including qPCR. Sampling was undertaken in 2020 and in 2021.

The results showed that wild bees were infected with DWV-A in a very high percentage (92,5-100%), whereas the level of infection in honey bees varied greatly depending on the year of study (35,71-96,77%). The mean intensity of infection in individual bees (both managed and wild) was higher in 2020 than in 2021, but honey bees carried a significantly higher intensity of infection at the individual level than wild bees. The influence of the environment (agriculture vs urban areas) was clearly visible only for honey bees in 2020. Namely, the prevalence of DWV-A and intensity of infection was significantly higher in urban honey bees compared to honey bees from the agricultural landscape.

Our study shows the very high prevalence of DWV-A in wild bees. It also highlights that the wild bees face a potentially significant threat from DWV-A so prevalent in honey bees and frequently infecting wild species. The population and community consequences of spillover between managed and wild species remains to be established.

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OVERCOMING CHALLENGES IN MEASURING BROOD RELATED VARROA RESISTANCE TRAITS BY COLONY-LEVEL PHENOTYPING OF MEAN VARROA REPRODUCTION RATE

Lefebre Regis, Ghent University, Ghent, Belgium; Claeys-Boúúaert David, Ghent University, Ghent, Belgium; Lina De Smet, Ghent University, Ghent, Belgium; de Graaf Dirk C., Ghent University, Ghent, Belgium

The feasibility and reliability of phenotyping brood-related *Varroa* resistance traits like non-mite reproduction (NMR), recapping and varroa-sensitive hygiene (VSH) often depends on several, sometimes non-manipulable factors. For example, the accuracy and precision of an estimated colony NMR score increases with the number of single-infested cells (SIC) analyzed during microscopic brood cell phenotyping, thus insinuating that a high *Varroa* infestation rate and number of SIC's is needed for NMR to be used as a selection parameter. In the frame of a honey bee colony selection programme, we microscopically phenotyped 387 different drone brood samples for NMR over a time frame of four consecutive years. However, only 18 brood samples contained between 20 and 30 SICs, which is still less than the overall-recommended 35 (Mondet et al., 2020). By approaching the data in a different way, we introduced a new holistic parameter called 'Mean Varroa Reproduction Rate' (MVRR). Simply, this parameter represents the average number of daughter mites per mother mite, which is calculated by dividing the total number of daughters by the total number of mother mites for a certain colony, including those from multiple infested cells. With only three parameters (total number of mothers, total number of daughters and number of opened cells), we showed that NMR and MVRR are strongly correlated, higher MVRR may result in higher infestation levels and plotting colony MVRR scores in function of their infestation level may give indications for both positive – and negative selection.

Keywords: *Varroa* reproduction, phenotyping, selection

DEFORMED WING VIRUS GENOTYPE B IS SPREADING ACROSS THE WORLD AND POTENTIALLY REPLACING GENOTYPE A

Paxton, Robert, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Schäfer, Marc, Institute of Infectology Medicine, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute, Greifswald – Island of Riems, Germany
Nazzi, Francesco, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italy
Zanni, Virginia, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italy
Annoscia, Desiderato, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italy
Marroni, Fabio, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università degli Studi di Udine, Udine, Italy
Bigot, Diane, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Laws-Quinn, Eoin, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Panziera, Delphine, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Jenkins, Christina, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Shafiey, Hassan, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

Deformed wing virus (DWV) is arguably the most serious pathogen of the honey bee (*Apis mellifera*) and a major cause of elevated rates of colony losses wherever it is transmitted by the varroa mite (*Varroa destructor*). There is good phylogenetic evidence that the original genotype A of DWV (DWV-A) was widespread across much of the world's honey bees, having been spread to the Americas and east Asia from a European origin. Australia remains the only large land mass with honey bees that are devoid of DWV (and varroa mites). A new variant of DWV, namely genotype B (DWV-B), was first described in Dutch varroa-infested honey bees in 2001. Using published and own data, we now find that DWV-B has increased markedly in prevalence across its native range of Africa, Europe and western Asia and seemingly replaced DWV-A in many of these locations. It has also been distributed to locations were A. mellifera is not native: North and South America around 2010 or later, and east Asia around 2015, where it also seems to be increasing in prevalence and potentially replacing DWV-A. An epidemiological model to describe the change in prevalence of DWV genotypes within a population suggests that interference between genotypes when co-infecting a host may be key to understanding the replacement of DWV-A by DWV-B. We argue that recombination may be the mechanism by which viral

genotypes compete with each other when co-infecting a host. Given evidence supporting the higher virulence of DWV-B over DWV-A, the ongoing replacement of DWV-A by DWV-B is bad news for beekeepers worldwide, who will need to be more attentive to the control of varroa mites, the major vectors of both genotypes of DWV.

Keywords: emergence, epidemiology, evolution

EFFECTS OF THE NEW VARROACIDAL COMPOUND LITHIUM CHLORIDE ON THE DEVELOPMENT OF HONEY BEE BROOD

Rein, Carolin, Apicultural State Institute Hohenheim, Stuttgart, Germany
Makosch, Marisa, Apicultural State Institute Hohenheim, Stuttgart, Germany
Renz, Julia, Apicultural State Institute Hohenheim, Stuttgart, Germany
Rosenkranz, Peter, Apicultural State Institute Hohenheim, Stuttgart, Germany

The treatment of the ectoparasitic mite *Varroa destructor* is indispensable in order to keep a healthy bee hive. As none of the admitted treatment options is fully satisfactory, there is a strong need for new acaricides with high efficacies, low contamination of bee products and good tolerability for bees and brood. With the discovery of lithium chloride (LiCl) we found a new compound with systemic mode of action which shows high efficacies against *V. destructor*, good tolerability for adult bees and because of its water solubility low risk for contaminations in wax. However, no data on larval toxicity are available so far.

Here we quantified the mortality of larvae fed with different concentrations of LiCl both in artificial reared larvae and in free flying colonies. In artificial reared larvae a concentration of 1 mM LiCl already led to a significant reduced survival probability whereas in hives 10 mM was still well tolerated. During a chronic application of 25 mM LiCl in hives, which represents the required concentration for an effective treatment , we recorded higher brood mortalities between 60% and 90%. However, a short term feeding of 2 days reduced the damages significantly so that there were no differences in brood-survival between control colonies and colonies treated with 25 mM LiCl.

We could also show, that the concentration of lithium in larvae (i) increased with higher concentrations of LiCl in food and (ii) with time of exposure. The highest lithium levels were measured in pre-pupal stages, shortly after the sealing of the brood cell (18.9 mg Li/kg). However, in 2-day-old larvae we only found traces of lithium indicating that pure worker jelly from the hypopharyngeal gland is lithium free.

These data show, that an application of LiCl in breeding colonies should be avoided but due to its high efficacy, lithium chloride could be a new option for the treatment of broodless hives.

Keywords: *Varroa destructor, Lithium chloride*, Brood damages

IMPACT OF AGRI-ENVIRONMENT SCHEMES ON PATHOGEN PREVALENCE AND SPILLOVER AMONG MANAGED AND WILD BEES

Pluta, Patrycja, General Zoology, Institute of Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
Czechofsky, Kathrin, Functional Agrobiodiversity, Department of Crop Sciences, University of Göttingen, Göttingen, Germany
Hass, Annika, Functional Agrobiodiversity, Department of Crop Sciences, University of Göttingen, Göttingen, Germany
Westphal, Catrin, Functional Agrobiodiversity, Department of Crop Sciences, University of Göttingen, Göttingen, Germany
Paxton, Robert, General Zoology, Institute of Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

Anthropogenic changes such as agricultural intensification lead to habitat conversion, monotonous food resources and increased exposure to harmful pesticides, all of which can jeopardize bee health and increase their vulnerability to pathogen infection. Agri-environment schemes have been implemented to combat these negative effects. Important measures include the re-establishment of semi-natural habitats, the sowing of flower strips and the shift to organic farming. To examine the impact of these agri-environment schemes on parasite prevalence in managed and wild pollinators, we collected honey bees (*A. mellifera*), bumble bees (*Bombus lapidarius, B. pascuorum, B. terrestris*) and solitary or primitively social bees (*Colletes* spp., *Andrena* spp., *Lasioglossum* spp.) at flowers from 16 study sites located along independent gradients of landscape composition that varied in the % area of organic farming, % area of semi-natural habitats, and area of annual flower strips within a 2 km radius. We screened individual bees for common bee pathogens: viruses (by qPCR): DWV-A, DWV-B, BQCV, SBV, ABPV; and eukaryotes (by standard PCR): Trypanosomes (*Crithidia* spp., *Lotmaria passim*), Neogregarines (*Apicystis* spp.) and Microsporidia (*Nosema bombi, N. ceranae*). The prevalence of pathogens was high in honey bees (98% were positive for at least one pathogen) and bumble bees (90% were positive for at least one pathogen), and moderate in solitary bees (60% were positive for at least one pathogen). Our results show that pathogen prevalence in bees was lower with an increasing percentage of organic crops and annual flower strips grown in the vicinity whereas it was higher with an increasing amount of semi-natural habitat in the vicinity. Moreover, the prevalence of pathogens in honey bees was positively related to that in bumble bees, suggesting pathogen spillover between these two groups. Some agri-environment schemes are clearly associated with low pathogen prevalence and might have a positive impact on bee health, but their benefits vary across bee taxa.

Keywords: agri-environment scheme, parasite, spillover

MULTIPLE EFFECTS OF LITHIUM CITRATE SYSTEMICALLY AND TOPICALLY APPLIED ON HONEY BEE COLONIES

Stanimirovic Zoran[1], Glavinic Uros[1], Jovanovic Nemanja M.[2], Ristanic Marko[1], Milojković-Opsenica Dušanka[3], Vejnovic Branislav[4], Stevanovic Jevrosima[1]

1 Department of Biology, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia;
2 Department of Parasitology, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia;
3 Department of Analytical Chemistry and Center of Excellence for Molecular Food Sciences, University of Belgrade - Faculty of Chemistry, Belgrade, Serbia;
4 Department of Economics and Statistics, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia

Lithium citrate (Li-cit) is one of promising lithium salts proposed for *Varroa destructor* control. In three experiments (E1, E2 and E3) performed on full-sized free-flying colonies, Li-cit was applied via food (in E1 and E2) or by trickling (in E3). Following concentrations were tested: 5, 10, 15, 20 and 25 mM (in E1), 5 and 20 mM (in E2), 5 and 10 mM (in E3). Varroacidal effect of Li-cit treatments was evaluated in E1 and E3. Residues of lithium were measured in E1 and E2, in samples of honey and wax taken nine months after treatment (in E1) or seven days post-treatment (in E2). Besides, in E3, honey bees were sampled 18 days after the Li-cit treatment for analyses of viral loads of four viruses, gene expression of immune-related and antioxidative enzymes and oxidative stress parameters. Acting systemically (in E1), all tested Li-cit concentrations expressed high varroacide efficacy (93.2–95.5%) and significantly ($p<0.01$) higher than negative and positive (amitraz-treated) controls. Contact varroacide efficacy (in E3) was even higher, being 96.85% and 96.80% (for 5 and 10 mM, respectively) and significantly ($p<0.001$) higher than negative and positive (oxalic acid treated) controls. Residues of lithium in wax matrices were not different (nine months post-treatment, 410-2314 µg/kg) or were significantly ($p<0.05$) lower (seven days post-treatment, 234.3–300 µg/kg) compared to negative control. Lithium residues in brood chamber honey were much higher in E1, nine months post-treatment (169.3–1756.0 µg/kg) than in E2, seven days post-treatment (19.2–27.8 µg/kg). In E2, lithium residues in honey intended for human consumption, were 26.9-33.7 µg/kg. In E3, loads of three honey bee viruses (ABPV, DWV and SBV) did not significantly change after the treatment, but CBPV load significantly ($p>0.01$) decreased in a group treated with 5 mM of Li-cit. Transcript levels of abaecin, apidaecin, defensin, vitellogenin, GST, CuZnSOD and MnSOD were significantly higher ($p<0.05$-0.001), while SOD, CAT, GST, MDA parameters were significantly lower ($p<0.05$-0.001) after the treatment with 5 and/or 10 mM of Li-cit. All presented results are important for the development of a new formulation for Varroa mite control.

Keywords: lithium citrate, residues, effects on mites and bees

PATHOGENS AFFECT WING MORPHOLOGY IN *APIS MELLIFERA* (L.) WORKERS

Tafi, Elena, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Nanetti, Antonio, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Cilia, Giovanni, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bogo, Gherardo, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Geometric morphometrics is used to study the shape of biological structures through the landmarks Cartesian coordinates. Insect wings are highly suitable for geometric morphometric analysis. In recent decades, the morphometric quantification of wing shape has become widely applied in several fields. In honey bees, geometric morphometrics is typically applied in subspecies identification. Honey bee wing morphology may vary depending on both genetic and environmental factors, the last including food availability, temperature, and *Varroa* infestation. Several pathogens infect the juvenile stages of the honey bee, so impairing its development. Consequently, an effect on wing morphology can be postulated. In the context of the project “BeeNet – monitoring the environment through bees and biodiversity”, in-depth information investigates for the first time the variations in honey bee wing morphology (size, shape, and fluctuating asymmetry) using the presence of the following pathogens as predictors: DWV, KBV, ABPV, CBPV, and *Nosema ceranae.* Approximately 600 worker forewings from ten apiaries in six Italian regions were analysed. Significant differences resulted in both wing shape and size between the infected and uninfected groups for all the pathogens. DWV, CBPV and N. ceranae were significantly associated with wing shape fluctuating asymmetry. DWV was significantly associated with fluctuating asymmetry in wing size, as well. These results highlight that honey bee pathogens may affect the wing morphology of honey bees. However, we cannot rule out a colony-mediated effect on the impairment suffered by the individuals. Further experiments need to clarify whether the effects on wing morphology result from a direct host-pathogen relationship. Artificial infections may help elucidate the importance of individual pathogens in the detected morphological alterations.

Keywords: Geometric morphometrics, viruses, *Nosema ceranae*

ACQUISITION AND PREVALENCE OF RNA VIRUSES IN NEWLY FIELD-EXPOSED BUMBLE BEES: A VIROME EXAMINATION

Yañez, Orlando, Institute of Bee Health, University of Bern, Bern, Switzerland
Schläppi, Daniel, School of Biological Sciences, University of Bristol, Bristol, United Kingdom
Maurer, Corina, Agroecology and Environment, Agroscope Reckenholz, Zürich, Switzerland
Bosco, Laura, Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland
Schauer, Alexandria, Institute of Bee Health, University of Bern, Bern, Switzerland
Koch, Michel, Division of Neurological Sciences, University of Bern, Bern, Switzerland
Seuberlich, Torsten, Division of Neurological Sciences, University of Bern, Bern, Switzerland
Neumann, Peter, Institute of Bee Health, University of Bern, Bern, Switzerland

Pest and pathogens are often closely associated with reports of insect declines. The intra- and inter-specific host shift of viral agents may play an important role in this context. These shifts have been widely demonstrated under laboratory conditions using managed bees. However, the dynamics of virus acquisition on field populations remain poorly understood. Given the frequency that virus host shifts may occur, newly introduced bees in an area may maintain their original virus background if the acquisition of infectious viruses are rare. Otherwise, frequent shift events may lead to changes in the virome composition. Here, we use RNA sequencing to investigate whether recently allocated field colonies of bumble bees (*Bombus terrestris terrestris*) obtain new viruses from local insect community.

In May 2017; two-week-old commercially reared *B. t. terrestris* colonies (N=40) were installed in the vineyards of Valais (Switzerland) with a minimal distance of 500 m between the colonies. Workers (N=5) were collected at day 0 and day 45 from each bumble bee colony. Additionally, samples were taken from surrounding managed honey bee colonies at day 90 (N=19). RNA was extracted and pooled for each of the three sampling sessions. Then, three libraries were prepared and sequenced using an Illumina SP flow cell (100 Mio reads/pool, 300 cycles). After filtering *in silico* the host genome derived sequences and plant virus sequences, analysis of the assembled contigs showed that the bumble bee virome from the first sampling was dominated by DWV (average read depths ~2x104), followed by *Bombus cryptarum densovirus* (~2x102) and Lake *Sinai virus* (~102). By day 45, the bumble bee virome had changed in composition and was dominated by *Castleton Burn virus* (~5x104) and *Mayfield virus-1* (~5x103), followed by *Duke bunyavirus* (~102). The virome of honey bees consisted mainly of DWV (~105) followed by *Varroa orthomyxovirus-1* (~5x102) and *Apis rhabdovirus-1* (~5x102). These results highlight substantial changes in the composition of RNA viruses in bumble bees. When exposed to the environment, the bumble bee virome changes to favour bumble bee-related viruses. More long-term field studies are required to understand virus transmission dynamics and their potential impacts on natural bee populations.

Keywords: *Bombus terrestris, Apis mellifera*, Virome

REDUCED PARASITE BURDEN IN FERAL HONEYBEE COLONIES

Kohl, Patrick L., Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
D'Alvise, Paul, Institute for Medical Microbiology and Hygiene, University Hospital Tübingen, Tübingen, Germany
Rutschmann, Benjamin, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
Roth, Sebastian, BEEtree-Monitor, Munich, Germany
Remter, Felix, Katholische Stiftungshochschule München, Munich, Germany
Steffan-Dewenter, Ingolf, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
Hasselmann, Martin, Department of Livestock Population Genomics, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Diseases caused by parasites are considered the main reason for managed honeybee (*Apis mellifera*) colony losses. It is unknown whether feral colonies that escaped from apiaries benefit from parasite-reducing mechanisms like swarming or suffer from high parasite pressure due to the lack of disease management. In the latter case they could act as parasite reservoirs and pose a risk to managed bee health. We compared the occurrence of 22 microparasites among managed (N=74) and feral (N=64) colony samples from Germany using qPCR. We distinguished five colony types (overwintered managed colonies, nucleus colonies, and hived swarms; overwintered and newly founded feral colonies) representing differences in colony age and management histories, two variables potentially modulating parasite prevalence. The number of parasite taxa per colony was significantly lower in feral colonies than in managed colonies. Parasite community compositions also differed, with Trypanosomatidae, Chronic bee paralysis virus and Deformed wing viruses A and B being clearly less prevalent and abundant in feral colonies. Parasite burden was lowest in newly founded feral colonies, intermediate in overwintered feral colonies and nucleus colonies, and highest in overwintered managed colonies and hived swarms. Our study confirms the hypothesis that swarming reduces parasite pressure in feral honeybee colonies. Furthermore, environmental differences between natural nest sites and apiaries must be involved in explaining the observed differences. We conclude that feral honeybee populations are unlikely to contribute significantly to the spread of bee diseases. Elucidating the parasite-reducing mechanisms of honeybees under feral conditions can lead to new beekeeping applications that improve the health of managed honeybees.

Keywords: Bee diseases, wild honeybees, swarming

A NEW FRONTIER FOR VISUALIZING THE IMPACT OF STRESSORS IN BEES: PROTEINS PICTURED BY MASS SPECTROMETRY

BULET Philippe, Institute for Advance Biosciences, CR UGA CNRS UMR5309 Inserm U1209, La Tronche, France; HOUDELET Camille, MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Palavas-Les-Flots, France; ASKRI Dalel, Plateforme BioPark d'Archamps, Archamps, France; ARAFAH Karim, Plateforme BioPark d'Archamps, Archamps, France; BOCQUET Michel, Apimedia, Pringy, Annecy, France

Among pollinating insects, bees play a critical role in boosting reproduction of wild and commercial plants, thus contribute to the preservation of plant biodiversity and sustainability of food webs. In the last few decades, domesticated and wild bees have been subjected to biotic and abiotic threats causing various health disorders. This presentation will focus on nosemosis, an infection caused by a single-cell microsporidian parasite Nosema being the causative agent, which chronically infects the digestive tract of honeybees. Therefore, developing solutions to improve bee health including nosemosis is increasingly necessary, but still lacking. Here, we focused our investigations on the development of Matrix-assisted laser desorption/ionization imaging (MALDI) molecular mass fingerprint (MFP) and its integration with MALDI imaging mass spectrometry (MALDI IMS) to monitor which tissues would be impacted in a comparison between Nosema-infected and non-infected Western honeybees *Apis mellifera*. MALDI IMS is a powerful technology used to investigate the spatio-temporal distribution of a huge number of molecules throughout a body/tissue section. Here, we report the use of MALDI IMS to follow the molecular impact of an experimental infection of *Apis mellifera* with the microsporidia *Nosema ceranae.* We performed representative MFP of selected tissues obtained by dissection. This was followed by MALDI IMS workflows optimization including specimen embedding and positioning as well as washing and matrix application. We recorded the local distribution of peptides/proteins within different tissues from experimentally infected versus non infected honeybees. As expected, a distinction in these molecular profiles between the two conditions was recorded from different anatomical sections of the gut tissue. More importantly, we observed differences in the molecular profiles in the brain, thoracic ganglia, hypopharyngeal gland, and hemolymph. We introduced MALDI IMS as an effective approach to monitor the impact of *N. ceranae* infection on *A. mellifera*. This opens perspectives for the discovery of molecular changes in peptides/proteins markers that could contribute to a better understanding of the impact of stressors and toxicity on different tissues of a bee in a single picture board.

Keywords: *Apis mellifera, Nosema*, Mass spectrometry imaging

SISTERS FROM ANOTHER FATHER – CAN HONEY BEE INTRA-COLONIAL DIVERSITY BUFFER THE IMPACT OF DISEASES?

Beaurepaire, Alexis, Institute of Bee Health, University of Bern, Bern, Switzerland (presenting)
Sann, Christina, Labogena, Jouy en Josas, France
Arredondo, Daniela, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Castelli, Lorelei, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Genchi-Garcia, Maria-Laura, Instituto Multidisciplinario de Biología Celular, La Plata, Argentina
Reynaldi, Francisco, Instituto Multidisciplinario de Biología Celular, La Plata, Argentina
Antunez, Karina, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Mondet, Fanny, INRAE, Avignon, France
Dalmon, Anne, INRAE, Avignon, France
Le Conte, Yves, INRAE, Avignon, France

Honey bees stand amongst the most polyandrous animals, with queens of *Apis mellifera* mating with 16 drones on average. This intra-colonial diversity is expected to enhance the resilience of honey bee colonies towards stressors, but empirical evidence showing this under natural conditions remains scarce. We here analyzed the infestation with *Varroa destructor* and infections with honey bee viruses across *A. mellifera* patrilines in order to investigate whether the presence of heterogenous worker genotypes in a honey bee colony can buffer the impact of these important biotic stressors. In a first experiment, we investigated the expression of several phenotypes associated with *V. destructor across A. mellifera* worker patrilines: i) infestation of brood, ii) number of foundresses in infested cells, iii) mite reproduction and iv) recapping. To do so, we first phenotyped 2627 pupae cells from six colonies for the above-mentioned traits. We then extracted the DNA of 486 of these pupae from three colonies and reconstructed their patrilines using microsatellite markers to compare the observed phenotypes across the different brood genotypes obtained. The results of this comparison showed no significant variation of the observed phenotypic traits across the pupae patrilines. In a second experiment, we used 108 mite-infested pupae from the same three genotyped colonies to quantify and compare the genome copies of five honey bee viruses and variants (ABPV, SBV, BQCV, DWV-A and DWV-B) across *A. mellifera* subfamilies. Only DWV-A and DWV-B were detected in the analyzed brood samples. Comparing the infestation levels with these two variants revealed significant differences across worker brood subfamilies within the colonies. Additionally, DWV-A and DWV-B also exhibited contrasting infection patterns in individual pupae, and showed different temporal infection dynamics. These intra-colonial heterogeneous viral infection patterns stress the need to better incorporate this level of complexity in

virus research, and call for more investigations of the molecular mechanisms behind the resistance against these important pathogens. Altogether, the results from these two studies suggest that high colony genetic diversity can be beneficial against virus infections, but may not help buffering the impacts of *V. destructor*.

Key Words: Host-Parasite Coevolution, Population Genetics, Honey bee Health

EXPLORING THE CLIMATIC DRIVERS OF HONEY BEE COLONY AND DISEASE DEVELOPMENT

Rowland, Ben, School of Natural and Environmental Sciences, Newcastle University, UK
Simcock, Nicola, School of Natural and Environmental Sciences, Newcastle University, UK
Budge, Giles, School of Natural and Environmental Sciences, Newcastle University, UK
Rushton, Steve, School of Natural and Environmental Sciences, Newcastle University, UK

We know little about how the climate affects honey bee populations, and even less about how meterlogical conditions impact honey bee disease. We used historical data on the location and timing of honey bee diseases across England and Wales and combined these with weather data to determine how meterological patterns contributed to the risk of honey bee disease. We used a Bayesian modelling technique to determine that weather had varying effects on the risk of European foulbrood, American foulbrood, chronic bee paralysis, varroosis, chalkbrood and sacbrood. Next we examined how climate projections for England might impact honey bee colony size. We used an individual-based model to simulate honey bee colonies under different weather conditions and highlighted which projected meterolocal changes may have the most detrimental impact on honey bee colony development.

Keywords: Honey Bee, Climate, Disease

VIRAL GENETIC LANDSCAPE IN THE HONEY BEE POPULATIONS OF THE AZORES ARCHIPELAGO

Lopes, Ana R., Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus Sta Apolónia, 5300-253, Bragança, Portugal
de Miranda, Joachim, Ekologicentrum, Swedish University of Agricultural Sciences, Uppsala, Sweden
Martín-Hernández, Raquel, Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla La Mancha, CIAPA de Marchamalo, Guadalajara, Spain; Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FSE/EC-ESF), Fundación Parque Científico y Tecnológico de Castilla—La Mancha, 02006 Albacete, Spain
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus Sta Apolónia, 5300-253, Bragança, Portugal

Honey bee decline can be attributed to many stressors, including pathogens and parasites, such as varroa and its vectored viruses. The global honey bee trade poses an important risk of pathogen dissemination. The arrival of varroa in the Azores because of (illegal) queen importations is clear evidence of that. Varroa was first detected on Pico in 2000, on Flores in 2001, and on Faial in 2008. The other six islands of the archipelago remain mite-free. Therefore, the Azores provide an interesting setting to study the impact of varroa in the viral landscapes, adding to previous studies. In July/August of 2014/2015 we sampled 402 colonies distributed across 8 islands. These were screened for DWV, BQCV and LSV by RT-qPCR and the positive samples were further examined by high-throughput sequencing (HTS) to uncover whether varroa has altered the viral genetic composition. Of the 8 islands, São Jorge and Terceira had no DWV-positive colonies and Pico and Flores showed the highest DWV prevalence. BQCV and LVS were detected on all the islands. The HST data indicated that DWV-A, -B, and -C variants were all present in the Azores, although with varying prevalences (DWV-A: 56%; DWV-C: 31.2%; DWV-B: 12.2%). The phylogenetic tree revealed a geographic pattern, in which most of the DWV variants from São Miguel and Santa Maria formed a clade with the DWV-C reference, whereas Pico, Faial, Flores, and Graciosa clustered with the DWV-A reference. Interestingly, the DWV-A sequences from Pico were more closely related with those from Faial than with those from Flores. If the DWV-A variant was introduced with varroa, then the illegal queen imports on Pico and Flores had independent origins, whereas the DWV-A variants on Faial might have originated from Pico. DWV-A and B have acquired a world-wide distribution in the wake of varroa, while variants DWV-C and DWV-D have become extremely rare, one plausible scenario is that DWV-C was one of the original genotypes in the Azores predating varroa and has been replaced by the variants A and B, due to their superior adaptation to varroa-mediated transmission. Also of interest is the distinct clade formed by the BQCV and LSV sequences of São Miguel and Santa Maria, as well as the independent cluster formed by Flores sequences supporting the previous scenario. All these findings will be discussed in this communication.

Keywords: Deformed Wing Virus, High-throughput sequencing, honey bee

HONEYBEE VIRUSES AND WHERE TO FIND THEM ACROSS THE PHYLOGENETIC TREE OF BEES

Tehel, Anja, Martin Luther Universität Halle-Wittenberg, Halle, 06120, Germany
Groom, Scott, University of Adelaide, Adelaide, 5000, Australia
Fung, Elisabeth, University of Adelaide, Adelaide, 5000, Australia
Gerth, Michael, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, 04103, Germany
Theodorou, Panagiotis, Martin Luther Universität Halle-Wittenberg, Halle, 06120, Germany
Paxton, Robert J., Martin Luther Universität Halle-Wittenberg, Halle, 06120, Germany

Viruses spilling over from honey bees into other flower visitors are suggested as one out of many possible reasons for wild bee decline. RNA viruses like deformed wing virus (DWV) or black queen cell virus (BQCV) are known to be highly prevalent in a variety of bumble bee species. However, despite their potential role in global bee decline, the occurrence of these viruses in wild bees, especially from genera other than *Bombus*, is poorly investigated.

We address viral spill-over from honey bees to wild bees and the role of viruses in wild bee species decline. We collected wild bees in Central Germany and screened them for 7 common RNA viruses. To clarify the taxonomy, DNA bar-coding was used. We found BQCV and DWV genotype B (DWV-B) at high prevalence in bee species belonging to the family Apidae but it was at lower prevalence in bee species of the families Colletidae, Halictidae and Andrenidae.

To help interpret patterns of viral prevalence across the phylogeny of bees, we experimentally infected pupae of different bee families collected in the wild, either Germany or Australia, to check for the ability of the virus to replicate in these potential new hosts.

Our data suggest that a phylogenetic component exists to viral susceptibility; honey bee-associated viruses may seem to be more prevalent for species in the family Apidae than for other phylogenetically more distant bee families. Also, we explore the potential role of Wolbachia, a bacteria that is known to be able to reduce virus infections in other insect taxa.

Keywords: RNA virus, multi-host pathogen, *Wolbachia*

AMERICAN FOULBROOD RISK ASSESSMENT ANTIMICROBIAL AND RESISTANCE: PARADIGM SHIFT FROM AN INDIVIDUAL HIVE TO A "HERD HEALTH MONITORING" IN SASKATCHEWAN, CANADA

Zabrodski, Michael[1]; Obshta, Oleksi[1]; Tasha Epp[1]; Wilson, Geoff[2] Kozii, Ivanna[1]; Thebeau, Jenna[1]; Sarah Biganski[1]; Masood, Fatima[1]; Raza, Fahim[1]; Jose, Midhun[1]; Silva, Marina[1]; Koziy, Roman[1]; Ruzzini, Antonio[1]; Wood, Sarah[1]; Simko, Elemir[1];

1 Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.
2 Ministry of Agriculture, Government of Saskatchewan, Prince Albert, Canada.

The North American beekeeping industry is heavily reliant on antimicrobial metaphylaxis for control of American foulbrood (AFB). In the face of growing international concern regarding antimicrobial resistance (AMR), large commercial beekeepers are inadequately prepared to reduce their reliance on antimicrobials without risk of significant economic losses to AFB. Current methodology to guide evidence-based antimicrobial use for AFB risk assessment relies on the sampling and testing of individual hives and is logistically impossible for large-scale, commercial beekeeping operations to implement.

To address this issue, we performed a small sampling study and confirmed that concentrations of AFB spores in samples of pooled, extracted honey (conveniently collected during routine, end-of-season extraction and representing multiple hives within a single sample) were reflective of the degree of spore contamination identified previously for individual hives. Accordingly, we demonstrated that pooled extracted honey was predictive of the spore contamination identified through individual hive testing and appeared to have prognostic value in assessing the risk of AFB at the yard or operation level.

Subsequently, we expanded testing of pooled extracted honey to 52 Saskatchewan commercial beekeepers representing approximately 75% of the province's 110,000 registered colonies. By correlating spore concentrations to the incidence of AFB, we established reliable prognostic threshold at 2 spores per gram of pooled extracted honey for low risk of AFB. In addition, we determined geographical distribution of *Paenibacillus* larvae resistant to oxytetracycline (rAFB-OTC) and identified an epidemiological nidus in a region highly populated by commercial beekeepers. Based on this transectional study, it seems that rAFB-OTC is present within a high percentage of tested samples of a few commercial beekeepers; however, the rAFB-OTC appears to be spreading to adjacent (i.e., neighboring) operations which is not surprising considering the overlap in foraging areas of adjacent bee yards of commercial beekeepers in this densely populated area. However, an alarming and unexplained finding is that the spread of rAFB-OTC is extending towards other geographically distant beekeeping areas in Saskatchewan. We anticipate that improved evidence-based antimicrobial use in apiculture will result in reduced use of antibiotics with subsequent reduction and spread of antimicrobial resistance in Saskatchewan beekeeping industry.

PATHOLOGY

POSTERS

HEALTH SCREENING OF LOCAL BEE BREEDS IN TÜRKIYE

Dilek Muz, Department of Virology, Faculty of Veterinary Medicine, Tekirdag Namık Kemal University, Tekirdag, Türkiye
Martín-Hernández, Raquel, Centro de Investigación Apícola y Agroambiental de Marchamalo, Marchamalo, Spain
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Nanetti, Antonio, Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca
in Agricoltura e l'Analisi dell'Economia Agraria, Roma, Italy
Mustafa Necati MUZ, Department of Parasitology, Faculty of Veterinary Medicine, Tekirdag Namık Kemal University, Tekirdag, Türkiye

Türkiye has the highest number of honey bee colonies and honey production in the Mediterranean countries. Türkiye is home to many *Apis mellifera* subspecies and various honey species. It is a partner of the Prima-supported MediBees project, which researches Mediterranean basin honey bees and their products. The interaction degree of bee genetics and environmental variables drives the sum of the yield characteristics and sustainable colony health in beekeeping. In order to improve the sustainable agricultural ecosystem, genetic characteristics of bee subspecies adopted in different regions of the Mediterranean country of Türkiye will be investigated in the MEDIBEES project. The presence and distribution of varroa in subspecies adapted to different climatic and geographical conditions will be investigated comparatively. In addition to yield characteristics based on subspecies and local differences, investigating any resistance to varroa is essential in establishing healthier bee colonies.

NO EFFECT OF TEMPERATURE ON THE PERFORMANCE OF TRICKLING BY OXALIC ACID

Garrido, Claudia, BeeSafe – Bee Health Consulting for Agriculture and Veterinary Medicine, Hamm, Germany
Russano, Maria, CONAPI Soc. Coop. Agricola, Italy
Nanetti, Antonio, CREA – Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy

Trickling is a common method to apply oxalic acid in varroa treatment, which is used successfully worldwide. The method was developed in the late 1990s as a winter treatment in broodless colonies. In a further advancement in the late 2000s, the method was adapted to a summer treatment by caging the queen before the treatment. We here review the original data from these experiments with regard to the influence of external temperature on the treatments.

In the winter experiment, seven groups of colonies (n=5 each) were treated with 4.2% oxalic acid in 60% sugar syrup, shifted by 7-14 days each. The medium temperature was assessed for each week. The efficacy of the treatments, as well as the colony condition after overwintering were assessed to evaluate the influence of the temperature.

In the summer experiments, the trickling method was assessed in southern and northern Italy. Over two years, a total of 46 colonies on five apiaries was treated with 4.2% oxalic acid in 60% sugar syrup (as the registered product ApiBioxal®) after caging the queen for 25 days. Again, efficacy and colony conditions were assessed before and after the treatment.

In the winter experiment, trickling oxalic acid consistently reached an efficacy over 95%, independently of the temperature during the treatment. Some of the groups were treated with temperatures down to -9°C. Only two out of 35 colonies did not survive the winter. The colony strength after winter did not significantly vary between groups.

In the summer experiments, the colonies were treated with temperatures up to 40°C after caging the queen. Again, the efficacy was consistently over 95%. The colony development was not affected by the caging or the temperature during the treatment.

We conclude that both efficacy and tolerability of oxalic acid treatments by trickling keep stable over a wide range of external temperatures.

Keywords: Varroa treatment, oxalic acid, temperature

MOLECULAR IDENTIFICATION OF *LOTMARIA PASSIM* IN SELECTED BUMBLEBEE SPECIES

Michalczyk, M, Sokół, R
Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn , Olsztyn, Poland

Bumblebees (*Bombus*) and honey bees(*Apis mellifera*) play very important roles in the ecosystem and the economy. They pollinate flowering plants, and insect pollination is essential in food production, which is why effective measures for conserving the populations of these useful insects are urgently required. There are around 300 bumblebee (*Bombus*) species in the world, 37 of them have been identified in Poland. The decline in the bumblebee population is attributed, inter alia, pathogens or the reduced availability of food resources due to the loss of natural habitats. One of the pathogens that infects honeybees is a protozoan from the *Trypanosomatidis* family. *Trypanosomatidae* are unicellular flagellates and parasites that infect mammals, insects even plants. At present, *L. passim* have been widely researched due to their detrimental effect on the behavior, physiology and immune function of bees. *Lotmaria passim* also can also occur in Polish bumblebee populations, so in this study, a Polish population of bumblebees was screened for the presence of *L. passim*. The experiment was performed on 13 adult bumblebees belonging to 4 species: *B. lapidarius* (2 workers, 2 males), *B. lucorum* (1 worker), *B. pascuorum* (5 workers, 1 male) and *B. terrestris* (2 workers). The insects were harvested found dead in the south of Poland. Genomic DNA were isolated from whole insects that were homogenized individually under sterile conditions in a mortar. *Lotmaria passim* were identified by PCR with HotStarTaq Plus Polymerase (Qiagen) and the HotStarTaq Plus Master Mix Kit (Qiagen). The following primers were used: LpCytb_F1 5'-CGAAGTGCACATATATGCTTTAC-3', LpCytb_R 5'-GCCAAACACCAATAACTGGTACT-3'. The reactions were run using a thermal profile consisting of a initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, primer annealing at 55°C for 45 seconds, and elongation at 72°C for 1 minute. After the completion of the last PCR reaction, the final extension step was conducted at 72°C for 10 minutes. *Lotmaria passim* was identified only in 1 insect of *B. pascuorum* (workers). Further research is needed to confirm the effect of concurrent pathogenic infections on the decline of bumblebee population. The role of *L. passim* and coinfections with other pathogens, including viral, bacterial and parasitic, in bumblebee should be investigated.

Keywords: *Lotmaria passim*, bumblebee, PCR

THE USE OF ENCAPSULATED ESSENTIAL OILS AGAINST *VARROA* AND *NOSEMA*

Charistos Leonidas, Department of Apiculture, Institute of Animal Science- ELGO 'DIMITRA', Nea Moudania, Greece.
Hatjina Fani, Department of Apiculture, Institute of Animal Science- ELGO 'DIMITRA', Nea Moudania, Greece.

Essential oils have been used against *Varroa* and *Nosema* for a long time in beekeeping. To stabilize the active compounds of the essential oils of several aromatic plants and to slowly release their desired properties a process called encapsulation was used. Using this process, the essential oils of oregano, lavender, fennel and clove, in equal parts, were encapsulated in nanoparticles and they were used against Varroa and Nosema, after fed to the honey bee colonies. Initially, 40 honey bee colonies were used per treatment group as follows: 3ml and 5ml of the active mixture diluted in 100 ml of sugar solution were administered to 20 honey bee colonies by trickling (4 times in weekly intervals), 3ml of the active mixture dilute in 1Lt of sugar solution was provided as feed to 10 honey bee colonies and untreated sugar solution was fed to the rest 10 colonies, used as control. The first results showed that the trickling of the 3ml/100ml of syrup or the 5ml/100ml of syrup were more effective in keeping the Nosema spores per bee in low numbers, compared to feeding the mixture in feeders containing the 1 Lt of syrup, while no effect was observed on varroa mortality. No significant difference was detected between the two above concentrations, although more experiments and repetitions are needed for secure conclusions. Lower concentrations (e.g. 2ml/100 ml of sugar solution) also used by trickling was not proved to be effective against Nosema parasite. This project has been financed by the Prefecture of West Macedonia of Greece, started in 2020.

Keywords: essential oils, encapsulation, nosemosis

EPIDEMIOLOGICAL ASSESSMENT OF HONEY BEE PATHOGENS IN EMILIA-ROMAGNA, ITALY

Cilia, Giovanni, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Tafi, Elena, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Zavatta, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Nanetti, Antonio, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Pathogens are major threats to the welfare of the managed honey bees. In recent decades, the interactions between infectious diseases and other factors like *Varroa* infestation, pesticide exposure, climate change and food shortage have led to colony collapse events worldwide. Monitoring plans have been established to improve our understanding of the honey bee health conditions and elucidate the contribution of individual risk factors in collapse events. In this context, the role of pathogens definitely needs more comprehensive investigation.

As a part of the Italian national monitoring project "BeeNet – monitoring the environment through bees and biodiversity", in the year 2021, 31 apiaries of the Emilia-Romagna region were assessed with molecular methods for both prevalence and abundance of DWV, KBV, ABPV, CBPV, *Nosema ceranae, Lotmaria passim, Crithidia mellificae*, and *Crithidia bombi*.

DWV was the most prevalent pathogen, followed by CBPV and *N. ceranae*. DWV and CBPV were the most abundant pathogens also. None of the three trypanosomatids (i.e., *L. passim, C. mellificae, C. bombi*) was detected. Different peaks of both pathogen prevalence and abundance were observed over the months, showing a seasonality likely related to the bee colony population dynamics and the trend of *Varroa* infestation level. Results also highlighted a potential correlation between pathogen prevalence in Emilia-Romagna and geographical longitude. DWV and *N. ceranae* appeared more prevalent in the eastern part of the region, which is characterized by proximity to the sea and higher annual average temperature, compared to the western areas.

This survey provides new knowledge on the epidemiological status of the managed honey bee colonies of the Emilia-Romagna region. The assessment of the health status of honey bees is important both to help the beekeepers keep the welfare of their colonies and to protect the wild pollinator populations, therefore contributing to the quality of the environment.

Keywords: pathogens, monitoring, welfare

QUANTITATIVE VIRUS DYNAMICS IN *APIS MELLIFERA* UNICOLOR COLONIES AFTER A NEW INVASION OF *VARROA DESTRUCTOR* IN LA RÉUNION ISLAND

Benoit Jobart[1,2], Hélène Delatte[3,4], Nicolas Cazanove[5], Olivier Esnault[5], Gérard Lebreton[1], Johanna Clémencet[2], Nicolas Blot[6]

1 CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France
[2] Université de la Réunion, 97400 Saint Denis, La Réunion France
3 CIRAD, UMR PVBMT, 101 Antananarivo, Madagascar
4 FOFIFA CENRADERU, DRA, 101 Antananarivo, Madagascar
5 GDS, 97418 La plaine des Cafres, La Réunion, France
6 Université Clermont Auvergne, CNRS, Laboratoire "Microorganismes: Génome et Environnement", Clermont–Ferrand, France

Honeybees suffer from several pathogens that affect their health and contribute to their decline in the world. On Reunion Island, a tropical island situated in the Indian Ocean with an endemic honeybee subspecies: *Apis mellifera unicolor*, suffered from the recent introduction of *Varroa destructor* in 2017. The invasion of the island was extremely fast, within three months all apiaries were infected, generating major health issues with high colony losses recorded. In addition to its spoliating action on the bee's fat bodies, the varroa mite is also known to lower its host's immunity and to be a vector of viruses such as DWV, which could increase the direct impact of varroa on colonies.

Here we investigated the dynamics of the three major viruses found (DWV, CBPV and BQCV) in La Reunion Island in a context of infestation by *V. destructor*. A total of 80 colonies of *A. m. unicolor* were monitored for two 1-year periods in two apiaries located on different altitudes. During these periods no acaricid treatments were carried out. Every month and for each colony, about 30 worker bees were collected for detection and quantification of viruses (DWV, CBPV, BQCV) by quantitative PCR. Colony strength and *V. destructor* infestation rate were measured.

The BQCV virus was detected throughout the year. Quantitative PCR revealed that the amount of DWV virus copies evolved in the same way as the varroa infestation in the colonies. Finally, lower incidences were found for CBPV and did not seem to be related to *V. destructor* load or colony strength.

All these results are discussed in the light of the local tropical beekeeping context.

Keywords: *A. m. unicolor*, pathogene dynamic, quantitative PCR

PHENOLOGY OF THE MICROSPORIDIA *NOSEMA CERANAE* IN FOUR MEDITERRANEAN COUNTRIES

Jabal-Uriel, Clara, CIAPA-IRIAF, Marchamalo, Spain
Bonjour-Dalmon, Anne, INRAE, Unité de Recherche Abeilles et Environnement, Avignon, France
Chejanovsky, Nor, Department of Entomology, ARO, The Volcani Institute, Rishon LeZion, Israel
Higes, Mariano, CIAPA-IRIAF, Marchamalo, Spain
Le Conte, Yves, INRAE, Unité de Recherche Abeilles et Environnement, Avignon, France
Lopes, Ana R., CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Pinto, M. Alice, CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Reyes-Carreño, Martiza-Regina, INRAE, Unité de Recherche Abeilles et Environnement, Avignon, France
Soroker, Victoria, Department of Entomology, ARO, The Volcani Institute, Rishon LeZion, Israel
Martín-Hernández, Raquel, INCRECYT-FSE/EC-ESF, CIAPA-IRIAF, Marchamalo, Spain

The presence and prevalence of the Microsporidia *Nosema ceranae* was assessed as part of the BEEHEAL project during a 2-year study period (2018-2019). Monitoring took place in six apiaries located in four Mediterranean continental apiaries: France, Israel, Portugal and Spain, and two insular apiaries located on Ouessant (France) and on São Miguel (Azores archipelago, Portugal). Besides honeybee sample collection every two months, parameters on colony strength, honey production, beekeeping management and climate were recorded. The pathogen was not detected on São Miguel Island during the study. Infection at the apiary level showed that most of the colonies were positive to *N. ceranae* except in mainland France, where the percentage of infected colonies was the lowest and it fluctuated, with an increase in August in both years. The percentage of infected honeybees per colony varied among apiaries, with Israel having the highest mean levels (32.39%). Colonies in Spain and Portugal showed a similar pattern with the highest levels of infection in April 2018. Ouessant Island had a high percentage of infected honeybees in 2018 and it decreased on 2019. Temperature was not correlated with *N. ceranae* infection, although high temperatures combined with high relative humidity were associated to high infection levels in Israel. Intra-colony percentage of infection was positively correlated with honeybee population when all apiaries were analysed together. Honey production was not related with *N. ceranae* infection. Regarding queen replacement, infection levels in spring 2019 in mainland Portugal and Spain decreased in those colonies that had replaced the queen the previous summer (August 2018). Colony mortality varied amo ng apiaries, with mainland France and Spain showing the highest colony losses (47.6% and 39.1%, respectively). However, this parameter was not correlated with the levels of infection. These results allowed to compare the N. ceranae phenology among different countries, showing that the infection is dependent on many factors. This information could assist in the development of beekeeping management strategies.

Keywords: intra-colony prevalence, climate and beekeeping management effect

DIRECT AND SENSITIVE DETECTION OF DEFORMED WING VIRUS USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) IN HONEY BEES

De Smet, Lina, Ghent University, Ghent, Belgium; de Graaf, Dirk C., Ghent University, Ghent, Belgium

The decline of honey bees is one of the biggest threats to biodiversity and the deformed wing virus is one of the main culprits. The deformed wing virus is transmitted by the ectoparasitic mite, *Varroa destructor.* Almost all efforts to tackle this double infection have so far focused on controlling the Varroa mite alone and not the viruses it transmits. In a recent study (de Graaf et al., 2020), we were able to show that the trait 'suppressed in ovo virus infection' (SOV) shows moderate heritabilities for the presence of multiple viruses simultaneously and for the presence of a single virus species. Colonies expressing the SOV trait appeared more resistant to virus infection as a whole, with fewer and less severe deformed wing virus infections. The implementation of this new trait in breeding programmes is recommended and therefore a simple and direct detection method for bee viruses is essential. The potential of loop-mediated isothermal amplification (LAMP) for bee virus detection was investigated and its implementation in beekeeping practice was explored. Primers sets for different bee viruses were developed. Currently, a colorimetric detection of deformed wing virus complex and chronic bee paralysis virus is being optimized. The ease with which this test can be performed together with the possibility of using crude extracts make it feasible to have this method performed as a field test by the beekeeper himself.

Keywords: diagnostics, virus, LAMP

TRYPANOSOMATIDS IN THE AZORES

Aguado-López, Daniel. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain
Maside, Xulio. CIMUS-IDIS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain.
Bartolomé-Hudsson, Carolina. CIMUS-IDIS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain.
Pinto, Alice. CIMO, Instituto Politécnico de Bragança, Bragança, Portugal.
Higes-Pascual, Mariano. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain
Martín-Hernández, Raquel. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain; Fundación Parque Científico y Tecnológico de Castilla—La Mancha (INCRECYT-FSE/EC-ESF), Albacete, Spain

The Azores is a group of islands on which there are still some islands free of *Varroa destructor*, making it the largest European territory with the highest number of colonies without the mite, which makes them unique locations for studying mite pre-colonization areas. In recent years, special attention has been paid to trypanosomatid infection in bees because of its possible role as pathogens. *Crithidia bombi, Crithidia mellificae* and *Lotmaria passim* are the most frequently species detected in honeybees. However, information on their distribution is still rather limited, and more so in varroa pre-colonization areas. In this study, 572 hives from the Azores archipelago and Madeira sampled in 2014 and 2015, and 89 samples collected in 2020 were analyzed to determine the trypanosomatid species. All honeybee samples were analyzed by PCR to detect the RPB1 gene of all trypanosomatid species. Afterwards, 93 positive samples were sequenced by Illumina MiSeq platform to determine which species were present. The 66.3% of colonies collected in 2014-2015 were positive in Madeira and 72% in the Azores. All the trypanosomatid sequences detected corresponded to *L. passim* both in islands with or without *V. destructor.* Two major haplotypes were found representing around the 98% of the total sequences obtained. As only a small percentage of trypanosomatid-positive samples were sequenced, all the remaining positive samples were analyzed by quantitative PCR (qPCR) using newly designed specific primers and probes, to confirm if L. passim was the only species in Azores and Madeira. This analysis confirmed *L. passim* as the main trypanosomatid species (98.1% in Madeira and 98.9% in Azores), however, *C. bombi* was also found in 34% of the positive samples from Madeira and just in 1.4%, in Azores. In samples collected in 2020 the prevalence was lower (31.5%), being *L. passim* the only species found. *C. mellificae* was not

found in any sample. The results suggest that *L. passim* and *C. bombi* have been present in *Apis mellifera* colonies in Azores since before the spread of varroa.

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COMPARISON OF DIFFERENT DNA EXTRACTION METHODS FOR THE STUDY OF PATHOGENS AND INTESTINAL BACTERIA IN HONEYBEES

Aguado-López, Daniel. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Buendía-Abad, María. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Jabal-Uriel, Clara. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Benito-Murcia, María. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Higes-Pascual, Mariano. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Martín-Hernández, Raquel. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain; Fundación Parque Científico y Tecnológico de Castilla—La Mancha (INCRECYT-FSE/EC-ESF), Albacete, Spain

DNA extraction is one of the critical points in molecular biology, since any error at this point will have amplified effects on subsequent procedures. In this work, we compared different DNA extraction methods to determine the most suitable for the detection of pathogens by PCR and of pathogens and intestinal bacteria by qPCR. Adult bee samples were collected from 30 colonies. Each sample (60 worker bees) was processed following 5 different extraction methods: i) **BS+PK**: using the BS96 DNA kit (Qiagen) in a BioSprint workstation (Tissues protocol) with a proteinase K incubation step; ii) **BS+P**: using BS96 DNA kit (cultured cells protocol) with protease treatment; iii) **TE**: bee macerate incubated in TE (Tris-EDTA) buffer (95°C, 15 min.); iv) **TE+PK**: adding a previous incubation with proteinase K; v) **TE+ATL+PK**: as the previous one but the proteinase k incubation made in ATL buffer. DNA template volumes used were 2.5 µl for PCR and 1 µl and 2.5 µl for qPCR. The most efficient methods to detect *Nosema ceranae* by PCR were BS+PK and TE+ATL+PK which detected 19 positive samples (11 samples were negative to all methods). By qPCR, the best results were observed using BS+PK, BS+P and TE methods both using 1 µl or 2.5 µl of DNA template, showing the BS+P method the highest limit of detection. For *Lotmaria passim*, the higher detection level by PCR was recorded using the BS+P method (22 positive samples) and the same by qPCR detecting the highest number of positive samples (n=23) and lowest Ct values. For gut bacteria (*Bartonella apis* and *Frischella perrara*), BS+PK (both 1µl and 2.5µl of DNA template) registered the highest number of positive samples (n=30) and together with BS+P showed the lowest Ct values. This study concluded that the most efficient DNA extraction methods were those carried out by BioSprint workstation although the incubation of samples in just TE buffer gave comparable results, being this latter a much more economical option. Regarding the volume of DNA template used, best results were obtained using 2.5 µl in conventional PCR and 1 µl in qPCR.

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CONTROL OF WAX MOTH BY MEANS OF ORGANIC ACIDS

Charrière Jean-Daniel; Schlagenhof Leon, Swiss Bee Research Center, Agroscope, Bern, Switzerland

The storage of frames that have contained brood or still contain pollen is problematic in practice as they can be attacked by the wax moth, *Galleria mellonella* in particular. To limit the damage to the drawn combs, different techniques or products can be applied. However, it is important that these treatments do not contaminate the wax so that it does not pose a risk to the bees or to the quality of the honey that will be stored in the combs later. These considerations have led to the banning of many chemical treatments in Europe and in Switzerland, only acetic acid is currently allowed. The way and the dosage recommended for practice to control *G. mellonella* with this organic acid are very empirical and we found no data on the effectiveness and mortality on the wax moth due to this method.

In a realistic field trial, we treated stacks of honey supers with liquid acetic acid according to the recommendations in the literature, we let the acetic acid vapours work for 120 hours and observed mortality on the different stages of the greater wax moth G. *mellonella* (egg, larva, cocoon, imago).

We have demonstrated very good efficacy of the treatment against eggs, cocoons and adult moths and partial mortality in larvae. We discuss the possibilities and limitations of this control technique. Practical recommendations are formulated to reach a good efficacy and to avoid risks for the users and risks of honey contamination.

IS THE BEEWOLF, *PHILANTHUS TRIANGULUM*, A NEW THREAT TO HONEY BEES ON THE CANARY ISLANDS?

De la Rúa, Pilar, Department of Zoology and Physical Anthropology, Veterinary Faculty, University of Murcia, Murcia, Spain

Beekeeping is a practice of special interest as a source of income for rural communities. Today, beekeepers face a variety of threats that endanger the health and therefore, the production of honey bees. These include the effect of invasive species which, lacking natural predators, can adapt to new ecosystems and spread to become pests. In this respect, islands deserve special consideration because local populations are more susceptible to the effects of this and other factors associated with honey bee colony decline.

Beekeeping in the Canary Islands has greatly developed in recent years and regional laws have been established to control the conservation of the local black honey bee, therefore, beekeepers are very sensitive to the introduction of new parasites or predators of honey bees. Recently, the apparently unintentional introduction of a wasp, *Philanthus triangulum* (commonly known as beewolf), has raised awareness among Canarian beekeepers. This wasp preys on honey bee workers which are the food source for the wasp larvae. Each female can capture more than 100 workers, so it could represent a potential threat for the viability of honey bee colonies located in areas with high wasp densities. This wasp has expanded rapidly across the Canary Islands.

I am providing here the results of a questionnaire addressed to beekeepers of Gran Canaria and La Palma consisting of questions on the presence and impact of the beewolf and its importance in relation to the main pest of honey bees, the Varroa mite. Of the 106 beekeepers who responded to the questionnaire, 90% think that a solution to the beewolf problem needs to be found, but only 29% would consider moving their hives to avoid its presence. At present, 23% are using some treatment against the wasp. On both islands, beekeepers consider the beewolf to be a very important problem for the beekeeping sector, but on Gran Canaria the problem is more pressing than on La Palma. In any case 57% of the beekeepers think that Varroa is still the most important problem faced by beekeepers in Gran Canaria and La Palma.

Keywords: invasive species, beekeeping, predator

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PROTEIN-BASED DIAGNOSTIC TOOL FOR DETECTION OF AMERICAN FOULBROOD AND THE DISTINCTION OF THE TWO MAIN GENOTYPES OF PAENIBACILLUS LARVAE

Ehrenberg, Sandra, Institute of Immunology, Friedrich-Loeffler Institute, Greifswald, Germany
Rüttinger, Lukas, Senova Gesellschaft für Biowissenschaft und Technik mbH, Weimar, Germany
Scholz, Friedrich, Senova Gesellschaft für Biowissenschaft und Technik mbH, Weimar, Germany
Schäfer, Marc, Institute of Infection medicine, Friedrich-Loeffler Institute, Greifswald, Germany
Kammerer, Robert, Institute of Immunology, Friedrich-Loeffler Institute, Greifswald, Germany

American foulbrood (AFB) is the most devastating bacterial brood disease of honey bees (*Apis mellifera*). AFB causes colony and economic losses worldwide and is a notifiable disease in many countries. Disease diagnosis is usually conducted via visual inspection. If a colony is suspicious, the disease has to be confirmed in the laboratory. Laboratory diagnosis can be labor intensive and time consuming. For better prevention of disease spread sensitive, genotype-specific and fast diagnostic tools are needed. To fasten diagnosis a lateral flow device (LFD) and a sandwich ELISA were developed to diagnose AFB and distinguish between the two main genotypes (ERIC I and ERIC II) of *Paenibacillus larvae*, the causative agent of AFB, in the field and in the laboratory.

Since ELISAs and LFDs are based on antibody detection of antigens (here: proteins), specific monoclonal antibodies (mAbs) for detection of *P. larvae* in general and for the ERIC-genotype differentiation were generated. The produced mAbs were characterized and the most suitable candidates were used for the establishment of a sandwich ELISA and the LFD. The developed assays are detecting *P. larvae* in general and the genotype ERIC II specifically, what makes ERIC-genotype differentiation possible.

The AFB sandwich ELISA detected all of the tested *P. larvae* field strains (n = 60), whereas the ERIC II sandwich ELISA detected all of the tested ERIC II field strains (n = 30) but none of the tested ERIC I field strains (n = 30). The same field strains were also tested in the LFD. After successful establishment of the assays they will be validated using field samples in the laboratory and directly with samples of colonies in the field.

Keywords: American foulbrood, diagnosis, ERIC-genotyping

TEMPORAL ANALYSIS OF THE PREVALENCE OF LOTMARIA PASSIM AND *APICYSTIS BOMBI* IN HONEY BEE COLONIES ON THE CANARY ISLANDS (SPAIN)

Sánchez-Aroca, Micaela; Martínez-López, Vicente; Muñoz, Irene; De la Rúa, Pilar

Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain.

Trypanosomatids and neogregarines are protists that infect vertebrates and invertebrates. In the case of bumble bees and honey bees they have been linked to population declines in recent decades. In honey bees, the trypanosomatid species was initially attributed as *Crithidia mellificae*, but following molecular analyses a new species, *Lotmaria passim* has been described. Subsequent studies have revealed a high prevalence of *L. passim* in diseased colonies compared to *C. mellificae*. As for the neogregarine species, *Apicystis bombi*, it is widely distributed in bumblebees while it is sporadically detected in honey bees.

A total of 293 honey bee colonies sampled during the years 1998, 2008, 2010, 2011, 2016 and 2017 in the Canary Islands were examined by molecular analysis. Of these, 32.42% were positive for *L. passim*, while no cases of *C. mellificae* were detected. The prevalence of *L. passim* ranged from 16.67% (2008) to 60.38% (2017), while only 2.05% were positive for *A. bombi* between 2008-2011.

Results revealed that *A. bombi* occurs sporadically while *L. passim* has a wide distribution in the Canary Islands and a higher prevalence in recent years. Moreover, the prevalence of *L. passim* was significantly higher in honey bees from La Palma than in those from Gran Canaria. This result could be due to the fact that colonies probably infected have been selected and disseminated in the framework of the conservation programme of the local ecotype of honey bee established in La Palma since 2001. All this highlights the need to implement pathogen analysis in the colonies to be included in the conservation programmes.

Keywords: Pathogens, Conservation, Honey bee ecotype

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THE PRESENCE OF THE FLIES (DIPTERA) IN HONEY BEE COLONIES KEPT IN LOG HIVES IN POLAND

Molasy, Bartłomiej, Warsaw University of Life Sciences , Warsaw, Poland
Gajda, Anna, Laboratory of Bee Diseases, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland
Mazur, Ewa, Laboratory of Bee Diseases, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

Beehives can be considered as small microcosm in which a variety of insects with various roles (detritivores, predators, scavengers, parasitoids) are presented, in addition to honey bees. Some dipteran species, being facultative parasitoids of *Apis mellifera*, have been highlighted. In order to detect the possible presence of these species, this research was carried out. Samples were taken from colonies living in close to natural conditions at four separated locations in Poland (Kłodzka Valley, Świętokrzyskie Mountains, Gołuchów park and Mazury forests), during two years 2021-2022. Debris and combs with brood from dead and alive colonies, inhabiting log hives, were examined. In 18 from 24 samples taken from Kłodzka Valley, puparies and adult flies were found. Preliminary classification of specimens indicates the presence of *Heleomyzidae*, *Drosophilidae*, *Phoridae* and *Mycetophilidae* families. It is supposed that puparies belonged to Heleomyzidae. In debris from two colonies from Świętokrzyskie Mountains, representatives of *Phoridae* were found. Possibly their larvae were also present on brood. In all six samples of debris from Mazury forests and Gołuchów park larvae and puparies were recorded. Puparies from Mazury were bred to adults and preliminary classified to *Heleomyzidae* family. Examination under microscope, with comparison to identification keys, was performed to indicate taxonomic affiliation of all adult flies. Further analyses to reach the species level of identification would be carried out in collaboration with expert taxonomists. The study showed that multiple specimens of different, potentially parasitoid species were present in 26 out of 32 samples. Acts of scavenging on dead honey bees were observed in 26 cases and were caused mainly by flies from *Heleomyzidae* family. Also brood infestation with probably *Phoridae* larvae was found in one sample. Despite the rather low damage caused by these flies per se, it seems to have importance. Considering that reproductive success and opportunities for parasitoid and scavenging behaviours are most frequent in weak or dying colonies. It is supposed that flies might be vectors, spreading the diseases. In conclusion, more intensive study of dipteran flies, associating honey bees, is required, before we would understand their relationships and their relevance.

Keywords: facultative parasitoid, *Apis mellifera*, *Diptera*

NANOFIBROUS MATRICES CONTAINING HOP LEAVES EXTRACTS FOR THE CONTROL OF AMERICAN FOULBROOD

Giménez Martínez, Pablo D.(1,2); Kloster, Gianina(2,3); Méndez, Carlos(4); Pegoraro, César(4); Medici, Sandra(1,2); Rivero, Guadalupe(2,3); Fuselli, Sandra R.(1,5)

1) Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM). CONICET-UNMdP. Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina.
2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos aires, Argentina.
3) Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA), Mar del Plata, Argentina.
4) Fares Taie Biotecnología, Mar del Plata, Argentina.
5) Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Buenos Aires, Argentina.

In recent years, natural compounds have been investigated for controlling beekeeping pathologies due to the presence of residues and the generation of resistance by synthetic compounds. Abundant sources of bioactive compounds are agro-industrial waste, which through the implementation of a circular bio-economy are given added value. An example of waste is the hops leaves (*Humulus lupulus*), whose flower is the important product for the brewing industry, so after its collection, the leaves generate a large amount of organic matter that is harmful to the environment.

Previous studies by our research group found that hydroalcoholic extracts from hop leaves have good antimicrobial activity against *Paenibacillus larvae* and they are not toxic to bees.

Electrospinning techniques are currently used for the preparation of nanofibers capable of immobilizing different types of active agents, which can be gradually released. In short, a high electric potential is applied to polymer-based solutions infused through a nozzle with a controlled rate, so that submicrometric fibers can be collected. The objective of this work is: 1) to prepare polymeric nanofibers containing hydroalcoholic extracts of hop leaves via electrospinning process, 2) to characterize the morphology, physicochemical and thermal properties by various techniques, 3) to analyze the antimicrobial activity against *P. larvae* and 4) to evaluate their toxicity to bees when consumed. Eudragit L100-55 was chosen as polymer matrix, given its selective dissolution at pH>5.5. The formation of uniform submicrometric fibers was corroborated by scanning electron microscopy (SEM) while the presence of carbonyl groups corresponding to the extracts was observed in the nanofibrous membranes by means of Fourier Transform Infrared Spectroscopy (FTIR-ATR). Regarding the antimicrobial activity against *P. larvae*, the growth of the strains was inhibited once the membrane was dissolved. Finally, both the loaded and control matrices showed no toxicity to bees when consumed.

These results are a potential new application of natural compounds in the hive that could provide antimicrobial activity without generating toxic residues in the commercial products.

Keywords: Nanofibers, Hop extract, American Foulbrood.

STUDIES ON CHRONIC BEE PARALYSIS: AN EMERGING DISEASE OF THE HONEY BEE

Budge, Giles, School of Natural and Environmental Sciences, Newcastle University, Newcastle, United Kingdom
Holder, Philippa, School of Natural and Environmental Sciences, Newcastle University, Newcastle, United Kingdom
Simcock, Nicola, School of Natural and Environmental Sciences, Newcastle University, Newcastle, United Kingdom
Woodford, Luke, School of Biology, University of St Andrews, St Andrews, United Kingdom
Evans, David, School of Biology, University of St Andrews, St Andrews, United Kingdom
Rushton, Steve, School of Natural and Environmental Sciences, Newcastle University, Newcastle, United Kingdom

Chronic bee paralysis is caused by an unusual and unclassified RNA virus known as chronic bee paralysis virus (CBPV). Individual honey bees suffering disease show characteristic shivering and shaking symptoms followed by paralysis and death. Colonies appear to transition very rapidly from being apparently healthy, to presenting with piles of thousands of dead bees outside the colony. We established field trials in apiaries with a history of chronic bee paralysis, and monitored colony symptoms fortnightly from March until July. We used quantitative real-time PCR to determine when and where the virus was first associated with any affected colonies. Our results indicate that the virus was killing honey bees from most colonies more than two months before any colony level symptoms were seen. Our data also indicate an ascertainment problem for beekeepers which means the early signs of the disease are missed. Overall our findings provide new evidence to inform our epidemiological understanding of this damaging and emerging disease.

Keywords: Chronic bee paralysis, CBPV, Disease

INTERACTIONS BETWEEN PREVALENT PATHOGENS OF HONEYBEES (*APIS MELLIFERA*)

Jabal-Uriel, Clara, CIAPA-IRIAF, Marchamalo, Spain
Bonjour-Dalmon, Anne, Unité de Recherche Abeilles et Environnement, INRAE, Avignon, France
Chejanovsky, Nor, Department of Entomology, ARO, The Volcani Institute, Rishon LeZion, Israel
Higes, Mariano, CIAPA-IRIAF, Marchamalo, Spain
Le Conte, Yves, INRAE, Unité de Recherche Abeilles et Environnement, Avignon, France
Lopes, Ana R., CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Pinto, M. Alice, CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Reyes-Carreño, Maritza-Regina, INRAE, Unité de Recherche Abeilles et Environnement, Avignon, France
Soroker, Victoria, Department of Entomology, ARO, The Volcani Institute, Rishon LeZion, Israel
Martín-Hernández, Raquel, INCRECYT-FSE/EC-ESF, CIAPA-IRIAF, Marchamalo, Spain

The aim of the BEEHEAL project was to determine the phenology and interactions of the microsporidia *Nosema ceranae* and 5 highly prevalent and pathogenic honeybee viruses (DWV, BQCV, ABPV, IAPV and CBPV) in four Mediterranean countries: France, Israel, Portugal and Spain. The prevalence and phenology of these pathogens was monitored in four continental apiaries (one in each country) and three insular apiaries located on Ouessant Island (France) and on São Miguel and Santa Maria Islands (Portugal). Colonies were sampled during a 2-year study period, and honeybees were collected and analysed once every two months. Microsporidia detection was performed by conventional triplex-PCR whereas viral loads were determined by RT-qPCR of the positive samples. Israel had the highest percentage of honeybees infected by *N. ceranae*, followed by Spain, continental Portugal, and Ouessant, whereas the levels were the lowest in France mainland and it was not detected on the Portuguese Islands. The countries with the higher prevalence of viruses were France and Israel whereas the apiaries in Portugal and Spain had a lower percentage of colonies and individual honeybees infected by viruses. Moreover, CBPV was not detected in the latter countries. The viral load of colonies and honeybees coinfected with two or more viruses was positively correlated. Data analysed from all apiaries together showed a negative correlation between the percentage of infected *N. ceranae* honeybees and the viral load. However, there was no correlation at the apiary level between *N. ceranae* and the viral load when analysed separately. Cross-tabulations showed a correlation in coinfection between *N. ceranae* with any of the three viruses: BQCV, IAPV and CBPV. In fact, honeybees coinfected by the microsporidia and one of these viruses had a higher viral load than honeybees infected only with the virus. This study showed that pathogen interactions differs greatly among countries and serves as a starting point for the development of epidemiological studies to unravel pathogen coinfections.

Keywords: viral load, microsporidia, coinfection

FORMULATIONS OF OXALIC ACID AGAINST *VARROA DESTRUCTOR* (DOSAGE, EFFICACY, DECOMPOSITION, RESIDUES)

Kanelis Dimitrios, Tananaki Chrysoula, Floudas Aggelos, Liolios Vasilios, Rodopoulou Maria-Anna
Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki, Thermi, Thessaloniki, Greece

The control of varroa (*Varroa destructor*) is one of the most crucial problems that beekeepers have to deal with. Usually, they apply on their bee colonies treatments including chemicals or natural substances such as organic acids. One of the most commonly used organic acids is the oxalic, which is applied through various formulations. In the present study, we evaluated the efficacy of the oxalic acid – glycerin strips method through time and identified the optimum concentration of oxalic acid in brood presence. According to the results, the optimum concentration of oxalic acid was 21% in water-glycerol solution (56.5: 43.5, v/v), applied to absorbent strips (60g oxalic acid, 100 mL H_2O, 130 mL glycerin, for 13 strips). The efficacy of this concentration was found more than 90% over a period of four years applications. In addition, the glycerin strip method was compared to others (using synthetic and organic acaricides) commonly used by beekeepers, such as vaporization and fumigation of oxalic acid. Although the vaporization showed good results against varroa, it can sometimes have negative effects on the bees, such as the loss of queens, while the chemical control led to reduced efficacy during the four years (lower than 80%). Moreover, the possible residuality of oxalic acid in honey was studied using high performance liquid chromatography (HPLC-DAD). The concentration of both oxalic acid and formic acid in honey samples was not affected by the presence of strips in the hives. Finally, it was observed that the decomposition of oxalic acid during the preparation of the strips is intense when the solution is heated to a high temperature. During the heating, apart from the decomposition of oxalic acid, there was also a simultaneous formation of formic acid and an unknown compound, which could not be identified by the present method of analysis.

Keywords: Oxalic acid, glycerin strips, decomposition, residues

CRUDE EXTRACTS OF THE FUNGUS *TALAROMYCES* SP. EXHIBIT ANTIVIRAL ACTIVITY AND IMPROVE THE SURVIVAL OF CHRONIC BEE PARALYSIS VIRUS INFECTED HONEY BEES

Vocadlova, Katerina, Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany
Lamp, Benjamin, Institute of Virology, Justus-Liebig-University, Giessen, Germany
Lee, Kwang-Zin, Pests and Vector Insect Control, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany
Vilcinskas, Andreas, Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany
Institute for Insect Biotechnology, Justus Liebig University, Giessen, Germany

Viruses are one of the main causes of honeybee colony declines worldwide. To conserve the pollination service in agricultural and natural ecosystems provided by bees, the approaches to control the spread of viruses and the possible antiviral treatments have been intensively studied. Several compounds of plant and fungal origin have been already demonstrated to exhibit an antiviral effect against honeybee viruses. In the present study, we focused on filamentous fungi colonizing the honeybee hive as a possible source of compounds affecting the viral infection in honeybees. We examined the effect of dietary addition of fungal extracts from seven *Talaromyces* sp. strains found in the hive-stored pollen on the CBPV infection in adult honeybees in laboratory conditions. The antiviral effect of the organic fungal extracts was subsequently examined *in vitro* using a feline cell culture model.

The addition of three of the tested crude extracts (strains B13, B18 and B30) into the diet ameliorated the viral infection by improving the survival of honeybees. The other tested extracts had either no (B11 and B49) or negative (B69 and B195) effect. In the cell culture model, one of the tested extracts (B18) inhibited the viral replication of FCV and FCoV and two extracts (B18 and B195) reduced the FCoV viral particles by about 90 and 99% respectively. The harmful effect of the extract B195 in bees might be caused by the presence of rubratoxins that can exhibit insecticidal activity. The effect of the extracts on the viral loads in honeybees and the chemical composition of the extracts have yet to be analyzed.

Our study implies some strains of *Talaromyces* sp. from honeybee food stores can be a source of compounds that ameliorate the viral infection and/or have an antiviral activity against. We assume the non-pathogenic fungi and their products in the food stores can be yet understudied factor affecting the survival of virus infected honeybees.

Keywords: *Apis mellifera*, filamentous fungi, antiviral activity

COULD BUTTERFLIES SPREAD BEE VIRUSES?

Pislak Ocepek, Metka; Institute of Pathology, Wild Animals, Fish and Bees; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Glavan, Gordana; Department of Biology; University of Ljubljana, Biotechnical Faculty; Ljubljana, Slovenia
Verovnik, Rudi; Department of Biology; University of Ljubljana, Biotechnical Faculty; Ljubljana, Slovenia
Šimenc, Laura; Institute of Microbiology and Parasitology; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Toplak, Ivan; Institute of Microbiology and Parasitology; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia

It is generally recognized that populations of pollinators are declining worldwide. Various disease-causing organisms are important factor in the loss of honeybee colonies, and some of these pathogens can be transmitted between different pollinator taxa of Hymenoptera. In our study, the possible transmission of bee viruses to butterflies (Lepidoptera, Papilionoidea) was investigated. A total of 120 butterflies and 40 honeybees were sampled on flowering plants at four different sites in Slovenia. In the laboratory, 10 specimens of the same family collected on the same day and at the same location were pooled, resulting in a total 12 pooled samples of butterflies and four pooled samples of bees. RNA detection of six bee viruses: acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), sacbrood bee virus (SBV) and Lake Sinai virus 3 (LSV3) was determined using a specific quantitative real-time RT-PCR assay.

Only four (33.3%) butterfly samples were completely negative for bee viruses. Two samples (16.7%) were positive for ABPV and LSV3, five samples (41.7%) were positive for BQCV, and seven samples (58.3%) were positive for SBV. No butterfly samples were positive for CBPV and DWV. For comparison, all bee samples were negative for CBPV, two were positive for DWV, and four bee samples were positive for ABPV, BQCV, LSV3, and SBV. However, when comparing Ct values between butterfly and bee samples, a much lower RNA copy number was observed in butterflies compared to positive bee samples. Therefore, we believe that bee viruses do not replicate in butterflies but were detected on their bodies as a result of visiting contaminated flowers. This is emphasised by the lack of positive butterfly samples for viruses, that were negative in bee samples, making infected bees a necessary condition for the presence of viruses in cohabiting butterflies. Given the low viral load, butterflies do not pose a threat to bees as potential vectors of pathogens. This is the first study on the presence of bee viruses in butterflies. Therefore, further studies are needed to determine the mode of pathogen transmission among these pollinators.

Keywords: bee viruses, Lepidoptera, pathogen transmission

DIAGNOSTIC VALUE OF THE MOLECULAR DETECTION AND QUANTIFICATION OF *PAENIBACILLUS LARVAE* SPORES IN HIVE DEBRIS SAMPLES

Pislak Ocepek, Metka; Institute of Pathology, Wild Animals, Fish and Bees; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Žvokelj Lucija; Institute of Pathology, Wild Animals, Fish and Bees; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Hočevar Barbara; Institute of Pathology, Wild Animals, Fish and Bees; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Rus Rene; Institute of Pathology, Wild Animals, Fish and Bees; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Kozar Monika; Institute of Pathology, Wild Animals, Fish and Bees; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Zajc Urška; Institute of Microbiology and Parasitology; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Kušar Darja; Institute of Microbiology and Parasitology; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia
Papić Bojan; Institute of Microbiology and Parasitology; University of Ljubljana, Veterinary Faculty; Ljubljana, Slovenia

American foulbrood (AFB) is one of the most severe brood diseases of honeybees. In Slovenia, several AFB outbreaks are reported every year. Long-term experience shows that the success of AFB control is compromised by late reporting of the first suspicion of the disease or its unsuccessful control by beekeepers without veterinary supervision. As a result, elimination of AFB outbreaks is time consuming and has significant economic impact. For early detection of potential AFB outbreaks and prevention of AFB clinical symptoms, we initiated a pilot project in which hive debris samples were subjected to molecular detection and quantification of *Paenibacillus larvae* spores.

Samples were collected from 400 apiaries during the winter and spring of 2022; beekeeper participation was voluntary. Half of the colonies from each apiary were sampled by pooling every 10 samples and analysing them using a previously described quantitative PCR assay with detection and quantification limits of 188 and 707 spores, respectively. Based on our previous experience, samples were then divided into four categories (A–D). Category A includes negative samples and samples with a log spore count <4.3. Apiaries in this category are unlikely to have disease outbreak in the near future. Samples with a log spore count between 4.3 and 5.6 were classified as category B – apiaries with an elevated spore count but no significant probability of disease outbreak in the near future. Samples with a log spore count between 5.6 and 7.3 were classified as category C - significantly elevated spore count with a probability of disease outbreak in the near future. Samples with a log spore count >7.3 were classified as category D – apiaries with a high probability that AFB clinical symptoms are already present. Up to 4% of apiaries tested were classified in this category. Detection and quantification of P. larvae spores in hive debris is the first step

in combination with good beekeeping practices toward preventive measures to reduce clinical outbreaks. The results were obtained within the Action programme in Beekeeping in the Republic of Slovenia for 2020-2022, funded by the state and the European Union.

Keywords: American foulbrood, hive debris, qPCR

INVESTIGATING THE VARIATION IN SUSCEPTIBILITY OF HONEY BEES TO CHRONIC BEE PARALYSIS VIRUS

Commandeur, Theodora, School of Natural and Environmental Sciences, Newcastle University, Newcastle, UK
Simcock, Nicola, School of Natural and Environmental Sciences, Newcastle University, Newcastle, UK
Rushton, Steve, School of Natural and Environmental Sciences, Newcastle University, Newcastle, UK
Budge, Giles, School of Natural and Environmental Sciences, Newcastle University, Newcastle, UK

Chronic bee paralysis is an emerging viral bee disease affecting honey bee (*Apis mellifera*) colonies globally. Until recently, it's effects were rare but severe, often leading to colony collapse. However, case numbers are increasing and in order to prevent further spread it is vital to understand transmission. A number of factors mediate viral susceptibility, but here we present the first study to investigate age and gut microbiota following oral exposure to chronic bee paralysis virus. Interestingly we found that age did play a role, with younger bees proving more susceptible to symptomatic infection. Additionally, DNA sequencing was used to identify the gut microbiota present in symptomatic and asymptomatic cohorts. These data, along with symptom progression and honey bee longevity were examined using statistical modelling techniques, providing a clearer picture for the infection and transmission of the chronic bee paralysis virus.

Keywords: virus, susceptibility, microbiota

DNA VIRUSES OF HONEY BEE

Kadlečková, Dominika, Department of Genetics and Microbiology, Faculty of Science Charles University, BIOCEV, Vestec, Czech Republic
Tachezy, Ruth, Department of Genetics and Microbiology, Faculty of Science Charles University, BIOCEV, Vestec, Czech Republic
Saláková, Martina, Department of Genetics and Microbiology, Faculty of Science Charles University, BIOCEV, Vestec, Czech Republic
Erban, Tomáš, Crop Research Institute, Prague, Czech Republic

Number of viruses have been identified to host honey bees; however, research focuses mainly on few highly pathogenic viruses manifesting visible clinical signs (e.g., Deformed wing virus). With the onset of next generation sequencing (NGS) methods many new viruses of bees were discovered. The virome of honey bees which consist of viruses of bees, plants, other eukaryotes, and bacteria is now explored in its complexity. One of the methodological shortcomings of virome studies in bees is the focus on RNA viruses. It is therefore not surprising that while number of RNA viruses were discovered, research reporting DNA viruses in bees is scarce. *Apis mellifera* filamentous virus (AmFV) is the only DNA virus described in honey bees and it is highly unusual in comparison to other insects that the virome consist of almost RNA viruses.

Therefore, we searched the NGS data of viromes (Sequence Read Archive, our data) that included DNA in preparation of samples. In the first step we tried to map our latest data against known insect infecting viruses (over 250 unique viruses from NCBI, wide variety of insects) without discovering any new virus. Further, we examined classification of contigs from our and additional studies. This investigation enabled us to identify nine unique and distinct complete *Parvoviruses* belonging to *Densovirinae*. These viruses are historically known to infect insects but have not been described in bees until present. One of the studies that we included in our analysis was composed from libraries made from many individual bees. Analysis of these sequences revealed low prevalence of those viruses. Apart from *Densovirinae* we found number of long contigs classified as different species of *Nudiviridae* family but with low amino-acid identity (+-40%) to different insect infecting species of *Nudiviridae*. They have a large genome which is difficult to sequence completely with standard NGS technique. Therefore, we will additionally examine publicly available sequences of DNA libraries to try to gain complete genome of this new viruses of bees. Results could help to determine prevalence of the newly discovered DNA viruses and their biological significance.

Keywords: Honey bee viruses, DNA viruses, *Parvoviridae*

This study was supported by CELSA [Metagenomic Analysis of Honey Bee (Apis mellifera) in the Czech Republic, 2017 to 2019], grant number QK1910018 of the Ministry of Agriculture of the Czech Republic (http://www.eagri.cz), and the MICOBION project funded from EU H2020 (number 810224).

RELIABILITY OF MORPHOLOGICAL AND PCR METHODS FOR THE OFFICIAL DIAGNOSIS OF *AETHINA TUMIDA* (COLEOPTERA: NITIDULIDAE): A EUROPEAN INTER-LABORATORY COMPARISON

Franco, Stéphanie, Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France
Cougoule, Nicolas, Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France
Tison, Amandine, Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France
Del Cont, Aurélie, Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France
Gastaldi, Cristina, Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France
ILC Consortium, European Reference Laboratory for Bee Health, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France
Duquesne, Véronique, Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis, France

The Small Hive Beetle (*Aethina tumida* Murray, 1867) is an invasive scavenger of honeybees. Originally endemic in sub-Saharan Africa, it is regulated internationally in order to preserve the areas still free from this species. To ensure the reliability of official diagnoses, the European Reference Laboratory for Bee Health organised an inter-laboratory comparison on the identification of *A. tumida* by morphology and real-time PCR, two types of methods used routinely to confirm outbreaks.

Twenty-two National Reference Laboratories in Europe participated in the study and analysed 12 samples with adult coleopterans and insect larvae. The performance of the laboratories was evaluated in terms of sensitivity and specificity. Sensitivity was satisfactory for all the participants and both types of methods, thus fully meeting the diagnostic challenge of confirming all truly positive cases as positive. Two participants encountered specificity problems. For one, the anomaly was minor whereas, for the other, the issues concerned a larger number of results, especially real-time PCR, which probably were related to inexperience with this technique.

The present comparison is, to our knowledge, the first organised at the international level on the official diagnosis of *A. tumida*. It demonstrated the reliability of official diagnosis, including the entire analytical process of *A. tumida* identification: from the first step of the analysis to the expression of opinions. The performed diagnostic tools, in parallel with field surveillance, are essential to managing *A. tumida* introduction in countries where its presence has not yet been detected and where early detection is crucial.

Keywords: diagnosis; inter-laboratory comparison; *Aethina tumida*

A SURVEY OF IMPORTANT HONEY BEE VIRUSES IN THE AZORES ARCHIPELAGO

Lopes, Ana R., Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus Sta Apolónia, 5300-253, Bragança, Portugal
de Miranda, Joachim, Ekologicentrum, Swedish University of Agricultural Sciences, Uppsala, Sweden
Martín-Hernández, Raquel, Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla La Mancha, CIAPA de Marchamalo, Guadalajara, Spain; Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FSE/EC-ESF), Fundación Parque
Científico y Tecnológico de Castilla—La Mancha, 02006 Albacete, Spain
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus Sta Apolónia, 5300-253, Bragança, Portugal

The Azores archipelago is an interesting place to study honey bee viruses and their associations with *Varroa destructor*, given that there are islands with (Pico, Flores, and Faial) and without the mite. With the goal of establishing a baseline for future viruses studies, here we sampled 494 colonies distributed across eight islands, in the summers of 2014/2015 and 2020. These samples were screened for the most important honey bee viruses (BQCV, CBPV, SBV, BeeMLV, and the LSV-, DWV-, and AKI-complexes) and the viral loads were determined by RT-qPCR for the positive samples. Of the seven viruses, only BeeMLV and the AKI-complex were not detected. The BQCV was found on all the islands with a high prevalence (>79%) with a wide range of viral loads. São Miguel showed the lowest median BQCV loads ($3.18x10^2$copies/µL) and Terceira the highest ($6.19x10^3$copies/µL). In the 2020 sampling, the BQCV loads showed a significant increase on São Jorge and Santa Maria. The LSV was also found on all the islands. Notably, the LSV prevalence increased significantly in 2020, but not the viral loads. Only Faial and Pico (both with varroa) had SBV, but there was no statistical difference in viral loads between the two islands. CBPV was detected on Pico, São Miguel, Graciosa, Terceira, and Faial, but only in a few colonies (5.3%), although with high viral loads. DWV was never found on São Jorge and Terceira in either sampling period. With the exception of Faial, DWV prevalence was higher on the islands with varroa (Pico and Flores) than on the varroa-free islands. The viral loads were not statistically different between islands with and without varroa for any of the viruses, suggesting that varroa is not the only factor contributing to infection. This study suggests that, in addition to varroa- and *Nosema ceranae*-free honey bee populations, some of the islands seem to harbor populations that are also free of some important viruses like DWV, CBPV, SBV, BeeMLV and AKI-complex.

Keywords: viruses, real-time qPCR, prevalence

SPATIO-TEMPORAL PATTERNS OF NOSEMA CERANAE IN THE AZORES ARCHIPELAGO

Lopes, Ana Rita, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
Martín-Hernández, Raquel, Laboratorio de Patología Apícola, IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Camino de San Martín, 19180 Marchamalo, Spain; Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FSE/EC-ESF), Fundación Parque Científico y Tecnológico de Castilla—La Mancha, 02006 Albacete, Spain
Higes, Mariano, Laboratorio de Patología Apícola, IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Camino de San Martín, 19180 Marchamalo, Spain
Henriques, Dora, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Nosema ceranae is a highly prevalent pathogen of *Apis mellifera*, which is distributed worldwide. However, there may still exist geographically isolated areas that remain free of this pathogen. While Nosema spp. spores have been identified in samples from the Azores archipelago since early 2000's, whether those spores matched *N. apis* or *N. ceranae* was unknown until this study. To address this question, we used molecular tools (multiplex PCR and real-time qPCR) to scrutinize 474 colonies sampled from eight islands in 2014/2015 and 91 from four islands in 2020. The findings revealed that *N. ceranae* was not only present but also the dominant species in the Azores. In 2014/2015, *N. apis* was rare and *N. ceranae* prevalence varied between 2.7% in São Jorge and 50.7% in Pico. In 2020, *N. ceranae* prevalence increased significantly ($p < 0.001$) in Terceira and São Jorge, which also showed higher infection levels. The spatio-temporal patterns suggest that *N. ceranae* colonised the archipelago recently, and it rapidly spread across other islands, where at least two independent introductions might have occurred. Flores and Santa Maria have escaped the *N. ceranae* invasion, and it is remarkable that Santa Maria is also free of *Varroa destructor*, which makes it one of the last places in Europe where the honey bee remains naive to these two major biotic stressors.

Keywords: *Nosema ceranae*, prevalence, Azorean honey bees

DYNAMICS IN VERTICAL TRANSMISSION OF VIRUSES IN NATURALLY SELECTED AND TRADITIONALLY MANAGED HONEY BEE COLONIES ACROSS EUROPE

Claeys Bouuaert, David; De Smet, Lina; Brunain, Marleen; de Graaf, Dirk C. - Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
Dahle, Bjørn; Oddie, Melissa Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway
Blacquière, Tjeerd; Panziera, Delphine - Bees@wur, Wageningen University & Research, Wageningen, The Netherlands
Dalmon, Anne - Abeilles et Environnement, INRAE, Avignon, France
Dezmirean, Daniel; Giurgiu, Alexandru - Department of Apiculture and Sericulture, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
Elen, Dylan - Department of Molecular Ecology & Evolution, School of Natural Sciences, Bangor University, Bangor, United Kingdom
Filipi, Janja - Department of Ecology, Agronomy and Aquaculture, University of Zadar, Zadar, Croatia
Gregorc, Aleš - Faculty of Agriculture and Life Sciences, University of Maribor, Pivola, Slovenia
Kefuss, John - Le Rucher D'Oc, Toulouse, France
Locke, Barbara; de Miranda, Joachim - Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
Parejo, Melanie - Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain
Pinto, M. Alice - Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

The ‘suppressed *in-ovo* virus infection’ trait (SOV) was the first trait applied in honey bee breeding programs aimed to increase resilience to virus infections, a major threat for colony survival. By screening drone eggs for viruses, the SOV trait scores the antiviral resistance of queens and its implications for vertical transmission. In this study, queens from both naturally surviving and traditionally managed colonies from across Europe were screened using a two-fold improved SOV phenotyping protocol. First, a gel-based RT-PCR was replaced by a RT-qPCR. This not only allowed quantification of the infection load but also increased the test sensitivity. Second, a genotype specific primer set was replaced by a primer set that covered all known deformed wing virus (DWV) genotypes, which resulted in higher virus loads and fewer false negative results. It was demonstrated that incidences of vertical transmission of DWV were more frequent in naturally surviving populations than in traditionally managed colonies, although the virus load in the eggs remained the same. Dynamics in vertical transmission were further emphasized when comparing virus infections with queen age. Interestingly, older queens showed significantly lower infection loads of DWV in both traditionally managed and naturally surviving colonies, as well as

reduced DWV infection frequencies in traditionally managed colonies when compared with younger queens. Seasonal variation in vertical transmission was found with lower infection frequencies in spring compared to summer for DWV and black queen cell virus. Together, these patterns in vertical transmission suggest an adaptive antiviral response of queens aimed at reducing vertical transmission over time.

DEVELOPING AN AFFORDABLE WHOLE GENOME SEQUENCING PIPELINE TO STUDY OUTBREAKS OF EUROPEAN FOULBROOD

Pufal, Hollie, School of Natural and Environmental Sciences, Newcastle University, UK
Tomkies, Victoria, Bee health laboratory, Fera Science Ltd, UK
Rushton, Steve, School of Natural and Environmental Sciences, Newcastle University, UK
Budge, Giles, School of Natural and Environmental Sciences, Newcastle University, UK

European foulbrood (EFB), caused by *Melissococcus plutonius*, is found on every continent where honey bees are kept. In the United Kingdom, EFB is subject to statutory controls. Multi-locus sequence typing (MLST) is a method that is used to map and monitor different strains of EFB at outbreak sites by comparing the DNA sequences of housekeeping genes. MLST results are valuable but the genetic information is limited to only four genes, whereas whole genome sequencing would provide more genetic information allowing a deeper understanding of national transmission events. We have developed a low-cost DNA extraction pipeline that uses Oxford Nanopore technologies to generate whole genome sequence information from each UK outbreak in 2020. Costs of the new pipeline are similar to those for MLST without the need for investment in expensive equipment, making our method accessible. Here we share some insights into the results.

Keywords: EFB, MLST, Sequencing

GENETIC STRUCTURE OF *VARROA DESTRUCTOR* POPULATIONS WITH DIFFERENT PYRETHROID RESISTANCE

Muñoz, Irene[1]; Millán-Leiva, Anabel[2]; Marín, Oscar[2]; Christmon, Krisztina[3]; vanEngelsdorp, Dennis[3]; González-Cabrera, Joel[2]; De la Rúa, Pilar[1]

1 Department of Zoology and Physical Anthropology, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain.
2 Institute BIOTECMED, Department of Genetics, Universitat de València, Valencia, Spain.
3 Department of Entomology, University of Maryland, College Park, MD 20742, USA.

The specialized obligate ectoparasite *Varroa destructor* is one of the major threats of *Apis mellifera*, as it induces immunosuppression, transmits viruses thus compromising the viability of honey bee colonies. Currently, regular control of the mite with acaricides is necessary to prevent colony losses. Pyrethroid acaricides like tau-fluvalinate and flumethrin have been used extensively for varroa control for many generations, leading to the evolution of resistance in many mite populations worldwide. In varroa, the most common mechanism involved in the loss of sensitivity to pyrethroids is the substitution of certain amino acids in their target protein, the voltage-gated sodium channel (VGSC), often referred to as *kdr*-type mutations.

In the present work, we have analyzed the genetic diversity and structure of *V. destructor* in populations from Europe and from the USA using microsatellite markers and characterized its susceptibility to pyrethroids by determining the presence of *kdr*-type mutations at position 925 of the VGSC. Our molecular analysis supports the hypothesis of genetically different varroa populations in Europe and the USA, which is also coincident with the different distribution of pyrethroid resistant alleles detected in previous studies. The results of this study also show genetic introgression events corroborating that the distribution of *kdr*-type mutations at position 925 of the VGSC is a consequence of unintentional anthropogenic movements of mites around the world when moving hives or honey bee packages without control of their parasitism.

This work can help to design varroa control methods that incorporate quarantine strategies and to avoid the emergence of resistant populations of *V. destructor* populations.

Keywords: Population genetics, *Varroa destructor*, Pesticide resistance

MOLECULAR GENOTYPING OF *PAENIBACILLUS LARVAE* STRAINS FROM FRANCE

LOUISE, PETIT, Bacteriology, Bee Pathology UNIT, ANSES (French Agency for Food, Environmental and Occupational Health & Safety), Sophia Antipolis, France
JEAN-CHARLES, THOMARAT, Bacteriology, Bee Pathology UNIT, ANSES (French Agency for Food, Environmental and Occupational Health & Safety), Sophia Antipolis, France
KARIM, SIDI-BOUMEDINE, Bacteriology, Bee Pathology UNIT, ANSES (French Agency for Food, Environmental and Occupational Health & Safety), Sophia Antipolis, France

The spore forming bacterium *Paenibacillus larvae* is responsible for American foulbrood, a severe infectious disease of honey bees (*Apis mellifera* and *Apis cerana*) which affects the brood and leads to the weakening and mortality of colonies in the most serious cases. The disease is well known to induce economic and ecological impacts, to be endemic in many regions of the world and to have limited possibilities of prophylaxis.

Currently, in France, routine clinical diagnosis is based solely on the detection of *P. larvae* in the brood samples. It has been shown that the severity of the infection can vary between strains of *P. larvae*. Indeed, the strains of *P. larvae* causing a slow death of the larvae present a genotype different from those inducing a quicker mortality of the larvae. However, data regarding the genotypes present in France are scarce or missing.

The aim of this study was to produce data and knowledge about the genotypes of *P. larvae* from a collection of strains isolated in France.

Briefly, genomic DNA was extracted from BHI based cultures of *P. larvae* isolated from different regions of France. Firstly, genomic characterisation and comparisons were performed using ERIC-PCR, MLST and MLVA analyses. Secondly, the discriminatory power of the three genotyping methods was evaluated.

This study allowed the identification of *Paenibacillus larvae* genotypes circulating in France from a short panel of our strain's collection. This work should be continued to obtain more data and it could facilitate the survey of the spread of a given isolate within and between apiaries.

Keywords: *Paenibacillus larvae*, American foulbrood, Genotyping

ANTIMICROBIAL DISK SUSCEPTIBILITY TESTING OF *PAENIBACILLUS LARVAE* ISOLATES FROM BOSNIA AND HERZEGOVINA FOR ANTIMICROBIAL RESISTANCE STATUS UNDER ONE HEALTH APPROACH

Santrac Violeta, Rujevic Dragana, Mitrovic Aleksandra,

PI Veterinary Institute of the Republic of Srpska „Dr Vaso Butozan Banja Luka,
Bosnia and Herzegovina

American foulbrood is an infectious bacterial disease of *Apis mellifera* brood that is enzootic on the territory of Bosnia Herzegovina for many decades in past. The disease is officially under the control of veterinary service and applicable measures are based on a strategy for testing and removing positive hives. American foulbrood is a prevalent notifiable disease and based on the legislation there are no registered antibiotics to cure the disease and a metaphylactic approach is not allowed.

In the work, we examine 76 randomly selected field isolates, frozen samples of *Paenibacillus larvae*, collected during the 6 years and check their sensitivity against 7 different antibiotics. Awareness was about finding signs of antimicrobial resistance risk. Research came as an additional option for verifying disease control indirectly to see the connection between antibiosis and possible antimicrobial resistance under beekeeping and veterinary management. The laboratory method was designed as disk susceptibility testing in vitro, based on requirements given in CSLI VET01 A4.

We found that average isolate sensitivity in millimeters was for: Oxytetracycline 30,2±6,3; Tylosin 30,5±6,6; Lincomycin 26,6±10; Streptomycin 24,5±8,2; Trimethoprim/sulfamethoxazole 27,4 ±10,6; Chloramphenicol 32,7±6,3; Nitrofurantoin 25,6±9,6;

Based on the given results we did not find empirical evidence of risky resistance among local *Paenibacillus larvae* isolates. Bacterial spores that can be found in local honey can’t be a risk for human health concerning the One Health approach, that was implemented through beekeeping and veterinary practice.

Keywords: *Paenibacillus larvae*, antimicrobial resistance, Bosnia and Herzegovina

BEE PATHOGEN PREVALENCE ACROSS A GRADIENT OF PLANT DIVERSITY

Buydens, Louella, Department of Plants and Crops, Ghent University, Ghent, Belgium
Meeus, Ivan, Department of Plants and Crops, Ghent University, Ghent, Belgium
Piot, Niels, Department of Plants and Crops, Ghent University, Ghent, Belgium
Smagghe, Guy, Department of Plants and Crops, Ghent University, Ghent, Belgium

Bee pathogens are naturally occurring within populations, but the existing equilibrium may be disrupted due to different drivers of bee decline, such as reduced forage availability and diversity. Reduced plant diversity may reshape pathogen transmission routes, since multi-host pathogens can be transmitted between bees via the shared use of flowers through external vectoring or an oral-fecal transmission route. Furthermore, reduced forage opportunities may weaken the tolerance and resistance mechanisms of bees against pathogens. Both altered transmission and immunity may change the pathogen dynamics within and between bee hosts, which could result in pathogens becoming another driver of wild bee decline.

In this exploratory study which is also part of NutriB^2 (an EU-BiodivERsA research project), the link between plant diversity and pathogen prevalence in wild bees is investigated. Wild bees were sampled during May and June 2020 from 9 different locations in Belgium and 42 locations in Germany along a gradient of plant diversity. The RNA of specimens of the solitary bee species *Andrena haemorrhoa*, the primitively eusocial species *Lasioglossum pauxillum* and several bumble bee species was extracted and screened with qPCR for the presence of viruses as well as unicellular pathogens. Furthermore, features of the observed plant-bee networks were compared across the plant diversity gradient in order to investigate possible bee pathogen transmission networks.

Keywords: Pathogens, Plant-bee network, Diversity

STRIPS WITH OXALIC ACID AND GLYCERINE: IS IT A RELIABLE WAY OF *VARROA* TREATMENT?

Danihlík, Jiří; Pinďáková, Eliška; Dostálková, Silvie; Petřivalský, Marek, Department of Biochemistry, Faculty of Science, Palacký Univesity Olomouc, Olomouc, Czech Republic
Dobeš, Pavel; Hurychová, Jana; Hyršl, Pavel, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Varroa mites (*Varroa destructor*) are the most significant worldwide cause of honey bee colony losses. In affected countries, beekeepers have developed strategies to prevent and treat *Varroa*-infested colonies. Those strategies are based on regularly monitoring Varroa infestation and applying biotechnological approaches or chemical products.

Oxalic acid is a relatively cheap and available organic acid and is one of the many active substances present in several products used for *Varroa* treatment. It has been used for decades now in the form of trickling or sublimation.

Trickling and sublimation act as a ‘flash’ treatment with limited long-term activity on colonies, especially in the summer season (Central Europe). Recently, a new formulation of a long-term application of oxalic acid was developed. Many beekeepers started experimenting with cellulose strips impregnated with oxalic acid and glycerine solution. The results reported by amateur scientists indicate that beekeepers are mostly based on the statement ‘I am satisfied with the treatment’. However, the data from the field on efficacy and side effects are vague; thus, we decided to set up an experiment to measure this application’s efficacy and possible side effects on the honey bee organism.

The experiment was performed in Czechia on 12 honey bee colonies, divided into 4 groups: no treatment, oxalic acid trickling, oxalic acid-glycerol strips, and flumethrin (Gabon). We measured the efficacy of the treatment and several parameters related to oxidative damage, longevity, immunity, and the digestive system. The oxalic acid-glycerol strips showed low efficacy against *Varroa* mites. However, they stimulated activation of the immune system. We observed higher antimicrobial activity of hemolymph and increased production of antimicrobial peptide apidaecin after oxalic acid-glycerol strips application compared to the other groups. All experimental groups observed no effects on the antioxidative and digestion systems.

This research was funded by The Ministry of Agriculture of the Czech Republic grant QK1910286.

Keywords: Immunity, Integrated Pest Management, Organic Acids

BEE VIRUSES IN *VESPA VELUTINA*

Rodríguez-Flores, M. Shantal, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Diéguez-Antón, Ana, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Escuredo, Olga, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Pinto, Alice, CIMO - Centro de Investigação de Montanha, Polytechnic Institute of Bragança, Portugal
Felicioli, Antonio, Department of Veterinary Sciences, University of Pisa, Italy
Mazzei, Maurizio, Department of Veterinary Sciences, University of Pisa, Italy
Seijo-Coello, M. Carmen Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain

Globalization favors the prevalence and spread, of many species playing an important role in the global spread of pathogens. Vespidae species are among the most successful invasive animals and prove to be an environmental concern. In recent years, it has been shown that honey bee viruses can infect other hosts, such as the Vespidae species, causing a spillover effect in the associated ecosystems. When some of these species become established, they change pathogenicity balances and alter the predator-prey interaction, changing the defense strategies of the native host species. One of the most relentless examples of the spread of invasive vespid species in Europe is *Vespa velutina*. The arrival in 2004 had a strong impact in beekeeping sector causing significant hive losses. The interaction between this hornet and the honey bee is an increasingly demonstrated factor in the transmission of viruses, affecting the health and strength of the colony. In *V. velutina*, the presence of replicative forms of 10 bee virus with different variants has already been confirmed in several countries (Dalmon et al., 2019; Marzoli et al., 2021; Yang et al., 2020). This work compiles the types of viruses that have been detected in *V. velutina*, as a consequence of its interaction with honey bees, and provides new replicative forms in the northwest of the Iberian Peninsula. Thus, the replicative forms of DWV and LSV were identified for the first time in individuals of *V. velutina*. The impact of *V. velutina* in the propagation of these viruses could help to better understand the incidence of viruses mediated diseases in honey bee colonies in the infected areas and to be an instrument in the development of strategic management plans.

Keywords: *Vespa velutina*, Beekeeping, Bee virus

INVESTIGATION OF SOME VIRUS DYNAMICS IN LOCAL BREED COLONIES BASED ON "APIS MELLIFERA LIGUSTICA" QUEENS ORIGINATED FROM ITALY AND "APIS MELLIFERA ANATOLIACA" QUEENS ORIGINATED FROM TÜRKIYE, APIARY IN THE "EAST THRACE OF TÜRKIYE"

Dilek Muz, Department of Virology, Faculty of Veterinary Medicine, Tekirdag Namık Kemal University, Tekirdag, Türkiye
Nanetti, Antonio, Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Roma, Italy
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Martín-Hernández, Raquel, Centro de Investigación Apícola y Agroambiental de Marchamalo, Marchamalo, Spain
Mustafa Necati MUZ, Department of Parasitology, Faculty of Veterinary Medicine, Tekirdag Namık Kemal University, Tekirdag, Türkiye

Türkiye is among the most critical countries in the world where commercial migratory beekeeping is carried out on a professional scale. "Queens" is suitable for the subspecies of honey bees preferred in Türk style; migratory beekeeping should adapt very well to different temperatures, drought and cold, and different plant flora. Over the years, the "Queen" preferences of thoroughly professional migratory beekeepers have partially enabled different subspecies of honey bees to establish bee colonies that are more resistant to climate change in geographic regions. In this study, queens of *A.mellifera ligustica* and *A. mellifera anatoliaca* subspecies will be compared, each adapted to different geographical and climatic conditions originally in Italy and Türkiye. In the apiary established in a particular and isolated area with a diameter of 10 km, in newly adopted colonies, loads of some honeybee viruses will be tested according to the months. At the point of comparison, its adaptation degree and success in "new climatic conditions" will be investigated.DWV and BQCV dynamics will be monitored, frequently preferred in colony health monitoring. This research will take place within the scope of MEDIBEES, which uncovers the genetic background of Mediterranean honey bee subspecies and aims to identify and understand their adaptation to Mediterranean environmental conditions. The assessment of local Mediterranean subspecies, the impact of climatic changes, and the impact on pathogen load will be discussed.

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ORAL PRESENTATIONS

CHAIRPERSONS
Jevrosima Stevanović
Silvio Erler

NITRIC OXIDE IS A KEY SIGNALING MOLECULE FOR ANTIMICROBIAL PEPTIDE PRODUCTION IN *APIS MELLIFERA*

Janků, Martina; Danihlík, Jiří; Žvátorová Zuzana; Petřivalský, Marek, Department of Biochemistry, Faculty of Science, Palacký Univesity Olomouc, Olomouc, Czech Republic

Honey bees, like other insects, do not have a specific part of the immune system. However, it has an evolutionarily improved system of innate immunity, which is the basis of defensive reactions against pathogens. After pathogen recognition by the host cell, several biochemical signalling reactions occur, including nitric oxide (NO) production. In general, NO is an important signalling molecule that maintains cellular homeostasis and is a crucial host defence molecule. In the invertebrate immune system, NO is generally thought to regulate insect immune responses through signalling pathways leading to activation of antimicrobial peptide (AmP) production or by a direct cytotoxic effect tending to the killing of invading pathogens.

NO's ability to induce the expression of a gene encoding AmP, as an activator and integral component of the Imd signalling pathway, has been described in some insect species. In *Apis mellifera*, initial studies on the involvement of NO in immune responses have shown that the number of NO-producing granulocytes, a type of hemocytes responsible for NO production, increases in hemolymph in bees during injury and is also involved in hemocyte activation upon detection of foreign particles. Thus, NO appears to act as a critical molecule for immune activation of hemocytes and as a messenger for further signalling during immune responses.

In this study, we focused on the relationship between NO levels and AmP production after stimulation of the bee immune system. Newborn honey bees were fed with sucrose supplemented with modulators of NO levels for one week, then were immunised by lipopolysaccharide, and changes in NO and its metabolites and AmP levels were analysed. The obtained data show that NO levels also lead to changes in the produced AmP levels. Our pilot study thus provides initial insights into regulating the humoral component of bee immunity by a signal molecule such as nitric oxide.

Keywords: Nitric oxide, Antimicrobial peptide, Humoral immunity

HUMORAL IMMUNE RESPONSE TO SELECTED PORPHYRINS IN *APIS MELLIFERA* HONEYBEES

Trytek Mariusz and Buczek Katarzyna, Department of Industrial and Environmental Microbiology, Maria Curie-Skłodowska University, Lublin, Poland
Zdybicka-Barabas Agnieszka, Wojda Iwona and Cytryńska Małgorzata, Department of Immunobiology, Maria Curie-Skłodowska University, Lublin, Poland
Borsuk Grzegorz, Institute of Biological Basis of Animal Production, University of Life Sciences, Lublin, Poland
Lipke Agnieszka, Department of Inorganic Chemistry, Maria Curie-Skłodowska University, Lublin, Poland
Gryko Dorota, Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland

Nosema ceranae infection causes energetic stress and decreases bee health by affecting the immune system, including down-regulation of immunoregulatory genes and disturbances in the host amino acid metabolism. Development of strategies that can efficiently stimulate the honeybee immune system while simultaneously being harmless to insects and humans is of great importance. Porphyrins are considered one of the most powerful anti-Nosema agents acting directly against microsporidia; however, their potential effects on the *Apis mellifera* immune system have yet to be explored.

The present study aimed to investigate the effect of porphyrins on humoral immune response in both healthy and *Nosema*-infected honeybees in conjunction with the infection level and bee survival. The level of phenoloxidase (PO) activity in the hemolymph and the expression of immune-related genes encoding antimicrobial peptides (AMP), i.e. abaecin, defensin, and hymenoptaecin, were determined after 24- and 48-h treatment with two protoporphyrin IX derivatives conjugated with aspartate [PP(Asp)2] and lysine [PP(Lys)2] moieties. Both porphyrins administered to the honeybees at the 100 µM concentration decreased the spore loads significantly (by 74%) in the infected individuals on day 12 post-infection. It was shown that porphyrins penetrate into the hemolymph from the digestive system of the honeybees and have an impact on their immunity without adverse effects on the honeybee lifespan. The non-infected honeybees treated with porphyrins showed significantly [even 11.3-fold] higher PO activity in the hemolymph than the non-infected control bees; however, no increase in the expression of AMP genes was observed. Completely different profiles of PO activity were observed in the infected and healthy bees and, for each porphyrin, the highest PO activity was associated with a lower level of AMP gene transcripts and vice versa. Our findings indicate that the immune response to porphyrins contributes to a drastic reduction of *N. ceranae* spores in porphyrin-treated honeybees. We suggest that, due to their immunomodulatory properties, porphyrins mobilize the honeybee immune system through induction of the optimal PO activity/AMP expression variant according to the varying level of nosemosis infection, which allows infected insects to improve their lifespan substantially.

Keywords: Immune response, *Nosema ceranae*, Porphyrins

WINTER HONEY BEE (*APIS MELLIFERA*) POPULATIONS SHOW GREATER POTENTIAL TO INDUCE IMMUNE RESPONSES THAN SUMMER POPULATIONS AFTER IMMUNE STIMULI

Dostálková, Silvie; Danihlík, Jiří; Škrabišová, Mária; Petřivalský, Marek, Department of Biochemistry, Faculty of Science, Palacký Univesity Olomouc, Olomouc, Czech Republic
Dobeš, Pavel; Kunc, Martin; Hurychová, Jana; Hyršl, Pavel, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
Havlík, Jaroslav, Department of Food Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Praha, Czech Republic
Titěra, Dalibor, Bee Research Institute at Dol, Libčice nad Vltavou, Czech Republic

In the temperate climates of central Europe and North America, two different honey bee (*Apis mellifera*) populations are commonly found in colonies: short-living summer bees and long-living winter bees. Besides the differences in their life span, each of these populations fulfils a different role within the colonies. For instance, summer bees emerge in spring and survive until summer, mainly foraging and providing nutrients for bee brood. Winter bees emerge in late August, and their primary role is overwintering and establishing a new generation of bees in the spring. Winter worker bees also have higher vitellogenin levels and more significant reserves of nutrients in their fat bodies than summer bees.

The differences between the immune systems of both populations are well described at the constitutive level; however, our knowledge of its inducibility is still very limited. In this study, we focused on the immune response of 10-day-old honeybee workers triggered in vivo by injecting heat-killed bacteria. This study was focused on honey bees that emerged and lived under hive conditions and evaluated immune response by measuring the antimicrobial activity of hemolymph, determining the relative gene expression of antimicrobial peptide genes and vitellogenin, quantifying antimicrobial peptide apidaecin, and determining vitellogenin concentration.

Responses to bacterial injections differed between summer and winter bees. Winter bees exhibited a more intense immune response, including higher expression of antimicrobial genes and antimicrobial activity, as well as a significant decrease in vitellogenin gene expression and its concentration in the hemolymph. The observed intense immune response in winter honeybees may contribute to understanding the relationships between colony fitness and infection with pathogens and its association with successful overwintering.

Keywords: Humoral immunity, Antimicrobial peptides, Honey bee Longevity

IMMUNITY

POSTERS

OXIDATIVE STRESS ASSOCIATED WITH VARROA PARASITISM: EVALUATION OF LIPID PEROXIDATION IN INFESTED BEES

Pinďáková, Eliška; Danihlík, Jiří; Dostálková, Silvie; Petřivalský, Marek, Department of Biochemistry, Faculty of Science, Palacký Univesity Olomouc, Olomouc, Czech Republic
Dobeš, Pavel; Hurychová, Jana; Hyršl, Pavel, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Varroa destructor mites are responsible for the most severe problems with honey bee health worldwide. They cause a disease named varroosis. The mites transmit a wide array of highly lethal viruses to honey bee colonies, typically the deformed wing virus (DWV). Bee responses to external and internal stressors include the production of reactive oxygen species (ROS), which occurs as a part of humoral immunity. High ROS production or impaired functions of the antioxidant system result in ROS accumulation and oxidative stress, leading to damage to essential biomolecules such as lipids, proteins, and nucleic acids. Under disturbed redox balance, cellular lipids represent a primary target of ROS-induced oxidative modifications. The process of lipid peroxidation can be assessed either at the level of reactive primary products, lipid hydroperoxides (LOOH) or at the level of stable terminal products represented by malondialdehyde (MDA).

This study aimed to determine lipid peroxidation levels in adult honey bees (*Apis mellifera*) after infestation by *Varroa destructor* mites during the pupal period. The collected bees were kept in plastic cages in a thermostat (34 ± 1 °C, 80 % relative humidity, dark) for 10 days and fed with a sucrose solution (1:1 w/v; sucrose/ water) and pollen ad libitum. Levels of LOOHs were quantified by the xylenol orange (FOX2) method, whereas MDA levels were determined by the thiobarbituric acid (TBARS) method.

Bee infestation resulted in significantly decreased levels of both lipid peroxidation parameters compared to the control non-infested group, with the same declining trend for LOOH and MDA. These results do not conform with the hypothesis that mite attack induces oxidative stress and damage in Varroa-infested bees. The observed decline of lipid peroxidation will be addressed in further studies.

This research was funded by The Ministry of Agriculture of the Czech Republic grant QK1910286.

Keywords: oxidative stress, lipid peroxidation, *Varroa destructor*

MODELING OF SEASONAL IMMUNE DYNAMICS OF HONEY BEES IN RESPONSE TO INJURY AND INJECTION OF HEAT-KILLED *SERRATIA MARCESCENS*

Hurychová Jana[1], Dostál Jakub[2], Dobeš Pavel[1], Kunc Martin[1], Eliáš Sara[1], Dostálková Silvie[3], Škrabišová Mária[3], Petřivalský Marek[3], Hyršl Pavel[1], Danihlík Jiří[3],

1 Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
2 Department of Mathematical Analysis and Application of Mathematics, Faculty of Science, Palacký University, Olomouc, Czech Republic
3 Department of Biochemistry, Faculty of Science, Palacký University, Olomouc, Czech Republic

The honey bee, *Apis mellifera*, is one of the main pollinators worldwide. In a temperate climate, seasonality affects the life span, behavioural, physiological, and immunological characteristics of honey bees, impacting their response to pathogens and parasites. In this study, we used Bayesian statistics and modelling to examine the immune response dynamics of summer and winter honey bee workers after immune challenge with heat-killed *Serratia marcescens*, an opportunistic honey bee pathogen. We investigated humoral and cellular immune reactions on a transcriptional and functional level by qPCR of selected immune genes, antimicrobial activity assay, and flow cytometric analysis of hemocytes. We observed an increase in the number of hemocytes in summer honey bees immediately after bacterial stimuli and their rapid reduction after 24 hours. This reaction was not observed in winter bees, whereas the increase in antimicrobial activity at the transcriptional and functional levels after injection was greater in winter bees than in summer bees. Our results support the hypothesis that the summer population mounts a cellular response to *S. marcescens*, while winter honey bees preferentially rely on humoral immune reactions. These differences point out the necessity to target distinct mechanisms to ensure better sustainability of beekeeping in a temperate climate. Moreover, we created a model based on our data using Bayesian statistics to show the dynamics of the honey bee immunity response to the pathogen that will be employed in the further research. This approach may lead to a more quality investigation of these mechanisms. This research was funded by The Ministry of Agriculture of the Czech Republic grant QK1910286.

Keywords: Bayesian statistics, seasonal changes, *Serratia marcescens*

NUTRITION

ORAL PRESENTATIONS

CHAIRPERSON
Mathieu Lihoreau

PERCEPTION, REGULATION, AND FITNESS EFFECTS OF POLLEN PHYTOSTEROLS IN THE BUMBLE BEE, *BOMBUS TERRESTRIS*

Nebauer, Carmen A., Plant-Insect Interactions, Department of Life Science Systems, Technical University of Munich, Freising, Germany
Schleifer, Marielle C., Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Würzburg, Germany
Ruedenauer, Fabian A., Plant-Insect Interactions, Department of Life Science Systems, Technical University of Munich, Freising, Germany
Leonhardt, Sara D., Plant-Insect Interactions, Department of Life Science Systems, Technical University of Munich, Freising, Germany
Spaethe, Johannes, Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Würzburg, Germany

The majority of flowering plants depend on insects for successful cross-pollination. To attract insect pollinators, plants typically offer nutritional rewards, like nectar and pollen, on which especially bee pollinators rely to successfully raise their offspring. Whereas nectar is the main source of carbohydrates, pollen provides all essential micro- and macronutrients, like proteins, lipids and sterols. Among others, sterols play a major role in maintaining metabolic functions and the structural integrity of cells and tissues. They are also involved in the ovarian development of bees. However, bees cannot synthesize sterols de novo but need to obtain via their food resources, in particular pollen. However, pollen phytosterols content can vary strongly between plant species with potentially strong effects on the health and reproductive fitness of bees.

We therefore hypothesized that (i) variation in pollen sterol content affects longevity and reproduction in bumble bees and (ii) can therefore be perceived via the bees' antennae prior to its consumption. To test our hypotheses, we performed feeding assays using pollen diets differing in sterol content, and we investigated sterol perception using differential chemotactile proboscis extension response (PER) conditioning.

We found that *B. terrestris* workers can perceive several sterols (cholesterol, cholestenone, desmosterol, stigmasterol, β-sitosterol) by means of their antennae, but cannot differentiate between them. However, when sterols were added to pollen, the bees did not differentiate between pollen differing in sterol content, suggesting that sterols are not perceived when being part of the complex chemical mixture represented by pollen. Unexpectedly, different sterol concentrations in pollen did also not affect pollen consumption, brood development and worker longevity. Our findings thus suggest that variation in concentrations of the tested sterols does not affect pollen foraging preferences or colony performance in *B. terrestris*. As we used naturally occurring and slightly higher concentrations as found in plant pollen, our findings indicate that bumble bees may not need to pay specific attention to variation in pollen sterol content, because encountered sterol concentrations might fully support their sterol requirements. Furthermore, higher concentrations, as used in this study, do not seem to have any detrimental effect on their survival and reproductive fitness.

Keywords: feeding assay; PER conditioning; phytosterols

ENSURING THE QUALITY AND SECURITY OF BEE FEED : THE CASE OF CANDY-BOARDS

BOCQUET Michel, Apimedia, ANNECY, France
SMODIŠ ŠKERL, Maja Ivana, Agricultural institute of Slovenia, Ljubljana, Slovenia
FALCÃO Soraia I., Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal
GIACOMELLI Alessandra, UNAAPI, Florence, Italy
CHLEBO Robert, Slovak University of Agriculture Nitra, Slovakia
QUAGLIA Giancarlo, Lifeanalytics, FLORAMO, Rocca De' Baldi,Italy

Honey bee colonies are strongly dependent on floral resources for their development and survival. These resources are often altered by landscape modifications and agriculture intensification in the context of multistressors pressure (climate changes, pests, parasites, pesticides). Accordingly, the beekeepers are increasingly using supplemental feeding to reduce risks for their colonies. At the scale of the beekeeping sector, a large offer of bee feed supplements or substitutes is proposed by the market with highly variable compositions and quality levels. It is why the COLOSS (Colony LOSSes, Honey Bee Research Association) NUTRITION Task Force members proposed the BeeQualiFeed project. It aims to promote honey bee feed control and monitoring by elaborating adapted methodologies and protocols in collaboration between laboratories and the different stakeholders .

In this study, we present the general approach used in the case of the Candy Boards as a model of bee feed, rather simple in composition and largely used among beekeepers as a supplement. The analytical part is discussed, from the choice of the samples (industrial and local production) to the parameters used and the global methodology applied to these samples. Additionally, we present the main stakes, and practical questions by each stakeholder (e.g. beekeepers, food companies, laboratories, consumers, policymakers). Finally, we explore the possible use of the results to elaborate guidelines to support and assist food companies and regulators to proceed the effective quality and safety control of honey bees' supplements and substitutes.

Key words: honey bee, nutrition, candy boards

NUTRITIVE EVALUATION OF BEE POLLEN COLLECTED IN A SUBURBAN AREA

Manganello Federico[1], Petrocchi-Jasinski Francesca[1], Cresta Eleonora[1], Lazzari Filippo[1], Danieli Pier Paolo[1]

1 Department of Agriculture and Forest Sciences (DAFNE), Tuscia University, Viterbo, Italy

Adequate pollen nutrition is a basic condition for good growth and development of honey bees (HB). However, it can be expected that locally environmental conditions can play a role on the nutrition of HB colonies through the diversity pollen. Two ex-hive trials were performed to evaluate the nutritive value of bee pollen collected in a suburban area (Viterbo, Central Italy) where an experimental apiary was established in the frame of PLANT-B, an European project funded by the PRIMA Foundation (grant n. 1812). From March to August 2021 pollen was collected monthly. A queen (50% membership to the subspecie *Apis mellifera ligustica* Spin.) was caged in a honeycomb and, and the comb with capped brood was moved to an incubator till the emergence of young workers. Groups of 30 0-24 h HB were collected and then moved into small cages provided with solid diets, water and sugar solution 50% w/w (SS50) *ad libitum*. The cages were incubated at 28°C and at 70% relative humidity. Six pollen-based diets (levelled on the dry matter) and two controls, no pollen or Haydak's formulation, were set up. Each thesis was replicated three times. All solid the diets were subjected to centesimal analysis to determine their composition. Every day for 2 weeks, deceased HB were counted and removed, and the consumption (μl/bee/day) of water and SS50 was recorded; solid diets were replaced every two days and the consumption (mg dry matter/bee/day) was measured. Survival rate and daily weight gain (DWG) were calculated at the end of the trials. The diet had a significant effect ($p < 0.01$) on DWG. In detail, the pollen of April, May, and July were the ones that showed higher HB weight gain at 14 days versus Haydak's diet and the no pollen thesis. In contrast, the treatment does not affect SS50, water consumption and survival rate. The higher dry matter intake correlated with DWG ($r = 0.70$, $p < 0.01$) and the DWG correlated with all the nutritional components, especially with crude protein ($p<0.01$). Concluding, the biological composition of pollen influences the DWG but further investigation must be conducted on field.

Keywords: *Apis mellifera*, Protein nutrition, Pollen

LINKING THE NUTRITIONAL STATES OF WILD BEES TO FLORAL RESOURCES AVAILABILITY IN EUROPEAN GRASSLANDS

Leroy, Clementine, Abeilles et Environnement, INRAE, Avignon, France
Brunet, Jean-Luc, Abeilles et Environnement, INRAE, Avignon, France
Butschkau, Susanne, Plant-Insect Interactions, Technical University of Munich (TUM University), Freising, Germany
Buydens, Louella, Department of Plants and Crops, Ghent University, Ghent, Belgium
Keller Alexander, Center for Computational and Theoretical Biology and Department of Bioinformatics, Biocenter, University of Würzburg, Würzburg, Germany
Leonhardt, Sara Diana, Plant-Insect Interactions, Technical University of Munich (TUM University), Freising, Germany
Parreno Alejandra, Plant-Insect Interactions, Technical University of Munich (TUM University), Freising, Germany
Smagghe Guy, Department of Plants and Crops, Ghent University, Ghent, Belgium
Henry Mickael, Abeilles et Environnement, INRAE, Avignon, France
Alaux Cedric, Abeilles et Environnement, INRAE, Avignon, France

All bees have in common that they rely on floral pollen and nectar for their growth, reproduction and survival. Nectar contains mainly carbohydrates that fuel the energetic demands for bees, and pollen that provides proteins, amino acids, lipids, and sterols essential for their reproduction and larval development. A decline in the diversity and abundance of floral resources in the environment is therefore expected to trigger a nutritional stress and consequently affect bee health. However, besides some studies on model species such as honeybees, little is known about the influence of floral resource abundance and diversity on the nutritional health of wild bees and thus their sensitivity to changes in floral resources at landscape-level.

To address this knowledge gap, we measured the nutritional state of bees at the community level (26 species from 4 families) collected in grasslands under different management intensities and characterized by three different floral diversity gradients in Belgium and Germany. We specifically analyzed the body content in proteins, glycogen and triglycerides since these components are essential for several life history traits (e.g. reproduction, immune functions, diapause) and their storage are expected to reflect the floral resources availability in the environment. These nutritional state indicators were then linked to landscape variables such as the floral resources density and diversity and the land use intensity.

By assessing the nutritional state of wild bee species, we can identify how different landscape change drivers, such as floral density, diversity, and management practices, affect different species. Ultimately, we aim to identify some indicator species providing early warning signals of reduced habitat quality, as well as to assess the consequences of such changes for related bee species. Our results should therefore help improving the management of floral resources in wild bee conservation programs.

Keywords: energetic metabolism, floral diversity, bee conservation

HIGH DIETARY OMEGA 6:3 RATIO DECREASES NURSING ACTIVITY AND ACCELERATES FORAGING ONSET

Danny F. Minahan and Sharoni Shafir

B. Triwaks Bee Research Center, Department of Entomology, Institute of Environmental Sciences, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem

Honey bees are eusocial insects that nest in large perennial colonies, and where workers demonstrate an age-based division of labor, with young workers performing tasks inside the hive, such as nursing immature bees in the larval stage, while older workers embark on foraging flights to collect resources. Pollen is an important source of macronutrients for developing bees, not only in the larval stage but also during the first 8-10 days following adult emergence. Omega 6 and omega 3 for example are essential fatty acids acquired from pollen and are typically balanced (omega 6:3 ratio = 1) in diverse pollen samples collected from honey bee hives. However, the ratio of omega 6:3 is variable among plant species, and in low resource diversity systems this may lead to an imbalanced omega 6:3 ratio in the honey bee diet, with negative consequences for individual and colony function. For example, bees raised on a high ratio diet (ratio = 5) demonstrate decreased larvae rearing success and shortened lifespan relative to bees fed a balanced diet. These findings suggest that bees fed a high ratio diet may contribute less to the nursing of larvae, while also transitioning to the foraging role earlier. We tested these hypotheses by marking 1-day old workers with uniquely identifiable barcodes and feeding them either a high ratio or balanced omega 6:3 diet until 8-days old, and then video monitoring the hive entrance and brood area of a common garden hive for which bees from both diet treatments were introduced. We observed that nurses fed a high ratio diet visited fewer larvae that were raised to the capping stage, made fewer visits to successfully capped cells, and visited larvae across fewer development stages than bees fed a balanced diet, while also foraging at a younger age, and having a shorter lifespan than bees fed a balanced diet. These findings suggest that an imbalanced omega 6:3 ratio in the honey bee diet may be one source of imbalanced nutrition contributing to colony losses generally, but also the colony collapse phenomenon more specifically.

Keywords: nutritional balancing, behavior, fatty acid

POTENTIAL OF DIETARY SUPPLEMENT TO ENHANCE HYGIENIC BEHAVIOUR OF HONEY BEE COLONIES AND THEIR COMBAT WITH MOST COMMON INFECTIONS

Stevanovic Jevrosima[1], Glavinic Uros[1], Jovanovic Nemanja M[2], Ristanic Marko[1], Dominikovic Nina[1], Vejnovic Branislav[3], Stanimirovic Zoran[1]

1 Department of Biology, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia;
2 Department of Parasitology, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia;
3 Department of Economics and Statistics, University of Belgrade - Faculty of Veterinary Medicine, Belgrade, Serbia;

Hygienic behavior of honey bee colonies is one of the many strategies they apply to effectively fight pathogens and presents their collective reaction to the presence of diseased brood. The data on the influence of diet supplementation on honey bee hygienic behavior are scarce. The aim of this study was to evaluate the effect of a dietary amino acid and vitamin supplement "BEEWELL AminoPlus" on hygienic behavior of 40 colonies and their combat with *Nosema ceranae* and honey bee-associated viruses. Five groups with 8 colonies in each were established: one supplemented and infected with *Nosema ceranae* and viruses, three not supplemented, but infected with *N. ceranae* and/or viruses, and one negative control group. During the study, honey bee trypanosomatids were also monitored knowing their common coexistence with *Nosema* sp. microsporidia. The supplement tested in this study expressed a positive influence on hygienic behaviour of colonies infected with *N. ceranae* and four viruses. In fact, starting from the day of the supplement application, the hygienic behaviour was significantly better expressed in each subsequent assessment time compared to previous one(s), contrary to non-supplemented groups, either infected or not. The supplement also helped colonies in terms of control of *N. ceranae* infection. Among infected groups, only the supplemented one remained *Lotmaria passim*-free throughout the study. To conclude, tested supplement enhances hygienic behavior of honey bee colonies and helps them fight the most common microsporidian and viral infections, but further studies are needed to confirm the potential of the supplement in the prevention of *L. passim* infection.

Keywords: diet, amino acid and vitamin supplement, hygienic behaviour

DO HONEY BEES' UPTAKE OR AVOID DIFFERENT ELEMENTS IN THEIR FOOD?

Zarić, Nenad, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Brodschneider, Robert, Institute of Zoology, University of Graz, Graz, Austria
Stanisavljević, Ljubiša, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Goessler, Walter, Institute of Chemistry, University of Graz, Graz, Austria

Insects including honey bees have different needs for minerals, metals and metalloids. Some of them, including Na, K, Ca, P, Fe, Zn, Mn, Cu, have known metabolic functions and are considered essential. While others, for example Al, Ba, Cd, As, Pb, Sr, have no known functions in bees and are considered non-essential. Until recently, elemental concentrations were measured in pooled and homogenized samples of honey bees. Most of them used honey bees as biomonitors of metal pollution in the environment. A recent study was the first to determine elemental composition of individual bees. In all of these studies whole bees were used. The aim of this study was to determine the origin of elements in honey bees. We wanted to determine how much of the elements in the whole honey bee are actually present in bees' tissues and how much were in their midgut, which would represent the food (mostly beebread). We dissected the honey bees, separating the gut from the rest of the body, and analyzed them separately for elemental concentrations. In addition, we have analyzed beebread from the same colonies, to determine the relationship of elements present in the food and in the body of the bee (excluding midgut which is presumed to contain mostly food). In this study we show that honey bees actively take up and accumulate Na, As, Se, S and Fe from their food (much higher concentration measured in bee body compared to beebread). While at the same time they can regulate the uptake of access amounts of Ba, B, Al, Cd, Sr, Mn, V, Ni, Co present in beebread in much higher amounts compared to the dissected body of the honey bee with the removed midgut.

Keywords: *Apis mellifera*, Metals, Beebread

EFFECT OF STORAGE TIME AND CONDITIONS ON THE MICROBIAL LOAD OF FRESH POLLEN

Liolios Vasilios, Kanelis Dimitrios, Rodopoulou Maria-Anna, Tananaki Chrysoula, Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki (AUTH), Thermi,Thessaloniki, Greece

In recent years, bee pollen has been widely used as a dietary supplement due to its high nutritional value. In Greece, bee pollen is mostly marketed and consumed in fresh form. However, the quality of the final product that reaches the consumer depends to a large extent, not only on the handling at the harvest stage but also on the post-harvest handling. Thus, in the present study we evaluated the effect of storage time and conditions on the change of the microbial load of bee pollen in its fresh form. The variation of microbial load was studied for Mesophilic Total Viable Count (MTVC), Yeasts and Moulds (Y&M), Enterobacteriaceae (ENT), Lactic Acid Bacteria (LAB) and *Escherichia coli*. Freshly collected pollen was evenly distributed in glass jars, which were stored at 25oC (room temperature), at 4oC and at -18oC. In the first case (25°C), the analyses were performed every seven days (0, 7, 14, 21, 28), while in the other two (4°C, -18°C) every 3 months (0, 3, 6, 9). During storage at -18°C, a decreasing trend of the MTVC, ENT and LAB counts was observed in percentages of 2.22%, 2.25% and 24.28% respectively. In contrast, Y&M counts showed an upward trend (29.8%) during the nine months of storage at -18°C. In none of the cases the levels of the examined microorganisms did exceed the permissible limits as defined in the food hygiene standards. On the contrary, during the storage of fresh pollen at 4°C, after six months, MTVC and ENT counts were higher than the legislative limits, with values of 5.14 log cfu/g and 2.34 log cfu/g, respectively, while during storage at 25°C, the largest change in the load of the examined microorganisms was observed, in the short period of four weeks. *E. coli* was not detected in any sample of fresh pollen, regardless of the storage time and conditions. Considering all the above, it can be suggested that the storage of fresh bee pollen be carried out at freezing temperature (-18°C).

Keywords: fresh bee pollen, microbial load, post - harvest practices

BUMBLEBEES ADJUST NUTRIENT COLLECTION TO TEMPERATURE VARIATION

Kraus, Stéphane, Research Center on Animal Cognition (CRCA), CNRS – University Paul Sabatier, Toulouse, France
Juliano Morimoto, School of Biological Sciences, The University of Aberdeen, UK
Devaud, Jean-Marc, Research Center on Animal Cognition (CRCA), CNRS – University Paul Sabatier, Toulouse, France
Lihoreau, Mathieu, Research Center on Animal Cognition (CRCA), CNRS – University Paul Sabatier, Toulouse, France

Temperature drives the behaviour and metabolism of bees. While social bees work hard to keep a stable in-nest temperature suitable for colony development, they can nevertheless suffer cold or heat stress under extreme climatic conditions. Understanding how bees adapt their foraging decisions to such temperature variations is crucial to both conservation and pollination. Here we studied the effect of temperature variation on the nutritional behaviour of bumblebees using experimental designs of nutritional geometry. Micro-colonies of bumblebees were given a choice between fully artificial diets varying in their ratios of protein, carbohydrates and lipids at several temperatures (20°C, 30°C and 35°C) for 10 days. Under all temperature regimes, bumblebees adjusted their collection of the three macronutrients nutrients along a trade-off between maximizing survival and egg-laying. However, this ratio varied with temperature. At the lowest temperature, bees tended to increase carbohydrate collection, likely favoring survival at the cost of fewer egg-laying. At the highest temperature, however bees did not seem capable to regulate food intake anymore. Our results suggest climate change can have a strong influence on bee behaviour and fitness.

Keywords: nutrition, artificial diets, temperature

NUTRITION

POSTERS

INVERTASE AND DIASTASE ACTIVITIES IN ROBINIA HONEYS FROM HUNGARY

Kiss, Tünde, Zajácz, Edit
Department of Apiculture and Bee Biology, Institute for Farm Animal Gene Conservation, National Centre for Biodiversity and Gene Conversation, Gödöllő, Hungary

Honey contains only small amounts of enzymes, however, they play an essential role in converting nectar to honey and they are also responsible for the quality of honey. The most important honey enzymes are invertase (α-D-glucosidase) and diastase (α- and β-amylase). Their sensitivity towards storage and heating is high, therefore both enzyme activities are used as indicators of honey quality.

The aim of this present study was to determine invertase and diastase activities in black locust (*Robinia pseudoacacia*) honey samples from different geographical regions of Hungary in order to obtain information about the quality of honeys, as there are no or few data available. 97 honey samples (in 2015: 34, in 2016: 32, in 2017: 31 samples) were collected directly from beekeepers from 3 different regions of Hungary (northern, north-eastern and south-western regions of Hungary) and were analysed. The botanical origin of honey samples were determined by melissopalinological analysis complemented by sensory and physicochemical analysis. The quality of honey samples was also studied by determination of hydroxymethylfurfural (HMF). The invertase/diastase ratio in the honeys was also determined. A high variability in the invertase activity of the individual honeys was detected, and the average invertase values for the regions also showed differences, which were influenced by the weather conditions of the respective years. The diastase activity values differed slightly in the individual honeys and also in the case of the average values of the different regions. The results showed that the mean invertase activities of Hungarian *Robinia* honeys were higher (in all three years and in all regions) than the European average reported by the International Honey Commission. Concerning diastase activity, the values did not differ significantly from the European average.

Keywords: *Robinia* honey, invertase, diastase

THE EFFECT OF SPERMIDINE SUPPLEMENTATION ON EXPRESSION OF DNA METHYLTRANSFERASE GENES (DNMT1A AND DNMT1B) IN HONEY BEES

Pihler Ivan[1], Kebert Marko[2], Radišić Predrag[3], Đorđievski Srđana[4], Čelić V. Tatjana[4], Vukašinović L. Elvira[4], Kojić Danijela[4], Purać Jelena[4]

1 Department of animal science, Faculty of Agriculture, University of Novi Sad, Serbia
2 Institute of lowland forestry and environment, University of Novi Sad, Serbia
3 Biosense institute, University of Novi Sad, Serbia
4 Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia

Honey bee (*Apis mellifera* L.) is one of the most important pollinators in the world whose number of colonies has been decreasing in the last few decades. For this reason, health of the honey bees has been the subject of many studies in recent years. In particular, research on nutritional supplements in the form of laboratory tests, with the aim of improving their health and immunity are coming into focus. In that sense spermidine appears as a potential candidate. Spermidine is a naturally occurring polyamine compound with various metabolic functions. Exogenous supplementation of spermidine prolongs the lifespan, increases resistance to stress in several model organisms and shows health promoting effects on aging and its comorbidities in humans. The mechanism behind spermidine is not fully understood. Induction of autophagy is proposed as the main, however other mechanisms are also described. Recent studies indicate that the changes in polyamine metabolism affect the concentrations of substances and enzyme activities involved in DNA methylation. The aim of this study was to test the effect of spermidine supplementation on expression of DNA methyltransferase (Dnmt) genes in honey bees. For this reason, bees of the same age were fed in controlled conditions for 17 days. In addition to the sucrose-fed control group, the diet of honey bees was supplemented with 0.1 mM and 1 mM spermidine. qPCR was performed with the specific primers for two genes: Dnmt1a and Dnmt1b, using RNA extracted from head and abdomen, as starting material. Results showed that supplementation with 0.1 mM spermidine significantly increased Dnmt1a gene expression in head and abdomen, while 1 mM spermidine increased Dnmt1a expression only in abdomen. On the contrary, spermidine supplementation did not affect Dnmt1b gene expression. The obtained results indicate that spermidine can affect the DNA methylation in honey bees, and consequently transcription of target genes. Further studies are necessary to determine the exact epigenetic changes induced by spermidine and mechanisms of its action.

Keywords: spermidine, epigenetic, DNA methyltransferase

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SEASONAL VARIATION IN MORPHOLOGICAL PARAMETERS OF *APIS MELLIFERA LIGUSTICA* FORAGERS IN A SOUTHERN TEMPERATE CLIMATE

Knoll, Stephane, Department of Animal Nutrition, University of Sassari, Sassari, Italy
Fadda, Valeria, Department of Animal Nutrition, University of Sassari, Sassari, Italy
Pinna, Walter, Department of Animal Nutrition, University of Sassari, Sassari, Italy
Varcasia, Antonio, Department of Parasitology, University of Sassari, Sassari, Italy
Scala, Antonio, Department of Parasitology, University of Sassari, Sassari, Italy
Cappai, Maria Grazia, Department of Animal Nutrition, University of Sassari, Sassari, Italy

Winter in temperate zones of the boreal hemisphere is characterized by low temperatures, reduced daylength and a lack of flowering plants. The combination of these environmental factors induces the cessation of brood rearing and transition of honey bees into their winter form. Winter bees effectively serve as a nutrient storage caste (through accumulation of vitellogenin: fat and protein), resulting in notable morphological alterations. Climate change and increasing environmental temperatures however have potentially caused continuous feed availability within a Mediterranean climate. Consequently, honey bee colonies might no longer require to switch to a broodless state for survival, as is the case in areas where temperatures commonly fall below 10°C in winter. For this reason, this study aimed to monitor seasonal morphological changes of forager bees (weight, total length, abdominal and thoracal length and width, cranial width and fat body size), with the goal to provide a better understanding of the activity and nutritional status of bees and to generate new insights on the summer to winter bee transition in correlation with seasonal alterations and climate change. Ten bees from Sassari (Italy) were sampled monthly over the transition from autumn-winter-spring (2019-2020). Data were analyzed in function of sampling date and environmental factors (temperature, daylength and feed availability) and revealed the presence of a winter-bee-like caste, characterized by increased bodyweight, abdominal length, cranial width and fat body size, solely in December. Reduction of ambient temperature and daylength had a positive effect on bodyweight, abdominal and thoracal length, cranial width and fat body size of bees. However, continuous feed availability and forgiving ambient temperatures during winter likely allowed for brood rearing year round, leading to a quick exit from the overwintering state. Although continuous activity of honey bees in warm temperate climates might seem beneficial, limited pollen availability and quality during cold months might cause excessive nutritive pressure leaving colonies in a vulnerable state. Therefore, climate change could represent a rising challenge for *Apis mellifera* in temperate regions.

Keywords: Winter bees, Morphometry, Climate change

EXPRESSION OF PROTEASE IN ADULT HONEY BEES FED WITH DIFFERENT PATTIES

Pavlović Ratko[1], Dojnov Biljana[2], Šokarda Slavić Marinela[2], Pavlović Marija[3], Tomić Nevena[2], Mišić Milan[2], Vujčić Zoran[1]

1 Department of Biochemistry, University of Belgrade – Faculty of Chemistry, Belgrade, Serbia.
2 Department of Chemistry, University of Belgrade – Institute of Chemistry, Technology and Metallurgy – National Institute of the Republic of Serbia, Belgrade, Serbia.
3 Department of Physical Chemistry, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia.

Feeding of honey bee (*Apis mellifera*) is a challenge for beekeepers and formulation of food supplements is improved continuously. When natural food sources are scarce or not available, supplemental foods are widely used to support and build up honey bee colonies. Influence of commercial (sugar) patty and the patties enriched with 12.5% pollen and 12.5% dried yeast on protease expression in honey bee adults is presented. This is part of a wider study aimed to compare the expression of digestive enzymes using different patties. Data collected in this study can be useful for development of higher quality of food supplements for honey bees.

Honey bees were kept in an incubator for 21 days, at a temperature of 35°C and at 80% humidity. In each cage there were one hundred bees and a piece of honeycomb. Midgut samples were taken after 7, 14 and 21 days, homogenized and analyzed for protein concentration and protease activity.

The highest protein concentration was detected in the midgut of pollen fed bees, and lowest in commercial patty fed group, determined by the Bradford method. Protease activity was the highest in bee's midgut fed with pollen patties, and the lowest in bees fed commercial patties which is shown by enzyme assay and by zymograms. There were different protease isoforms present in bees fed pollen, yeast and commercial patties, but the major isoforms were the same.

The observed decrease in protease activity over time in all groups is probably due to the transition to foraging tasks. Reduced protease activity in the gut of bees fed commercial patties is due to a lack of proteins or some other inducers present in food.

Because pollen is honey bee natural food, we conclude that food supplements that induce similar enzyme expression as pollen (dried yeast) can be considered as appropriate food substitution, because it is superior to supplements which induce very different enzyme expression.

Keywords: protease, honey bee, nutrition.

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UNIFLORAL HONEYS IN HUNGARY FROM SPRING TO LATE SUMMER

Déri Helga
Department of Apiculture and Bee Biology, Institute for Farm Animal and Gene Conservation, National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

In the honeys, in addition to the dominant species, the pollen of other plant species can also be found that bloom in the same period in the foraging area. The botanical origin of honey can be determined by the percentage of the total pollen content of each honey sample by plant species, which points not only the main food sources of honey bees, but also the apicultural significance of each plant species. By evaluating the samples of the given period together, it can be identified the plant species which are the most characteristic of the examined foraging area. This is the geographical origin of honey. In this work the main sources of nectar for honey bees are represented based on the microscopic examination of 8 unifloral honeys from Hungary 2020-2021. Pollen from 30-100 plant species per period was detected in the samples, of which approximately 2/3-3/4 was nectariferous. Presumably due to the selective collection behavior and flower fidelity of the honey bee that there were only 2-8 species per honey of which pollen constituted more than 3 % of the total pollen content so determinate their botanical origin and organoleptic properties. These are also our most valuable bee pasture plants. In March, the main nectar sources were dogwood-, willow-, maple-, and Prunus species. April is the gathering time of the economically important rapeseed honey, followed by the acacia honey in May. Summer honeys (e.g. false indigo, linden, sweet chestnut, phacelia, sunflower) were rich in plant species and honeydew elements were also characteristic for them. At the end of summer, the number of flowering plant species providing nectar and pollen for bees decreased. Asteraceae family was the main source of nectar. As can be seen from the above, the bee-pasture species providing unifloral honeys which typify Hungary as a geographical area come primarily out of our agricultural crops or stock forming tree species in the forests.

Keywords: nectar sources, unifloral honeys, microscopic pollen identification

EFFECT OF DIET ON HONEY BEE HAEMOLYMPH AMINO ACID COMPOSITION

Tafi, Elena, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Sagona, Simona, Department of Pharmacy, University of Pisa, Pisa, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Galloni, Marta, Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy
Bogo, Gherardo, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Casini, Lucia, Department of Veterinary Science, University of Pisa, Pisa, Italy
Barberis, Marta, Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy
Nepi, Massimo, Department of Life Sciences, University of Siena, Siena, Italy
Felicioli, Antonio, Department of Veterinary Science, University of Pisa, Pisa, Italy

Amino acids (AAs) are an abundant class of nectar solutes, and they are involved in the attractiveness to flower visitors. Among them, often proline is the most abundant protein AA, and γ-amino butyric acid (GABA) and β-alanine are the two most abundant non-protein AAs. These three AAs are well known to affect insect physiology, being involved in flight metabolism, neurotransmission and phagostimulation. In the context of the project "BeeNet – monitoring the environment through bees and biodiversity", in-depth information investigates the effects of artificial diets enriched with either β-alanine, GABA, or proline on feed intake, survival, and haemolymph composition in honey bees of two different ages (i.e., newly emerged and foragers). Feed intake was not affected by dietary AAs, contrarily to survival. Indeed, β-alanine increased the newly emerged bee survival rate, while both proline and GABA reduced it. Variation in the haemolymph concentration of β-alanine, GABA, and proline occurred only in newly emerged bees, according to the diet and the time of haemolymph sampling. Furthermore, results highlighted differences in haemolymph composition between newly emerged and forager bees before the beginning of the experiment. This work showed a greater susceptibility of young bees to dietary AAs than older bees. The variations in the concentrations of AAs in the haemolymph reflect either the accumulation of AAs taken with diet or the existence of metabolic pathways that may lead to the conversion of dietary AAs into different ones. This preliminary investigation could be an initial contribution to studying the complex dynamics that involve haemolymph protein and non-protein AAs and their effects on honey bee physiology.

Keywords: proline, non-protein amino acids, *Apis mellifera*

EFFECT OF DIETARY PROTEIN LEVELS ON PHENOLOXIDASE ACTIVITY IN HONEY BEE *APIS MELLIFERA IBERIENSIS*

Dzul, Daniel[1]; Muñoz, Irene1; Garrido, Mª Dolores[2]; Serrano, José[1]

1 Department of Zoology and Physical Anthropology, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain.
2 Department of Food Technology, Nutrition and Bromatology, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain.

Nutrition is one of the factors affecting the immune system and the health of honey bees (*Apis mellifera*). The immune system responds to the attack of pathogen microorganisms by various mechanisms such as the use of phenoloxidase, an enzyme that acts against bacteria, fungi, and viruses by melanisation. Previous studies have indicated that providing artificial food to honey bees increases the protein concentration in the haemolymph, the number of immune system cells and phenoloxidase activity. The latter can be determined by measuring the melanisation rate after a certain time of controlled feeding.

In this work, newly emerged worker bees (*Apis mellifera iberiensis*) were collected and randomly distributed into six boxes of 100 individuals each. The honey bees were fed with different dietary protein content (0, 3, 6, 9, 12 and 15%) and were kept for 15 days in an incubator at 32°C and 68% relative humidity. Haemolymph from five honey bees per treatment (10 µl in total) was collected every three days for measurement of phenoloxidase activity.

Results showed that the highest increase in phenoloxidase activity was recorded in honey bees with six days of age consuming diets containing 6% and 9% protein. An increase in enzyme activity was also observed when six-day-old honey bees were fed diets containing 3% protein and in honeybees with nine days of age consuming diets with 6%, 9% and 12% protein content. Phenoloxidase activity decreased with increasing honey bee age and dietary protein content.

These results indicate that providing artificial feed to honey bees at early age, when pollen is scarce in the field, can improve the immune system and prevent diseases. The application of this practice by beekeepers can improve health, pollination services and honey bee production.

Keywords: Immune system, Pathogens, Nutrition.

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MICROBIOTA

ORAL PRESENTATIONS

CHAIRPERSONS
Asli Özkirim
Eva Forsgren

MOVING COLONIES TO A SEMINATURAL AREA PROMOTES MORE NATURAL HONEY BEE MICROBIOMES

Gorrochategui-Ortega, June, Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain
Muñoz-Colmenero, Marta, Dept. of Genetics, Physiology and Microbiology, Complutense University of Madrid, Madrid, Spain
Kovačić, Marin, Faculty of Agrobiotechnical Sciences, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
Filipi, Janja, Dept. of Ecology, Agronomy and Aquaculture, University of Zadar, Zadar, Croatia
Puškadija, Zlatko, Faculty of Agrobiotechnical Sciences, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
Kezić, Nikola, Dept. of Fisheries, Apiculture and Special Zoology, University of Zagreb, Zagreb, Croatia
Parejo, Melanie, Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain
Büchler, Ralph, Landesbetrieb Landwirtschaft Hessen, Kirchhain, Germany
Estonba, Andone, Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain
Zarraonaindia, Iratxe, IKERBASQUE, Bilbao, Spain

Honey bee health and its gut microbiome are interconnected. External stressors can induce microbial dysbiosis and compromise bee health. Also noteworthy are the multiple niches present in hives, each with distinct microbiotas and all coexisting, which we termed “apibiome”. Thus, we hypothesized that 1) the bacterial communities of beehives located in areas with different degrees of anthropization differ in composition, and that 2) due to interactions between the multiple microbiomes within hives, changes in the community of a niche would impact the bacteria present in other hive sections. We characterized the bacterial consortia of different hive niches (bee gut, bee bread, hive entrance and internal hive air) of 43 colonies from 3 different environments (agricultural, semi-natural and natural) through 16S rRNA amplicon sequencing. Agricultural samples presented a decreased community evenness and increased phylogenetic diversity, indicating abnormal proliferation of bacteria. The genus Arsenophonus emerged as a bioindicator of anthropization level, gradually decreasing its abundance from agriculture to the more natural environments. Functional profiles of gut and hive entrance, as determined by predictive PICRUSt2 analysis, indicated increased recruitment of anti-stress responses in agriculture. Finally, translocation of anthropized beehives to a semi-natural environment drove their apibiome towards less stressed and more natural states, suggesting a new management strategy towards promoting colony health.

Keywords: beehive, bacteriome, anthropization

THE IMPACT OF VAIRIMORPHA (NOSEMA) CERANAE NATURAL INFECTION ON HONEY BEE (*APIS MELLIFERA*) AND BEE BREAD MICROBIOTA

Georgi Irini[1#], Asoutis Didaras Nikos[1#], Nikolaidis Marios[2], Charistos Leonidas[3], Hatjina Fani[3], Amoutzias D. Grigoris[2] and Mossialos Dimitris[*1]

1 Microbial Biotechnology-Molecular Bacteriology-Virology Laboratory, Department of Biochemistry & Biotechnology, University of Thessaly, GR-41500 Larissa, Greece.
2 Bioinformatics Laboratory, Department of Biochemistry & Biotechnology, University of Thessaly, GR-41500 Larissa, Greece.
3 Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA', Nea Moudania, Greece
# Equal Contribution
* Correspondence: mosial@bio.uth.gr; Tel.: +30-241-056-5270

Honey bees (*Apis mellifera*) are essential pollinators of crops. Over the past two decades honey bees face new challenges including climate change and new pathogens, such as *Vairimorpha* (*Nosema*) *ceranae*, which synergistically might cause a syndrome designated as Colony Collapse Disorder (CCD). The population of colonies suffering from CCD is continuously declined and at the end only the queen bee with some remaining worker bees survive inside the hive. Often, high loads of *Vairimoprha* (*Nosema*) *ceranae* spores are detected in collapsed colonies. Moreover, diagnosis and prognosis of Nosemosis is still a challenge for beekeepers. In this study, a metataxinomic approach (Next-Generation Sequencing) has been employed in order to investigate the effect of *V.* (*Nosema*) *ceranae* natural infection (spore counts >2,500,000 per bee) on gut microbiota (bacteria and fungi) of adult *A. mellifera* bees as well as microbiota of stored beebread (BB) compared to hives having low spore counts (<40,000 per bee). Alpha-diversity analysis revealed for the first time, a significant decrease in the number of observed species (unique Operating Taxonomic Units) of both bacterial and fungi sequences, in bees and BB samples from heavily infected colonies compared to those with low spore counts, except for one sample demonstrating the lowest bacterial diversity. Plausibly, overall reduction of microbial diversity might lead to dysbiosis and promote CCD. Furthermore, the fungal genera *Podosphaera* and *Blumeria* were completely absent and significantly reduced (in terms of relative abundance) respectively in BB samples collected from heavily infected hives. The biological importance of these findings remains elusive. However, the overall reduction in microbial diversity as well as the alteration in relative abundance of certain fungal genera in BB samples from heavily infected hives might serve in prognosis (biomarkers) of colony collapse and warrants further investigation.

Keywords: *Vairimorpha* (*Nosema*) *ceranae*, *Apis mellifera*, nosemosis, microbiota, bee bread

THE HONEY BEE GUT MICROBIOME – A DIVERSITY PROFILE OF DIFFERENT GUT PARTS AND SEASONS ACROSS EIGHT EUROPEAN COUNTRIES

Basler, Nikolas, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium
de Graaf, Dirk C., Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
De Smet, Lina, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
Matthijnssens, Jelle, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

As a highly productive pollinator of flowering plants, the honey bee (*Apis mellifera*) plays an important role in ecology and agriculture. However, managed honey bee colonies are suffering from a number of stressors, including pathogens, parasites, and the use of pesticides. Studying and improving the health of honey bees is therefore of great ecologic and economic interest. Previous studies have shown that the honey bee gut houses a stable bacterial microbiome of only approximately eight core species. On the other hand, recent studies described a higher diversity in bacteria on the sub-species level as well as in bacteriophages (or phages, i.e. viruses that infect bacteria). Additionally, it has been shown that the gut microbiome affects the bees' development, immune signalling and behaviour.

This project is part of the EU-funded Innovative Training Network VIROINF (grant agreement No 955974) which revolves around virus-host interactions and aims to facilitate the connections between the fields of virology and bioinformatics. Honey bees from eight different European countries (Belgium, France, Germany, Netherlands, Portugal, Romania, Switzerland and United Kingdom) were collected during spring, summer and autumn 2020 as part of the EU-funded B-GOOD project (grant agreement No 817622). From each sample, midguts, ileums and rectums of 10 individual bees have been pooled and processed to generate Illumina libraries for shotgun sequencing. From the resulting data, the diversity of the microbiome was assessed based on species-level abundances as well as shared fractions of single-nucleotide variations (SNVs) in orthologous core genes within species. Preliminary analysis of the core species abundance profiles suggests that the rectum contains the entire bacterial community, of which ileum and midgut contain nested subsets. Unlike species abundances, SNV profiles within some core species appear to separate by geographic origin.

These findings will also inform future experiments to gain more insight into the complex interactions between the honey bee, its gut bacteria and their viruses. We thereby aim to contribute to a better understanding of honey bee health and disease.

Keywords: Metagenomics, Bacteria, Diversity

CLIMATE AND BEE HOST AFFECTS MICROBIAL COMMUNITY IN SOLITARY BEES ON A CONTINENTAL SCALE

*Hettiarachchi, Gowri Amanda, Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium
*Tuerlings, Tina, Laboratory of Agrozoology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
Weekers, Timothy, Agroecology Lab, Université Libre de Bruxelles, Brussels, Belgium
Vereecken, Nicolas J. , Agroecology Lab, Université Libre de Bruxelles, Brussels, Belgium
Michez, Denis, Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium.
Vandamme, Peter, Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium
Smagghe, Guy, Laboratory of Agrozoology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

*Co-first authors

Bee communities are known to be shaped by environmental conditions. Wild bee diversity specifically is sensitive to ecosystem changes, due to the spectrum of specialism and lack of support systems in agriculture. Gut health is known to impact plasticity and resilience in the changing conditions of the Anthropocene. While microbial composition can greatly impact this sensitivity, it is understudied in solitary bees, but is essential for advancing animal health management.

In this talk, the microbial community of key solitary bee species in fruit pollination is characterized, and influences of anthropogenic filters are identified. The gut microbiota of eight solitary bee species sampled in apple orchards along a European climate gradient was characterized through 16S rRNA and ITS2 amplicon sequencing and broad PCR screening of protozoa. Considering different qualitative and quantitative explanatory variables, the differential microbial community was mostly explained by sampling location and host species. In several bee species, correlations between microbial community and climate, landscape or bee community parameters are found. Despite the environmental variation, solitary bee species also seem to have characteristic core microbiota. Interactions between the three microbial groups as a third layer in this study indicate the complexity of this ecosystem. Bacterial and fungal diversity is suggested to be related to parasite infection, in which certain bacterial and yeast species are associated with infected and infection-free bees. This talk further untangles the complex wild bee ecosystem and the immense effect of the environment on presence and prevalence of microbial species, and shows the need to fill the immense knowledge gap to sustain the undervalued role of wild bees for agriculture and biodiversity.

Keywords: Microbiota – pollinators – climate

INSIGHT INTO WILD BEES MICROBIOME USING AMPLICON SEQUENCING TECHNIQUE

Żmuda, Aleksandra, Institute of Botany, Jagiellonian University, Kraków, Poland
Kolasa, Michał, Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland
Nobis, Agnieszka, Institute of Botany, Jagiellonian University, Kraków, Poland
Kierat, Justyna, Kraków, Poland
Mazur, Ewa, Department of Pathology and Veterinary Diagnostics, Warsaw University of Life Science, Warsaw, Poland
Szentgyörgyi, Hajnalka, Institute of Botany, Jagiellonian University, Kraków, Poland

There are more than 2000 bee species described in Europe and our knowledge about their biology and especially their microbiome is still limited. In other insect taxa, microbiome abundance and composition correlates with environmental variables, whereas in bees we lack such data. Our aim was to test the applicability of amplicon sequencing of targeted bacterial marker genes in different bee species and to describe the abundance and composition of their microbiome in correlation with environmental factors.

We collected bees in June-July 2020 and 2021 in southern Poland in three anthropogenic landscape types: agricultural, rural, and urban, each represented by four study plots. On 1km transects, we collected flowers together with their pollinators, which we then identified to species level. We used those data to calculate diversity indices. Next, 106 bees (agricultural: 29, rural: 46, urban: 31) belonging to 65 species were selected as pilot samples to establish microbiome composition using customised amplicon sequencing of the targeted marker gene 16S. We did not include the honeybee in the microbiome survey.

The microbiome was very diverse across bee species. We aligned 16S sequences to 2093 bacterial OTUs, of which 25% were already described as honeybee symbionts. In 90% of all samples we identified a facultative symbiont, *Wolbachia*, described as a beneficial symbiont mitigating the course and possible effects of viral infection. We calculated the correlation between eight environmental and seven microbial parameters for each landscape type. In rural sites, none of the correlations was statistically significant. In agricultural landscapes, we found three significant negative correlations between honeybee abundance and total abundance of bacteria, total abundance of symbionts, and percentage of symbionts to total bacteria. In urban sites, there were significant negative correlations between Wolbachia abundance and plant/bee Shannon-Wiener index or species richness. Moreover, abundance of honeybees caught on transects positively correlated with the percentage of *Wolbachia* in the whole microbiome. Our research has also demonstrated that the amplicon sequencing technique works appropriately for a wide range of bee species.

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MICROBIOTA

POSTERS

HONEYBEE GUT MICROBIOTA MODULATION IN EXPERIMENTALLY INFECTED WORKERS WITH *VAIRIMORPHA CERANAE*

Aguado-López, Daniel. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Urbieta-Magro, Almudena. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Rodríguez-Gómez, Juan Miguel. Departamento de Nutrición y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid (UCM), Madrid,
Higes-Pascual, Mariano. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain;
Martín-Hernández, Raquel. IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain; Fundación Parque Científico y Tecnológico de Castilla—La Mancha (INCRECYT-FSE/EC-ESF), Albacete, Spain

Vairimorpha ceranae is an obligate intracellular parasite that infects the ventricular cells of honeybees. Previous studies showed that the youngest adult worker bees were more susceptible to the infection than the older ones. In this work we analyse how the age of parasite infection interferes in the establishment of gut bacterial communities, as there is growing evidence for the role of an adequate microbiota in bee health. For this purpose, the main bacteria that are part of the normal gut microbiota of honeybees were analysed by qRT-PCR in adult workers of different ages. Those bees had been infected in the laboratory with the same number of *N. ceranae* spores and euthanized after 7 days. Results were compared with those from uninfected bees as a control group. *N. ceranae* spore solution used to infect the bees was also analysed to check for the presence of bee gut bacteria. A preliminary analysis of the results showed that *Gilliamella apicola, Lactobacillus* Firm-4 and Firm-5, *Bifidobacterium asteroides* and *Bartonella apis* were part of the gut microbiota in all bees (both infected and controls) of all ages. However, *Snodgrassella alvi, Frischella perrara* and *Bombella apis* were not detected at some ages. There was a higher number of bees positive for all bacteria when the bees were infected just after emergence. Spore suspensions were negative to all bacteria. In terms of bacterial load, the highest level of *G. apicola* was found in the bees infected just after emergence (both infected and not). *S. alvi* and *Lactobacillus* Firm-5 had higher loads when the bees were infected at day 11 and 14 a.e. *B. asteroides* load was higher in older infected bees and *B. apis* in older controls. *Lactobacillus* Firm-4 load was highest in bees infected at one day a.e. and *F. perrara* load was highest in bees infected at day 4 and 14 a.e. *B. apis* recorded its highest load on bees infected at day 13 a.e. These results suggest that experimental infection with *N. ceranae* may influence the establishment of the honeybee gut microbiota by modulating it.

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Keywords: Microbiota, *Nosema*, Infection

MECHANISM OF PROTECTION OF THE MICROBIOTA AGAINST CRITHIDIA BOMBI IN *BOMBUS TERRESTRIS*

Blasco-Lavilla, Nuria, Department of Zoology and Anthropology, University of Murcia, Murcia, Spain
López-López, Alejandro, Department of Invertebrate Evolution, Jagiellonian University, Kraków, Poland
De la Rúa, Pilar, Zoology and Anthropology Department, University of Murcia, Murcia, Spain
Barribeau, Seth, Department of Evolution, Ecology and Behaviour, University of Liverpool, United Kingdom

The microbiota of the commercial bumblebee species *B. terrestris* and *B. impatiens* contribute to their resistance against the common virulent trypanosomatid parasite *Crithidia bombi.* Previous studies have found different mechanisms by which the microbiota protect these insects: gut bacteria influence the immune response in honey bees and bumblebees. The gut symbionts *Gilliamella apicola* and *Snodgrassella alvi* form a biofilm on the bumblebee gut wall that might complicate *C. bombi* adhesion, and the symbiont *Lactobacillus bombicola* acidifies the gut sufficiently to inhibit *C. bombi* growth. However, the exact mechanisms by which gut microorganisms protect the host remain unknown.

Here, we test two hypotheses: that the gut microbiota 1) protects the host by stimulating its immune response or 2) protects the host by competing for resources with the parasite within the gut. To test these, we experimentally reduced the microbiota of *B. terrestris* workers and then experimentally re-established the normal gut community with microbiota supplements or left them microbially diminished. We then exposed them to an infectious dose of *C. bombi*, characterised the expression of four immune genes and determined the composition of gut microbiota after infection.

Although we do find that bees with supplemented microbiota were protected against *C. bombi*, we did not detect elevated immune gene expression that could alone explain this protection. Nevertheless, we found a higher relative abundance of *Lactobacillus* bacteria in the gut of infected workers and a negative correlation of this genus with the genera *Gilliamella* and *Snodgrassella*. Therefore, our results point to a displacement of bumblebee endosymbionts by *C. bombi* that might be caused by competition for resources between the parasite and the microbiota within the gut.

Keywords: *Bombus terrestris, Crithidia bombi*, microbiota

^{1}H-NMR METABOLITES ANALYSIS OF WORKER BEE GUTS (*A. MELLIFERA*) AFTER APPLYING BACTERIAL STRAIN SUPPLEMENTS

Lee, Saetbyeol, Food science, Czech University of Life Sciences Prague, Prague, Czechia
Killer, Jiri, Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Prague, Czechia
Kamler, Martin, Bee Research Institute Dol, Libcice nad Vltavou, Czechia
Titera, Dalibor, Bee Research Institute Dol, Libcice nad Vltavou, Czechia
Havlik, Jaroslav, Food science, Czech University of Life Sciences Prague, Prague, Czechia

Numerous studies have demonstrated that gut microbiota plays critical roles in host physiology, metabolism, health, and immunity. In honey bees, gut microbiota associates with improved honey bee health. This study used proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to analyze the metabolic characteristics of worker bees' gut depending on the supplementation of potentially probiotic strains. Newly emerged, age-synchronized bees were collected from the brood comb. They were kept in cages (100 per cage), and fed with a sugar cake containing one of four bacterial strains $15*10^9$ cells (*Lactobacillus helsinborgensis, Lactobacillus kimbladii, Bifidobacterium asteroides*, and *Bifidobacterium coryneforme*) for five days. Additionally, a germ-free sterile group was fed only sugar cake. The sugar cake ingredients and cage were gamma-irradiated for sterility. On the fifth day, each group of bees was color-labeled on their thorax and transplanted into five different colonies. On the tenth day, we re-collected the color-labeled bees together with randomly selected nurse bees at the brood combs from each colony. Five bees in each group were used for the analysis. Individual guts were homogenized and extracted with methanol and analyzed by NMR-based metabolomics coupled with chemometric analysis, covering approx. 50 metabolites. The statistical analysis was conducted in MetaboAnalyst.

Transplanted bees showed significant differences from the randomly selected nurse bees as seen in an Orthogonal Partial Least Squares Discriminant Analysis (Q^2=0.62, R^2Y=0.76), showing lower concentrations of an unknown singlet at 2.83 ppm, trehalose, proline, and other metabolites. Linear models with covariate adjustments were conducted to explore the effect of probiotic supplementation on gut metabolite profiles and showed that the germ-free group had significantly higher beta-alanine and proline concentrations than in the treatment groups ($P < 0.005$). However, the authors cannot overlook that these results may be attributed to different food collection patterns amongst the hives, stress during the transplanting process, and microbiota development and maturation. The information gathered from this study provides new insights into honey bee gut function.

This research is a part of the grant project (PROBEE: QK21010088), supported by the Ministry of Agriculture of the Czech Republic.

Keywords: *Apis mellifera*, Gut metabolites, Nuclear magnetic resonance

MAPPING POST-ECLOSION DEVELOPMENT OF THE HONEY BEE GUT AT SINGLE-CELL RESOLUTION AND IMPACT OF THE MICROBIOME

Campos, Ana Claudia; Kwong, Waldan. Microbial Genomics and Symbiosis, Instituto Gulbenkian de Ciência, Oeiras, Portugal

The microbiome is known to affect gut development as well as host immunity. The cells of the gut lining are the major interface between animal hosts and most microorganisms they encounter. In bees, the presence of the microbiome and gut organ integrity are important in maintaining health of the host, both for nutritional processing and in defense against pathogens. In our proposed work, we will examine how the adult bee gut develops in the presence of their normal microbiome, through the lens of gene expression.

To do this, we will use single cell RNA sequencing (scRNA-seq) to 1) identify the types of cells in the bee hindgut, 2) determine the spatial localization of these cells, 3) identify shifts in their expression at different timepoints during post-eclosion development, and 4) determine the influence of the microbiome on normal gut cell development. In achieving these objectives, we will create for the first time a detailed cellular map of the bee gut, integrating spatial, temporal, and symbiotic perspectives.

This will provide a framework for future studies investigating host responses to microbial and environmental perturbations, which will help us to develop better targeted interventions to improve bee health.

Keywords: Gut development, Gut microbiota, scRNA-seq

AMERICAN FOULBROOD AND LACTIC ACID BACTERIA IN SWEDISH HONEYBEES

Nilsson, Anna, Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
D'Alvise, Paul, Department of Livestock Population Genomics, Institute of Animal Science University of Hohenheim, Stuttgart, Germany.
Milbraith, Meghan, Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
Forsgren, Eva, Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

American foulbrood (AFB) is a widespread honeybee brood disease affecting apiculture worldwide. It is caused by the spore forming bacterium *Paenibacillus larvae*, and is not only lethal to individual larvae but also to entire honeybee colonies. Honeybee-specific lactic acid bacteria (hbs-LAB) belonging to the honeybee microbiome have been shown to have an inhibitory effect on *P. larvae in vitro.* Here, we wanted to look at the relationship between the composition of hbs-LAB in honeybees and AFB. Honeybee samples were collected in Sweden 2018 from areas with AFB from 41 colonies with AFB, asymptomatic colonies with *P. larvae* and colonies without *P. larvae*. The microbiome composition of hbs-LAB was analysed using a high-throughput qPCR platform together with specific primers designed and optimised for 11 hbs-LAB (nine lactobacilli and two bifidobacteria). The hbs-LAB composition and the abundance of each hbs-LAB was compared between different outbreaks and colonies with different AFB status. While we found small differences in the abundance of the individual hbs-LABs and a higher diversity in colonies with *P. larvae* than in the colonies without the pathogen, no differences in abundance or diversity between colonies with or without AFB symptoms. *Apilactobacillus kunkeei,* which has been suggested as the most common and abundant hbs-LAB microbiome, was only identified in 29.3% of the samples. The results shows that there are differences in the hbs-LAB microbiome, but further studies are needed to better understand the relationship between hbs-LAB and AFB, and what other factors that affects the hbs-LAB microbiota.

ECOTOXICOLOGY, PESTICIDES

ORAL PRESENTATIONS

CHAIRPERSONS
Alpar Barsi
Johannes Lückmann

DETERMINATION OF TOXIC AND POTENTIALLY TOXIC ELEMENTS USING ICP-OES IN HONEY AS A BIOINDICATOR OF THE POLLUTION OF THE ENVIRONMENT

Arsić, Biljana, Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Niš, Republic of Serbia
Kostić, Danijela, Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Niš, Republic of Serbia
Georgijev, Aneta, Timomed d.o.o., Knjaževac, Republic of Serbia
Tošić, Snežana, Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Niš, Republic of Serbia

Honey is a food rich in minerals, vitamins, and phenolic compounds. Their determination in honey samples is proof of their authenticity and quality today. Minerals significantly contribute to the conductivity of honey. However, not all elements in honey have positive values for our health. Some of them are toxic or potentially toxic, and therefore, honey is a bioindicator of the pollution of the environment. Selected honey samples from the territory of Serbia were analysed on toxic and potentially toxic elements (Al, As, Pb, Cd, Hg) using ICP-OES. The samples for ICP-OES were prepared using four different methods. The highest found concentration among the investigated samples of aluminium was 9.43 mg/kg, lead 0.43 mg/kg, arsenic 2 mg/kg, cadmium 0.034 mg/kg, and mercury 0.22 mg/kg. Our found aluminium, lead, arsenic and cadmium concentrations are higher than those in Romanian honey samples. Similar results to ours for lead were found in honey samples from Greece where like in our case, some values exceeded the new recommended value for lead (0.1 mg/kg) but were less than the previously used limit value (1 mg/kg). Aluminium values in investigated Greek honey samples were like our found values, and arsenic was similar or lower than ours.

Keywords: Honey, ICP-OES, Bioindicator

TRANSLOCATION OF TEBUCONAZOLE BETWEEN BEE MATRICES AND ITS POTENTIAL THREAT ON HONEY BEE (*APIS MELLIFERA* LINNAEUS) QUEENS

Raimets, Risto,[a] Mänd, Marika,[a] Naudi, Sigmar,[a] Bartkevics, Vadims,[b] Smagghe, Guy,[c] Karise, Reet,[a]

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia,[a], Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes Street 3, Riga, LV-1076, Latvia,[b], Department of Crop Protection, Ghent University, B-9000 Ghent, Belgium,[c]

Various pesticide residues have been found in different bee products. Nevertheless, there is few information whether and in which concentrations pesticides translocate between different bee products. Majority of scientific studies are focusing on insecticides effects on honey bee (*Apis mellifera* L.) workers, while fungicides and honey bee queens have received little attention. Developing honey bee queen receives non-contaminated food (royal jelly) from nurse bees. However, there is little knowledge of how and in which concentrations lipophilic fungicides translocate from one bee matrice to another.

The aim of current study was to determine fungicide tebuconazole translocation from queen cell wax to RJ, queen larvae and newly emerged queens and to evaluate its potential hazard to queens.

The results showed that fungicide tebuconazole successfully translocated from wax to royal jelly with strong dilution effect. Nevertheless, no residues were detected in queen larvae and newly emerged queens. Residue concentrations detected do not possess any direct threat to honey bees, however these might still cause changes in bee homeostasis.

Keywords: Translocation of pesticides, Tebuconazole, Honey bee queen

A REVIEW ABOUT PESTICIDE RESIDUES IN LARVAL FOOD JELLY OF THE WESTERN HONEY BEE *APIS MELLIFERA*

Wueppenhorst, Karoline, Institute for Bee Protection, Julius-Kuehn Institute, Braunschweig, Germany
Eckert, Jakob H., Institute for Bee Protection, Julius-Kuehn Institute, Braunschweig, Germany
Steinert, Michael, Institute for Microbiology, Technische Universität Braunschweig, Braunschweig, Germany
Erler, Silvio, Institute for Bee Protection, Julius-Kuehn Institute, Braunschweig, Germany

Honey bees are of public interest because of their pollination service, and production of honey, wax, and propolis. Further, they are considered as model organisms in pesticide risk assessment on pollinators. The stored nectar and pollen serve as nutritional source for nurse bees to produce the food jelly for queen, worker, and drone larvae. However, different bee-related products like honey, bee bread, wax, and pollen have been proven to be contaminated with pesticides and contaminants might be transferred into the larval food jelly. The study aimed to review and assess the amount of contaminants remaining in jelly, to evaluate factors influencing their occurrences, and to deduce risk for larvae. Therefore, the current literature dealing with residue analysis of pesticides in food jelly was summarized and analyzed. All studies determined residues in royal jelly, and only one in worker jelly. The application and exposure method are the main factors influencing if residues remain detectable in jelly. It was demonstrated that 30 out of 176 analyzed pesticides were detectable in a range of 0.005 to 3860.25 ng/g in different royal jelly samples, which corresponds to 0.00001% to 58% pesticide transfer into the jelly. All detected concentrations were predominantly below toxicological values for bee larvae, but sub-lethal effects should not be neglected. Nevertheless, there are still knowledge gaps about the contamination pathway of pesticides, dilution or accumulation factors within the hive, degradation time in bee-related matrices, and impact on larval physiology, which should be filled to allow for sufficient protection levels of honey bees.

Keywords: royal jelly, contamination flow, larval development

ANALYZING PESTICIDE RESIDUES IN DAMAGED BEEHIVES IN ISRAEL

Afik, Ohad, The Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan, Israel

Honeybees are the main pollinators of agricultural crops. However, they also suffer from unintentional damage due to the use of agricultural pesticides. Implausibly, it isn't rare to find beehives damaged by pesticide application at their pollinated crops. Israel is unique for its' wide variety of crops grown in a limited area, emphasizing this challenge, and creating a great risk for the local beekeeping industry. Every year, we assume that hundreds of hives are damaged, recognizable by a sudden accumulation of dead bees at the hive entrance. Often the colonies survive these events, nonetheless they are generally left in a weakened state. During the past five years, 42 poisoning events were investigated following beekeeper complaints, warranting a wide scan of chemical residues in dead bees using GC/MS and LC/MS. More than 30 different chemicals were detected, including mainly insecticides, but also herbicides and fungicides, with a maximum of seven chemicals in a single sample. The most common chemicals were herbicides oxyfluorfen and diuron, which are not considered to be toxic to honeybees, and DMPF, an amitraz byproduct. Among the insecticides considered to be highly toxic to bees, imidacloprid and methiocarb were the most common, as well as abamectin, bifenthrin and methomyl - all found in multiple samples. Reports from beekeepers occurred year-round but were most common during springtime and early summer from March to June. Based on these findings, we tried to assess the main risks for the bees and develop recommendations to beekeepers and farmers aimed at reducing damage to beehives. For instance, while imidacloprid is widely used in Israeli agriculture and hard to avoid, methiocarb damage is almost exclusively related to early blooming peaches and nectarines. Therefore, recommendations to remove beehives from these orchards during early spring, together with orchard growers shifting to night spraying or beehive-friendly insecticides, may reduce bee damage, while allowing effective treatment of the orchards. Mapping the main risks for bees and providing efficient solutions is a critical step towards maintaining our diverse agriculture, while preserving healthy beehives sufficient to answer local pollination needs.

Keywords: Insecticides, Imidacloprid, Methiocarb

A SINGLE DOSE, BUT LONG TERM EFFECT: THE CASE OF SULFOXAFLOR IN HONEYBEES

Barascou Lena[1], Requier Fabrice[2], Sene Deborah[1], Crauser Didier[1], Le Conte Yves[1], Alaux Cedric[1]

1 Abeilles et Environnement, INRAE, Avignon, France
2 UMR Évolution, Génomes, Comportement et Écologie, Université Paris-Saclay, CNRS, IRD, Gif-sur-Yvette, France

Pesticide risk-assessment guidelines for honeybees (*Apis mellifera*) generally require determining the acute toxicity of a chemical over the short-term through fix-duration tests (e.g. 48 hr.). However, potential long-lasting or delayed effects resulting from a single dose exposure are often overlooked, although alteration of some bee physiological and/or developmental processes may have life-long consequences. To investigate this question, we exposed young honeybee workers to a single sublethal field-realistic dose of a neurotoxic pesticide, sulfoxaflor, at one of two amounts, at the moment when they initiated orientation flights (preceding foraging activity). We then tracked in the field their flight activity and lifespan with bee counters. We showed that both amounts of sulfoxaflor administered reduced the total number of flights but did not affect bee survival and flight duration. When looking at the time series of flight activity, effects were not immediate but delayed until foraging activity with a decrease in the daily number of foraging flights and consequently in their total number. The results of our study therefore blur the general assumption in honeybee toxicology that acute exposure results in immediate and short-term effects and call for long-term recording and/ or time-to-effect measurements, even upon exposure to a single dose of pesticide.

Keywords: pesticides, delayed effects, foraging flights

EFFECT OF SDHI FUNGICIDE DURING PREMATURE AND REPEATED EXPOSURE ON *APIS MELLIFERA*

Desclos Le Peley Victor; Laboratory of Ecology and Biology of Interactions (UMR CNRS 7267) ; Université de Poitiers ; France
Grateau Stephane; Experimental Unit Bees, Landscapes, Interactions and cropping Systems (APIS); National Research Institute for Agriculture, Food and the Environment (INRAE); Surgères; France
Raboteau Daniel ; Experimental Unit Bees, Landscapes, Interactions and cropping Systems (APIS); National Research Institute for Agriculture, Food and the Environment (INRAE); Surgères; France
Moreau-Vauzelle Carole; Experimental Unit Bees, Landscapes, Interactions and cropping Systems (APIS); National Research Institute for Agriculture, Food and the Environment (INRAE); Surgères; France
Aupinel Pierrick; Experimental Unit Bees, Landscapes, Interactions and cropping Systems (APIS); National Research Institute for Agriculture, Food and the Environment (INRAE); Surgères; France
Requier Fabrice; Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie; Gif-sur-Yvette; France
Richard Freddie-Jeanne; Laboratory of Ecology and Biology of Interactions (UMR CNRS 7267); Université de Poitiers; France

Bees provide a critical function of pollination that supports crop yield and biodiversity. However, bee populations are declining worldwide due to multiple factors acting separately or in combination, such as loss of habitat and resources, pests (viruses, bacteria, parasites), and pesticide exposure. The increase number of threats to bees is source of concern among scientists, governments and citizens.

The sublethal effects of pesticides on non-target species are complex to study and likely underestimated. For instance, the SDHI (succinate dehydrogenase inhibitor) fungicides negatively affects non-target organisms such as earthworms and some insects with consequences on reproduction and metabolism. Sublethal consequences could lead to loss of capacities and a decrease in longevity, which is more challenging to evaluate than direct mortality. Boscalid, SDHI fungicide, is wildly applied on field crops such as rapeseed or fruit trees. Honey bees (*Apis mellifera*) are exposed to this molecule at different stage, of their life (development, and adults) and repeatedly through their diet. Our objectives are to evaluate the Boscalid sublethal effects during premature and recurrent exposure. We thus compared the life history of adult honey bees previously exposed or not to boscalid. Moreover, we also compared individuals exposed at the larval stage under laboratory conditions and at the emergence and at three different time of the season. To do so, bees were individually marked with RFID (Radio-Frequency IDentification) microchips and their activity followed in field conditions. In total we marked and followed 3299 workers and monitored foragers activities (number and duration of flights) and life history traits (age of first exit, lifespan). . We will present our results on the effect of Boscalid exposure on lifespan and their foraging activities. We will discuss these results in the context of global increase in pesticide consumption. The results will help understanding of the consequences of pesticides on non-target organisms and inform towards recommendations of management practices for the conservation of bees, pollination services and biodiversity in farmlands.

Keywords: Larvae rearing; Flight activities; Fungicides

PESTICIDE RESIDUES IN *APIS MELLIFERA* BEEBREAD COLLECTED IN THE FIRST YEAR OF THE ITALIAN MONITORING PROJECT BEENET

Dettori, Amanda, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bogo, Gherardo, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Caringi, Valeria, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Medrzycki, Piotr, CREA Research Centre for Agriculture and Environment, Bologna, Italy

“BeeNet – monitoring the environment through bees and biodiversity” is a scientific research project which monitors bees, in order to define the qualitative conditions of the agro-environment. BeeNet is composed by two monitoring networks (i.e. Honey Bee Network and Wild Bee Network) that can provide an overview of all bees and the environment where they live. The Honey Bee Network is composed of approximately 370 monitoring stations (five hives each), distributed throughout the Italian territory. By means of field observations and laboratory analyses, the project evaluates several parameters of colony health and growth (i.e. pest and pathogens, pollen quality, pesticide residues, and colony assessment), together with environmental parameters inside/outside the hives. Pesticide residues, assessed through multi-residual analysis (GC-MS/MS and LC-MS/MS) of beebread stored in the hive by honey bees, provide indications on pesticide presence in the environment. Beebread is collected twice a year during spring (March and June), the period characterized by the highest treatment pressure in agriculture. To assure high representativity of the samples, for each station beebread is collected from at least three points in three different frames of each hive. Here we present the results of the multi-residual analyses of beebread samples collected in the framework of the Honey Bee Network and the colony loss incidents reported by beekeepers, during the first year (2021) of the project. Results show that most of the samples contained residues of at least one pesticide, some reaching up to approximately 20 different compounds, and highlight the presence of several active ingredients which are currently not approved for use in the EU.

Keywords: honey bees, plant protection products, pollen

CATCH CROPS AND ECOLOGICAL FOCUS AREAS ARE THEY UNDERESTIMATED PESTICIDE EXPOSURE ROUTES FOR POLLINATORS?

HAUTIER Louis, Unité Santé des plantes et forêts, Centre wallon de Recherches agronomiques, Gembloux, Belgium
SAN MARTIN Gilles, Unité Santé des plantes et forêts, Centre wallon de Recherches agronomiques, Gembloux, Belgium
BRUNEAU Etienne, Centre Apicole de Recherches et d'Information CARI, Belgium
ROUSSEAU Gilles, Unité produits de protection, de contrôle et résidus, Centre wallon de Recherches agronomiques, Gembloux, Belgium
SIMON-DELSO Noa, BeeLife, Bruxelles, Belgium

Pesticides are well identified as potential bee's stressors. To reduce stress and protect bees, identification of resources contaminants and exposure ways is crucial. In a previous study, we detected residue of pesticides in pollen pellets harvested weeks before the overwintering. Whereas at this time crop spraying is limited, at least one-pesticide residues contaminated almost half of samples with notably the presence of the fungicide boscalid and the insecticide dimethoate. Palynological analyses revealed these contaminations could be predicted by the presence of *Phacelia tanacetifolia* pollen. Lacy phacelia is used by the farmers as catch crop or more recently as ecological focus areas (EFA). To assess this potential exposure route by catch crop flowers, samples of flower tops of white mustard were collected in the Walloon region, Belgium. In the autumn of 2017 and 2018, 64 and 50 catch crop flowers field were sampled. In addition, honeybee colonies were also placed in some fields to harvest pollen pellets during the catch crop blooming. The multiresidues analyses by LC-MS/MS and GC-M/MS confirmed contamination by mainly: fungicides like cyproconazole or azoxystrobin but also herbicides like flufenacet, chlorotoluron and insecticides like dimethoate or imidacloprid. According to the physicochemical characteristic of pesticide and agricultural uses, the contamination by systemic products is most probably explained by the persistence in the soil of products applied in spring on the main crop. For non-persistent products, drift from a neighbouring field could largely explain the residues detected on these untreated surfaces. At the end of the season, bees can therefore be exposed to various pesticides through catch crops or EFA. This exposure is all the more important, as the food resources available to bees at the end of the season are limited. In addition, these resources are stored and used throughout the winter. This raises the question of the risk to winter bees of chronic exposure to pesticide mixtures via beebread or honey from catch crop or EFA.

Keywords: honeybee, flowers, pollen pellets

VALIDATION OF THE HOMING FLIGHT TEST BY OECD TO ASSESS THE EFFECTS OF LOW DOSES BEFORE PESTICIDE HOMOLOGATION

Fourrier, Julie, ITSAP-Institut de l'Abeille, UMT PrADE, INRAE UR 406 Abeilles et Environnement, Avignon, France
Aupinel, Pierrick, INRAE, UE APIS, Centre de recherche Nouvelle-Aquitaine-Poitiers, Surgères, France
Moreau, Carole, INRAE, UE APIS, Centre de recherche Nouvelle-Aquitaine-Poitiers, Surgères, France
Chevallereau, Colombe, INRAE, UE APIS, Centre de recherche Nouvelle-Aquitaine-Poitiers, Surgères, France
Henry, Mickaël, INRAE, UR 406 Abeilles et Environnement, UMT PrADE, Avignon, France
Decourtye, Axel, ITSAP-Institut de l'Abeille, UMT PrADE, INRAE UR 406 Abeilles et Environnement, Avignon, France

Until now, the risk assessment on honeybees before pesticide registration was mainly based on the direct mortality of individuals (lethal effects). With the revision of pesticide risk assessment on the honeybee by European authority (EFSA, 2013), a European ring test was conducted from 2015 to 2019 with 11 laboratories to test a methodology assessing the effects of sublethal doses of a plant protection product on the homing capacity of forager bees. Homing success is measured by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology. Results reproducibility was assessed in different contexts for the method validation. The main steps of the protocol finalized in 2019 consists in i) the capture of foragers coming from a site located at 1 km (+/- 100 m) from the colony to ensure that the bees have a prior knowledge of the pathway back to the colony, ii) oral exposure of the RFID-tagged foragers to 3 sublethal doses of a reference insecticide molecule (thiamethoxam) or not (control) in the laboratory, iii) release of the tagged foragers on the known site and record of the homing success at the hive entrance with the RFID system for a period of 24 hours after release. The ring test showed the sensitivity of the method for measuring the effects of low doses of pesticides. In the last year, 75% of the laboratories successfully conducted the test at the acceptance thresholds decided as validity criteria and by determining a No Observable Effect Level (NOED) on the homing success as a test endpoint. In 2018 and 2019, we also assessed the variability of the homing flight ring test results by considering the explanatory factors in particular for bees exposed to the insecticide. These factors include the landscape context and climatic conditions (Henry et al. 2014) or the health status of the colonies, in particular Varroa load (Monchanin et al. 2019). An aggravating effect of Varroa on the rate of homing failure of the bees exposed to the doses with effect was observed in 2018. The method is now validated and available online (GD OECD n° 332, 2021).

Keywords: sublethal doses, ring test, homing flight

DIFFERENT AGONISTS ACTIVATE DIFFERENT SIGNALING PATHWAYS IN *APIS MELLIFERA* AND *VARROA DESTRUCTOR* OCTOPAMINE RECEPTORS

Anaïs Bertaud[1,2], Thierry Cens[1], Matthieu Rousset[1], Jean-Baptiste Thibaud[1], Claudine Ménard[1], Janique Guiramand[1], Michel Vignes[1], Michel Vivaudou[2] & Pierre Charnet[1]

1 Pharmacochemistry of synaptic transmission and neuroprotection group, IBMM, Montpellier, France
2 Channels group, Institut de Biologie Structurale, Grenoble, France

Octopamine receptors (OctoR) are G protein-coupled receptors specific to invertebrate, which are expressed in many neurons in *Apis mellifera*. They control or regulate neuromuscular transmission, learning, memory or responses to odorant by modulating responses of odorant receptors, interneurons or motoneurons. In honeybee, 5 types of OctoR have been identified, with functional homology to α or β adrenoreceptors, able to activate different intracellular signaling pathways (Gs, Gi/o, Gq), although no precise characterization has been done yet. Amitraz, a synthetic OctoR agonist is used extensively in the hive worldwide to fight against the *Varroa destructor*, a parasite mite partly responsible for the increasing number of colonies collapse disorders recorded last years. The specificity of amitraz for the honeybee or varroa OctoR is however is not really known as well as the precise pharmacology and signaling profiles of the mite OctoRs.

We have cloned the 5 honeybee and 2 varroa OctoR and expressed them in oocytes to (1) analyze their pharmacological profile (octopamine, tyramine, dopamine and amitraz) and (2) identified the signaling pathway they activate.

Our data show that these OctoR can activate multiple G-protein dependent pathways (Gi/o, Gs, Gq) but can also activate directly (G-protein independent) a cation channel that is tentatively identified as TRPC in Xenopus oocyte. The presence of these different types of responses is dependent upon the type of receptors expressed and the agonist used.

We now verify the existence of these OctoR-specific signaling pathways in bee neurons from the antennal lobes and mushroom bodies.

PAN-EUROPEAN ASSESSMENT OF THE EXPOSURE OF HONEYBEES, BUMBLEBEES AND SOLITARY BEES TO PESTICIDE RESIDUES DETECTED IN BEEBREAD AND POLLEN STORES

Kiljanek, Tomasz[1], Goliszek, Milena[1], Małysiak, Marta[1], Burek, Olga[1], Łusiak, Patrycja[1]

1 Department of Pharmacology and Toxicology, National Veterinary Research Institute, Puławy, Poland

One of the main objectives of the PoshBee project is a Pan-European assessment of the exposure of honeybees, bumblebees and solitary bees to pesticides. In eight European countries (Spain, Italy, Germany, Switzerland, Ireland, UK, Estonia and Sweden), a network of flowering sites of winter oilseed rape and apple trees was set up and colonies of honeybees, bumblebee nests and solitary bees were placed in the vicinity. During this time, farmers carried out unchanged crop protection. After the end of the flowering period, samples of beebread were taken from the bee colonies, and pollen stores collected from the nests of bumblebees and solitary bees. The samples were then analysed to determine the pesticide residues.

In a first step, each sample was analysed using a specially developed multiresidue method allowing the simultaneous determination of 261 pesticides that are active substances of plant protection products or varroacides and 6 PCB congeners [1]. Pesticide residues were detected in more than 98% of the 320 samples analysed. In total, residues of 146 pesticides from different application groups (fungicides - 53, insecticides - 50, herbicides - 28, acaricides - 7, growth regulators - 4, varroacides - 3, PCBs - 1) were detected in bee bread/pollen store samples. In half of the samples tested residues of at least 10 pesticides were detected at the same time, with significant differences between results for samples from different countries tested. At the same time, residues of up to 32 different pesticides were detected in a sample of bee bread/pollen store.

The samples were further tested for glyphosate, AMPA and glufosinate-ammonium residues using another dedicated method. Residues of glyphosate or AMPA were detected in approximately 25% of the 288 beebread/pollen store samples tested. These results rank glyphosate nineteenth among the pesticides most frequently detected in the samples tested.

A preliminary toxicological assessment of the results obtained indicates a risk of exceeding the lethal doses (LD_{50}) in the scenario of exposure of bees to the maximum concentrations of some of the detected pesticides.

[1] Kiljanek T. et al. Miniaturized multiresidue method for determination of 267 pesticides, their metabolites and polychlorinated biphenyls in low mass beebread samples by liquid and gas chromatography coupled with tandem mass spectrometry. Talanta 235 (2021) 122721.

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Keywords: Pesticides, beebread, exposure assessment

EFFECTS OF FUNGICIDE EXPOSURE ON REPRODUCTION IN *APIS MELLIFERA*: AN INTEGRATIVE STUDY

Pineaux, Maxime[1,2], Gomes, Elisa[1,2], Aupinel, Pierrick[1], Richard, Freddie-Jeanne[2]

1 Unité expérimentale d'entomologie, INRAE, Surgères, France
2 Laboratoire Ecologie et Biologie des Interactions UMR CNRS 7267, Université de Poitiers, Poitiers, France

In increasingly anthropized ecosystems, organisms face multiple environmental stressors that have consequences on their health, fitness and behavior. Among them, pesticides are a major threat to biodiversity due to direct mortality or sublethal effects. Among sublethal effects on non-target organisms such as pollinator insects, pesticides can reduce flight and orientation abilities. However, the negative effects of pesticides on reproduction have been poorly studied. Such effects can nevertheless play a crucial role in the drastic decline of pollinator populations, especially in social insects for which a single individual, the queen, produces all offspring. In this study, we investigated the consequences of boscalid exposure on the reproduction of honeybee (*Apis mellifera*) queens, for which successful mating flights are essential to reproductive success and therefore colony performance. Boscalid is a fungicide that inhibits the activity of succinate dehydrogenase (SDHI) and hence alters mitochondrial respiration in fungi, but also in non-target organisms such as honeybees. Workers exposed to boscalid exhibit higher mortality, possibly due to flight and orientation impairment. However, consequences of boscalid exposure on queens are still unknown. We investigated these consequences at both individual (on queen reproductive quality and physiology) and colony levels. We found that field-relevant doses of boscalid, as a pure form and in commercial formula, disrupted mating flights with increased queen mortality and reduced sperm count in the spermatheca. Queens' exposure to boscalid also had an impact on the colonies they established (on brood production, quantity of stored pollen and *Varroa destructor* infection). Gene expression analyses suggest that these longer-term effects on colony performance might come from a disruption in energy reserves allocated to the eggs. Our integrative study (from queen physiology to colony development) therefore provides an important insight into the many impacts of pesticides on reproduction in non-target insects.

Keywords: honeybee queen, reproductive quality, boscalid

ARE WE TESTING INSECTICIDES CORRECTLY? EVALUATING INSECTICIDES WITH HIGH-RESOLUTION MOLECULAR APPROACHES

Witwicka, Alicja, Biology Department, Queen Mary University of London, London, UK
López-Osorio, Federico, Biology Department, Queen Mary University of London, London, UK
Pracana, Rodrigo, Biology Department, Queen Mary University of London, London, UK
Wurm, Yannick, Biology Department, Queen Mary University of London, London, UK

Social and solitary bees are important insect pollinators, yet their populations are declining because of intensified agriculture and the use of insecticides. Many authorised insecticides cause severe sub-lethal effects to multiple bee species, showing that the methods used in toxicity tests are inadequate. Toxicity assessments are typically based on the survival of so-called surrogate species (mainly *Apis mellifera* workers) under acute, short-term exposure. In real-life scenarios, insects experience long-term exposure to low doses of insecticides. Moreover, post-exposure effects observed in wild pollinators and the surrogate species may differ due to differences in life histories and physiologies. Using RNA-seq, we show (1) that the effects of exposure cannot be directly extrapolated between species and (2) that the effects of acute, short-term exposure are not comparable to field-realistic, chronic exposure.

Because many insecticides including neonicotinoids and their replacements target nicotinic acetylcholine receptors (nAChRs), we first examined the expression of these receptors. Each nAChR consists of five subunits, but a bee genome contains 10-15 subunit genes. In consequence, species can produce multiple receptor types with different shapes, functions, and affinity to insecticides. We find that the expression of individual subunits differs substantially between tissues, developmental stages, and castes of *Bombus terrestris* and *Apis mellifera*, providing a mechanistic explanation for the diverse effects of nAChR targeting insecticides.

Second, we present differences in gene activity between brains of *Bombus terrestris* exposed to three cholinergic insecticides: Clothianidin, Acetamiprid, and Sulfoxaflor. We compare chronic, low-dose exposure and acute, high-dose exposure. We show that the effects of insecticides on gene expression vary significantly between pesticides, but also between chronic and acute exposure.

Overall, our work highlights how high-resolution molecular approaches can improve our understanding of the effects of insecticides on bee health. Such toxicogenomic approaches, which are widely used in assessments of drugs for humans, are the best way forward to test the effects of insecticides on non-target species. We anticipate that such work can provide essential information for regulatory bodies and improve insecticide safety assessments.

Keywords: insecticide assessments, transcriptomics, toxicogenomics

NEONICOTINOIDS, CANOLA AND HONEY BEES IN SASKATCHEWAN, CANADA

Wood, Sarah; Kozii, Ivanna; Silva, Marina; Silva, Roney; Klein, Colby; Zabrodski, Michael; Dvylyuk, Ihor; Koziy, Roman; Epp, Tasha; Ferrari, Maud; Simko, Elemir.

Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

Saskatchewan, Canada, is a worldwide leader in canola production and Saskatchewan is home to a vibrant beekeeping industry which supports Saskatchewan agriculture through pollination services. Saskatchewan is also a global leader in the use of neonicotinoid insecticides, with some of the highest residues of the neonicotinoid insecticides in honey and pollen reported worldwide. Discrepancy in the scientific literature regarding the effects of neonicotinoid residues on non-target insects such as honey bees has fostered government re-evaluation of neonicotinoid use in Canada. We investigated chronic exposure of honey bee colonies to the high levels of neonicotinoid residues found in this province. We found that chronic exposure of nucleus colonies to 20 ng/g neonicotinoids significantly decreased colony weight gain (honey production) by 30% and decreased adult bee cluster size by 21%. Chronic exposure to 100 ng/g thiamethoxam significantly decreased overwinter survival of strong fall colonies by 55%, with 20 ng/g thiamethoxam resulting in a statistically nonsignificant 15% decrease in overwinter survival. In addition, we evaluated larval survival, reproductive fitness and histopathology of honey bee queens exposed to to incremental doses (0, 5, 50ng) of the neonicotinoid thiamethoxam (THI) applied directly to individual late larvae of honey bee queens. The 5ng dose represents the highest calculated environmental level of exposure for honey bee queen larvae in Saskatchewan. Morphometric evaluation revealed that the total area of mandibular gland epithelium in queens exposed to 5 and 50ng THI was reduced by 12.1% and 26.2% respectively, the latter being statistically different from control. Decreased mandibular gland size may alter pheromone production which could in part explain previously observed negative effects of THI on the reproductive fitness of queens. We also found that late larval exposure to THI reduced larval and pupal survival and decreased sperm viability in mated queens. These changes may interfere with queen development and reproductive longevity. In light of these findings, chronic environmental exposure of honey bees to neonicotinoids should be maintained at levels below 20 ng/g to ensure Saskatchewan honey bee colonies and Saskatchewan agriculture continue to thrive.

ECOTOXICOLOGY, PESTICIDES

POSTERS

IMPACT OF PESTICIDE MIXTURES ON PHYSIOLOGICAL BIOMARKERS: A COMPARISON BETWEEN THE AFRICANIZED HONEY BEE *APIS MELLIFERA* AND NATIVE STINGLESS BEES

TAVARES, Daiana Antonia[1]; GRELLA, Tatiane Caroline[1]; DORIGO, Adna Suelen[1]; NOCELLI, Roberta Cornélio Ferreira[2]; MALASPINA, Osmar[1].

1 São Paulo State University (UNESP), Institute of Biosciences, Department of General and Applied Biology, Rio Claro, São Paulo, Brazil.
2 Center for Agricultural Sciences, Department of Natural Sciences, Mathematics and Education, Federal University of São Carlos, UFSCar, Araras, São Paulo, Brazil.

The importance of bees for the maintenance of natural systems and for agroecosystems is widespread. In Brazil, in addition to the exotic subspecie Africanized *Apis mellifera* there is a diversity of native stingless bees such as *Melipona scutellaris* and *Scaptotrigona postica*. Seeking to understand the reduction of pollinators most studies whit pesticides carried out are with the honey bee *A. mellifera*, however, it is not known exactly whether the effects observed for this species can be extrapolated to other native species. Aiming to understand if the effects on foragers of Africanized honey bees *A. mellifera* are similar to the effects on native stingless bees foragers, we evaluated the impact of realistic field concentrations (0.7; 1.9 and 0.2 μg/L respectively) of three common pesticides: imidacloprid (I) glyphosate (H) and pyraclostrobin (F) isolated, in binary or ternary mixtures on the physiological biomarkers. After oral exposure and determination of the mean lethal time (TL_{50}) for each bee species and for each exposure, the enzymes modulation was evaluated: acetylcholinesterase (AChE), carboxylesterase (CaE-3), glutathione-S-transferase (GST), glutathione peroxidase (GP), glutathione reductase (GR), alkaline phosphatase (ALP) and glucose-6-phosphatase dehydrogenase (G6PDH) covering different physiological functions (metabolic, detoxification and oxidative stress). Among the main results we found that the three bee species studied were differently impacted, through the different enzymes evaluated. In general, for all species, a major effect on the modulation of biomarkers in the mixtures is notable than when the bees were exposed to isolated pesticides. A particularity was found for *S. postica*, it was the only species to have modulation of AChE in the binary mixture (insecticide + fungicide) and full abdomen CaE-3 in the fungicide and all mixtures. GST proved to be an efficient biomarker because in all biological compartments analyzed (with the exception of the abdomen of *M. scutellaris*) it was modulated. It is possible to assume that the results found here for Africanized *A. mellifera* cannot be extrapolated to the other species studied, since each one has a particularity. Furthermore, considering our data, *S. postica* can be considered a viable species for standard assessments in Brazilian ecotoxicology studies.

Keywords: Detoxification 1, imidacloprid 2, ecotoxicology 3

CHRONIC TOXICITY OF COPPER AND ZINC TO HONEYBEES (*APIS MELLIFERA CARNICA*)

Glavan, Gordana; Department of Biology; University of Ljubljana, Biotechnical Faculty; Ljubljana, Slovenia
Božič, Janko; Department of Biology; University of Ljubljana, Biotechnical Faculty; Ljubljana, Slovenia

Environmental contamination with metals can negatively affect honeybee colonies. Copper (Cu) and zinc (Zn) salts are frequently used as fungicides and leaf fertilizers. Here we evaluated the effects of 10-days chronic oral exposure to different concentrations of Cu and Zn on honeybee survival and feeding rate. Also, 24 hours two-choice feeding experiments was performed to test feeding preference or avoidance for Cu and Zn in sucrose solution. We found that mortality increased in honeybee workers in a concentration-dependent manner and that Cu (LC_{50} = 66 mg/L) is more toxic than Zn (LC_{50} = 144 mg/L). No difference in feeding rate of bees treated with different concentrations of Cu was observed, however, feeding rate decreased with the elevation of the concentration of Zn. We found that honeybees prefer Zn containing sucrose solutions compared to the control diet, whereas honeybees tend to avoid Cu spiked sucrose solution. The preference for Zn elevates the possible risk for honeybee intoxication in Zn polluted environment. In conclusion, our results indicate that honeybee workers could suffer detrimental effects when they are exposed to ecologically relevant concentrations of Cu and Zn.

Keywords: CuSO4, ZnCl2, LC_{50}

ACTIVE BUMBLEBEES AS PASSIVE SAMPLERS

Benner, Lena, Institute for Environmental Research, RWTH Aachen University, Aachen, Germany
Luisa Coder, Institute for Environmental Research, RWTH Aachen University, Aachen, Germany
Benjamin Daniels, Institute for Environmental Research, RWTH Aachen University, Aachen, Germany
Felix Stibany, Institute for Environmental Research, RWTH Aachen University & gaiac Research Institute for Ecosystem Analysis and Assessment at RWTH Aachen University e.V., Aachen, Germany
Martina Roß-Nickoll, Institute for Environmental Research, RWTH Aachen University, Aachen, Germany
Andreas Schäffer, Institute for Environmental Research, RWTH Aachen University, Aachen, Germany

The decline of insect populations is gaining increased public attention, as the lack of key pollinators becomes a pressing issue for agriculture. Natural habitats for pollinators such as bees and syrphids are being lost due to fragmentation, land-use changes and the application of pesticides. This forces pollinators into urban surroundings, where they find small scale habitats in parks, gardens, and other green infrastructure. In man-made landscapes, anthropogenic activities lead to diverse pollutant emissions. In both habitats, pollinators are expected to be exposed to these pollutant profiles during their lifetime, e.g. during foraging flights. Until now, most research has focussed on honeybees, while data on pollutant loads of non-Apis pollinators is rare. *Bombus terrestris* (bumblebee) is a common pollinator and has a more spatially restricted foraging behavior. This makes it well suited as a bioindicator for representing the exposure of pollinators to chemical profiles at high spatial resolution.

In this study, commercial *B. terrestris* hives were placed at six locations in and around Aachen, Germany, in urban and agricultural surroundings. Foragers were caught when returning to the hive after an exposure period. Individual bumblebees were extracted using a modified QuEChERS method and analyzed for 25 target pesticides using HPLC-MS/MS.

At every sampling location, at least one pesticide was found. The results show that the individual pesticide load of bumblebees is variable between individuals from the same site (ranging from no pesticides up to seven pesticides). This could be caused by individual foraging decisions and indicate complex pollution patterns in small scale habitat structure. No location or land-use specific pollution patterns were found. Rather, pesticide residues changed with sampling time. The ubiquitous presence of pesticides shown in this study should be considered when evaluating the risks to pollinators in Europe.

This study could be a starting point for future research projects extended to chemical monitoring with non-Apis pollinators and the development of ecotoxicological test systems suitable to assess the sublethal effects of the pollutant load to so far only minor considered non-Apis pollinators. This could close the gap between sublethal effects in the field and observed effect thresholds of standardized acute toxicity tests.

Keywords: Bumblebees, Pesticides, Biomonitoring

HONEYBEE CAV4/DSC1 CHANNEL: DIFFERENT FROM CLASSICAL NAV AND CAV CHANNELS

Anais Bertaud[1]; Lisa Soussi[1]; Thierry Cens[1]; Matthieu Rousset[1]; Claudine Ménard[1]; Claude Collet[2]; Jean-Baptiste Thibaud[1]; Mohamed Chahine[3]; Pierre Charnet[1];

1: IBMM, UMR 5247 CNRS, Univ. Montpellier, France
2: INRAE, UR 406, Abeilles et Environnement, Avignon, France
3: Univ. de Laval, Québec, Canada

We have recently cloned a channel homologous to DSC1 (drosophila sodium channel 1) in the honeybee. Despite sequence similarity with voltage-dependent Na^+ channel, we demonstrate a strong calcium (Ca^{2+}) permeation, and suggested that AmSC1 may belong to a new type of voltage-dependent calcium channel (VDCC), now named CaV4.

Identification of a particular sequences for the selectivity filter (DEEA), for inactivation domain (MFL, homologous to the IFM of the NaV channel) and a C-terminus sequence containing a calmodulin (Cam)-binding site led us to analyze the singular permeation and the voltage and calcium-dependent inactivation properties of this new channel as well as its sensibility to pyrethroides. Its voltage-dependence of activation and deactivation suggest an important role in the Ca influx during action potential.

A COMPARISON OF THE IMPACTS OF TECHNICAL GRADE GLYPHOSATE AND A GLYPHOSATE-BASED FORMULATION ON THE DIGESTIVE TRACT PROTEOME AND MICROBIOTA OF *BOMBUS TERRESTRIS*

Cullen, Merissa, Department of Biology, Maynooth University, Maynooth, Kildare, Ireland
Bliss, Liam, Department of Biology, Maynooth University, Maynooth, Kildare, Ireland
Stanley, Dara, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland
Carolan, James, Department of Biology, Maynooth University, Maynooth, Kildare, Ireland

Agrochemical pesticides have been identified as one of the primary drivers of pollinating insect decline, via effects on non-target organisms. Although considerable research exists on the impact of insecticides on insect pollinators, such as bees, little is known about the effects of non-insecticidal pesticides. One of the most widely used pesticides worldwide is the broad-spectrum herbicide glyphosate, which due to its mode of action of shikimate pathway inhibition in plants and microbes, is thought to have little effect on non-target organisms such as insects which lack this pathway. Glyphosate is usually applied to plants as part of a formulation, mixed with co-formulants, which increase the efficacy of the pesticidal active ingredient and are assumed to have negligible impacts on living organisms. Ambiguity around the regulatory risk assessment of co-formulant effects on arthropods makes evaluating the true impact of glyphosate on insects difficult.

This research provides new insights into the effects of glyphosate on the bumblebee *Bombus terrestris* at the molecular and cellular level. Using mass spectrometry-based proteomics and next-generation sequencing, we characterized the effects of glyphosate (GLY) and the glyphosate-based formulation, Roundup Optima+® (RU), chronic oral exposure on the *B. terrestris* digestive tract proteome and microbiota. Our results revealed a ‘glyphosate-conserved’ effect, with oxidative stress regulation, metabolism and cell structural integrity altered in the *B. terrestris* digestive tract after exposure to both treatments. However key distinctions between treatments were observed including impacts on endocytosis, fatty acid degradation and mitochondrial transport proteins, attributable to the co-formulant ingredients present in RU. Both glyphosate treatments showed little effect on bacterial species of the microbiota. However, RU but not GLY, led to statistically significant alterations to Ascomycete fungi, suggesting that one or more co-formulants cause dysbiosis in *B. terrestris* digestive tract fungal communities. Overall, our findings suggest that i) glyphosate affects bees at the molecular level; ii) co-formulants are responsible for additional effects which cannot be attributed to glyphosate alone and iii) the effect of glyphosate on the *B. terrestris* digestive tract proteome is more than likely a result of direct damage to digestive tract cells rather than knock-on effects from microbiota alterations.

Keywords: Glyphosate, Proteomics, Microbiota

EFFECTS OF FUNGICIDE PYRACLOSTROBIN ON THE FAT BODY AND PERICARDIAL CELLS MORPHOPHYSIOLOGY IN AFRICANIZED HONEY BEE

Domingues, Caio[1,2], Inoue, Lais[2], Gregorc, Aleš[1], Silva-Zacarin, Elaine[3], Malaspina, Osmar[2]

1 Faculty of Agriculture and Life Sciences, University of Maribor, Hoče, Slovenia
2 Centro de Estudos de Insetos Sociais, São Paulo State University - "Júlio de Mesquita Filho", Rio Claro, Brazil
3 Laboratório de Ecotoxicologia e Análise de Integridade Ambiental, Federal University of São Carlos, Sorocaba, Brazil

Managed honey bees (*Apis mellifera*) are daily exposed in agricultural settings or wild environments to multiple stressors such as pesticides, habitat loss, parasites, disease, and others. Currently, fungicide residues are increasingly present in bees' pollen and nectar and can harm colonies' production and survival. Our study aimed to evaluate the effects of residual concentrations of pyraclostrobin on the morphophysiology of the fat body and pericardial cells of Africanized honey bee foragers. The parameters analyzed were the morphometry of oenocytes and pericardial cells and their histopathological changes. The workers were divided into different experimental groups (20 bees in four replicates per group): control (CTL), solvent control (CAC), pyraclostrobin 0.125 ng/μL (FG1), pyraclostrobin 0.025 ng/μL (FG2) and pyraclostrobin 0.005 ng/μL (FG3). Bees in control groups received syrup (50% water + 50% sugar, w/w) without adding fungicide. After five days of oral exposure (*ad libitum*), the bees were dissected and prepared for histological and morphometric analysis. The results showed a significant decrease in the oenocyte's surface area for bees exposed to all pyraclostrobin concentrations compared to CTL and CAC groups ($p<0.0001$). The bees from FG1 ($p<0.0001$) and FG2 (p 0.0001) groups presented reduced surface area of pericardial cells compared to controls, and FG3 groups, being that FG1 showed a smaller size. There was no difference in pericardial cells surface area between FG3 and control groups. The fungicide pyraclostrobin also induced sublethal effects with increased vacuolization, condensed nuclei of oenocytes, and disruption of the cytoarchitecture of trophocytes, suggesting the death of both cells in FG1 and FG2 groups. Bees exposed to FG3 group only oenocytes vacuolization. FG1 bees induced extensive cellular changes in pericardial cells when comparing FG3 or changes observed in untreated, control bees. Bees from the FG2 group showed peripheral vacuolization and pyknotic nuclei, but the cytoarchitecture of pericardial cells was not compromised as observed in FG1. Additionally, immune system cells were observed through the fat body in FG1 group. The results of our study showed harmful effects of fungicide pyraclostrobin concentrations at the individual bee cellular level, which potentially harms colony level.

Keywords: *Apis mellifera*, Biomarkers, Strobilurin fungicide

THE EFFECT OF ACLONIFEN ON INTESTINAL HEALTH IN FORAGER HONEYBEES

Ozfen, Egehan Onat, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey
Sevin, Sedat, Ankara University Faculty of Veterinary Medicine, Pharmacology and Toxicology, Ankara, Turkey
Ceylan, Ahmet, Ankara University Faculty of Veterinary Medicine, Histology and Embryology, Ankara, Turkey

Honeybee (*Apis mellifera*) is the world's most important single species of pollinator in natural ecosystems and a key contributor to natural ecosystem functions. In addition to bee diseases and pests, pesticide use accelerates bee health and colony collapse. Thousands of hives are damaged every year as a result of the intensive and unconscious use of pesticides. In poisoned bees, body movement weakens, behaviors that fail to fly are observed, followed by paralysis and death. Due to climate change and drought events experienced in recent years, hunger and colony collapses are observed in bees. Beekeepers choose cultivated plants to be least affected by this situation. Among the cultivated plants, especially sunflower comes to the fore. One of the most common used herbicides in sunflowers in the world and in our country is Aclonifen. The LD50 dose of Aclonifen in bees (The LD50 10 days was 71.21 µg a.s. bee/day in the chronic toxicity test) was determined. When the literature is examined, it has been observed that there is no study on the effect of bee intestinal health. Field bees were used in our study. Oral toxicity studies were carried out with cage experiments under laboratory conditions, and histopathological examinations of the intestines of the bees were carried out after 10 days. 4 doses and 5 control groups were used in the study. LD50 as a dose; LD50/5; LD50/10; The LD50/20 has been chosen. Acetonitrile, which is used as the solvent of Aclonifen, was used in the control groups. Acetonitrile ratio was used in the amounts used in drug doses. The structural organization of the midgut wall was found similar in all control groups except asetonitril group. Although, epithelial cell morphology and peritrophic membrane (PM) integrity in whole control groups displayed no alterations, in the asetonitril group there was some differences in the formation of PM. In LD50 experimental group, the majority of the epithelial cells showed signs of degeneration such as the presence of densely granulated vacuoles in the cytoplasm or invisible nucleus. The negative effect of Aclonifen on the intestinal health of honey bees has been confirmed by the results obtained.

Keywords: Aclonifen, Forager Honeybees, Histopathology

CHARACTERIZATION OF THE EFFECTS OF THE INSECTICIDE DELTAMETHRIN ON NEURONAL SODIUM CHANNELS AND MUSCULAR CALCIUM CHANNELS FROM THE HONEY BEE *APIS MELLIFERA*

Kadala, Aklesso, UR 406 Abeilles et Environnement, INRAE, Avignon, France.
Kaabeche, Mahira, UR 406 Abeilles et Environnement, INRAE, Avignon, France.
Charreton, Mercédès, UR 406 Abeilles et Environnement, INRAE, Avignon, France.
Charnet, Pierre, UMR 5247, Institut des Biomolécules Max Mousseron (IBMM), CNRS - Université de Montpellier 2, Montpellier, France.
Collet, Claude, UR 406 Abeilles et Environnement, INRAE, Avignon, France.

Honey bees are important pollinators and are often described as indicator species for the quality of the environment. Domestic honey bees have suffered significant losses over the last decades and insecticides are clear contributing factors to this decline. Bees intoxicated with deltamethrin, a widely used pyrethroid insecticide, exhibit symptoms of strong neuromuscular alterations. In order to better understand the modes of action of this molecule on bees, we characterized its effects on isolated antennal neurons and skeletal muscle fibers. Using the patch-clamp electrophysiological method, we monitored the effects of deltamethrin on voltage-gated sodium channels (NaVs) and on voltage-gated calcium channels (CaVs), which are responsible for action potentials in neurons and insect muscle fibers, respectively. Under voltage-clamp, deltamethrin clearly impeded neuronal NaVs function by slowing down their closure. The sodium deactivation current was altered in both a use-dependent and a concentration-dependent manner. Conversely, in muscle fibers, preliminary observations suggest that ionic current amplitudes through CaVs are not altered by similar concentrations of deltamethrin. Further analysis of the CaVs biophysical properties is needed to confirm this latter result and in particular to fully describe their voltage-dependency in the presence of the insecticide.

Keywords: Deltamethrin, Antennal neurons, Skeletal muscle fibers

BACILLUS THURINGIENSIS SSP. *AIZAWAI* − OBSERVATIONS ON HONEY BEES AND DISTRIBUTION IN COLONY MATRICES UNDER FIELD CONDITIONS

Erler, Silvio, Institute for Bee Protection, Julius Kühn- Institute (JKI) – Federal Research Centre for Cultivated Plants, Braunschweig, Germany
Charlotte Steinigeweg, Institute of Geoecology, Technische Universität Braunschweig, Braunschweig, Germany
Hannes Beims, Institute for Apiculture, Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Celle, Germany
Jakob H. Eckert, Institute for Bee Protection, Julius Kühn- Institute (JKI) – Federal Research Centre for Cultivated Plants, Braunschweig, Germany
Martina Janke, Institute for Apiculture, Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Celle, Germany
Ina P. Wirtz, Institute for Bee Protection, Julius Kühn- Institute (JKI) – Federal Research Centre for Cultivated Plants, Braunschweig, Germany
Dania Richter, Institute of Geoecology, Technische Universität Braunschweig, Braunschweig, Germany
Jens Pistorius, Institute for Bee Protection, Julius Kühn- Institute (JKI) – Federal Research Centre for Cultivated Plants, Braunschweig, Germany
Abdulrahim T. Alkassab, Institute for Bee Protection, Julius Kühn- Institute (JKI) – Federal Research Centre for Cultivated Plants, Braunschweig, Germany

Microbial pest control products are commonly applied worldwide as alternatives to avoid potential adverse effects of chemical plant protection products. Here, we aimed to evaluate the biosafety of a commercial product containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS-1857) using four different approaches: 1) laboratory chronic exposure to evaluate the survival of adult and larval bee, 2) in-hive feeding under field conditions to examine the effect of B. t. on brood development and the core gut microbiome of adult bees, 3) semi-field colony-feeding to determine contamination levels of B. t. spores in various matrices, and 4) a field trial with spray application in a bee-attractive crop to estimate potential environmental accumulation and exposure of honey bee colonies.

Adult bee and larval survival were negatively affected after chronic exposure depending on the tested concentrations; however, pollen feeding to adults promote survival of treated bees and delay the effects. Under colony conditions, treated colonies showed a higher brood termination rate and a significantly lower normalized abundance of the core gut microbiome in worker bees. B. t. spores were detectable in all matrices at different concentrations, decreasing over time under semi-field conditions. High spore levels were present in honey sacs and pollen pellets immediately after application. No spore reduction was seen in stored matrices like nectar and bee bread.

In conclusion, the pest control product containing *B. t.* strain ABTS-1857 showed a negative effect on exposed bees under laboratory as well as field conditions, for instance on colony development and caused dysbiosis of the gut microbiome. However, further field-realistic exposure studies in bee attractive crops are needed to evaluate the potential risk of such products on honey bees.

Keywords: *Bacillus thuringiensis*, microbiome, microbial pest control

BEYOND BEES: TOXICITY OF A NEONICOTINOID INSECTICIDE IN THREE DIPTERAN POLLINATOR

Martins, Cátia A.H., DISTAL, University of Bologna, Bologna Italy.
Azpiazu, Celeste, CSIC, Universitat Pompeu Fabra, Barcelona, Spain.
Francati, Santolo, DISTAL, University of Bologna, Bologna, Italy.
Dindo, Maria Luisa, DISTAL, University of Bologna, Bologna, Italy.
Bosch, Jordi, CREAF, Universitat Autònoma de Barcelona, Barcelona, Spain.
Burgio, Giovanni, DISTAL, University of Bologna, Bologna, Italy.
Sgolastra, Fabio, DISTAL, University of Bologna, Bologna, Italy.

Pollinators comprise a widely diverse group of insect species that are essential for ecosystem functioning and crop pollination. However, in agricultural environments, where their pollination services are often most needed, pollinators are commonly exposed to pesticides. Pesticide exposure is considered one of the main drivers of pollinator population losses worldwide. Unfortunately, information on the effects of agrochemicals on insect pollinators is mainly based on a single species, the western honey bee, *Apis mellifera*. Some studies have unveiled important differences among bee species in sensitivity to pesticides, but information on non-bee pollinators is to date mostly lacking. Dipterans are a highly diverse insect order, comprising about 160,000 species, including some important pollinators. In this study we assess the sensitivity of three dipteran species to a neonicotinoid insecticide (Confidor®, imidacloprid). We worked with adult females of two hoverflies, *Sphaerophoria rueppellii* and *Eristalinus aeneus* (Syrphidae), and a parasitoid fly, *Exorista larvarum* (Tachnidae). We adjusted contact exposure protocols available for honey bees and calculated the median lethal dose (LD_{50}) at 48h, to later compare our results with those available for nine bee species (social and solitary). To do so, we used the species sensitivity distribution (SSD) approach as a refinement of the pesticide effects to pollinator populations. We found that the three dipterans show wide differences in sensitivity to imidacloprid, with LD50s increasing in the following order: *S. rueppellii* < *E. larvarum* < *E. aeneus*. The SSDs showed that 60% of the species included in the model, one of which *S. rueppellii*, were more sensitive to imidacloprid than *A. mellifera*. However, when accounting for body weight, all three dipterans showed higher tolerance to imidacloprid than A. mellifera and 6 other bee species. Our results may suggest a phylogenetic component in pesticide sensitivity of the dipteran species, particularly to imidacloprid, that would need further investigation.

Keywords: Non-bee pollinators, species sensitivity, acute exposure

DETERMINATION OF THE CHRONIC MEDIAN LETHAL DOSES (LD50) OF TWO COMMON SYNTHETIC ACARICIDES IN *APIS MELLIFERA*

Benito-Murcia, María, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Marchamalo, Spain.
Alonso-Prados, Elena Unidad de Productos Fitosanitarios, Instituto Nacional de Investigación y Tecnología Agraria (INIA), Madrid, Spain.
Botías, Cristina, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Martín Hernández, Raquel, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Aguado-López, Daniel, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Jabal-Uriel, Clara, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Higes, Mariano Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.

There is mounting evidence that acaricide residues are among the most prevalent xenobiotic compounds present in honey bee colonies worldwide, being the pyrethroid tau-fluvalinate and the organophosphate coumaphos two of the most frequently detected in hive matrices. These two synthetic acaricides are commonly used in beekeeping worldwide to control the parasitic mite *Varroa destructor*. Numerous studies have evaluated the effect of acute contact exposure of honey bees to these two compounds, yet there is little information regarding the effect of chronic oral exposure. In this study, the calculation of the chronic LD50s of tau-fluvalinate and coumaphos was performed for adult bees. To do this, we followed the recommendations established in the guideline No. 245 of the OECD, slightly modified to optimize the correct dissolution of these highly hydrophobic compounds and to warrant the exposure of honey bees to the nominal concentrations tested. Honey bee individuals of maximum two days post-emergence were fed ad *libitum* with X different concentrations of tau-fluvalinate and X different concentrations of coumaphos in an exposure period of 10 days. Each concentration tested was administered to three replicates containing 10 bees, performing three assays for coumaphos (two in spring 2020 and one in autumn 2021), and two assays for tau-fluvalinate (one in spring 2020 and one in autumn 2021). According to the LD50 values obtained, coumaphos is more toxic than tau-fluvalinate through oral exposure in honey bees. In addition, the results suggest a higher susceptibility of autumn bees than spring bees to both compounds, entailing a high risk for the colony, since the mandatory treatments to control Varroa mites, which are usually based on these active substances, are commonly applied in the autumn.

Keywords: tau-fluvalinate, coumaphos, chronic toxicity test

RISK ASSESSMENT OF ACETAMIPRID FORMULATION ON SOLITARY BEES USING OMICS APPROACH

Shcherbachenko, Elena, Institute for Environmental Studies, Faculty of Science, Charles University, Prague, Czechia; Crop Research Institute, Prague, Czechia
Erban, Tomáš, Crop Research Institute, Prague, Czechia

Pollinators are potentially exposed to a number of xenobiotics in the environment. The most important group of hazardous substances are plant protection products (PPPs) that are used by farmers and small gardeners. Prior to placing on the market, PPPs undergo a thorough evaluation to minimize negative effects on the environment and non-target organisms. In recent years, the evaluation of chronic effects on non-target organisms, such as pollinators has gained in importance. Honey bees following with bumblebees are the most widely used pollinator species in the risk assessment; however, many other bee species are necessary to protect. Among them, solitary bees are fundamental for pollination services and the preservation of biodiversity. Therefore, solitrary bees are necessary include in the risk assessment. Their response to environmental stresses including pesticide exposure can be different from that of the eusocial honey bees and bumblebees, at least due to different genome, life strategy and the social organization.

In the risk assessment is necessary to consider not only the most relevant non-target organisms, but also advances in analytical methods and scientific knowledge. Various state-of-the-art analytical approaches are currently used to investigate side effects on bees. Indeed, high-throughput technologies facilitate identifying the hidden side effects, because many markers can be observed in one sample.

Our research is focused on the evaluation of the effect of sublethal doses of pesticides on solitary bees employing the OMICs approach. As a model species, we used the red mason bee (*Osmia bicornis*), which has been domesticated. We tested the effect of acetamiprid. The exposure was via soil application using Careo sticks formulation. The bees were exposed in field cages in which the flowering *Phacelia tanacetifolia* was placed. The distribution and fate of acetamiprid in the soil substrate, plant and bee females was analyzed. Using UHPLC-MS/MS was identified the possible hazardous acetamiprid metabolite IM-2-1 in plant tissues. However, high-throughput shot-gun proteomic analysis indicated a very low risk of acetamiprid for *Osmia bicornis*.

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Keywords: Pesticides, Acetamiprid, Pollinators

BIOMARKERS DETERMINATION IN ADULT BEES EXPOSED TO LETHAL DOSES OF TWO ACARICIDES TAKING AN IN VIVO AND IN VITRO APPROACH

Benito-Murcia, María, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Botías, Cristina, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Martín Hernández, Raquel, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Aguado-López, Daniel, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Jabal-Uriel, Clara, Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Higes, Mariano Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA-IRIAF), Marchamalo, Spain.
Martínez-Morcillo, Salomé, Toxicology Unit, Veterinary School, University of Extremadura, Caceres, Spain.

Due to the significant and often irreversible impacts of human activity on ecosystems, there is a growing need to develop tools to monitor the effects of pollution on organisms. The evaluation of the physiological and functional integrity of individuals requires tools that act as indicators of exposure to environmental stress factors. Therefore, biomarkers reveal information about the quality of the environment in terms of contamination by xenobiotics and are defined as the observable measures at the biochemical, molecular, cellular, and physiological levels that show exposure to pesticides, among others. Honey bees are considered suitable species to measure environmental pollution since they are frequently exposed to pesticide mixtures through their foraging activity on cultivated and wild plants. In addition, the regular application of veterinary drugs to control *Varroa destructor* parasitization leads to the accumulation of acaricide residues in different hive matrices. Actually, different studies reported that acaricides are the most prevalent residues in wax and pollen stored in bee colonies, with coumaphos and tau-fluvalinate being among the most frequently detected. Therefore, due to the persistence of these active substances in the hive, it is essential to investigate the effects of chronic exposure on honey bee health. In this study, the effect of exposure to the chronic median lethal dose (LD50) of coumaphos and tau-fluvalinate was evaluated by analysing the activity of biomarkers related to neurotoxicity and biotransformation in adult honey bees (*in vivo assays*). In addition, the toxicity of coumaphos and tau-fluvalinate was assessed in bee tissues using a range of concentrations of both compounds including the LD50 values (in vitro assays). Dimethoate was used as a reference substance and the biomarkers analyzed were acetylcholinesterase (AChE), carboxylesterase (CbE), glutathione-S-transferase (GST), and malondialdehyde formation (MDA). Overall, this study shows that chronic exposure to tau-fluvalinate and coumaphos alters the activity of enzymes related to neurotoxicity and biotransformation of xenobiotics. Moreover, the IBR was higher in coumaphos than in tau-fluvalinate, indicating that the organophosphate is more toxic than the pyrethroid, both in terms of lethality and alteration of the activity in the biomarkers used.

Keywords: biomarkers, chronic toxicity test, acaricides

ELECTRICAL PROPERTIES AND INTRACELLULAR CALCIUM SIGNALING IN CARDIOMYOCYTES FROM THE HONEY BEE HEART

KAABECHE, Mahira, INRAE UR 406 Abeilles & Environnement, Avignon, France.
CHARRETON, Mercedes, INRAE UR 406 Abeilles & Environnement, Avignon, France.
KADALA, Aklesso, INRAE UR 406 Abeilles & Environnement, Avignon, France.
ROUSSET, Matthieu, CNRS UMR 5247 Institut des Biomolécules Max Mousseron Université de Montpellier 2, Montpellier, France.
CENS, Thierry, CNRS UMR 5247 Institut des Biomolécules Max Mousseron Université de Montpellier 2, Montpellier, France.
BOIS, Patrick, Laboratoire Signalisation et Transports Ioniques Membranaires CNRS Université de Poitiers, Poitiers, France.
CSERNOCH, Laszlo, Department of Physiology Faculty of Medicine University of Debrecen, Debrecen, Hungary.
SZENTESI, Peter, Department of Physiology Faculty of Medicine University of Debrecen, Debrecen, Hungary.
CHARNET, Pierre, CNRS UMR 5247 Institut des Biomolécules Max Mousseron Université de Montpellier 2, Montpellier, France.
COLLET, Claude, INRAE UR 406 Abeilles & Environnement, Avignon, France.

In insects, circulation of haemolymph is driven by the autorhythmic contraction of the dorsal heart vessel located in the abdomen. In a previous study, a recently authorized class of insecticides was found highly toxic when honey bees were exposed through the dorsal abdomen, suggesting direct cardiotoxic effects. In order to explore cardiotoxicity, we developed enzymatical isolation of heart cells from bees. Their function was explored with traditional electrophysiological methods (current-clamp, voltage-clamp, intracellular microelectrode) and calcium imaging. Cell parameters were consistent with the in situ morphology observed in intact heart. Action potentials obtained under current-clamp with a patch pipette resembled action potentials recorded with intracellular electrodes in intact heart. Under voltage-clamp, ionic currents were recorded and their basic pharmacology was studied. Dynamic confocal microscopy was used to assess calcium homeostasis. Intact cardiomyocytes from honey bee may help better understand the excitability of insect heart and cardiotoxicity of insecticides.

Keywords: Insecticide, Heart, Excitability

STRESS CAUSED BY PESTICIDES IN THE BEES' FAT BODY

GRELLA, Tatiane Caroline[1]; TAVARES, Daiana Antonia[1]; NOCELLI, Roberta Cornélio Ferreira[2]; MALASPINA, Osmar[1].

1 São Paulo State University (UNESP), Institute of Biosciences, Department of General and Applied Biology, Rio Claro, São Paulo, Brazil.
2 Center for Agricultural Sciences, Department of Natural Sciences, Mathematics and Education, Federal University of São Carlos, UFSCar, Araras, São Paulo, Brazil.

Bees contribute to the cross-reproduction of plants, increasing the quality of fruits and seeds. Among the bees we have *Apis mellifera* and *Melipona quadrifasciata*. During pollination, bees are exposed to pesticides, such as the insecticide imidacloprid and the herbicide glyphosate, alone and in combination. Exposure can cause damage at the cellular level, such as an increase in heat shock protein (HSP), among which we have HSP70 and HSP90. HSPs can be used as biomarkers to monitor cellular responses and various environmental stimuli. The present study aimed to analyze the effects of sublethal concentrations of the insecticide imidacloprid, and the herbicide glyphosate, isolated and combined in the fat tissue cells of *A. mellifera* and *M. quadrifasciata* through the techniques of HSP70 and HSP90. The bees were collected and fed sucrose solutions containing different concentrations of isolated and combined pesticides (0.0075ng imidacloprid/µL and 0.0025ng glyphosate/µL). After 48 hours, 15 bees from each group were anesthetized and dissected to collect the fat body. Then the material went through a protocol for analysis under a confocal microscope, and the results were analyzed. From the results obtained, we observed that among the groups analyzed, the group exposed to the mixture was the one that presented the highest value in the expression of HSP70 for *A. mellifera* and the lowest for *M. quadrifasciata*. As for HSP90, we observed that the group exposed to glyphosate had a higher value of protein expression in *M. quadrifasciata* and the lowest for *A. mellifera*. The increased expression of HSP may be an indication of an attempt to protect the fat body and consequently the innate immune system. Studies show that environmental stressors, such as pesticides, can affect the immune capacity of bees. From the results we can conclude that exposure to imidacloprid and glyphosate caused an increase in the expression of HSP70 and HSP90 and put the immune system of bees at risk.

Keywords: *Apis mellifera* 1; *Melipona quadrifasciata* 3, HSP

Grants: 2017/21097-3 and 2019/20109-3 São Paulo Research Foundation (FAPESP)

EFFECTS OF INSECTICIDE MIXTURES ON ATP LEVELS IN RED MASON BEE (*OSMIA BICORNIS*)

Filipiak, Zuzanna, Institute of Nature Conservation, Polish Academy of Sciences, Krakow, Poland.
Misiewicz, Anna, Institute of Nature Conservation, Polish Academy of Sciences, Krakow, Poland.
Bednarska, Agnieszka, Institute of Nature Conservation, Polish Academy of Sciences, Krakow, Poland.

Concerns over the potential effects caused by pesticides to pollinators have led to numerous studies, however most of them focused on the pesticides examination in isolation and/or on managed honeybees. The mixture effects of pesticides are still poorly recognized, especially for non-Apis bees which, in contrast to honeybees, are not included in any standard procedures for ecological risk assessment of pesticides. Moreover, the effects of pesticides at environmentally relevant concentrations may not cause mortality, but still can imply sublethal effects affecting physiological condition of organisms. Notably, perturbation in energetic metabolism may have an effect on behavior and flight performance in bees. In present study we tested the effects of three agrochemicals representing different insecticide types (Dursban 480 EC with the organophosphate chlorpyrifos, Sherpa 100 EC with the pyrethroid cypermethrin, and Mospilan20 SP with the neonicotinoid acetamiprid) on whole body ATP levels in the females of *Osmia bicornis*. The bees were exposed topically in triple mixture using a full factorial design with three environmentally relevant concentrations of each insecticide: 0, 0.2, 0.4 × RAC (Recommended Application Concentrations) for Dursban 480 EC and 0, 0.5, 1 × RAC for both Sherpa 100 EC and Mospilan 20 SC. Within 4 days of exposure, Sherpa 100 EC caused increased ATP levels when applied in isolation and in tripe combination with Mospilan 20 SC and Dursban 480 EC. However, when Sherpa 100 EC was combined only with Dursban 480 EC at its highest RAC, ATP levels decreased with increasing Sherpa 100 EC concentration. This suggest that presence of Mospilan 20 SC in the mixture modified the physiological response of bees when exposed to highest RAC of Sherpa 100 EC and/or Dursban 480 EC and that pesticides with different mode of actions can affect bee energetic metabolism differently when applied either in isolation or in combination.

This study was supported by National Science Centre, Poland (UMO-2017/26/D/NZ8/00606).

Keywords: solitary bee, toxicity, metabolism

EFFECTS OF SEASONALITY AND LANDSCAPE COMPOSITION ON POLLEN COLLECTED BY HONEYBEES

Malagnini Valeria, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Cappellari Andree, Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Legnaro (PD), Italy
Marini Lorenzo, Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Legnaro (PD), Italy
Livia Zanotelli Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Gino Angeli, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Claudio Ioriatti Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Loris Tonidandel Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Fontana Paolo, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy

The honeybee is the most important and widespread managed pollinator. Its diet is based on nectar and pollen, and since the quality of pollen varies among plant species, bees must have access to diverse pollen sources to assure colony health. Only landscapes with high floristic diversity, e.g., related to the presence of semi-natural areas, can therefore guarantee adequate resources for honeybees. Besides the availability of heterogeneous pollen, also the amount of agricultural area can have a strong effect bee health, due to pesticide contamination in pollen.

This work aimed to explore how the composition of pollen and pesticide residues in pollen collected by honeybees were modulated by seasonality and landscape heterogeneity in Northern Italy. We selected 13 locations, where we placed two honeybee colonies from which we collected pollen samples every month during the whole flowering season over two years. For each pollen sample, we determined pollen type composition and diversity and the Pollen Hazard Quotient (PHQ), which provides a measure of potential pollen toxicity. Finally, we determined the cover of the main habitat types in 3-km radius buffers around the sampling locations.

Honeybees, despite being extremely generalist, focused their foraging activity on a few plant species, which changed throughout the season. Landscape composition did not affect pollen diversity from April to August, however, in September the diversity increased with increasing semi-natural areas. While honeybees were able to collect heterogeneous pollen independently of landscape composition up to mid-summer, the scarcity of floral resources in late summer turned semi-natural areas into key habitats. Only 6 pollen samples out of 136 were pesticide-free. We detected more than 100 compounds, mainly fungicides.

PHQ increased with increasing proportion of certain categories of agricultural areas, in particular apple orchards, and decreased with increasing semi-natural areas.

Our research highlighted the scarcity of floral resources in late summer and the resulting importance of semi-natural habitats for honeybees. Since pollen quality can affect the response of bees to pesticides, beekeepers should evaluate landscape composition before placing beehives, to maximise floral resources and the diversity of pollen collected by bees, while minimising the likelihood of pollen contamination by pesticides.

Keywords: foraging behavior, pesticides, pollen composition

ATRAZINE EXPOSURE PERTURBS THE GUT MICROBIOTA, REDUCES POLLEN CONSUMPTION AND ALTERS FUNCTIONAL GENE EXPRESSION IN HONEYBEES

Kang, Wang, Microbial Genomics and Symbiosis Group, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Atrazine is a widely used pesticide and the effect of this xenobiotic on the microbiota metabolism has been reported in many insects. However, it is not clear whether atrazine negatively affects the gut microbiota in honeybees. To address this research gap, we evaluated the effects of atrazine exposure on size and composition of honeybee gut communities, and showed that treatment with sublethal atrazine severely increased beta diversity of composition and reduced

the size of gut microbiome. Moreover, we assessed other indicators associated with gut microbes: pollen consumption and global genes expression in gut. Importantly, exposure to higher concentrations of atrazine (37.3 mg/L) significantly decreased pollen consumption compared to exposures to lower concentrations of pesticide (3.73 mg/L) and in control groups. When compared with control honey bees, several functional genes associated with cytochrome P450, peroxisome and lysosome were up-regulated in both low and high exposed groups. Additionally, we observed a reduction in expression of 93 genes, in bees exposed to lower atrazine concentrations. This expression downregulation extended to 384 genes in bees exposed to higher concentrations of the pesticide. The overlapping down-regulated gene list was enriched in insect genes involved in hormone biosynthesis, protein processing in endoplasmic reticulum, glutathione, and pyruvate, propanoate, cysteine and methionine into the KEGG signaling pathways. Thus, exposure of bees to atrazine perturbs the honeybee microbiota, reducing pollen consumption and leading to alterations in functional genes expression, which may influence the honeybee effectiveness as pollinators, and possibly weak colony health.

Keywords: atrazine, honeybee, microbiota

ENVIRONMENTAL EFFECT COUPLED WITH OXALIC ACID TREATMENT ALTERS GUT BACTERIAL BUT NOT FUNGAL COMMUNITIES

Gorrochategui-Ortega, June, Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain
Muñoz-Colmenero, Marta, Dept. of Genetics, Physiology and Microbiology, Complutense University of Madrid, Madrid, Spain
Baquero, Candela, Dept. of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country, Leioa, Spain
Parejo, Melanie, Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain
Galartza, Egoitz, ERBEL
Zarraonaindia, Iratxe, IKERBASQUE, Bilbao, Spain
Estonba, Andone, Applied Genomics and Bioinformatics, University of the Basque Country, Leioa, Spain

One of the most important *Apis mellifera* pathogens is *Varroa destructor*, causing colony losses worldwide and requiering annual/recurring treatments. A common treatment for management of these mites is oxalic acid (OA). Ubiquitous in nature and typically synthetized and consumed by microorganisms (e.g. bacteria and fungi), OA treatment is considered ecological. Nonetheless, the effect of oxalic acid on the honey bee microbiome (intrinsically linked to bee nutrition and immune response) is unclear, and scarce data exists regarding the OA effect on the honey bee gut microbiome and other microbial communities associated with diverse hive niches. We hypothesize that treatment with OA would 1) impact bacteria and/or eukaryotic community diversity and composition, 2) have a dose-dependent effect, and 3) be beehive niche specific. Herein, we studied the effect of three OA treatments (tricking at 2.1%, tricking at 4.2%, and sublimation through Varrox®) upon microbial communities associated to worker gut and hive bee bread through amplicon sequencing. The V4 16S rRNA and the ITS2 gene were studied for bacteria and eukaryote identification, respectively. Preliminary results of the 20 colonies studied show that besides apiary location greatly influencing bacterial and eukaryotic communities, OA induced slight compositional changes in gut microbiomes. In particular, a reduction of the potential pathogen *Nosema ceranea* was detected. However, treatment also induced a reduction of the overall bacterial diversity in the same colonies, which might reduce hive resilience and resistance towards opportunistic microbial colonizers and endanger honey bee hives. Considering the detected effect, we propose a measured use of OA treatments to maintain the equilibrium between reduction of pathogens and of native and beneficial bacteria.

Keywords: *Varroa destructor*, oxalic acid, microbiome

ASSESSING SYNERGISM AND TIME-REINFORCED TOXICITY OF AN INSECTICIDE-FUNGICIDE MIXTURE IN THREE BEE SPECIES

Sgolastra Fabio, Dipartimento di Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum Università di Bologna, Bologna, Italy.
Bogo Gherardo, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Focks Andreas, Institute of Mathematics, Osnabrück University, Germany
Medrzycki Piotr, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Molowny-Horas Roberto, CREAF, Universitat Autònoma de Barcelona, Bellaterra, Spain.
Jordi Bosch, CREAF, Universitat Autònoma de Barcelona, Bellaterra, Spain.

In agricultural environments, bees are exposed to combinations of pesticides, notably insecticides and fungicides. Systemic insecticides applied during pre-bloom are frequently found in the pollen and nectar of bee-attractive crops, and fungicides are routinely applied during bloom. In addition, under field conditions, bees may be exposed to pesticides for long periods, either due to the persistence of some chemicals in the environment or to multiple applications within their foraging range, or even through contaminated food stores accumulated in the nest. However, multi-residue and long-term exposure scenarios are not accounted for in current regulatory risk assessment schemes, which are based on the single-product single-crop paradigm. This is an important shortcoming because different pesticides can interact synergistically and their toxicity can be reinforced by long-term exposure. Moreover, current pesticide risk assessment relies on a single species, *Apis mellifera*, as a surrogate for the 20.000 bee species described worldwide. Yet, depending on their life history, bee species show strong differences in exposure and sensitivity to pesticides. In this study we test the chronic oral toxicity of the neonicotinoid insecticide clothianidin (CLO) alone and in combination with the EBI fungicide propiconazole (CLO+PRO) on three bee species (*Apis mellifera, Bombus terrestris* and *Osmia bicornis*). Our objective is to establish whether the insecticide shows time-reinforced toxicity (TRT) and, if so, whether TRT is influenced by the presence of the fungicide. Because SBI fungicides and neonicotinoids are known to interact synergistically, we hypothesize that ingestion of PRO will enhance TRT of CLO. *Osmia* species have been shown to be particularly sensitive to neonicotinoids. For this reason, we hypothesize a higher level of TRT in this species. *O. bicornis* was the most sensitive species to CLO and CLO+PRO. In all three species, the interaction between CLO and PRO was dose-dependent with synergism and antagonism observed at high and low doses, respectively. Prolonged exposure to CLO+PRO led to increase toxicity in the three species but clear time-reinforced toxicity (TRT) occurred only in *O. bicornis*. Our results underscore the need to test prolonged multi-residue scenarios and to account for differential pesticide sensitivity among bee species in risk assessment schemes.

Keywords: *Apis mellifera, Bombus terrestris, Osmia bicornis*

INVESTIGATION OF EUROPEAN FOULBROOD IN COLONIES PROVIDING BLUEBERRY POLLINATION SERVICES IN CANADA

Jenna M. Thebeau[1], Allyssa Cloet[1], Dana Liebe[1], Fatima Masood[2], Ivanna V. Kozii[1], Colby D. Klein[1], Michael W. Zabrodski[1], Sarah Biganski[1], Oleksii Obshta[1], M. Fahim Raza[1], Midhun S. Jose[1], Marina C.B. Silva[1], Roman V. Koziy[1], Antonio Ruzzini[2], Igor Moshynskyy[1], Larhonda Sobchishin[1], Geoff Wilson[3], M. Marta Guarna[4], Eric M. Gerbrandt[5], Elemir Simko[1] and Sarah Wood[1]

1 Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4
2 Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4
3 Ministry of Agriculture, Government of Saskatchewan, 141-800 Central Drive, Prince Albert, Saskatchewan S6V 6Z2
4 Agriculture and Agri-Food Canada, Beaverlodge, Alberta, T0H 0C0
5 British Columbia Blueberry Council, Abbotsford, British Columbia V2T 1W5

Canada is the second largest commercial producer of blueberries in the world. Honey bee pollination is essential for high quality and quantity of blueberry production in North America. Unfortunately, an eledged increased incidence of European foulbrood (EFB) disease has been reported in honey bee colonies used for commercial blueberry pollination in British Columbia and Atlantic Canada. We have used *in vitro* and *in vivo* experimental models of EFB to investigate potential predisposing factors for European foulbrood during blueberry pollination with specific focus on certain aspects of the host, pathogen and environment. We tested the effect of fungicide exposure, various *Melissococcus plutonius* isolates, blueberry pollen and bee population on larval susceptibility to European foulbrood. We tested effects of fungicides on larval susceptibility to EFB in vitro and found that larvae chronically exposed to a combination of four, formulated fungicides used in commercial blueberry production had significant increase in mortality when infected with *M. plutonius* compared to infected controls that were not exposed to fungicides. At the same time, exposure of infected larvae to the individual fungicides did not result in an increased larval mortatlity when compared to infected and non-exposed larvae to fungicides. We compared *in vitro* pathogenicity of *M. plutonius* strains isolated from blueberry-pollinating and non-blueberry pollinating colonies affected by EFB and found no difference in *in vitro* pathogenicity among these strains, suggesting that eledged increased incident of EFB in blueberry-pollinating colonies is not associated with a potentially enhanced virulence/pathogenicity of *M. plutonius* isolates from blueberry-pollinating versus non-blueberry pollinating colonies. Using our in vivo experimental EFB model, we also compared effects of beebread obtained from blueberry-pollinating versus non-blueberry pollinating colonies on larval susceptibility to EFB and found that larvae in colonies exposed to blueberry-beebread were higher mortality after experimental infection with *M. plutonius* when compared to similarly infected larvae in colonies exposed to polyfloral beebread. Effects of size of bee population on larval susceptibility to *in vivo* experimental infection with *M. plutonius* is currently being investigated. Based on these data, it seems that certain aspects associated with blueberry pollination may predispose larval susceptibility to EFB; however, further investigation is needed using both *in vivo* EFB experimental models and natural cases to confirm our *in vitro* data.

Keywords: fungicide, European foulbrood, virulence, blueberry pollination, honey bee, *Melissococcus plutonius*

IMPACTS OF PESTICIDES ON BUMBLEBEE COLONIES

Thompson, Linzi Jay, School of Agriculture and Food Science, University College Dublin, Ireland
Stout, Jane, Department of Botany, Trinity College Dublin, Ireland
Olsson, Ola, Department of Biology, Lund University, Sweden
Stanley, Dara, School of Agriculture and Food Science, University College Dublin, Ireland

Fungicides and herbicides are the most applied pesticide groups in the world. There is little research into how these pesticides may effect bees, particularly at the colony level. We investigated how colonies receiving glyphosate (herbicide) or prothioconazole (fungicide) treatment fared when placed outside, receiving no supplemental nectar or pollen.

Over a four-week period the activity levels of the colonies were recorded, colonies were weighed regularly. At the end of the experiment the number of workers and males were counted and measured. We found that prothioconazole has a significant effect on the weight and activity of colonies but we do not see this reflected in colonies receiving glyphosate treatment. These results indicate that when bees are exposed to the fungicide prothioconazole it may have detrimental effects.

POLLINATION AND BEE FLORA

ORAL PRESENTATIONS

CHAIRPERSON
Arnon Dag

BOTANICAL IDENTIFICATION OF HONEYBEE-COLLECTED POLLEN SAMPLES: A COMPARISON BETWEEN PALYNOLOGY AND DNA METABARCODING

Quaresma, Andreia, CIMO, Instituto Politécnico de Bragança, Portugal
Brodschneider, Robert, Institute of Biology, University of Graz, Austria
Brusbardis, Valters, Latvian Beekeepers' Association, Latvia
Gratzer, Kristina, Institute of Biology, University of Graz, Austria
Hatjina, Fani, Ellinikos Georgikos Organismos DIMITRA, Greece
Keller, Alexander, Ludwig-Maximilians-Universität Munich, Germany
Kilpinen, Ole, Danish Beekeepers Association, Denmark
Pietropaoli, Marco, IZSLT, Italy
Vejsnæs, Flemming, Danish Beekeepers Association, Denmark
van der Steen, Jozef, Alveus AB Consultancy, Netherlands
Pinto, M. Alice, CIMO, Instituto Politécnico de Bragança, Portugal
INSIGNIA consortium

Numerous studies have sought to gain a broader understanding on honeybee biology and health by examining botanical diversity of pollen loads transported by foragers into the colony. This goal has been addressed by identifying pollen loads by light microscopy. However, because this method is time consuming, expert-knowledge dependent and frequently lacks taxonomic resolution, it is being replaced by alternative approaches. DNA metabarcoding is one such alternative. Pollen metabarcoding can achieve high taxonomic resolution and, because it is based on high-throughput sequencing (HTS), it allows simultaneous analysis of large numbers of samples at a faster pace and lower cost than microscopy. While it is broadly acknowledged that metabarcoding can retrieve accurate lists of plant species from pollen analysis, there is no clear consensus as to what extent it produces reliable estimates on their relative abundances. Herein, we compared the two approaches on 145 pollen-samples collected in ten European countries. The samples were homogenized and split into two sets. One was analysed by palynology experts and the other was subjected to HTS, using ITS2 as barcode. Classification of the sequencing reads was done at the taxonomic level allowed by palynological data (genus or family) using ITS2 databases constructed for each country. The databases were curated to minimize misidentifications due to incorrectly annotated sequences downloaded from GenBank. Diversity analysis retrieved higher values for metabarcoding (Shannon index median =1.37) than for palynology (median=0.98), suggesting its higher power in detecting pollen sources. Analysis of the relative abundances showed a median (IQR) correlation (Mantel test) between the two approaches of 0.63 (0.14), ranging from 0.23 to 0.84. The highest correlations were observed for Italy (0.84), Portugal (0.83), and Denmark (0.71), while Ireland (0.37) and Greece (0.23) had the lowest correlations. These findings indicate that the ITS2 databases need to be further enriched, especially for countries like Greece. Greek flora is poorly represented in GenBank and therefore in our database. Our findings suggest that DNA metabarcoding can be an alternative to palynology, especially after large-scale sequencing efforts to improve ITS2 databases.

Keywords: Pollen DNA metabarcoding, Preservation bias, Citizen science

IMPACT OF THE "FARMING WITH ALTERNATIVE POLLINATORS" APPROACH ON CROP POLLINATOR POLLEN DIET

Sentil, Ahlam, Laboratory of Zoology, University of MONS, Mons, Belgium and Loboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco
Wood, Thomas James, Laboratory of Zoology, University of MONS, Mons, Belgium
Lhomme, Patrick, Laboratory of Zoology, University of MONS, Mons, Belgium and Loboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco
Hamroud, Laila, Laboratory of Zoology, University of MONS, Mons, Belgium and Loboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco
El Abdouni, Insafe, Laboratory of Zoology, University of MONS, Mons, Belgium and Loboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco
Ihsane, Oumayma, Laboratory of Zoology, University of MONS, Mons, Belgium and Loboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco
Bencharki, Youssef, Laboratory of Zoology, University of MONS, Mons, Belgium and Loboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco Rasmont, Pierre, Laboratory of Zoology, University of MONS, Mons, Belgium
Christmann, Stefanie, Laboratory of entomology, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco
Michez, Denis, Laboratory of Zoology, University of MONS, Mons, Belgium

Pollinators are facing declines at a global level. One of the main factors driving this decline is insufficient access to floral resources due to habitat loss and degradation that can affect both diet generalist species as well as those with more restricted floral preferences. Here we evaluated the effect of a novel mitigation strategy in agricultural ecosystems, Farming with Alternative Pollinators (FAP) on the pollen diet of crop pollinators. The approach dedicates 25 % of the cropped area to Marketable Habitat Enhancement Plants (MHEP) that attract pollinators, natural enemies of the crops and provide farmers with income. We assessed the effect of the approach on pollen diet of faba bean (*Vicia faba*) and pumpkin (*Cucurbita maxima*) flower visitors in four different regions in Morocco during 2018 and 2019 by comparing control fields (monoculture) and FAP fields in 13 trials and 101 sites. Results from 25 wild bee species show that almost two third of the species carrying or collecting pollen when visiting pumpkin flowers and half of the species carrying or collecting pollen when visiting faba bean flowers gathered this pollen from two or more host plants (i.e. MHEP, main crop and/or wild plants) and displayed a wide dietary breadth. Pollen grains from the main crops were poorly represented on the female scopae, indicating that crops

were mainly visited for nectar. Hence, crop flower visitors may require alternative pollen sources to meet their nutritional needs. The number of pollen genera collected by flower visitors and the dietary breadth of crop flower visitors did not show a significant increase in response to FAP management. Among the selected MHEP, sunflower (*Helianthus annuus*) was the pollen resource for pumpkin flower visitors. In faba bean, flower visitors collected pollen from coriander (*Coriandrum sativum*) and canola (*Brassica napus*). Our study sheds light on the importance of characterizing the pollen diet and the foraging behavior of crop pollinators to identify appropriate plant species that complement their food, maintain and conserve their populations.

CROP FERTILIZATION AND HONEYBEE POLLINATION ACTIVITY

Dag Arnon, Gilat Research Center, Volcani Institute, Ministry of Agriculture, Israel

Fertilization is a major farming practice intended to support crops' nutritional demands. However, there is almost no information about the effect of fertilization on a critical aspect of fruit-trees farming; pollination activity. In our recent studies, we identified two contrasting fertilization effects on the attractiveness of the crop to pollinating honeybee.

In avocado, we found high concentration of potassium in nectar (ca. 4,000 pp"m). Such high concentration, reduce its attractiveness to the pollinating honeybee as have been demonstrated in controlled experiments with sugar solutions. In the field, in avocado trees grown in containers, reduced potassium applications increase bee activity and fruit set.

In almond, we found that increased phosphorous fertilization in the summer, improves carbohydrates conversion to almond nectar in spring, subsequently attracting more bees for cross-pollination and fruit-set.

In conclusion, crop nutrition may play a major role on its attractiveness to pollinating bees and the consequence fruit set and yield.

ATTRACTIVENESS OF SELECTED PLANT SPECIES FOR WILD BEES: TESTING FOR THE REGIONALLY SUITED FLOWER STRIP MIX FOR SERBIA

Angelovski, Andrijana, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Raičević, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Bila Dubaić, Jovana, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Ugrenović, Vladan, Institute of Soil Science, Belgrade, Serbia,
Filipović, Vladimir, Institute of Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia,
Ćetković, Aleksandar, Faculty of Biology, University of Belgrade, Belgrade, Serbia,
Plećaš, Milan, Faculty of Biology, University of Belgrade, Belgrade, Serbia

Oilseed rape is important crop in Europe and is used in food production, as a biofuel and as animal feed, and its production in Serbia is steadily increasing. Various studies have shown that oilseed rape yield is dependent on the pollination service, primarily provided by bees. Therefore, it is important to support high diversity of wild bees and other pollinators in agricultural landscapes. In many European countries agri-environmental schemes aim to mitigate negative effects of biodiversity loss by protecting remaining natural habitats or promoting the sowing of wildflower strips. The main concept of flower strips around the crops is to attract pollinators (and other beneficial insects), from where they can spill-over into the crops and provide important ecosystem services. Currently, there are no such agri-environmental schemes in Serbia and the knowledge on the effectiveness of various wildflower species for attracting the pollinators is lacking. Within the framework of EcoStack project, we studied the attractiveness of 24 flowering plant species for wild bees, to estimate their potential for use in flower strips for improved oilseed rape production. We established experimental field at the Institute of Medicinal Plants Research "Dr Josif Pančić" where we planted oilseed rape strip surrounded by flower species planted in separate quadrats, arranged into four randomized transects. During the period May–July 2021, wild bees were sampled three times by timed hand-netting during each plant's flowering phase. We recorded 533 bees within 16 genera, of which 7 genera had about 95% of the cumulative number. The most attractive plant species was *Phacelia tanacetifolia,* regarding the highest number of detected bee genera and the highest bee abundance. Other prospective plant species were *Cynara scolymus, Calendula officinalis, Coriandrum sativum, Pimpinella anisum, Lotus corniculatus, Althaea officinalis* and *Onobrychis viciifolia.* Due to marked phenological differences, relatively few species flowered during the whole study period with the most of the plants in flower during June, and with the majority of bees active in that period. Our results should provide the baseline for further development and design of regionally suited flower mixes for the improved conservation of pollinator diversity in Serbian agricultural landscapes.

Keywords: pollinators, oilseed rape, EcoStack project

HOW MUCH NECTAR SUGARS AND POLLEN ARE AVAILABLE FOR POLLINATORS IN AN AGRICULTURAL LANDSCAPE? A CASE STUDY FROM SE POLAND

Jachuła Jacek, Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland
Denisow Bożena, Department of Botany and Plant Physiology, University of Life Sciences, Lublin, Poland
Wrzesień Małgorzata, Department of Botany, Mycology and Ecology, Maria Curie-Sklodowska University, Lublin, Poland

A decline in the numbers of pollinating insects has been recorded for many years in various parts of the world. Among the important reasons for this phenomenon, a shortage of food resources and/or a discontinuity in the food supply are indicated.

Long-term floristic analyses were conducted in man-made non-cropped habitats (non-forest woody vegetation, road verges, railway embankments, field margins, fallows) in an agricultural landscape of Lublin Upland (SE Poland). Detailed study on nectar and pollen production in the most frequent entomophilous plant species allowed to assess the spatial and seasonal distribution of food supply in man-made habitats. Using the previously collected data on sugar and pollen productivity of forests, meadows/pastures, orchards and winter rape crops, the total mass of sugars and pollen available in the landscape was calculated. Landscape-scale distribution of food resources over the flowering season was presented and sugar and pollen resources were compared to the dietary requirements of honey bees and bumble bees.

At the landscape scale, on average 985.1 ± 591.4 kg/km2 of sugars and 958.7 ± 409.1 kg/km2 of pollen was available. It was found that a large part (ca. 38%) of the sugar resources was provided by the flora of man-made non-cropped habitats, followed by winter rape crops (ca. 33%) and forest vegetation (15%). In the case of pollen, natural and semi-natural areas (forests and meadows/pastures) offered ca. 44% of the total resources, winter rape crops- ca. 35%, and man-made non-cropped habitats- ca. 18%. Especially high productivity of sugars and pollen was recorded in April and May, during the flowering period of forest, meadow and cultivated species (orchards, rape). Food shortages were observed in March and June. From July to October, 71-98% of available sugars and 66-99% of pollen were derived from man-made non-cropped habitats.

A spatial and temporal diversity of habitats (natural, semi-natural and man-made non-cropped areas) is essential to provide sufficient sugar and pollen resources and their continuity for pollinators. Man-made non-cropped habitats are an important addition to the pollinator food resources and significantly contribute to the alleviation of food stress, especially during periods of intense pollinator insect development.

Keywords: forage flora, food resource continuity, pollinating insects

"YOU ARE WHAT YOU EAT " – THE INFLUENCE OF POLYPHAGOUS AND MONOPHAGOUS DIET ON THE FLIGHT PERFORMANCE OF BEES

Hollens-Kuhr, Hilke, University of Muenster, Institute of landscape ecology, Muenster, Germany
Krüger, Jula-Klarissa, University of Muenster, Institute of landscape ecology, Muenster, Germany
Buchholz, Sascha, University of Muenster, Institute of landscape ecology, Muenster, Germany

Bees are known as the most important pollinators and are crucial for sexual reproduction of many angiosperms and crops. Due to anthropogenic land use change and the resulting fragmentation as well as the increase in monocultures, many bee species have to resort to a less diverse flower supply. A restricted flower supply may have strong negative effects on fitness of bees, for example on sexual fitness, which potentially results in reduced reproduction rates leading to extinction of populations. Furthermore, a reduced physiological fitness can cause a decreased body mass or lower movement performance which can also decrease reproductive success. Limited bee movement performance may cause a decline of potential forage areas with decreasing abundance, diversity and nutritional quality of available hostplants which may likely weaken stress resistance, sexual and physiological fitness of the bee population. Furthermore, a reduced movement performance of bees may negatively affect plants as well: due to reduced flower visitation rates, pollination and gene flow across landscape is limited and facilitates extinction of plant species.

Despite the brisance of the topic, there is still a big knowledge gap about the consequences of restricted flower supply on bee´s fitness. Therefore, we investigated the influence of polyphagous and monophagous diets on the flight performance of wild bees using *Bombus terrestris* as a model organism for polyphagous pollinators. For the monophagous treatment *Centaurea cyanus, Phacelia tanacetifolia* or *Sinapis arvensis* were grown in two mesocosms each. For the polyphagous treatment we used three mesocosms with a mix of each of the three plant species. In each mesocosm we introduced one bumblebee colony. After nine to twelve days of poly- or monophagous feeding flight performance was studied using flight mills. A flight mill is based on magnetic levitation technology and velocity, distance and periodicity of a bee´s flight was measured for a maximum of 20 minutes.

The study showed that the polyphagous-fed bumblebees flew longer and further than the monophagous-fed bumblebees. Hence, an unbalanced diet due to low flower supply leads to decreasing flight performance which may have dramatical consequences for pollination services capabilities and fitness of bee populations.

THE IMPACT OF COCOA INTENSIFICATION ON POLLINATING INSECTS IN FOREST ECOSYSTEMS IN GHANA

Boakye Richard[1,2] White Blanaid[3] Combey Rofela[4] Stanley Dara[1,2]

1 School Of Agriculture and Food Science, University College Dublin, Dublin, Ireland
2 Earth Institute, University College Dublin, Belfield, Dublin, Ireland
3 School Of Chemical Sciences, DCU Water Institute, Dublin City University, Dublin, Ireland
4 School Of Biological, Earth And Environmental Sciences, University Of Cape Coast, Ghana

Cocoa (*Theobroma cocoa*), a key ingredient of chocolate, is an important commercial crop plant which supports the livelihoods of an estimated forty to fifty million people around the world who are fully or partially involved in its cultivation. Income generated from cocoa export helps sustain the economies of many cocoa producing countries, particularly developing nations. As a crop that thrives primarily in the tropical rainforest, cocoa expansion has resulted in the conversion of large expanses of tropical rainforest to agricultural land for cocoa production. Presently more than 6 million hectares of rich tropical rainforests have been lost to cocoa cultivation in West Africa where seventy percent of the world's cocoa is produced. Even though habitat degradation and land use intensity have been implicated in global pollinator declines, little is known about the effects of cocoa expansion on bees and other pollinating insects whose key roles as ecosystem service providers to crops and wild plants are well documented. To understand the impacts of cocoa expansion on bees and other insect orders, we investigated how the abundance, species richness and diversity of bees, and the abundance of other insect orders, respond to land use intensity in Bia West District which is one of the leading seventy-one cocoa producing districts in Ghana. Eighteen study sites which varied along a gradient of the amount of natural forest within a 2 km landscape radius were surveyed on four occasions using both ground and aerial pan traps during dry and rainy seasons. Results suggest differential responses of bees and other insect orders to decreasing natural forest cover. This has implications for both the management of pollination services to crops, and the conservation of bee and insect diversity in the region.

Keywords: COCOA PRODUCTION, LAND USE, POLLINATORS

HOW DIFFERENT AGRO-ECOSYSTEMS IMPACT WILD BEES AND FLOWER COMPLEXITY RELATIONSHIP

Ranalli, Rosa, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Zavatta, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Flaminio, Simone, CREA Research Centre for Agriculture and Environment, Bologna, Italy. – Laboratory of Zoology, University of Mons, Mons, Belgium.
Giovanetti, Manuela, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Quaranta, Marino, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Modern agricultural techniques lead to an extreme simplification of the environmental features, reducing complexity of the habitats and biodiversity, while increasing parasites, plant and bee diseases linked to the crops. In this study was calculated Floral Complexity Index (FCI) in order to investigate the relationship between plant and wild bee communities in agro-ecosystems with different characteristics. The FCI is based on floral morphology, considering traits such as corolla's depth, size, symmetry and shape, and the inflorescence structures, all characteristics closely affecting plant-pollinator relations.

In the project "BeeNet – monitoring the environment through bees and biodiversity", the monitoring activities were carried out in 2021, March to October, in 5 Italian regions of the network, from north to south: Veneto, Piemonte, Emilia-Romagna, Campania and Puglia, comparing in each one two agro-ecosystem patterns, one intensive and one semi-natural in protected areas

This study investigates wild bees species, through transects and specimens collection, and floral composition, through FCI, both subjected to main biodiversity indices calculation and Network Analysis. Modularity is useful to assess the degree of specialization in sampling sites and to highlight hub species among plants and pollinators and the dynamic of their interactions.

Monitoring confirms that better conditions for the subsistence of plant and wild bee communities occur in semi-natural agro-ecosystems. Analysis of biodiversity indices, modularity and direct field observations lead to conclude that communities of plants and wild bees in semi-natural agro-ecosystems more robust and stable, and rich in biodiversity. On the contrary, in intensive agroecosystems, plant-pollinator communities are simpler and more fragile.

Keywords: Flower Complexity Index, Wild Bees, Network Analysis

OZONE AIR POLLUTION AFFECTS CROP POLLINATORS AND POLLINATION

Rollin, Orianne, CARI (Beekeeping Research and Information Centre), Louvain-la-Neuve, Belgium, & Centre for Ecology Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal;
Aguirre-Gutiérrez, Jesús, School of Geography and the Environment, University of Oxford, Oxford, UK;
Yasrebi-de Kom, Izak A.R., Department of Medical Informatics, Amsterdam UMC, Amsterdam, The Netherlands;
Garratt, Michael P.D., Centre for Agri-Environmental Research, University of Reading, Reading, UK;
de Groot, Arjen, Wageningen Environmental Research, Wageningen University & Research, Wageningen, The Netherlands;
Kleijn, David, Plant Ecology and Nature Conservation Group, Wageningen University, Wageningen, The Netherlands;
Potts, Simon G., Centre for Agri-Environmental Research, University of Reading, Reading, UK;
Scheper, Jeroen, Plant Ecology and Nature Conservation Group, Wageningen University, Wageningen, The Netherlands;
Carvalheiro, Luísa G., Departamento de Ecologia, Universidade Federal de Goiás, 74001-970 Goiânia, Brasil;

Human driven environmental changes increase the concentrations of polluting reactive compounds in the troposphere, such as ozone and nitrogen oxides. These changes lead to biodiversity losses and alter plant physiology and plant-pollinator interactions, essential for pollination services, with potential consequences for agricultural production. Here we used 133 unique sampling events from NW Europe to investigate how air pollution (ozone and nitrogen oxides) and other sources of nitrogen is related to pollinator visitation rate and their contribution to agricultural production, also considering possible interactive effects with landscape quality and pesticide input. We showed that ozone modulates the effect of pesticide exposure and temperature on crop pollinators, increasing the probability of negative impacts on crop pollination. Indeed, when ozone levels are highest, the strength of the effect of pesticide on pollinators is more than double then when ozone levels are intermediate. This indicates that air pollution should be considered in management plans and policies aiming to safeguard biodiversity and promote more sustainable food production practices.

Keywords: Crop pollination, bees, ozone pollution

INTRASPECIFIC SIZE SHIFTS IN GENERALIST BUMBLEBEES AND FLOWERS LEAD TO LOW FUNCTIONAL CONSEQUENCES

Reverté, Sara. Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium
Gérard, Maxence. INSECT Lab, Division of Functional Morphology, Department of Zoology, Stockholm University, Svante Arrhenius väg 18b, 11418, Stockholm, Sweden
Bodson, Maxime. Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium
Descamps, Charlotte. Earth and Life Institute–Agronomy, UCLouvain, Croix du Sud 2, 1348 Louvain-la-Neuve, Belgium
Gosselin, Matthias. Laboratory of Entomology, Haute École Provinciale de Hainaut - Condorcet, 11, Rue Paul Pastur 7800 Ath, Belgium
Jacquemart, Anne-Laure. Earth and Life Institute–Agronomy, UCLouvain, Croix du Sud 2, 1348 Louvain-la-Neuve, Belgium
Louvieaux, Julien. Applied Plant Ecophysiology Laboratory, Haute École Provinciale de Hainaut - Condorcet, 11, Rue Paul Pastur 7800 Ath, Belgium
Smagghe, Guy. Laboratory of Agrozoology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
Vandamme, Peter. Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium
Vereecken, Nicolas J.. Agroecology Lab, Université libre de Bruxelles (ULB), Avenue FD Roosevelt 50, B-1050 Brussels, Belgium
Michez, Denis. Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du Parc 23, 7000 Mons, Belgium

Body size is a trait that can affect plant-pollinator interaction efficiency and plant reproductive success. We explored the impact of intraspecific size shifts on the interactions between pollinators and flowering plants under controlled conditions. We considered two development conditions leading to the production of large and small flowers of *Borago officinalis* and *Echium plantagineum*. We also used the natural variability of worker size within bumblebee colonies to isolate small and large workers. We performed a fully crossed experiment with the two flower sizes of each plant species, and the two sizes of bumblebee workers. Our results show that the size of both partners did not affect bee foraging behavior in most of the evaluated parameters, and both bee sizes were equally efficient in depositing pollen. Significant differences were found only in the lifelong pollen deposition in small flowers of *B. officinalis*, with the greatest quantity of pollen deposited by small bees. We did not find a relationship between pollinator size and plant fitness. Our results suggest that generalist plant-pollinator interactions may be resilient to global changes, but potentially damaged under certain combinations of local conditions.

Keywords: flower size, bee size, plant-bee interaction

WILD BEES IN VILLAGES: EFFECTS OF HABITAT TYPE AND FLORAL RESOURCES

Schulze, Sonja, Daniela Kessner-Beierlein, Alicia Bender, Annika Schöninger, Ingolf Steffan-Dewenter, Andrea Holzschuh, Department of animal ecology and tropical ecology, University of Würzburg, Germany

In the last decades, wild bees experienced massive declines due to habitat loss in agricultural landscapes and the intensive use of pesticides. Whereas wild pollinators are widely studied in agricultural habitats, parameters affecting pollinator diversity and abundances in villages have been less studied. Although green spaces and gardens in villages have the potential of being refuges for wild bees, it remains unclear which village habitats are suited best for wild pollinators. To better understand the importance of typical village habitats and if differing floral resources in village habitat types are drivers for varying effects on the abundance and richness of different pollinator groups, we recorded bees and hoverflies on 200 sites in 40 villages in Northern Bavaria, Germany. We conducted five sweep net surveys between April and August 2020 in five habitats per village: private gardens, farm gardens, green public areas, fallow lands, and cemeteries. Moreover, we recorded the number of flowering plant species and flower cover. In total we found 229 wild bee species, including 18 bumble bee species, which together make up for 44% of all Bavarian wild bee species. Our results show that village habitats differ in pollinator diversity and abundance, flowering plant species diversity and flower cover. In cemeteries we found the highest number of flowering plant species and flower cover as well as the highest numbers of wild bees, honeybees, hover flies. Total pollinator diversity and the numbers of bumble bees were highest in farm gardens. Moreover, we found that the number of flowering plant species drives the differences in habitats. With these results we highlight the value of villages for native pollinators and conclude that high quality village habitats should be a target for future conservation measures to restore wild pollinator diversity.

DO INSECT POLLINATION SERVICES DEPEND ON CLIMATE? AN INTERNATIONAL FIELD STUDY ON APPLE ORCHARDS

Riva, Clémence, Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France
Apostoloudis, Aggelos, Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA', Nea Moudania, Greece
Bevk, Danilo, Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
Bouchot, Claire, Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France
Filipi, Janja, Department of Ecology, Agronomy and Aquaculture, University of Zadar, Croatia
Hatjina, Fani, Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA', Nea Moudania, Greece
Kolega, Šimun, Department of Ecology, Agronomy and Aquaculture, University of Zadar, Croatia
Kovačić, Marin, Department of Plant Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia
Manthos, Ioannis, Department of Nut Trees, Institute of Plant Breeding and Genetic Resources, ELGO 'DIMITRA', Lamia, Greece
Noureddine, Adjlane, Department of Agronomy, faculty of Sciences, University of Boumerdes, Algeria
Puškadija, Zlatko, Dept. of Plant Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia
Sotiropoulos, Thomas, Department of Deciduous Fruit Trees, Institute of Plant Breeding and Genetic Resources, ELGO 'DIMITRA', Naoussa, Greece
Stipešević, Bojan, Department of Plant Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia
Theodorakidis, Evanggelos, Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA', Nea Moudania, Greece
Yamina, Haider, Department of Agronomy, faculty of Sciences, University of Boumerdes, Algeria
Žunič Kosi, Alenka, Department of Organisms and Ecosystems Research, National Institute of Biology, Vecna pot 111, Ljubljana, Slovenia
Requier, Fabrice, Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France

Over the last century, there is increasing recognition that pollination deficits are limiting crop yields worldwide. Current trends in insect pollinator decline can intensify crop pollination deficits and thus challenge food security. It is however still unclear whether climate change affects crop pollination deficits. We tested this hypothesis in the frame of the European SafeAgroBee (PRIMA) project, which aims at safeguarding agroecosystem resilience under climate change through efficient pollination and sustainable beekeeping. We performed standardized pollination experiments on 29 apple orchards (*Malus domestica*) distributed along a climate gradient in the Mediterranean Basin covering five countries: Algeria, Croatia, France, Greece and Slovenia. Apple is a globally important crop that highly depends on insect pollination. By comparing the fruit-set of flowers visited by insects and the fruit-set of flowers excluded from insect visitations, we assessed how climate impacts the service provided by insect pollinators. We also performed manual supplementation of pollen to these pollination treatments to assess the potential climate dependence of pollination deficits in apple production. We will present our results of pollination deficit effects on fruit-set and how climate interplays with pollination-driven productions. We will discuss these results in a context of climate change and how climate could impact crop pollination services, with a particular interest of suggesting management practices that would support the conservation of insect pollinators in farmlands.

Keywords: Apple; Insect pollination; Crop yield

LAVENDER AND HONEY BEES: POLLINATION AND BEE PRODUCTS

Tananaki Chrysoula, Kanelis Dimitrios, Liolios Vasilios, Rodopoulou Maria-Anna, Kovousoglou Evgenia
Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki, Thermi, Thessaloniki, Greece

Lavandula angustifolia Mill, because of the essential oil production has a great economic importance in cosmetics, food manufacturing and pharmaceutical industry. In addition, it is considered as a plant with a special interest in the production of beekeeping products. However, few studies there has been on the benefits of lavender plants (pollen, nectar) to honeybees, bees' behaviour and plant-pollinator interactions. In the present work applying field experiments, the effectiveness of *Apis mellifera* in pollination of *Lavandula angustifolia* and in yield and composition of its essential oil was studied, in relation to the population density of honeybees and the distance from the crop. The possibility of honey and pollen production from lavender cultivations was evaluated and the characteristics of the produced honey were investigated. The results showed that honeybees prefer to visit lavender at noon and closer to the hive, collecting nectar and small quantities of pollen. The visits of bees on lavender flowers effect on its pollination, increasing the yield of the essential oil and the weight of the seeds. No quality differences were found in the volatile profiles of the produced essential oils. The analysis of the physicochemical and microscopical characteristics of lavender honey shown that it has a moderate moisture content and diastase activity, very low electrical conductivity and HMF concentrations, and is characterized as light-colored honey.

Keywords: pollination, lavender honey, *Lavandula angustifolia*, essential oil

FORAGING DISTANCES, HABITAT PREFERENCES AND SEASONAL COLONY PERFORMANCE OF HONEYBEES IN CENTRAL EUROPEAN FOREST LANDSCAPES

Rutschmann, Benjamin, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
Kohl, Patrick L., Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
Steffan-Dewenter, Ingolf, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany

1. Woodland is considered as native habitat of the Western honeybee (*Apis mellifera*) and is assumed to provide important pollen and nectar sources. However, resource supply might be spatially and temporally restricted and landscape-scale studies in European forest regions are currently lacking.
2. Capitalizing on the unique communication behaviour, the waggle dance, we investigated honeybee foraging in a deciduous forest region in southern Germany. We analysed 2022 waggle dances performed by bees of twelve colonies placed at locations with varying degree of forest cover (50-99% at a 2 km radius) from March–August 2019, thereby identifying foraging distances and habitat preferences over almost an entire foraging season). By connecting dance information with colony weight recordings, we estimated the contribution of the different habitat types to colony weight gain.
3. Foraging distances generally increased with the amount of forest in the surrounding landscape. Yet, this effect strongly depended on the season and was more pronounced for pollen than for nectar foraging. Even though colonies in forest-dominated landscapes had to fly further, colony weight was not affected by forest cover.
4. Compared to expectations based on the proportions of different habitats, colonies foraged more frequently in grasslands and cropland than in deciduous and coniferous forests, with late summer being an especially difficult period for pollen foraging in forests. During a phase of colony weight gain in early summer, the use of forests for nectar/honeydew foraging was close to the expectation, highlighting forests as an important source of carbohydrates during short periods of the year.
5. *Policy implication*. The ecological and economic value of managed forest as habitat for honeybees and other wild pollinators could be significantly increased by the continuous provision of floral resources, especially for pollen foraging. We therefore recommend diversifying forest stands with insect-pollinated trees, permitting secondary successions in forest gaps, and at larger scales, creating forest landscapes with high habitat diversity.

Keywords: Central European forests, habitat preferences, wild-living honeybees

INTELLIGENT POLLINATION FOR SUSTAINABLE INCREASES IN CROP YIELDS AND GLOBAL FOOD SECURITY

Kordic Evans, Sandra, Canetis SRL, Bagni di Lucca, Italy

A growth in the volume of pollinator dependent crops combined with intensive farming techniques has stimulated development of a new agricultural industry - managed bee pollination services. However, as yet, no technologies are available for monitoring bee pollination, and no data or tools that farmers can use to support pollination management decisions. iPollinate is an EU funded R&D project which is developing a IoT sensor that provides real time monitoring of the foraging rate of honeybee colonies used for crop pollination services. The sensor is an optical bee counter that is fitted to a beehive which continuously monitors bees leaving and arriving at the hive. The results for testing of the sensor accuracy and field trials performed over the past two seasons in California almond pollination are presented. The data collected have yielded a number of colony parameters such as foraging force at any given time, total number of foraging trips, mortality in the field and daily flight profiles, all directly related to the efficiency of pollination service provided. Current methods for assessing pollination efficiency rely on meteorologically driven models which, while indicative, do not deal with borderline conditions effectively, be it climatic conditions or the colony strength inconsistencies. Our data have shown that flight hours of bees in almonds can vary drastically from those derived from models as they are subject to an interplay of factors that include not only weather conditions and colony strength but also colony composition, energy reward available to foragers and even the microclimate differences between seemingly identical geographic areas. Real time information allows for timely intervention where necessary thus mitigating inadequacies in pollination. While almonds form the model crop in this project the same principle is being applied to other crops such as sunflowers, strawberries etc. Our results demonstrate that continuous tracking of bee colonies' foraging activity provides unprecedented insight into colony behaviour and hence the effectiveness of pollination. This will create a new data driven, technology enabled business model for the delivery of Precision Pollination Services, helping farmers to optimise pollination efficiency and crop yields.

Keywords: bee hive monitoring, bee counter, precision pollination

SEED MIX PERFORMANCE AND FUNCTIONAL NETWORK ROLES AS A FRAMEWORK TO SELECT KEY POLLINATOR RESOURCES

Uta Müller, Neal M. Williams
Department of Entomology and Nematology, University of California Davis, Davis, CA

Current native bee declines have, among other factors, clearly been attributed to habitat loss. Mitigation approaches are trying to establish or enhance habitat by plantings of wildflowers, which are often done by seed mix applications. Whereas assessment of plant performance in a variety of restoration efforts has been done so far, there is a lack of evaluation in pollinator seed mixes. Moreover, plant species choices for seed mixes are predominantly based on abundance and diversity visitation data which might not encompass the interaction diversity of plant-pollinator-networks, another crucial component of biodiversity. Therefore, the selection of "key pollinator resources" could be extended with regards to functional network roles.

I am presenting results from two different experiments that independently assessed the performance of a set of 28 native Californian wildflowers in seed mix applications over 3 years and pollinator visitation in monoplantings of the same plant species.

Within seed mixes, plant species' performance according to maximum floral area and phenological coverage clearly differed with certain taxa found to dominate, or only establish short-term or not be competitive at all. Only a small set of 5 species persisted with high performance over the whole 3 years.

In contrast to applying abundance-diversity metrics functional network roles enabled the identification of network cores and species supporting specialized pollinators' interactions. Both functional roles are characteristic in natural plant-pollinator networks and therefore could present key characteristics for plant choices with regards to restoring mutualistic plant-pollinator networks with wildflower habitat plantings. Plant species' network roles changed over the seasons arguing for a focus on seasonality in key pollinator resource selection. Only a subset of species of high functional importance in networks proved to be successful in seed mix applications. The results emphasize the need to evaluate wildflower plantings and prioritize species of high performance for multiple criteria in current and future applications.

POLLINATION AND BEE FLORA

POSTERS

POLLEN IDENTIFICATION BY ITS2 METABARCODING: CURATION OF THE SEQUENCES RETRIEVED FROM GENBANK TO BUILD A REFERENCE DATABASE

Quaresma, Andreia, CIMO, Instituto Politécnico de Bragança, Portugal
Keller, Alexander, Cellular and Organismic Networks, Faculty of Biology, Ludwig-Maximilians-Universität Munich, Germany
Rufino, José, CeDRI, Instituto Politécnico de Bragança, Portugal
van der Steen, Jozef, Alveus AB Consultancy, Netherlands
Pinto, M. Alice, CIMO, Instituto Politécnico de Bragança, Portugal

A powerful way of studying the quality of the environment is by examining the pollen collected by honey bees (*Apis mellifera*) as it contains information on available plant sources, spatial and temporal floral diversity, as well as on chemical contaminants. This entails botanical identification of pollen which has typically been addressed by classical palynology, a costly approach that often provides low taxonomic resolution, is time-consuming, labour intensive, and requires plant taxonomy expertise. However, with high-throughput sequencing becoming increasingly affordable, pollen metabarcoding is gaining momentum, and it is a promising alternative to classical palynology. But one of the main drawbacks of pollen metabarcoding is the lack of good quality reference databases for the barcode of choice. BCdatabaser (Keller et al. 2020) was developed to automatically generate a standardized database for the ITS2 barcode from the primary sequence database GenBank. While using BCdatabaser to construct an ITS2 reference database for identification of bee-collected pollen, we noticed several misidentified sequences retrieved from GenBank, which would impact identification accuracy. There were two types of problems: plant sequences that were assigned to the wrong plant species and fungi sequences that were identified as plants. To overcome these issues, we developed scripts in bash and R to curate an ITS2 reference database for pollen identification purposes. These scripts allowed us to identify the Fungi sequences retrieved from GenBank for subsequent removal from the database, to perform a pairwise alignment of all the sequences using vsearch v2.14.1 (Rognes et al., 2016) and, then to remove all the sequences with low identity percentage using an iteration process in R v4.1.2. The database curation is automated therefore enabling easy update of the ITS2 database to take advantage of the new sequences that are regularly deposited in GenBank.

Keywords: Pollen DNA metabarcoding, ITS2 database curation, honeybee

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BOMBUS TERRESTRIS: I CAN OPEN THE BLOCK BARRIERS!

Özenirler, Çiğdem, Hacettepe University Faculty of Science Department of Biology
Aytekin, Ahmet Murat, VP University of Mons Department of Zoology Belgium

Bombus terrestris is the most common bumblebee species used in pollination. As a researcher, sometimes you have to count bumblebees to evaluate the foraging performance of pollinators on target plants-especially in agricultural production. It can be done directly-counting the number of bees foraging on a specific flower, branch, plant or a whole tree or indirectly, counting the foragers going through in or out of their hives. The hives we worked on had two tunnels opening to the outside. The counters' working principle is closing one of the tunnels with a block barrier. Within this system the individuals go out from the hive through these block barriers and can enter just from the other open hole. By the help of barriers, only the foragers going out are counted.

During the field observations it was observed that some individuals could open the block barriers with their forelegs from the opposite sites. Because of this, contractionary to the system, an individual counted for two times.

To witness such a behavior was a failure for our study. On the other hand, it was a different learning performance in the colony. We do not know the possible effects of this variation among the foragers. But for the future studies, natural variation in learning ability and the evolutionary causes and consequences of this variation between individuals could be studied deeply.

Keywords: commercial bumblebees, learning, counter

ARE BUMBLEBEE REVISITS OF PROBED FLOWERS ERRORS IN FLOWER CHOICE, OR ARE THEY DETERMINED BY EXTRAORDINARY NECTAR REPLENISHMENT IN NECTAR-RICH FLOWERS? THE CASE OF INVASIVE ALIEN *IMPATIENS GLANDULIFERA*

Najberek Kamil, Kosior Andrzej, Solarz Wojciech
Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland

Decrease in efficiency of bumblebee feeding may result from revisits to flowers that were already probed. Moreover, it may affected the plant pollination service. In 2021 we revealed that bumblebee revisits in probed flowers of *Impatiens glandulifera* (the best nectar-rewarding plant in Europe) increased with air temperature. The result was particularly significant for bumblebee workers. In turn, at temperatures > 37°C, bumblebee males revisited probed flowers less often than workers.

In this experiment, however, we did not test whether the revisits are errors in flower choice or rather they are determined by fast nectar replenishment in *I. glandulifera* flowers. The first scenario would be alarming in terms of global warming because workers provide food for larvae, thus any decrease could pose a serious threat to the survival of bumblebee colonies and also for the plants that they pollinate. In turn, the second scenario would indicate that under high temperatures workers exceed males in foraging efficiency.

Although males do not feed larvae, they participate in pupal incubation; moreover the traits of males (e.g., sperm quality) determine queens' longevity and reproductive success. If flower revisits increasing with air temperature are errors, then in a hot weather males may be more efficient in foraging than females. On the other hand, if revisiting of probed flowers is determined by fast nectar replenishment, then the role of males in a colony could be even higher. It would indicate that at temperatures < 37°C males are more efficient in recognizing refilled flowers than workers.

At the EurBee 9 we will provide data on ability of nectar replenishment of *I. glandulifera* flowers under high temperatures. It will allow to address the research question whether the revisits, influenced by rising air temperature, were errors in flower choice, or else they were determined by effective nectar replenishment.

Keywords: Bumblebee, Himalayan balsam, Error in flower choice

IDENTIFICATION, CHARACTERIZATION AND PILOT MAPPING OF HONEY BEE FLORA OF THESSALONIKI REGION, GREECE

Rodopoulou Maria-Anna, Kanelis Dimitrios, Liolios Vasilios, Tananaki Chrysoula,
Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki, Thermi, Thessaloniki, Greece

The increase in number of bee colonies along with the reduction of bee flora makes the transferring of bee hives to areas with diverse flowering plants a necessary condition, so that the bee colonies are successfully productive. Thus, the mapping bee flora can be an important tool that will help beekeepers in the transferring schedule of bee hives, as to date it is based mainly on personal experiences and observations. The aim of the present study was the pilot mapping of beekeeping plants in an area of the prefecture of Thessaloniki (area of 25Km^2) with on-site recording in the field and sampling for the identification of plant species. For the estimation of the population size of the surrounding flora, we used a semi-quantitative scale with four classes, adapted from Witting, where class I contained scarcely located taxa and class IV the dominant taxa (covering at least 30% of the collection site), while for the identification of pollen grains the melissopalynological analysis was applied. The results of the recording were depicted through the GeoJSON format on maps containing also information on pollen grain morphology and plant identification. The mountainous and semi-mountainous areas were dominated by shrubs and trees, namely *Quercus* sp. (scale: IV), *Pinus* sp. (scale: IV) and *Castanea sativa* (scale: III), and the lowlands by *Sinapis* sp. and *Papaver rhoeas* (scale: IV), while *Carduus* sp. was recorded in smaller numbers (scale: II). *Cistus* sp. and *Paliurus spina-christi* were recorded in semi-mountainous areas (scale: III), while *Vitex agnus-castus was* found mainly in areas with increased humidity regardless of altitude (scale: I). The results of the present research and their application to a greater extent with the use of GIS will contribute to further knowledge of bee flora in wider areas, for the successful transferring of bee hives to targeted flower zones.

Keywords: bee florarecording, bee flora diversity, bee flora maps

HONEY BEE-COLLECTED POLLEN FOR BOTANICAL IDENTIFICATION VIA ITS2 METABARCODING: A COMPARISON OF PRESERVATION METHODS FOR CITIZEN SCIENCE

Quaresma, Andreia, CIMO, Instituto Politécnico de Bragança, Portugal
Brodschneider, Robert, Institute of Biology, University of Graz, Austria
Gratzer, Kristina, Institute of Biology, University of Graz, Austria
Gray, Alison, Department of Mathematics and Statistics, University of Strathclyde, UK
Keller, Alexander, Cellular and Organismic Networks, Faculty of Biology, Ludwig-Maximilians-Universität Munich, Germany
Kilpinen, Ole, Danish Beekeepers Association, Denmark
Rufino, José, CeDRI, Instituto Politécnico de Bragança, Portugal
van der Steen, Jozef, Alveus AB Consultancy, Netherlands
Vejsnæs, Flemming, Danish Beekeepers Association, Denmark
Pinto, M. Alice, CIMO, Instituto Politécnico de Bragança, Portugal

While classical palynology has been the method of choice to assess botanical diversity of bee-collected pollen for multiple purposes, DNA metabarcoding is emerging as a powerful alternative being able to achieve high taxonomic identification accuracy. Moreover, DNA metabarcoding allows analysis of hundreds of samples in a single high-throughput sequencing run, therefore offering unprecedented scale in citizen science projects. Biases in metabarcoding can be introduced at any stage of sample processing and preservation is at the forefront of the pipeline. Hence, it is important to test how sample preservation influences quality and quantitative performance of pollen metabarcoding. While in metabarcoding studies pollen has typically been preserved at –20°C (FRZ), this is not the best method to be applied by citizen scientists. In this study, we compared the freezing method with ethanol (EtOH), silica gel (SG) and room temperature (RT) for preservation of pollen collected from hives in Austria and Denmark. The pollen was stored for ~4 months before analysis. DNAs were extracted with a food kit, and their quality and concentration measured. The quality of most of the DNA extracts exhibited an absorbance ratio close to the optimal 1.8, with RT samples from Austria showing poorer quality than FRZ and SG samples ($P < 0.027$). DNA concentration also showed statistical differences, with EtOH samples producing lower yields than RT and FRZ samples in both countries and SG in Austria ($P < 0.042$). However, the floral composition (as expressed by richness, relative abundance and Shannon diversity) inferred from ITS2 high-throughput sequencing was not impacted by the preservation methods in both countries. While freezing and ethanol are normally used for archiving tissue for molecular applications, desiccation is cheaper and an easier method to use regarding both storage and transportation. Since SG is less dependent on ambient humidity and less prone to contamination than RT, we recommend SG for preserving bee-collected pollen for metabarcoding. SG is straightforward for laymen to use and so it is a robust preservation method for widespread application in citizen science studies.

Keywords: Pollen DNA metabarcoding, Preservation bias, Citizen science

USING DNA METABARCODING IN POLLEN SAMPLES FOR THE IDENTIFICATION OF FLORAL SPECIES

Karapetsi, Lefkothea, Institute of Applied Biosciences (INAB), Centre for Research and Technology (CERTH), Thessaloniki, Greece
Pratsinakis, Emmanouil, Laboratory of Agronomy, School of Agriculture, Aristotle University of Thessaloniki (AUTH), Thessaloniki, Greece
Kanelis, Dimitrios, Laboratory of Apiculture-Sericulture, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece
Liolios, Vasilis, Laboratory of Apiculture-Sericulture School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece
Tananaki, Chrysoula, Laboratory of Apiculture-Sericulture, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece
Madesis, Panagiotis, Laboratory of Molecular Biology of Plants, School of Agricultural Sciences, University of Thessaly, Volos, Greece

During the last decade numerous studies have highlighted the importance of DNA metabarcoding in pollen samples collected from pollen traps and honey, to identify floral visitation across honeybees. DNA metabarcoding provide researchers a powerful tool for a large-scale identification of known and unknown taxa within a mixed pollen sample using specific DNA barcode markers and high-throughput sequencing. In the presented study, 40 pollen samples collected from pollen traps that were attached in selected beehives around Peristera (Thessaloniki region). Pollen samples were preserved at -20°C, until DNA extraction was performed according to the protocol from NucleoSpin™ Plant II kit (Macherey-Nagel, Germany). PCR amplification of extracted DNA was performed using primers that target the ITS2 region. Multiplexing and library preparation was performed according to Illumina's 16S Metagenomics Protocol with modifications using the Nextera® XT Index Kit. The libraries' pool was loaded on the Illumina Miseq (2 x 300 bp) using a MiSeq® Reagent Kitv3. Raw sequence data was processed using the DADA2 ITS Pipeline within Galaxy. The starting point was a set of Illumina-sequenced paired-end fastq files that have been split ("demultiplexed") by sample. The product is an amplicon sequence variant (ASV) table, providing records of the number of times each exact amplicon sequence variant was observed in each sample. We also assigned taxonomy to the output ITS sequence variants using the UNITE database. The external tool Phyloseq (R package), along with dada2 were used to visualize the results of our analyses: bar-plots for abundance, sequence complexities, quality plots, error plots. Ten most abundant plant families detected were: Asteraceae, Ericaceae, Araliaceae, Rosaceae, Papaveraceae, Brassicaceae, Fabaceae, Rhamnaceae, Oleaceae and Scrophulariaceae. Most abundant plant species belonged to genus: *Crepis, Echium, Trifolium, Paliurus, Conium, Brassica, Papaver, Erica, Rubus, Xanthium, Hedera* and *Portulaca*.

Keywords: ITS2, floral visitation, abundance

BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.) AS A MELLIFEROUS PLANT

Zajácz Edit, Déri Helga, Sisa Anita, Donkó Kata Sára, Rácz Tímea
Department of Apiculture and Bee Biology, Institute for Farm Animal Gene Conservation, National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

In Hungary, *Robinia pseudoacacia* plays a particularly important role among bee pasture plants. The amount of nectar produced by the varieties and its sugar content determine the honey production, which is influenced by a number of factors. Of these, we represent the effect of the experimental sites and some microclimatic factors on nectar production. During 2018-2019, we examined the nectar production of 31 black locust cultivars at 9 sampling sites. The studied varieties were selected for late-flowering, industrial and apicultural purposes. Our results show, that the amount and sugar value of nectar were less affected by the weather factors than its sugar concentration. Based on the mass and sugar value of nectar, the examined cultivars were divided into 3 groups. Late-flowering varieties secreted significantly less nectar with lower sugar values than those of which selected for industrial and apicultural uses. The highest sugar concentrations (50-60%) were measured in those sites where the air temperature was above 20 °C, while in the coldest production areas with 15 °C average temperature and rainy weather, the sugar content reached only 26-37%. The insect attractiveness of false acacia was also determined by the fact that its flowers constituted racemose inflorescences. Therefore, its apicultural value was also estimated by calculating the nectar and sugar yield per raceme. Likewise to the nectar volume per flower, nectar yield per inflorescence was the lowest in the late flowering cultivars (24.68-45.41 mg/ raceme) and the highest in the apicultural varieties (91.13-115.06 mg/ raceme). Sugar yield per racemose was paralleled with nectar sugar value. In Hungary, black locust is planted in large areas. Not only the apicultural varieties, but the nectar of industrial and late-flowering cultivars is also attractive to honey bees. This is because the sugar content of the floral nectar exceeded the 10% threshold value above which bees feel the nectar sweet, even under various sampling sites and changeable weather conditions. Selection of the late-flowering cultivars or which produce more nectar is very useful for beekeeping because in the future they could be utilized as multipurpose varieties (apiculture and forestry). This work was supported by the VEKOP-2.1.1-15-2016-00166 tender.

Keywords: *Robinia*, nectar, apicultural value

A COMPARISON OF RECORDING METHODS OF BEEKEEPING PLANTS FLOWERING

Liolios Vasilios, Kanelis Dimitrios, Rodopoulou Maria-Anna, Tananaki Chrysoula,
Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki, Thermi, Thessaloniki, Greece

The knowledge of the bee flora of an area and the prediction of the flowering period of target plants are necessary and basic for the development of the beekeeping, contributing significantly to the planning of bee hives transferring and the reduction of production costs. At the same time, climate change and its impact on the flowering of beekeeping plants creates additional problems and leads to failures in the transferring schedule of the bee hives. Thus, the aim of the present study was to investigate the use of pollen traps as an easy and reliable tool for recording the flowering period of beekeeping plants in an area. The estimation of the flowering days was based on the presence of the pellets in the pollen traps, while for the evaluation of the accuracy of the results, at the same time on-site recordings were made in the area around the beehives. Experimental apiaries were placed in two areas of the Prefecture of Thessaloniki and *Olea europaea*, *Cistus* sp., *Paliurus spina-christi, Sonchus asper, Erica angustifolius, Ephedra sp., Trifolium pratense, Verbascum* sp., *Carduus* sp. and *Portulaca oleracea* were used as target plants. The collected pollen pellets were separated by color and identified through microscope. The maximum flowering (flowering rate> 80%) in all cases coincided with the maximum presence of the respective pellets in the pollen traps. Specifically, in *O. europaea*, the maximum flowering corresponded to a participation rate of 41.11% of the total collected pollen in the trap, in *Cistus* sp. 44.75%, in *P. spina-christi* 67.95%, in *S. asper* 4.17%, in *E. angustifolius* 41.84%, in *Ephedra* sp. 47.98%, in *T. pratense* 2.97%, in *Verbascum* sp. 3.54%, in *Carduus* sp. 5.07% and in *P. oleracea* 18.40%. Although the preference of bees in terms of the harvested quantity of pollen is influenced by many parameters (e.g. abundance of plants, plant supply, etc.), it seems that the estimation of the period and the maximum flowering can be predicted with great accuracy with the use of pollen traps.

Keywords: pollen traps, blooming season, bee flora recording

STUDY OF BEE FAMILIES RAISED IN AN URBAN ENVIRONMENT

Tsvetelina Nikolova[1*], Elena Tsvetkova[1]

1 Forestry University, Sofia, Bulgaria
* c.alipieva@abv.bg

The article is in the results of a scientific project investigating an alternative to traditional beekeeping, enabling the cultivation of *Apis mellifera* in urban conditions. In Bulgaria, beekeeping is a traditional activity in all areas of the country. In the last few years, there has been a tendency for more and more beekeepers to look after their bees in cities and within populated areas. Sofia is a good and preferred city for beekeeping. About 200 beekeepers have been raising their bees within the city for the past two years. One of the goals of the project is to track and prove that the urban environment is suitable for raising bee colonies and the production obtained from them is suitable for human consumption. For this purpose, 10 bee colonies were used in settlements of different intensity. The influence of the urban environment on the development of bee colonies, their strength and honey productivity was tracked. The results obtained by us provide opportunities for popularizing urban beekeeping and prove that bee colonies develop well in urban conditions.

Key words: Urban environment, Urban beekeeping, Environmental conditions, Pollution, *Apis mellifera* L., Honey bee

AUTOMATIC DETERMINATION OF COLOUR DIVERSITY ON MOBILE PHONE IMAGES OF POLLEN TRAPS

Borlinghaus, Parzival, Institute for Operations Research, Karlsruhe Institute of Technology, Karlsruhe, Germany

Pollen is known to be the only source of proteins and fats for honey bees. Therefore it is an important part of the honey bees' nutrition. They are essential for brood care and a good indicator of the quality of a site. It is also known that a diverse diet makes colonies more robust, also in relation to winter losses. In this work, an app is presented that allows to quantify the pollen from a pollen trap and to determine its colour diversity in an automatic way. The colour diversity is closely related to the actual plant diversity. This correlation allows conclusions to be drawn on the quality of a site and on biodiversity in general. In this way, the app provides beekeepers with important information about the well-being of their colonies, while scientists can benefit from aggregated information about local biodiversity. The app is available as a web app on all devices.

Keywords: citizen science, pollen diversity, honey bee nutrition

PAN-EUROPEAN RESEARCH NETWORKS

ORAL PRESENTATIONS

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Mark Brown
Dirk de Graaf

UPDATE OF THE RED LIST OF THE BEES OF EUROPE: AN OVERVIEW

Boustani, Mira, Laboratory of Zoology, Université de Mons, Mons, Belgium
Michez, Denis, Laboratory of Zoology, University of Mons, Mons, Belgium

In the recent years, the observed decline of many bee species at the European level has drawn the attention to the key ecological role they play as crop pollinators. Consequently, monitoring wild bees has moved to the forefront of action plans for biodiversity conservation. Efficient conservation actions require many tools to inform the best course of action. One example is Red Lists, that are becoming one of the core elements of environmental decision making. The first Red List of bees of Europe was finalised in 2014, setting the baseline for pan-European wild bee status monitoring. Almost a decade later, the knowledge of the European bee fauna has moved forward, and many species require an update of their status. In addition to singling out the species that are threatened with extinction at the regional level, the Red List allows for a compilation of taxonomic and ecological information that back-up the species spatial occurrences.

We present here the ongoing work on the European Red List of bees update, presenting the contributors network, the methods used to manage the database, the main challenges, and the importance of a species-level approach. The priority of this update is gathering and validating occurrences for the Data Deficient species of the 2014 assessments in order to enhance their chances of acquiring a status.

Keywords: Regional Red List, Wild bees, European fauna

THE HORIZON 2020 B-GOOD PROJECT OF THE EUROPEAN COMMISSION PAVES THE WAY TOWARDS HEALTHY AND SUSTAINABLE BEEKEEPING WITHIN THE EUROPEAN UNION

de Graaf Dirk C., Ghent University, Ghent, Belgium, Coby van Dooremalen, Wageningen Research, Wageningen, Netherlands, Marten Schoonman, Stichting BEEP, Driebergen-Rijsenburg, Netherlands, Kirsten Leufgen, SciProm, Saint-Sulpice, Switzerland, Teodor Metodiev, Pensoft Publishers, Sofia, Bulgaria, José Paulo Sousa, Universidade de Coimbra, Coimbra, Portugal, Christopher John Topping, Aarhus University, Aarhus, Denmark, Wim Verbeke, Ghent University, Ghent, Belgium, Lina De Smet, Ghent University, Ghent, Belgium

The B-GOOD project aims to test and implement a common index for measuring and reporting honey bee health status (= Health Status Index, HSI), which will aid risk assessors, authorities and the plant protection and veterinary medicines industries to measure health status in real time and across geographical locations, as well as evaluating the effect of (beekeeping) management decisions and actions. Data collection on colony (health) status related components occurs according to a 3-tiered process that spans three bee seasons. New innovative tools will soon be available and will provide additional information about, among other things, vibrational communication, gas composition in the hive and the genetic predisposition of resilience of the colony. Our socio-economic study has identified 18 key attention points for policy and strategy development. We have laid the foundation for a dynamic landscape model across the EU, capturing the major floral resources. We will soon be able to make the HSI operational capitalising on machine learning and modelling.

POLLINATOR HUB: COLLECTIVE APPROACH TO UNDERSTAND POLLINATOR TRENDS AND THREATS

Simon Delso N, Gocs K, Susanj G, San Martin G, Rubinigg M

BeeLife European Beekeeping Coordination, Avenue Louise 209/7, 1050 Brussels, Belgium

It is well established that insect pollinators' numbers, diversity and density have severely decreased over recent years in Europe and worldwide. These trends are worrying when considering that nearly 90% of flowering plants depend on pollination by animals to some extent, while more than 75% of leading global food crops do, and in Europe, more than 80% of food crops rely to some extent on invertebrates for pollination. Therefore, many sectors and actors depend on pollinators and pollination for their activity, ranging from beekeepers, providers of pollination services, farmers and the agricultural production of pollinators-dependent crops and related agri-food sector; to research on pollinators, pollination and assessment of the environment through them. As a consequence, bees and other insect pollinators are taking increasing relevance in the public debate and growing efforts have been put over the years to understand these trends and the factors that may affect them. Related data have been/is produced by different institutions and actors, with various purposes and in multiple formats, making it impossible to obtain a clear picture of the situation and more importantly, the possibilities to reverse the trends. In Europe, stakeholders came together to share data. The EU Bee Partnership was then created and these stakeholders are steering a consortium of partners developmenting an innovative technology in the field of data connectivity and dissemination applied to the environment: The PollinatorHub(.eu). The project includes activities of technical development (software and infrastructure development) and data and quality management (data acquisition, management, processing, integration, visualisation, security). The PollinatorHub platform aims to transform the existing segregated data into the reference tool for those seeking information or whose activity depends on pollinators and pollination, and need to adapt their activities to manage the drivers determining their fate.

MALDI BEETYPING®: A TOOL FOR BEE HEALTH MONITORING BASED ON A “BLOOD” TEST, AN INNOVATIVE APPROACH WITHIN THE H2020 POSHBEE PROJECT

Askri, Dalel, Plateforme BioPark d’Archamps, Archamps, France

PoshBee[1] is an interdisciplinary project for bee health monitoring. The aim of PoshBee is to study the effects of pesticides, pathogens, nutrition and their interactions on bee health, with a focus on three major pollinators (*Apis, Bombus and Osmia*) and using a multiscale experimental design (field, semi-field and laboratory). We analysed bee haemolymph (insect blood) and developed the MALDI BeeTyping® approach[2] to generate molecular mass fingerprints (MFPs) as signature of abiotic and biotic stressors impacts on individual bees. The MFPs are transformed in reference mass spectra representative of stressor(s) models that will serve to evaluate the health status of the bee. To exemplify the benefits of this approach for beekeepers, results from field experiments will be presented and discussed. In brief, we evidenced specificities in MFPs from haemolymph of *Apis* species sampled from different geographical environments and exposed or not to chemical treatments. Using MALDI-BeeTyping®, we were able to gradate the molecular impact within/between bees collected in different crop environments across eight European countries. In addition, we developed a proteomics approach to identify and characterize different biological pathways that were found modulated in response to different stressors (e.g. pathogens, pesticides, poor nutrition), and their interactions. Ultimately, we performed label-free proteomics analyses based on laboratory samples, using high resolution LC-ESI-MS/MS and Gene Ontology to quantify the proteins markers that were found modulated within the altered pathways. By combining these two proteomics approaches we are providing an integrated marker database. Getting insight into the proteome changes of exposed bees to different stressors will allow (i) to improve our knowledge on the impact of these stressors, (ii) to decipher the molecular mechanisms in which various proteins may play a major role, and (iii) to propose markers of diagnosis and/or prognosis for the risk assessment of bee health and to evaluate the impact of stressors (abiotic and biotic). Finally, MALDI BeeTyping® may represent a cost- and time-effective tool to support beekeepers and sanitary services in their practices along with a user-friendly tool to integrate referenced mass spectra derived from individual haemolymph analyses into a bee health card readable by beekeepers and policy makers.

Keywords: Bee health, MALDI BeeTyping, Proteomics

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BIO-MONITORING OF ENVIRONMENTAL POLLUTION USING THE CITIZEN SCIENCE APPROACH

van der Steen Jozef, Alveus AB Consultancy, Netherlands
Amaral Joana, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Baveco Hans, Wageningen Environmental Research, Wageningen, Netherlands
Blanco Muñoz Patricia, Universidad de Almeria, Almeria, Spain
Brodschneider Robert, University of Graz, Graz, Austria
Brusbardis Valters, Latvian Beekeepers' Association, Jelgava, Latvia
Buddendorf Bas, Wageningen Environmental Research, Wageningen, Netherlands
Carreck Norman, Carreck Consultancy Ltd, Limited, West Sussex, United Kingdom
Charistos Leonidas, Department of Apiculture, ELGO 'DIMITRA', Nea Moudania, Greece
Danneels Ellen, Ghent University, Gent, Belgium
de Graaf Dirk, Ghent University, Gent, Belgium
Díaz Galiano Francisco José, Universidad de Almeria, Almeria, Spain
Fernandez-Alba Amadeo, Universidad de Almeria, Almeria, Spain
Ferrer Amate Carmen, Universidad de Almeria, Almeria, Spain
Formato Giovanni, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri" , Roma, Italy
Gómez Ramos María José, Universidad de Almeria, Almeria, Spain
Gratzer Kristina, University of Graz, Graz, Austria
Gray Alison, University of Strathclyde, UK
Hatjina Fani, Department of Apiculture, ELGO 'DIMITRA', Nea Moudania, Greece
Henriques Dora, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Kasiotis Konstantinos, Benaki Phytopathological Institute, Athina, Greece
Kilpinen Ole, Danmarks Biavlerforening, Sorø, Denmark
Lopes Rita, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Martínez Bueno María Jesús, Universidad de Almeria, Almeria, Spain
Murcia Morales María, Universidad de Almeria, Almeria, Spain
Pietropaoli Marco, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri" , Roma, Italy
Pinto M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Quaresma Andreia, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Rufino José, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Roessink Ivo, Wageningen Environmental Research, Wageningen, Netherlands
Vejsnaes Flemming, Danmarks Biavlerforening, Sorø, Denmark
Zafeiraki Effrosyni, Benaki Phytopathological Institute, Athina, Greece

Honeybee colonies are excellent bio-samplers of biological material such as nectar, pollen, and plant pathogens, as well as non-biological material such as pesticides or airborne contamination. The INSIGNIA-EU project aims to design and test an innovative, non-invasive, scientifically proven citizen science environmental monitoring protocol for the detection of pesticides, microplastics, heavy metals, and air pollutants by honey bee colonies http://insignia-eu.eu. In the pilot INSIGNIA project (2018-2021), a protocol was developed and tested for citizen-science-based monitoring of pesticides using honeybees. As part of the project, biweekly pollen was obtained from sentinel apiaries over a range of European countries and landscapes and analysed for botanical origin, using state-of-the-art molecular techniques such as metabarcoding. An innovative non-biological matrix, the "APIStrip", was also proved to be very efficient for detecting the residues of 273 agricultural pesticides and veterinary products, both authorized and unauthorized. The data collected are used to develop and test a spatial modelling system aimed at predicting the spatially-explicit environmental fate of pesticides and honeybee landscape-scale pollen foraging, with a common underlying geo-database containing European land-use and land-cover data (CORINE), the LUCAS database (landcover) supplemented with national data sets on agricultural and (semi-) natural habitats.

After a call by the European Commission, a new 2 years project was granted aiming to present a comprehensive pan-European environmental pollution monitoring study with honey bees. Although pesticides used in agriculture, are a known hazard due to their biological activity, other pollutants, have even been recognized as such, for which we have not been aware of their impact for many years. An example is air pollution which increased while our societies industrialized and is currently regarded as the single largest environmental health risk in Europe (https://www.eea.europa.eu/). Unfortunately, other pollutants such as heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, airborne particulate matter, and microplastics have also reached our environment. The outcome of this project will provide the first standardized EU-wide monitoring of all types of environmental pollutants with honey bee colonies. The project is funded by the EU, under the N° 09.200200/2021/864096/SER/ ENV.D.2 contract.

Key words: Bio-monitoring, citizen science, environmental pollutants

SAFEGUARDING AGROECOSYSTEM RESILIENCE UNDER CLIMATE CHANGE THROUGH EFFICIENT POLLINATION AND SUSTAINABLE BEEKEEPING

Hatjina Fani, Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA',, Nea Moudania, Greece.
Adjlane Noureddine, Department of Agronomy, Faculty of Sciences, University of Boumerdes, Algeria.
Bevk Danilo, Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia.
Bohorič Ladeja, Dept. of Animal Production, Agricultural Institute of Slovenia, Slovenia.
Bourdon Sarah, Department of Civil, Environmental, Architectural Engineering and Mathematics, University of Brescia, Via Branze 43, 25123, Brescia, Italy.
Charistos Leonidas, Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA',, Nea Moudania, Greece.
Darazi Dalida, Department of Plant Protection, Lebanese University, Faculty of Agricultural Sciences Deckwane, Lebanon.
El-Obeid Dany, Department of Basic Sciences, Lebanese University, Faculty of Agricultural Sciences Deckwane, Lebanon.
Filipi Janja, Department of Ecology, agronomy and Aquaculture, University of Zadar, Croatia
Garavini Gabriele, ACME21, Faenza, Italy.
Issa Dimitria, Department of Basic Sciences, Lebanese University, Faculty of Agricultural Sciences Deckwane, Lebanon.
Kolega Šimun, Department of Ecology, Agronomy and Aquaculture, University of Zadar, Croatia
Kovačić Marin, Dept. of Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia.
Manthos Ioannis, Department of Nut Trees, Institute of Plant Breeding and Genetic Resources, ELGO 'DIMITRA', Lamia, Greece.
Marinč Andraž , Dept. of Animal Production, Agricultural Institute of Slovenia, Slovenia.
Mole Katarina ,Dept. of Animal Production, Agricultural Institute of Slovenia, Slovenia.
Papadakis Michael ,TERRA SPATIUM SA, Athens, Greece.
Pasi Roberto, ACME21, Faenza, Italy.
Prešern Janez , Dept. of Animal Production, Agricultural Institute of Slovenia, Slovenia.
Puškadija Zlatko , Dept. of Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia.
Requier Fabrice, Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France.
Riva Clémence, Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France.
Semeniuk Julia, ACME21, Faenza, Italy.
Simonetto Anna, Department of Civil, Environmental, Architectural Engineering and Mathematics, University of Brescia, Via Branze 43, 25123, Brescia, Italy.
Sotiropoulos Thomas, Department of Deciduous Fruit Trees, Institute of Plant Breeding and Genetic Resources, ELGO 'DIMITRA', Naoussa, Greece.

Sperandio Giorgio, Department of Civil, Environmental, Architectural Engineering and Mathematics, University of Brescia, Via Branze 43, 25123, Brescia, Italy.
Stathopoulos Vassilios, TERRA SPATIUM SA, Athens, Greece.
Stavrinides Menelaos, Department of Agricultural Sciences, Cyprus University of Technology, Limassol, Cyprus
Stipešević Bojan, Dept. of Plant Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia
Gilioli Gianni, Department of Civil, Environmental, Architectural Engineering and Mathematics, University of Brescia, Via Branze 43, 25123, Brescia, Italy.
Rizk Ziad, Department of Bacterial and Parasitology, Lebanese Agriculture Research Institute LARI, Fanar, Lebanon
Tannoury Abdo, Department of Environmental microbiology and compost, Lebanese Agriculture Research Institute LARI, Fanar, Lebanon.
Yammouni Dany, Department of Apiculture, Lebanese Agriculture Research Institute LARI, Fanar, Lebanon.

The overall objective of SafeAgroBee is to contribute to adaptation and mitigation of the effects of climate change and other drivers negatively influencing the sustainability and the resilience of the agricultural system in the Mediterranean basin, ensuring the income of farmers and food security. In SafeAgroBee we focus on beekeeping and pollination provided by both wild and managed bees as important drivers in ruling food security and human existence. To this aim, SafeAgroBee will specifically address the following: 1) examine the resilience of bee pollinators (Apis and non-Apis bees) on a changing environment towards pollination services and productivity by a) documenting wild and domesticated bee contribution to the pollination of key crops; b) determining the carrying capacity of several crops as a novel approach for bee productivity and c) by projecting historical climatic data and bee related data in today's conditions; 2) investigate the adaptability of local bee populations and the application of optimal practices under climate change in order to ensure sustainable beekeeping by monitoring the development and the performance of local populations and their resistance to diseases; 3) support the development of mitigation strategies ensuring the health of the bees and provide advice for the beekeepers; 4) develop innovative monitoring tools and precision apiculture systems for advanced data acquisition by building on sounds, bee movements and heat detection, also enhancing business potential; 5) test and validate novel models for predicting the health of the bees, as for example the Health Status Index, as well as their productivity in terms of honey and pollination services based on two strong conditions, i) on previous experience, developments and knowledge and ii) on the large data sets with very accurate data and from diverse climatological conditions; 6) adopt a Citizen Science approach to interact and collaborate with all stakeholders and the citizens thus performing a multi-actor approach at all levels; Furthermore, to better understand the knowledge, the opinion and the expectations farmers and beekeepers have from each other as well as from their business perspectives, under the concept of pollination services provided by bees, we conducted a large socio-economics citizen-science study, the 1st in its kind in Mediterranean countries.

Key words: Sustainability, adaptation, pollination

COLOSS 3.0: A PLATFORM FOR GLOBAL COLLABORATION IN HONEY BEE SCIENCE

Neumann, Peter, Institute of Bee Health, Vetsuisse Faculty, University of Bern and Agroscope, Bern, Switzerland.
Bouga, Maria, Lab of Agricultural Zoology & Entomology, Agricultural University of Athens, Athens, Greece
Brodschneider, Robert, University of Graz, Institute of Biology, Graz, Austria.
Carreck, Norman, Carreck Consultancy Ltd., Woodside Cottage, Shipley, UK and Laboratory of Apiculture and Social Insects, University of Sussex, UK
Chantawannakul, Panuwan†, Bee Protection Center, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
Dall'Olio, Raffaele*, BeeSources - beekeeping consultancy, Bologna, Italy
Dietemann, Vincent, Swiss Bee Research Center, Agroscope, Bern, Switzerland and Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland
Fabricius Kristiansen, Lotta, SLU RådNu–National Competence Center for Advisory Services Swedish University of Agricultural Sciences, Sweden
Gajda, Anna Laboratory of Bee Diseases, Institute of Veterinary Medicine, Department of Pathology and Veterinary Diagnostics, Warsaw University of Life Sciences – SGGW, Warsaw, Poland
Gregorc, Aleš, University of Maribor, Faculty of Agriculture and Life Sciences, Slovenia.
Laurino Daniela, no current affiliation
Ozkirim, Asli, Department of Biology, Faculty of Science, Hacetteppe University, Ankara, Turkey
Pirk,Christian W.W., Social Insects Research Group (SIRG), Department of Zoology & Entomology, and Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Republic of South Africa
Soroker, Victoria, Laboratory of honey bee health, Institute of Plant Protection, Agricultural Research Organization ARO, Israel
Williams, Geoffrey, Department of Entomology and Plant Pathology, Auburn University, Auburn, USA

†deceased
*corresponding author raffaele.dallolio@gmail.com

The COLOSS consortium, 1940 scientists from 109 countries (30.06.2022), www.coloss.org

COLOSS (prevention of honey bee COlony LOSSes) is a non-profit association of scientific professionals dedicated to high quality honey bee research and extension activities worldwide, with the goal to improve the well-being of these important pollinators. Everyone who supports and works towards the mission of COLOSS is welcome to join. Since COLOSS strictly follows a bottom-up approach, members are welcome to propose

new core projects, task forces, BEEBOOK content and new ideas for joint experiments. At the moment we maintain three core projects and eight task forces. Some task forces have been running for years conducting joint multinational experiments and projects, while more recent ones are dynamically addressing novel concerns of the beekeeping industry. The Monitoring Core Project is delivering its data on colony losses regularly. The B-RAP Core Project particularly aims to bridge research results to stakeholders, including beekeepers, thereby fostering an inclusive network environment. As part of the continued effort to standardize methods of honey bee research, new BEEBOOK volumes and chapters are in preparation and previous ones are being updated. Due to a novel grant scheme, our association in its 3.0 phase can now implement new activities and strengthen ongoing ones. We plan to create additional core projects and task forces, for example aiming at addressing other Apis species and further mapping honey bee pests and diseases worldwide. To support young career researchers with emphasis on developing countries, COLOSS "Ricola awards for excellence" are offered annually. Through the above mentioned activities, our mission is to provide robust answers to questions on honey bee health, with as many COLOSS members participating as possible, ideally across the entire globe.

Keywords: COLOSS, research network, honey bee health

POSHBEE: A H2020 CONSORTIUM FOR BEE HEALTH

Brown, Mark, Dept of Biological Sciences, Royal Holloway University of London, Egham, UK

In this talk, I will present PoshBee, a European H2020 consortium project. We aim to understand the interplay of agrochemicals, nutrition, and pathogens on the health of honey bees, bumble bees, and solitary bees. I will present results from across our array of experiments, and development of tools for monitoring bee health.

HONEY BEE WATCH: A CITIZEN-SCIENCE INITIATIVE TO EMPOWER RESEARCH ON WILD AND UNTREATED SURVIVING *APIS MELLIFERA* COLONIES

Arrigo Moro[1], Fabrice Requier[2], Alexis Beaurepaire[1,3], M. Alice Pinto[4], Rosa Maria Licon Luna[5], Ginevra Celani[6], Raffaele Dall'Olio[7], Keith A. Browne[8], Steve Rogenstein[9], Jutta Buschbom[10], Grace P. McCormack[6]

1 Vetsuisse Faculty, University of Bern, Institute of Bee Health, Bern, Switzerland
2 Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France
3 Agroscope, Swiss Bee Research Centre, Bern, Switzerland
4 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Portugal
5 Wild Bees Project, Crozet, France
6 Resilient Bee APS, Anghiari, Italy
7 BeeSources, Bologna, Italy
8 Evolution and Systematics Laboratory, Zoology, School of Natural Sciences, University of Galway, Ireland, H91 TK33
9 The Ambeessadors, 10551 Berlin, Germany
10 Statistical Genetics, Ahrensburg, Germany

The high losses of *Apis mellifera* colonies in recent decades pose a risk for biodiversity conservation and food security since they impact the survival of this species in the wild as well as within the beekeeping sector. Research aimed at understanding these losses has mostly focused on investigating how managed colonies respond to stressors and has placed particular emphasis on the use of therapeutic treatments aimed at reducing mortalities. Yet, despite significant advances in our understanding of the problem, and the sustained efforts made for its mitigation, dramatic losses persist to this day. Such a situation calls for the collection of novel data to help guide future research in finding more sustainable solutions. Identifying and characterising colonies surviving unassisted in the wild, as well as untreated in apiaries, will expand our understanding of how honey bee populations can develop resilience to current stressors through natural selection. However, as existing data on such colonies is insufficient and not harmonised, more efforts should be focused on collaboration and jointly developing methodologies to acquire more information on their distribution and survival mechanisms. Here we present Honey Bee Watch, a citizen-science initiative born from the COLOSS Survivors Task Force, which is currently building an international coalition of experts and developing digital scientific tools to collect data on naturally surviving colonies worldwide. Through an online platform, 731 reports of *Apis mellifera* colonies surviving in the wild or untreated in apiaries have been collected to date. The data obtained from these reports will be discussed in light of their capacity to inform and orient future honey bee research and beekeeping strategies. Moreover, the

opportunities for collaborative research on surviving colonies made available by Honey Bee Watch, and the possibility of extending the use of the tools developed by this initiative for the conservation of other *Apis* species, will also be presented. By creating a network of experts and the largest data repository on wild and surviving untreated colonies, Honey Bee Watch will support research aimed at the sustainable mitigation of colony losses, empower honey bee conservation initiatives, and foster the protection of biodiversity.

Keywords: Citizen-science; Wild colonies; Naturally surviving colonies

EUROPEAN INITIATIVES ON TAXONOMIC RESOURCES AND CAPACITY BUILDING FOR WILD BEE MONITORING

Michez, Denis, Laboratory of Zoology, University of Mons, Mons, Belgium
Reverté, Sara, Laboratory of Zoology, University of Mons, Mons, Belgium
Rosa, Paolo, Laboratory of Zoology, University of Mons, Mons, Belgium

Several studies have now shown at different spatial scale that certain species of the wild bee fauna are in decline in Europe. In response, some European countries and the European commission are implementing action plans to mitigate negative population trends. To evaluate the efficiency of these actions we need an accurate estimation of population. One of the first actions proposed is therefore an ambitious monitoring program, which involves the development of taxonomic / recognition tools and training to use them. Several projects started in 2021 and concern the whole of European continent. We will present here the initiatives on taxonomy (ORBIT project) and on capacity building (SPRING project). ORBIT and SRPING are three-years projects commissioned by the General Directorate for Environment of the European Commission. Orbit aims to develop taxonomic resources for facilitating European bee inventory and monitoring. SPRING aims to strengthen the taxonomic capacity in EU Member States, and support preparation for the implementation of the EU Pollinator Monitoring Scheme "EU-PoMS", notably by organising training sessions for different level of expertise.

Keywords: Wild bees, Taxonomy, Initiatives

PAN-EUROPEAN RESEARCH NETWORKS

POSTERS

INTERACTIONS OF DIFFERENT PESTICIDES AND *N. CERANAE* INFECTION IN THE HONEY BEE *APIS MELLIFERA*

Urueña, Álvaro, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain.
Blasco-Lavilla Nuria, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain.
De la Rúa, Pilar, Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain.

Bee health is compromised by many factors such as the use of pesticides in agriculture and the various diseases that can affect it. Multiple studies have shown that these factors can interact producing a synergistic effect that can compromise the viability of honey bee colonies. This study analyses the effect of the interactions between different agrochemicals and the microsporidium *Nosema ceranae* on mortality, sugar consumption, immune gene expression and detoxification genes in one of the western subspecies of honey bee (*Apis mellifera iberiensis*). For this purpose, workers were infected with *N. ceranae* and subjected to a sugar-water diet with field concentrations of the pesticides sulfoxaflor, azoxystrobin and glyphosate.

Results showed that mortality increased in workers exposed to sulfoxaflor alone but decreased markedly when workers were fed sulfoxaflor in combination with the other two pesticides. On the other hand, increased sugar intake and altered immune and cytochrome P450 genes expression were observed when sulfoxaflor-exposed workers were infected with *N. ceranae*. None of the pesticides affected *Nosema* spore production in the gut of the workers.

Taken together, our results suggest that lethal and sublethal effects of sulfoxaflor depend on the interaction with other pesticides and Nosema infection, and highlight the importance of studying the interaction between different stressors to ensure healthy honey bees.

Keywords: Pesticides, *Nosema ceranae, Apis mellifera*

Funding The study was funded by the European Horizon 2020 research and innovation programme under grant agreement No 773921, PoshBee Project (www.poshbee.eu).

DETERMINING THE EFFECTS OF RADIO-FREQUENCY ELECTROMAGNETIC FIELDS (RF-EMF) EXPOSURE ON BIODIVERSITY, DEVELOPMENT & FITNESS OF INSECTS

Thielens Arno, Department of Information Technology, Ghent University, Ghent, Belgium,
Bouga Maria, Department of Crop Science, Agricultural University of Athens, Athens, Greece,
Charistos Leonidas, Department of Apiculture, Institute of Animal Science- ELGO 'DIMITRA', Nea Moudania, Greece,
Froehlich Juerg, Fields at Work GmbH, Zurich, Switzerland,
Hatjina Fani, Department of Apiculture, Institute of Animal Science- ELGO 'DIMITRA', Nea Moudania, Greece,
Stavrinides Menelaos, Department of Agricultural Sciences, Cyprus University of Technology, Limassol, Cyprus,
Thanou Zoi, Department of Crop Science, Agricultural University of Athens, Athens, Greece,
Tsagkarakis Antonios, Department of Crop Science, Agricultural University of Athens, Athens, Greece,
Varnava Andri, Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol, Cyprus,
Zahner, Marco, Fields at Work GmbH, Zurich, Switzerland,
Huss Anke, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

The ongoing introduction of new telecommunication technologies (3G, 5G now, 6G in the future) comes with changes in exposure patterns and potential biological interaction of the radio-frequency electromagnetic fields (RF-EMFs) emitted by telecommunication networks with humans and ecosystems.

The Horizon Europe project 101057216, Exposure To electromAgnetic fIelds and plaNetary health (ETAIN) aims to develop and validate approaches to assess the impact of existing and novel communication technology from a planetary health perspective, while exploring options for exposure reduction and interacting with the public and stakeholders about exposure levels and possible associated risks.

Among other exposure outcomes, we will focus on insect biodiversity and fitness with relation to exposure to RF-EMFs emitted by telecommunication networks, with special focusing on pollinators, such as bees, at risk of peak exposures close to powerful antennas. The aims of the project for bees are:

- To define thresholds for wellbeing, health, behaviour and productivity of honeybee colonies under 3G and 5G exposure
- To measure possible changes in the fitness of a solitary bee species under 3G and 5G exposure
- To record possible impacts on insect biodiversity under 3G and 5G exposure
- To create digital models for dosimetric analysis of bees and other insects to 3G and 5G networks.

□ To give recommendations to policy makers in terms of sustainable management of honey bee and wild bee populations.

From a planetary health perspective, RF-EMF effects on insects may also have indirect effects on human health and the economy (e.g. via pollination). ETAIN is expected to generate scientific evidence of RF-EMF exposure effects on human, ecosystem and planetary health, feeding into policies and current regulatory frameworks. The ETAIN project is part of the European cluster on EMFs and health.

Keywords: Electromagnetic fields, Exposure, Biodiversity

LIFE VAIA: VALUING AFFORESTATION OF DAMAGED WOODS WITH INNOVATIVE AGROFORESTRY

Fontana Paolo, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Malagnini Valeria, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy

Storms, floods, fires: the occurrence of extreme climatic events, with the dramatic repercussions they have on our territories, is the testimony of climate change in recent decades. The VAIA storm that hit North East Italy in 2018 caused extensive damage to mountain areas and their delicate ecosystems. Extreme weather events quickly create open spaces where there were forests. Agroforestry is a solution that can provide for the exploitation of the new habitat, both by introducing temporary crops and through the implementation of beekeeping. In fact, in these environments we can observe the rapid and abundant appearance of a nectarous and polleniferous flora. With a duration of five years and a total budget of €6 million, of which €3 million has been allocated by the European Commission, the LIFE VAIA project aims to develop an innovative approach based on the application of "temporary" agroforestry measures (15/20 years). This strategy makes it possible to invest in the production of sustainable and low-impact products, increasing biodiversity and the sustainable use of resources. The main actions financed by LIFE VAIA concern the reproduction and cultivation of wild blueberries and other "wild" small fruits, food and medicinal plants in forest ecosystems, as well as the enhancement of beekeeping production in forest areas destroyed by storms and other extreme climate events. To monitor how the actions of the LIFE VAIA project will impact bees composition and diversity, pan traps, bee hotels and pollen collection will be used before and during the project.

Keywords: beekeeping, Apoidea, non *Apis* bees

SUBSPECIES AND THEIR RESILIENCE TO CLIMATE CHANGE FOR THE IMPROVEMENT OF SUSTAINABLE AGRO-ECOSYSTEMS

Raquel Martín-Hernández, CIAPA-IRIAF, Marchamalo, Spain
Antonio Nanetti, CREA- AA, Bologna, Italy
M. Alice Pinto, CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Noureddine Adjlane, UMBB-DZ, Bourmedes, Algeria
Nizar Haddad, NARC, Amman, Jordan
Chadi Hosri, Beirut, Lebanon
Mustafa Necati Muz, Tekirdağ, Turkey
Marion Zammitt Mangion, University of Malta, Msida, Malta
Ahmad Yousef Daour, Amman, Jordan
Soledad Sagastume, CIAPA-IRIAF, Marchamalo, Spain
MEDIBEES consortium

Beekeeping provides a livelihood for hundreds of thousands of beekeepers in the Mediterranean area. This activity relies on a number of different indigenous subspecies, adapted to the very diverse and harsh conditions of the region. Climate change is expected to increase the stress factors affecting bees, especially in this region, reducing both pollination efficiency and production potential. Unfortunately, our ability to address this problem is limited by the incomplete knowledge of the natural adaptation mechanisms developed by the different subspecies. In order to increase the knowledge base for future selection programs to improve bee populations for environmental changes, the MEDIBEES project is being developed. It includes 9 partners from 8 Mediterranean countries on all three shores of the Mediterranean, covering 10 local subspecies of A. mellifera, which represents a remarkable and poorly known proportion of the genetic diversity. The project aims to: a) unravel the differential genetic background of Mediterranean subspecies, b) understand their adaptation to local conditions, and c) characterize their resistance to climate change. To achieve the objectives, colonies belonging to the local subspecies will be studied phenotypically to determine their behavior under environmental conditions, covering survival, sensitivity to pests/pathogens, behavior, physiology and reproduction which will be completed by gene expression and transcriptomic assays. In addition, complete genomes of field and laboratory samples will be sequenced to find genes putatively involved in adaptation and to develop new genetic tools to characterize honey bee populations according to their resistance to environmental stress factors. This effort will encourage the use of local subspecies, to make them more attractive and avoid importing foreign breeds, and will lay the foundation for future selection programs. Besides, the valorization of honey by both promoting its use and developing quality labels, and the evaluation of beekeeping by-products as modifiers of soil fertility and biota are also approached to help the beekeepers improve the sustainability of their farms in an economical and environmental sound manner.

This project is part of the PRIMA program supported by the European Union

Keywords: Climate change adaptations, honeybee conservation, resistance to stressors

B-GOOD WP1: MAKE THE LIVES OF BEES AND BEEKEEPERS EASIER

Tehel, Anja, Martin Luther Universität Halle-Wittenberg, Halle, 06120, Germany
Claeys-Boúúaert, David, Ghent University, Ghent, 9000, Belgium
Dall'Olio, Raffaele, BeeSources di Raffaele Dall'Olio, Bologna,40132, Italy
Flener, Claude, Suomen Mehiläishoitajain liitto, Helsinki, 00130, Finland
Giurgiu, Alexandru, Universitatea de Stiinte Agricole si Medicina Veterinara Cluj Napoca, Cluj Napoca,400372, Romania
Godeau, Ugoline, Institut National de la Recherche Agronomique, Avignon,84914, France
Kumar, Tarun, The Nottingham Trent University, Nottingham, NG11 8NS, UK
Matthijs, Severine, Sciensano, Brussels, 1180, Brussels
Moro, Arrigo, Universität Bern, Bern, 3012, Switzerland
Paxton, Robert J., Martin Luther Universität Halle-Wittenberg, Halle, 06120, Germany
Schäfer, Marc O., Friedrich-Loeffler-Institut – Bundesforschunginstitut für Tiergesundheit, Greifswald - Insel Riems, 17493, Germany
Schoonman, Marten, Stichting BEEP, Driebergen-Rijsenburg, 3972 LK, Netherlands
Simões, Sandra, Universidada de Coimbra, Coimbra, 3000-456, Portugal
Ulgezen, Zeynep N., Wageningen Research, Wageningen, 6708 PB, Netherlands
van Gennip, Pim, Stichting BEEP, Driebergen-Rijsenburg, 3972 LK, Netherlands
de Graaf, Dirk C., Ghent University, Ghent, 9000, Belgium
van Dooremalen, Coby, Wageningen Research, Wageningen, 6708 PB, Netherlands

B-GOOD is an acronym that stands for Giving Beekeeping Guidance by cOmputatiOnal-assisted Decision making. It is an H2020 project funded by the European Commission and is about healthy and sustainable beekeeping. B-GOOD focuses on finding a Health Status Index (HSI, inspired by EFSA's Healthy-B toolbox 2016), which is a categorization of the health of a bee colony based on various indicators from within and around the beehive as well as socioeconomic conditions. Much data will be collected automatically, creating an almost continuous stream of data that will form the basis for determining and validating the algorithm that calculates the HSI.

The B-GOOD work package structure has 10 entities. WP1 contributes to the operationalization of the HSI by collecting data on different health components related to the bee colony under experimental and field conditions. Here detailed scenarios/protocols for end-users are developed in order to ensure harmonization of the data and sample collection during and after the project. The big data set is collected according to a 3-tiered approach in which the field of activity is gradually expanded. Tier 1 takes place at the level of the bee institutions; eight B-GOOD partners who have the expertise and infrastructure to keep bees. In Tier 2, five B-GOOD partners guide neighbouring local beekeepers in monitoring three of their colonies. The third and final tier engages beekeepers at the pan-European level. Data collection includes 1) automated data flow through the hive BEEP-base sensor system, 2) beekeeper observations at the hive itself logged through the BEEP-app and 3) the analyses for diseases, pesticides and worker bee genotypes performed on samples collected several times a year.

Keywords: honey bee, health status index, beekeeping management

BEEKEEPING IN THE MEDITERRANEAN: A MEDIBEES SURVEY ON PRACTICES, TRENDS AND CHALLENGES

Marion Zammit Mangion, University of Malta, Msida, Malta
Raquel Martín-Hernández, CIAPA-IRIAF, Marchamalo, Spain
Antonio Nanetti, CREA- AA, Bologna, Italy
M. Alice Pinto, CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
Noureddine Adjlane, UMBB-DZ, Bourmedes, Algeria
Asmaa Anwer, Egypt
Nizar Haddad, NARC, Amman, Jordan
Chadi Hosri, Beirut, Lebanon
Mustafa Necati Muz, Tekirdağ, Turkey
Ahmad Yousef Daour, Amman, Jordan
Soledad Sagastume, CIAPA-IRIAF, Marchamalo, Spain
MEDIBEES consortium

MEDIBEES is a PRIMA funded project that aims to identify honeybee colonies and subspecies that are most resilient to climate change and common pathogens throughout the Mediterranean region. Honeybees are critical to sustainable agricultural systems and the project aims to contribute to sustainability in the context of the increased desertification of the region due to climate change.

As part of this project an intensive investigation of beekeeping was carried out within the consortium, with the objectives of 1) understanding the main practices in place, 2) identifying trends and challenges faced by beekeepers. This would help guide the consortium in framing research questions that are rooted in the requirements of the stakeholders. A questionnaire was therefore developed and circulated among beekeepers from Algeria, Egypt, Italy, Jordan, Lebanon, Malta, Portugal, Spain and Turkey in late June 2021. To date, over 1300 detailed responses have been collected. This study has yielded an important first profile of the nature of the apicultural industry for many of these participating countries. In this presentation, a description of the socio-demographics and the diverse management practice in place among beekeepers across the Mediterranean will be presented. In addition, important first-time data on the distribution of hives, the main sources of losses including current biotic and abiotic threats, the characteristics and ecological trends of both native and introduced honey bee subspecies in the different geographical regions will be described as experienced by the major stakeholders ie beekeepers. Finally, notably and overwhelmingly, beekeepers from the Mediterranean region list changes in weather patterns as the major factor affecting colony losses and profitability. This was followed closely by indiscriminant use of agricultural insecticides and urbanisation.

QUANTITATIVE GENETICS, REPRODUCTION AND BEE BREEDING

ORAL PRESENTATIONS

CHAIRPERSON
Pierre Giovenazzo

A SNP-BASED PATERNITY ASSIGNMENT TEST TO EVALUATE THE EFFECTIVENESS OF HONEYBEE MATING STATIONS

Parejo, Melanie, Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa, Spain
Galartza, Egoitz, Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa, Spain
Zarraonaindia, Iratxe, IKERBASQUE Basque Foundation for Science, Bilbao, Spain
Estonba, Andone, Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa, Spain

Honeybee queens mate multiple times in flight away from their colonies at so-called drone congregation areas (DCAs). Geographically isolated mating stations are widely used throughout Europe, in an effort to guide the mating of the virgin queens with selected drone-producing colonies. To test the reliability of such mating stations, we developed a paternity assignment test based on the recently published genotyping tool for subspecies assignment that includes 4165 SNPs. Paternity is assigned based on two metrics (number of mismatch alleles and kinship) calculated between worker offspring and possible "fathers".

We first genotyped samples with known parent-offspring relationships to set sensible assignment thresholds, and then applied the method to the *A. m. iberiensis* mating station in the Ataun valley, Basque Country, Spain, in 2021. To this end, drone pool samples of the drone-producing colonies from the station and other possible "father" colonies in the valley were genotyped (N=47), as well as 96 worker offspring of queens mated at the station and 36 drones collected in the air at the DCA.

Out of the 96 worker samples, we could assign 72 (75%) to the tested "fathers", most of which matched with the selected drone-producing colonies of the mating station, while 25% were of unknown patriline. Six workers were assigned to "father"-colonies at the entrance of the Ataun valley, at ~8 km from the mating station. Out of the 36 drones collected in the air, 34 (94.4%) were assigned to drone-producing colonies, while only two drones were unrelated to any tested "father" colony. Our results show that despite the drone-producing colonies seemingly saturating the DCA, other drones are still successful in mating with the virgin queens in the Ataun valley.

Our honeybee paternity assignment test is able to identify first-degree relations (parent-offspring) and assign worker bees to their putative "father". Such results are useful for apiary management regarding the selection of suitable mating station locations and the positioning of drone-producing colonies therein. Our SNP-based paternity assignment test will thus be extremely valuable to evaluate the effectiveness of mating stations across Europe, key to advancing toward set breeding goals.

Keywords: paternity, mating station, SNPs

IDENTIFICATION OF HONEY BEE INTERCASTES USING WING MEASUREMENTS

Tofilski, Adam, Łopuch, Sylwia, Department of Zoology and Animal Welfare, University of Agriculture in Krakow, Krakow, Poland

Honey bee (*Apis mellifera*) females usually develop into two distinct castes: queens or workers. However, in some circumstances there can be produced intermediate individuals called intercastes. This happens occasionally during queen rearing or during in-vitro rearing of honey bees. One of the morphological features which differs between queens and workers are wings. In this study we verify if geometric morphometrics of forewings can be used to discriminate queens, workers and intercastes. We have used specimens obtained by standard methods of queen breeding and compared them with workers. Forewings of the specimens were dissected and their images were obtained. On the wing images there were indicated 19 landmarks. Their coordinates were aligned using Procrustes procedure.

Principal Component Analysis of the aligned coordinates revealed that in the graph of first two principal components the specimens obtained by queen breeding formed two clusters of points. It was assumed that the two clusters contained: typical queens and intercastes. The forewings of intercastes were significantly smaller than those of queens but bigger than those of workers. The wing shape differed significantly between queens, intercastes and workers. Intercastes were more similar to workers than to queens in terms of both forewing size and shape. We have used also Mahalanobis distance between the shape of the left and the right wing to assess forewing asymmetry. The intercastes were less asymmetrical than queens but more asymmetrical than workers.

Canonical Variate Analysis allowed to successfully classify (with cross validation) all specimens as queens, intercastes or workers. The data presented here show that wing measurements can be used to detects intercastes. This can be used for assessment of quality of honey bee queen produced by queen breeding.

Keywords: intercastes, wing, geometric morphometrics

A BREAKTHROUGH IN THE INSEMINATION OF BEE QUEENS

Dziechciarz Piotr, Olszewski Krzysztof, Borsuk Grzegorz
Subdepartment of Apidologie, Institute of Biological Basis of Animal Production; Faculty of Animal Sciences and Bioeconomy
University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland

In natural conditions, the queen is inseminated during the mating flight and can copulate with up to 44 drones, which is determined by polyandry. Since natural copulation in bees takes place in the air, it is impossible for humans to control the selection of the paternal side in the breeding process. Breeding of honeybees is possible either in isolated drone areas or with the use of instrumental insemination of queens. In Poland, the instrumental insemination method is applied to fertilize approx. 23 000 bee queens in 72 registered breeding apiaries.

Therefore, a new liquid for the instrumental insemination needle/capillary has been developed. It is based on physiological saline combined with spermathecal fluid. Additionally, the method for filling the capillary during the instrumental insemination procedure has been modified, which ensures insemination without the use of air buffer between the drone sperm and the liquid in the needle/capillary. The liquid and the insemination method have been granted patent protection (P.431980).

The experiment was carried out on 10 queens, which were instrumentally inseminated with the new liquid and the new method and kept in six-comb Dadant hives.

It turned out that all queens started oviposition after 14 days without any oviposition acceleration treatments. The control group of 10 queens was inseminated with 0.9% saline with air buffer to protect the sperm cells against contact with saline. The control queens started oviposition after 20 days, and the effectiveness of the instrumental insemination treatment reached 74%. All the queens were sisters. They were inseminated with a single dose of 8 µl semen at 7 days of age.

The new insemination liquid significantly improves the insemination process, as it can and should be in direct contact with drone sperm. During the collection of semen into the insemination needle/capillary, spermatozoa probably begin to move in the insemination needle. This largely improves the process of instrumental insemination of bee queens and ensures oviposition that does not require any additional treatments, which saves time for preparation of instrumentally inseminated queens and improves bee breeding considerably. Therefore, we offer our cooperation.

Keywords: instrumental insemination, bee queen, bee breeding

IMPACTS OF INDOOR MASS STORAGE OF TWO DENSITIES OF HONEY BEE QUEENS (*APIS MELLIFERA*) DURING WINTER ON QUEEN SURVIVAL, REPRODUCTIVE QUALITY AND COLONY

Levesque, Mireille, Biology Department, Laval University, Quebec City, Canada
Rousseau, Andrée, Beekeeping Sciences, Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Deschambault, Canada
Giovenazzo, Pierre, Biology Department, Laval University, Quebec City, Canada

Spring imports of honey bee (*Apis mellifera* L.) queens are essential to replace winter colony losses in Canada, but contribute to the spread of treatment-resistant strains of pathogens and undesirable genetic traits. A possible alternative to these imports is the mass storage of queens during winter. By overwintering a strong colony (queen bank) containing large numbers of mated queens isolated in cages, beekeepers could acquire local queens early in the spring. In this study, we tested the efficacy of overwintering queen banks at two different queen densities (40 and 80). Mated queens were stored in the upper brood chamber of a strong queenless colony from October 2019 to April 2020. Queen survival, reproductive quality and colony performance were assessed in early spring. In the 40-queen banks (40 QB, n=5), 74.2 % of queens survived the 6-month overwintering period, while 42.1 % of queens survived in the 80-queen banks (80 QB, n=5). When compared to queens overwintered free in their colony, queens from bank colonies were smaller and lighter in early spring, but had similar sperm viability and sperm count. Overwintering queens in banks did not have an impact on their acceptance in a nucleus colony, but reduced their oviposition in the initial weeks following their introduction. After several days in nucleus colonies, queens from banks had regained a size and weight similar to that of queens overwintered normally, suggesting that they could perform well over a complete beekeeping season. This study achieved promising results and highlights the potential of this technique for the beekeeping industry in Canada and worldwide.

Keywords: honey bee queen, queen storage, queen reproductive quality

GENETIC SELECTION OF THE HONEY BEE (*APIS MELLIFERA* L.) IN A NORTHERN CLIMATE

Maucourt, Ségolène, Department of Biology, Laval University, Quebec, Canada
Fortin, Frédéric, Quebec Pork Development Center, Quebec, Canada
Robert, Claude, Department of Animal Science, Laval University, Quebec, Canada
Giovenazzo, Pierre, Department of Biology, Laval University, Quebec, Canada

Genetic improvement programs have resulted in spectacular productivity gains for most animal species in recent years. The introduction of quantitative genetics and the use of statistical models have played a fundamental role in achieving these advances. For the honeybee (*Apis mellifera*), genetic improvement programs are still rare worldwide. Indeed, genetic and reproductive characteristics are more complex in honeybees than in other animal species, which presents additional challenges for access genetic selection. In recent years, advances in informatics have allowed statistical modelling of the honeybee, notably with the BLUP-animal model, and access to genetic selection for this species is possible now. The aim of this project was to present the genetic progress of several traits of interest to the Canadian beekeeping industry (hygienic behavior, honey production and spring development) achieved in our selection program since 2010. Our results show an improvement of 0.30% per year for hygienic behavior, 0.63 kg per year for honey production and 164 brood cells per year for spring development. These advances have opened a new era for our breeding program and sharing this superior genetic available to beekeepers will contribute to the sustainability and self-sufficiency of the beekeeping industry in Canada.

VARROA DESTRUCTOR INFESTATION LEVEL CAN BE PREDICTED BY BROOD CELL RECAPPING RATE AND MITE NON-REPRODUCTION IN HONEYBEE COLONIES (*APIS MELLIFERA* L.)

Morin, Marie-Lou, Département de biologie, Université Laval, Québec, Canada

The genetic selection of honeybees (*Apis mellifera*) offers the beekeeping industry the possibility of combating the Varroa destructor parasite and thus reducing its dependence on acaricides. Indeed, some bee populations have heritable hygienic behavioural traits that allow them to resist Varroa infestation. However, the links between these different behavioural traits are not yet well defined, which limits genetic progress. In this context, we measured the following behavioural Varroa resistance traits: freeze-kill brood assay (FKB) and pin-kill brood assay (PKB), varroa-sensitive hygiene (VSH), pupae removal, mite non-reproduction (MNR) and recapping activity. Correlations between these measures show that two are negative and significant: (1) between the recapping of cells infested with Varroa and the total number of recapped cells and (2) between the recapping of cells infested with Varroa and varroa-sensitive hygiene (VSH). We also selected the best predictive model of varroa infestation levels using the "step-wise" approach based on the AIC criterion. Our model revealed that MNR and FKB were significantly related to the varroa population levels with a negative relationship, while recapping was significantly related to mite infestation levels with a positive relationship. Thus, a higher MNR or FKB score is significantly related to low levels of mite infestation in colonies at the end of August, while a higher recapping activity is significantly related to a high level of mite infestation. Our work indicates that recapping behavior could be a useful trait to aid the selection of Varroa-resistant bee lines but that further work needs to be done to better understand its significance.

Keywords: Hygienic behavior, Mite non-reproduction, Varroa resistance

MORPHOLOGICAL ANALYSIS OF THE HONEY BEE (*APIS MELLIFERA*) FOR THE IDENTIFICATION AND CHARACTERIZATION OF GENETIC LINES

Justine Sénéchal[a], Ségolène Maucourt[a], Marilène Paillard[b], Pierre Giovenazzo[a]

a Department of Biology, Laval University, Québec, Canada
b Beekeeping Department, Centre de recherche en sciences animales de Deschambault, Québec, Canada

The honey bee (*Apis mellifera*) is an exogenous species in Canada that does not tolerate long winters well. The significant mortalities related to this problem make the Canadian beekeeping industry dependent on imports of queens and bees, which prevents the natural adaptation of the species. In order to encourage a genetic heritage adapted to local needs, our beekeeping research center produces between 12 and 15 lines of bees resulting from an annual genetic selection based on criteria of productivity, hardiness and resistance to diseases. The objective of this research was to verify whether certain anatomical and morphological traits make it possible to distinguish and characterize 6 of these genetic lines. The traits measured are the geometry of the wing veins, the length of the left forewing and the length of the proboscis. The analysis of the geometric morphometry of the wing veins shows significant differences between all the lines ($P < 0.001$). In addition, we found significant differences between lines for proboscis length ($P < 0.001$) and left forewing length ($P < 0.001$). The presence of variance in the expression of these traits within these lines indicates that selection pressure could be applied. For example, the selection of bees with a long proboscis would allow the development of specific lines for the pollination of lowbush blueberries whose flower nectaries are difficult to access for honey bees.

QUANTITATIVE GENETICS, REPRODUCTION AND BEE BREEDING

POSTERS

A PRELIMINARY ASSESSMENT OF MORPHOLOGICAL IDENTITY OF SOME *APIS MELLIFERA LIGUSTICA* (SPIN) POPULATIONS COLLECTED IN CENTRAL AND SOUTHERN ITALY

Danieli Pier Paolo, Manganello Federico, Lazzari Filippo, Vetturini Tiziana, Petrocchi-Jasinski Francesca, Cresta Eleonora

Department of Agriculture and Forest Sciences (DAFNE), Tuscia University, Viterbo, Italy

The genetic integrity of populations belonging to the Italian subspecies *Apis mellifera ligustica Spin.* (AML) is considered to be at risk and the identification, through morphometry, of strains with the characteristics of the pure subspecies is essential for programs devoted to their conservation and valorisation. In this study, as part of the Plant-B, an European Project funded by PRIMA Foundation (grant n. 1812), a morphometric survey was carried out to verify the belonging to the AML subspecies of 41 different bee colonies, gently provided by ten beekeepers located different areas of the Central and Southern Italy. The morphometric analysis was carried out with the determination of 18 biometric traits on the front wings, hind legs and tergites. The wings were mounted on anti-newton slides, then scanned at a resolution of 9600 dpi. Through the use of the DrawWing software, the coordinates of the junction points of the wing ribs on each pair of wings were determined. With the aid of a stereo microscope, the two rear legs of the samples were photographed and the following measurements were then determined: length of the femur, tibia, and length and width of the metatarsus. To identify the colour and profile of the pigmented bands of the T2 and T3 tergites, the Goetze scale was taken as a reference. Statistical belonging of each honey bee sample was defined in terms of membership degree of each colony the sample was taken out to the subspecies AML. The global membership of the 41 colonies was 40.5% (± 12,9%), with minimum and maximum values of 16.7% and 77.8%, respectively. Only in 26.8% of colonies a membership of at least 50% emerged, and of these colonies 36.4% had a value that exceeds 61%. In line with other previous studies, these preliminary results show that the genetic heritage of the Italian bee in managed colonies can be extremely variable being subjected to gene flow from other subspecies or hybrids. Notwithstanding, these preliminary outcomes suggest that there are true possibilities to find honey bee colonies in Central and Southern Italy that highly match the morphometric standard of AML for setting up strategic breeding plans for conservation purposes at regional and/or national level.

Keywords: *Apis mellifera ligustica* Spin., Morphometric characterization, Honey bee subspecies

SIMULTANEOUS MAINTAINING OF BEE COLONIES ON COMBS WITH SMALL AND STANDARD CELLS RESULTS IN BEHAVIORAL OVERDOMINANCE

Dziechciarz Piotr[1], Borsuk Grzegorz[1], Strachecka Aneta[2], Olszewski Krzysztof[1]

1 Subdepartment of Apidology, Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, Poland
2 Department of Invertebrate Ecophysiology and Experimental Biology, University of Life Sciences in Lublin, Poland

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The efficiency of a honey bee colony is the result of the cooperation of many worker bees, which shifts into the value of colony's functional traits. We observed that placing in the nests of colonies kept on combs built on a wax foundation with standard cells (size 5.50 mm), the combs with small cells (size 4.90 mm) resulted in a significant increase in the value of such traits as the number of cells with brood in spring and honey yield, compared to colonies kept only on combs of one type (small or standard cells). We believe that the reasons for this phenomenon into the cooperation of workers reared in these two types of combs placed in the same colony, because the effect of this cooperation resembles heterosis, we called them behavioral overdominance.

In order to estimate the effect of colony keeping on the number of brood cells in spring and honey yield, we compared three groups of colonies: SMC - kept only on combs with small cells (size 4.90 mm), STC - kept only on combs with standard cells (size 5.50 mm), SMC + STC - kept simultaneously on combs with small and standard cells, combs ratio 1: 1. Each group consisted of 8 colonies kept in the Dadant Blatt hives, with 10 combs in the brood chamber.

The way of keeping the colonies significantly influenced the number of brood cells and the honey yield. SMC + STC w colonies usually had significantly more brood cells than SMC and STC ($p = 0.05$, Tukey's test) (2020, 1st measurement: SMC = 12566, STC = 11559, SMC + STC = 152244; 2nd measurement: SMC = 33393 , STC = 30851, SMC + STC = 40170; 2021, 1st measurement: SMC = 18933, STC = 13775, SMC + STC = 22642; 2nd measurement: SMC = 47584, STC = 38978, SMC + STC = 47808. Faster spring development of SMC + STC was the result of the greater strength of these colonies in the fall. The SMC + STC colonies also produced significantly more honey than SMC and STC ($p = 0.05$, Tukey's test) (2020: SMC = 20.18 kg, STC = 27.53 kg, SMC + STC = 42.77 kg; 2021: SMC = 20.50 kg, STC = 25.42 kg, SMC + STC = 33.55 kg.

The higher productivity of colonies kept simultaneously on combs with small and standard cells may be due to a different division of labor of worker bees than in colonies kept on only one type of comb (small or standard cells).

Keywords: small-cell combs, behavioral overdominance

EVALUATION OF CONTROLLED MATING SUCCESS IN HONEYBEES

Moškrič, Ajda, Animal Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
Prešern, Janez, Animal Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
Aleksovski, Goran, Company for Applied Research and Permanent Education in Agriculture, Skopje, Macedonia
Bubnič, Jernej, Animal Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
Dahle, Björn, Norwegian Beekeepers Association, Kløfta, Norway
Kojek, Manca, Animal Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
Kovačić, Marin, Faculty of Agrobiotechnical Sciences Osijek, Croatia; Centre for applied life sciences Healthy Food Chain Ltd, Osijek Croatia;
Marinč, Andraž, Animal Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
Mole, Katarina, Animal Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
Pavlin, Anja, Biotechnical faculty, University of Ljubljana, Slovenia
Pavlov, Borče, Company for Applied Research and Permanent Education in Agriculture, Skopje, Macedonia
Puškadija, Zlatko, Faculty of Agrobiotechnical Sciences Osijek, Croatia; Centre for applied life sciences Healthy Food Chain Ltd, Osijek Croatia
Uzunov, Aleksandar, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University in Skopje, Macedonia
Andonov, Sreten, Swedish University of Agricultural Sciences, Uppsala, Sweden

A crucial precondition for a successful breeding program in honeybees is controlled mating. EEA and Norway Grants Fund for regional cooperation project Joint Effort For Honey Bee Conservation and Selection - BeeConSel aims to establish methods for successful mating control of honeybees in Croatia (HR), Macedonia (MK), and Slovenia (SI). In order to evaluate the success of the mating control the reliable and cost-effective method for paternity determination is needed. Using microsatellite genotyping we assessed the paternity from various sources of DNA from three different field set-ups. The queens were open-mated in either presence or absence of the drone-producing colonies (DPCs). In addition, the Jo Horner mating method in the absence of DPCs was tested. For each colony, we sampled successfully mated queen and around 30 worker pupae. From each DPC, we sampled each queen or alternatively the drone pupae. Sources for molecular paternity analysis included tissue from each queen and pupae, as well as spermathecae from successfully mated queens. To avoid sacrificing the queen, the wing tips were sampled as an alternative source of DNA. All the samples were genotyped using five microsatellite loci. Our results show that sampling from mated queens and their brood is the least costly approach among those tested but without the knowledge about the genotypes of the DPCs also bears uncertainty about the origin of the drones participating in the mating. Surprisingly, genotyping the spermatheca did not improve the identification of the patrilines. The approach where drone-producing queens were genotyped was the costliest but ensured reliable paternity determination. Wing tips may be a suitable alternative source of queen's DNA although the genotyping success rate was lower in comparison to other tissues. Different field set-ups also resulted in various numbers of patriline detected and various contribution of known drones in mating, providing additional evidence for evaluation of mating control success.

Keywords: honeybees, mating control, paternity determination

SUCCESSFUL INDOOR MASS STORAGE OF HONEYBEE QUEENS (*APIS MELLIFERA*) DURING WINTER

Rousseau, Andrée, Apiculture, Centre de recherche en sciences animales de Deschambault, Deschambault, Canada
Giovenazzo, Pierre, Département de biologie, Université Laval, Québec, Canada

The production of young, mated honeybee queens (*Apis mellifera*) is essential to replace dead queens or to start new colonies after wintering. Mass storage of mated honeybee queens during winter and their use the following spring is an interesting strategy that could help fulfill this need. In this study, we investigated the survival, fertility, and fecundity of young, mated queens stored massively in queenless colonies from September to April (eight months). The queens were kept in environmentally controlled rooms at temperatures above and below cluster formation. Young mated queens were produced at the end of the summer 2018 by 3 breeding operations (N total = 630 queens). Queens have been wintered individually in small cages and grouped together in a queen bank (40 queens/bank) in an environmentally controlled room kept at either 6°C, 11°C or 16°C from November 2018 to April 2019 (N=5 queen banks per temperature). The results show that indoor mass storage of mated queens can be achieved with success when queen banks are stored above cluster temperature. Significantly higher survival of queens was measured at temperature above cluster formation : 86.3% of the queens survived at 16 °C compared to 56.9% and 55.5% at 6 °C and 11 °C degrees respectively. Surviving queens wintered at different temperatures above or below cluster formation had similar fertility (sperm viability) and fecundity (egg laying and viable worker population). This study shows the potential of indoor overwintering of honeybee queen banks. The technique we describe could be applied on a commercial scale by beekeepers and queen breeders.

Keywords: Queen storage, Queen wintering, Queen banks

FACTORS THAT AFFECT HONEYBEES' SWARMING

Tananaki Chrysoula, Kanelis Dimitrios, Liolios Vasilios, Rodopoulou Maria-Anna

Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki, Thermi, Thessaloniki, Greece

Swarming is a natural way of reproduction for bees and a complex phenomenon affected by many factors, such as hive's overpopulation, insufficient amount of the queen's pheromone, high temperature and queen's genetic predisposition. The aim of this study was to evaluate the genetic predisposition and the lack of space as factors in the occurrence of the swarming through the application of field experiments. Thus, groups of equal bee colonies were created and received the same treatments during the experiment, until the appearance of the phenomenon, while all the queens of each group were sisters to each other. The created groups consisted of colonies with 2-year-old queens coming from natural swarming (Group A) and queen breading (Group B), 3-year-old queens (Group C) and 4-year-old queens (Group D) coming from queen breading. The following treatments were applied to these bee colonies: a. supplementary feeding, b. removal of empty space frames, c. artificial increase of the population. During the experiment the growth rate (population and brood frames), the supplies rate, the colonies' behavior and the time of appearance of the phenomenon were recorded. According to the results, the queens that came from natural swarming (Group A) showed a significant increase in both their food stocks and population and swarmed in a short period of time. The 3-year-old queens swarmed after the queen lacked space for laying eggs, but a month later than the queens of the first group. Finally, the queens of groups B and C did not swarm even when there was intense crowding in the hive, lack of laying eggs space and intense bee feeding. In conclusion, it was found that the genetic predisposition of bee colonies is the most important factor for the occurrence of the phenomenon, while factors such as the lack of laying space influence the swarming at a lower extent.

Keywords: swarming, bee reproduction, genetic predisposition

HONEY BEE NUPTIAL FLIGHTS UNDER VARIOUS ENVIRONMENTAL CONDITIONS

Uzunov, Aleksandar, UKIM & CARPEA, Skopje, MK
Kovačić, Marin, FAZOS & CALIS, Osijek, HR
Puškadija, Zlatko, FAZOS & CALIS, Osijek, HR
Andonov, Sreten, SLU, SE
Dahle, Bjørn, NBA, NO
Wegener, Jakob, ex-LIB, DE
Büchler, Ralph, LLH, DE
Prešern, Janez, KIS, SI

Nuptial flights of honey bee queens are performed mid-air at the very beginning of the queen bee's life. For this reason, it is very important that sexual partners are of sufficient quality and quantity which allow the colony to survive and prosper. The duration and the success rate of the flights can indicate the distance to the drone congregation area (mating place).

The focus of the EEA & Norway Grants Fund for Regional cooperation project BeeConSel is mating control as a key in guaranteeing the genetic gain in selection and conservation programs for honey bees (conservation via utilization). In the project scope we are testing several approaches to obtain controlled mating. Namely, geographical isolation, isolation by saturation (biological isolation) and temporal isolation are being tested in partner countries in a desire to evaluate the suitability of certain approaches.

In the first year, we tested the geographic isolation method in situations with and without the presence of drone-producing colonies (DPCs). We observed nuptial flights of 87 virgin queens: 30 in Croatia (HR), 35 in Macedonia (MK) and 22 in Slovenia (SI) on potential isolated sites. On average, at different locations, queens performed nuptial flights during a period of 1.8 days in HR, for 1.6 to 4.3 days in MK and for 1.6 to 2.4 days in SI. Number of nuptial flights also differed: the lowest was in Slovenia (Krma, 2.3) and the highest in MK (Belicki, 8.8). Flights, when queens returned without a mating sign, were two/threefold shorter than successful ones, e.g., returning with a mating sign. The successful flights were shortest in locations with DPCs present (SI - Krma, 11 min and HR - Batina, 14 min). Mating success was related to the presence of DPCs, but even at locations chosen for their putative isolation from known apiaries, unexpectedly high mating success was achieved, albeit at cost of longer flight durations (MK - Belicki, 42 %, 22 min; SI - Vrata, 70 %, 26 min).

Keywords: mating control, nuptial flights, breeding

DIFFERENTIAL EFFECTS OF A JUVENILE HORMONE ANALOGUE ON OVARY DEVELOPMENT IN BUMBLE BEES ACCORDING TO CASTE, SPECIES AND HIBERNATION STATUS

Enya Wynants[1], Cédric Van Dun[2], Tom Wenseleers[2], Felix Wäckers[1] & Annette Van Oystaeyen[1]

1 R&D department, Biobest Group NV, Westerlo, Belgium
2 Laboratory of Socio-ecology and Social Evolution, University of Leuven, Belgium

Juvenile hormone (JH) plays an important role in insect development and reproduction. In bumble bees (*Bombus* sp.), juvenile hormone acts as a gonadotropin and is involved in oogenesis and ovary development. In this study, the effect of administration of a juvenile hormone analogue, methoprene, on ovary and colony development was studied for two model bumble bee species: B. terrestris and *B. impatiens*. Methoprene was topically applied in different concentrations, after which ovary development, time to oviposition, and colony foundation success were evaluated in both hibernated and non-hibernated mated gynes. Additionally, the effect of topical application of methoprene on ovary development was also evaluated in workers of both species. Our results indicate that for both bumble bee species, application of methoprene activates ovary development of non-hibernated mated gynes, while it has no effect on ovary development in hibernated mated gynes that readily developed eggs without the application of methoprene. Noteworthy, non-hibernated *B. impatiens* gynes required a significantly higher dose of methoprene to activate ovary development than *B. terrestris* gynes. The differential effect of methoprene according to hibernation status could be explained by lower natural JH levels of non-hibernated gynes compared to hibernated gynes, as hibernated gynes naturally experience a rise in JH levels after hibernation. For both species, colony foundation (i.e. oviposition) and colony development was not accelerated when mated gynes were hibernated. For *B. terrestris*, a comparison was made between hibernated and non-hibernated gynes, showing a significant delay of oviposition by more than 40 days when gynes did not hibernate, even when methoprene was administered. Despite methoprene's significant positive effect on ovary development in non-hibernated gynes, it did not imitate hibernation with respect to time to oviposition or colony foundation success. Additionally, methoprene did not influence ovary development in workers of both species.

Altogether, our results highlight that juvenile hormone is involved in the development of ovaries in gynes but that it does not seem to play a direct role in the physiological process of egg-laying. The results further indicate that ovary development in bumble bee workers is not initiated by a rise in juvenile hormone.

Keywords: bumble bees, juvenile hormone, ovary development

A BETTER UNDERSTANDING OF BIOLOGICAL TRAITS OF THE TROPICAL HONEYBEE SUBSPECIES *APIS MELLIFERA UNICOLOR*

Benoit Jobart[1,2], Hélène Delatte[3,4], Damien Decantes[5], Olivier Esnault[5], Gérard Lebreton[1], Nicolas Blot[6], Johanna Clémencet[2]

1 CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France
2 Université de la Réunion, 97400 Saint Denis, La Réunion France
3 CIRAD, UMR PVBMT, 101 Antananarivo, Madagascar
4 FOFIFA CENRADERU, DRA, 101 Antananarivo, Madagascar
5 GDS, 97418 La plaine des Cafres, La Réunion, France
6 Université Clermont Auvergne, CNRS, Laboratoire "Microorganismes: Génome et Environnement", Clermont–Ferrand, France

The honeybee *Apis mellifera* has shown a great intraspecific diversity, with that, a variability of its life history traits. In European countries the biological cycle of brood is well documented while the tropical subspecies remains poorly known. For instance, colonies in temperate regions have evolved to overwintering with a cessation of brood rearing. In tropical regions where the majority of subspecies belonging to the lineage A, honeybee colonies seem to raise brood permanently. This different brood dynamics from temperate regions also implies different dynamics of pathogens like *Varroa destructor*. However, in *A. m. capensis*, a subspecies of lineage A, the post-capping period is much shorter than for European subspecies and contributes to a natural resistance to *V. destructor*.

Here we studied the annual brood cycle and the post-capping period of an African lineage subspecies in a tropical and insular environment. A total of 80 colonies of *A. m. unicolor* were monitored for two 1-year periods in two apiaries located on different altitudes in La Réunion. We found that *A. m. unicolor* raised their brood all year long and the post-capping period was 280.1 ± 0.12 h. Review of post-capping period of different subspecies showed that African lineage post-capping periods were significatively shorter than these of European lineage and *A. m. unicolor* post-capping period belonged in an intermediate cluster (hybrid). The knowledge of life history traits of *A. m. unicolor* may have implications for beekeeping practices and be considered in *V. destructor* resistance breeding programs.

Keywords: *A. m. unicolor*, Brood dynamic, Post-capping period

COMMUNICATION AND CHEMICAL ECOLOGY

ORAL PRESENTATIONS

CHAIRPERSON
Ulrich Ernst

DISPARATE SOCIAL STRUCTURES LEAD TO DISPARATE CHEMICAL COMMUNICATION SYSTEMS

Hefetz Abraham, Ruppin Academic Center, School of Marine Sciences, Israel; School of Zoology, Faculty of Life Sciences, Tel Aviv University, Israel

Reproductive division of labor is largely manifested by complex communication system between the fertile queen and her sterile workers. The prevailing hypothesis underlying this phenomenon is that workers forego their reproduction if they gain in doing so sufficient inclusive fitness. This kin-selection-based consideration largely depends on the social structure of the species. In systems with multiply mated queens (e.g., honeybees), reproductive monopoly and worker sterility are maintained by active policing of reproductive workers. In systems with singly mated queens (e.g., bumblebees), in contrast, workers' gain from direct fitness may surpass their gain from inclusive fitness and therefore they should be selected to compete with the queen and among nestmate over reproduction.

Accordingly, in honeybee the queens advertise their dominance by producing an idiosyncratic suit of esters as fertility signal in Dufour's gland. However, hopelessly queenless workers that have activated ovaries also produce these esters, corroborating their role as fertility signals. In bumble bees, workers are distinct from the queen by exhibiting a sterility signal. At the competition phase, workers initiate ovary activation despite the presence of the queen, with concomitant aggressive competition among them over male production. However, not all workers can successfully reproduce, creating two worker "castes", reproductive and non-reproductive. The latter, therefore, can gain at least inclusive fitness by caring to their sisters' brood. Analysis of Dufour's gland secretion revealed clear distinction between these two "worker castes". The sterile workers possess a suit of esters that disappears with ovary activation. Behavioral assays further showed that the secretion act as an appeasement, protecting sterile workers from aggressive attack by their reproductive nestmates. There is also a correlation between ester occurrence and foraging, strengthening the hypothesis that the esters signal "I am out of the competition and care for your brood".

The fact that closely related bees use similar (but not identical) suits of esters, but which have an opposite social, context-dependent, messages, emphasize the evolution parsimony of pheromones in social insects.

Keywords: Fertility signals, honeybees, bumblebees

WHAT DO WE KNOW ABOUT CHEMOSENSING OF *VARROA DESTRUCTOR*?

Nganso T. Beatrice[1], Eliash Nurit[1,2], Mani Kannan[1], Selah Noa[1], Altman Yam[1], Rafaeli Ada[1], Mikheyev Alexander S.[2,3] and Soroker Victoria[1]

1 Agricultural Research Organization, the Volcani Center, Rishon LeZion, Israel
2 Okinawa Institute of Science and Technology, 1919-1 Tancha Onna-son, 904-0495 Okinawa, Japan
3 Australian National University, Canberra, ACT, 2600, Australia

Keywords: host detection, olfaction, behavior

Varroa destructor is considered as the most important threat for honey bee (*Apis mellifera* L.) causing colony losses almost worldwide. This is an obligatory ectoparasitic mite that eavesdrops on its host chemical cues for feeding and reproduction. The mite is apparently able to discriminate between chemical cues from different colony members: adults and brood. However, the building blocks of their chemosensory machinery remain unresolved. To reveal the components of its chemosensory machinery, we used organ blocking techniques as well as gene silencing, transcriptomic and proteomic analyses along with behavioral studies. Altogether, our studies revealed that although the major olfactory organ is located in the Varroa forelegs, the RT-qPCR studies suggested that some putative chemosensory genes are expressed in both gnathosoma and forelegs. Mechanically blocking of the mites' forelegs reduced their ability to select and identify a bee host. RNA-interference (RNAi) studies revealed the involvement of at least two putative odorant binding proteins from the Niemann-Pick type C2 group in *Varroa* host chemosensing. The structure and function of the Varroa chemoreception network will be discussed.

Keywords: Chemosensation; honeybee; chemosensory genes; mite

RESPONSIVENESS TO INHIBITORY SIGNALING CHANGES AS A FUNCTION OF COLONY SIZE IN WESTERN HONEY BEES (*APIS MELLIFERA*)

Broccard-Bell, Heather, Division of Biological Sciences, Section of Ecology, Behavior, and Evolution, University of California San Diego, La Jolla, USA,
Hsiung, Kevin, Division of Biological Sciences, Section of Ecology, Behavior, and Evolution, University of California San Diego, La Jolla, USA,
Pasberg, Patrick, Department of Mechanical Engineering, Section of Biomimetics, Westphalian University of Applied Sciences, Bocholt, Germany,
Broccard, Frederic, Institute for Neural Computation, University of California San Diego, La Jolla, CA 92093, USA,
Nieh, James, Division of Biological Sciences, Section of Ecology, Behavior, and Evolution, University of California San Diego, La Jolla, USA

Honey bee colonies, like other types of biological collectives, use both excitatory and inhibitory communication signals to flexibly respond to an ever-changing world. In this study, we focused on one pair of vibrational signals: the waggle dance and the stop signal. Workers use the excitatory waggle dance to recruit colony mates to favourable locations, whereas the inhibitory stop signal is used to reduce waggle dancing for degraded sites. We explored the relationship between colony size and the responsiveness of waggle dancers to stop signals. Specifically, we exposed colonies of different sizes to artificial inhibitory signals and quantified the resulting reduction in waggle dancing. We hypothesized that small colonies need to maintain foraging, even if it is subopitmal, and thus predicted that inhibition would be weaker in small colonies than in large colonies. Our results supported our prediction. We discuss our findings with respect to the natural history of *Apis mellifera,* and in more general terms of biological network structure and function.

CONTRIBUTIONS OF CHEMICAL ECOLOGY TO A BETTER UNDERSTANDING OF SOCIAL IMMUNITY IN THE HONEY BEE

Mondet, Fanny, UR 406 Abeilles et Environnement, INRAE, Avignon, France
Le Conte, Yves, UR 406 Abeilles et Environnement, INRAE, Avignon, France

Despite their high susceptibility to invasions and disease spread, many social animals such as the honey bee have evolved mechanisms that allow effective defence at the group level. These collective strategies play a role at society level that parallels that of an immune system, and the social immunity they confer provide the basis for colony resistance to pathogens and parasites, such as varroa and other diseases. How compromised individuals are accurately diagnosed, and how the collective actions of bees undertaking the immune strategies are regulated at colony level pose many unsolved challenges. It is however known that chemical communication, which plays a central role in homeostasis maintenance within colonies, also plays a key role in host-parasite interactions and in defence against pathogens in general. We will present the current knowledge on the contribution of chemical ecology to social immunity, as well as our latest findings on the mechanisms of hygiene against varroa parasitised brood. This presentation will highlight the input of an in-depth understanding of host defence mechanisms to provide essential tools not only to predict, but also to mitigate against consequences of invasive species events.

Keywords: Social immunity, chemical communication, *Varroa destructor*

A NEW HYPOTHESIS: VOLATILE JUVENILE HORMONE PRECURSOR MAY BE THE TRIGGER OF VARROA'S REPRODUCTION IN THE HONEYBEE BROOD CELLS

Aurori Cristian, (1) Apiculture and Sericulture Department, Faculty of Animal Science and Biotechnology, University of Agriculture Sciences and Veterinary Medicine, Cluj-Napoca, Romania
Giurgiu Alexandru-Ioan, (1) Department of Biology, University of Copenhagen, Copenhagen, Denmark
Conlon Benjamin H., Department of Biology, University of Copenhagen, Copenhagen, Denmark
Kastally Chedly, Department of Forest Sciences, University of Helsinki, Helsinki, Finland,
Mirică Adina–Mădălina, (1) Molecular Ecology Department, Institute of Biology/Zoology, Martin-Luther-University Halle-Wittenberg, Halle an der Saale, Germany
Dezmirean Daniel S., (1) Molecular Ecology Department, Institute of Biology/Zoology, Martin-Luther-University Halle-Wittenberg, Halle an der Saale, Germany
Routtu Jarkko, Molecular Ecology Department, Institute of Biology/Zoology, Martin-Luther-University Halle-Wittenberg, Halle an der Saale, Germany
Aurori Adriana, Advanced Horticultural Research Institute of Transylvania, University of Agriculture Sciences and Veterinary Medicine, Cluj-Napoca, Romania

The reproductive behavior of Varroa is associated with a tight synchronization between the moment of cell infestation and the stage of brood development; with the drones offering a larger post-capping time frame in which Varroa can activate its ovary. Unidentified, yet volatile, compounds emitted by the brood and acting as cues for Varroa right before its first meal are considered to have a major role in that process. We analyzed the expression of the genes related with the ecdysone (Ecd) and juvenile hormone (JH) of the worker and drone larvae at five time points after cell capping. The Ecd related genes showed a later activation of ecdysone in drones compared to the worker larvae. Simultaneously, the level of the JH related genes was higher in drones. A higher level of JH during the last larval instar indicates a prolongation of the larval spinning stage and this fact was confirmed by the expression of the fibroin genes which was extended in drones, offering a window for Varroa's ovary activation. The analysis of possible orthologs of JH parthway in Varroa combined with the analysis of their expression at 8 and 30 hours post-capping revealed a possible inactive ortholog of *jhamt* gene which is involved in methyl farnesoate (MF) synthesis. All the metabolites of the JH pathway are volatile pheromones in insects. Their physicochemical properties are also compatible with a possible role of kairomones for Varroa. The most interesting seems to be the MF which is the JH-like hormone in Acari but its role in Varroa was never studied. Reduced fertility could be linked with a critical level of JH-like volatiles from the brood cells. Our findings help explain the lack of reproduction of Varroa in fast pupating honey bees and also its reduced fertility in small subspecies of honey bee which having a small body size do not supply the Varroa with the necessary amount of volatile. Further studies are needed in order to fully confirm this hypothesis.

Keywords: Drone – worker larvae, Kairomone cues, Methyl farnesoate

VOLATILE ORGANIC COMPOUND EMISSION OF VARROA INFESTED HONEY BEE BROOD CELLS

Amélie, Noël, INRAE UR 406 Abeilles et Environnement, Avignon, France
Charlène, Dumas, INRAE UR 406 Abeilles et Environnement, Avignon, France
Emilien, Rottier, INRAE UR 406 Abeilles et Environnement, Avignon, France
Dominique, Beslay, INRAE UR 406 Abeilles et Environnement, Avignon, France
Guy, Costagliola, INRAE UR 1115 Plantes et Systèmes Horticoles, Avignon, France
Christian, Ginies, INRAE UMR 408 Sécurité et Qualité des Produits d'Origine Végétale, Avignon, France
Yves, Le Conte, INRAE UR 406 Abeilles et Environnement, Avignon, France
Fanny, Mondet, INRAE UR 406 Abeilles et Environnement, Avignon, France

Varroa destructor is well known as a worldwide threat for honey bee colonies (*Apis mellifera*). A colony infested with the mite can collapse within a few months. Varroa infestation increase with an exponential tendency along the beekeeping season, and without treatment colony survival is compromised in most cases. However, some honey bee colonies have developed resistance to the parasite and are able to control the mite infestation rate. To achieve this, they have developed specific behaviours, such as VSH (*Varroa Sensitive Hygiene*), a form of hygienic behaviour that is directed towards varroa-infested brood cells. Bees performing this behaviour detect parasitized brood cells, uncap and clean their contents, thus disrupting mite reproduction. Several semiochemicals have been shown to be released from varroa-infested brood cells targeted by VSH behaviour and to trigger this behaviour. These chemicals harbour long carbon chains and a low volatility. In this study, we focus on the study of volatile organic compound (VOC) emissions from these same cells, as such highly volatile semiochemicals could allow the information about parasitism to be disseminated on a large scale in the nest. Under this hypothesis, compounds with lower volatility would complement VOC role by helping refine the specific detection of parasitized cells. We compared VOC emissions by *Varroa destructor* parasitized versus non-parasitized brood cells in a non-targeted approach, using solid phase microextraction (SPME) methods and gas chromatography coupled with mass spectrometry (GC-MS). We identified several VOC that characterise the health status of parasitized and non-parasitized brood cells. These candidate VOC were then tested in the field to characterise their hygienic behaviour triggering ability in workers. These findings increase our understanding of how honey bees fight brood diseases such as varroosis with hygienic behaviour.

Keywords: *Varroa destructor*, chemical ecology, Volatile organic compounds

HONEYBEE WORKERS DISPLAY TASK-RELATED AND SUBSPECIFIC PATTERNS IN THEIR CUTICULAR HYDROCARBON PROFILES

Rodríguez, Daniel, Department of Animal Ecology and Tropical Biology, Würzburg, Germany.
Scheiner, Ricarda, Department of Behavioral Physiology and Sociobiology, Würzburg, Germany.
Schmitt, Thomas, Department of Animal Ecology and Tropical Biology, Würzburg, Germany

Different *Apis mellifera* subspecies present natural selection-shaped local adaptive traits in response to climatic conditions and habitats they face in their own native distribution ranges. Such local adaptations of honeybee subspecies are of special importance under the current climate change scenario, as it can affect their distribution range and/or drive new competitive relationships between them or with other species. One of these local adaptive traits is the cuticular hydrocarbons (CHC), which prevent desiccation and mediate intra- and interspecific communication. The effectiveness of the functions of the CHC depends on their composition, which is influenced by environmental factors (e.g. climate). Short-chain hydrocarbons, as well as unsaturated and methyl-branched hydrocarbons, act as chemical cues but conform a less efficient desiccation barrier than long-chain, saturated hydrocarbons. Thus, the composition of CHC might be the result of a trade-off between water-loss prevention and communication necessities. In *A. mellifera*, the individual CHC profile is also influenced, among other factors, by the task-performance. Previous works have found that task-related variation of the CHC is to some extent age-independent and could serve as task recognition cues among the workers. The present study analyzes the CHC profiles of honeybee workers of five subspecies (*A. m. carnica, A. m. ligustica, A. m. macedonica, A. m. iberiensis,* and *A. m. ruttneri*) in winter and summer, regarding their task performance. Here we provide evidence of the consistent variation in the CHC composition of honeybee workers among different subspecies and tasks. The differences in the composition of the CHC profiles of honeybee workers seem to mainly occur in compounds that might have a communication function, but still could affect the water-loss prevention. The results exposed here indicate that the composition patterns in the CHC profile of honeybee workers respond to the trade-off between the prevention of water-loss and communication.

Keywords: honeybee ecology, cuticular hydrocarbons, climate adaptation

MONITORING BEES

ORAL PRESENTATIONS

CHAIRPERSON
Manuela Giovanetti

WINTER HONEYBEE COLONY MORTALITY IN FRANCE: RESULTS FROM A RETROSPECTIVE NATIONAL SURVEY

Laurent Marion, Unit of Honey bee Pathology, ANSES, Sophia Antipolis, France
Bicego Quentin, GDS Centre, Châteauroux, France
Boucher Samuel SNGTV - LABOVET CONSEIL, Les Herbiers, France
Carles Sophie, French National Research Institute for Agriculture, Food and the Environment, Lyon, France
Chaume Jacques, GNTSA, Hautes-Alpes, France
Giraud Florentine FNOSAD, Les Houches, France
Meziani Fayçal, Service des actions sanitaires, DGAl, Toulouse, France
Nozieres Emma, ADA France, Paris, France
Orlowski Muriel, Service Santé et Protection Animales, DDPP Drôme, Valence, France
Sourdeau Cédric, Veterinary and phytosanitary expertise Department, DGAl, Angers, France
Vallon Julien, Institut de l’Abeille, Avignon, France
Wendling Sebastien, Animal Health Bureau, DGAl, Paris, France

Since 2018 and following reports of significant honeybee colony losses during winter 2017-2018 from several regions in France, a retrospective national survey is launched every year to estimate winter mortality rate and to record the opinion of beekeepers on their winter losses and the possible causes along with information on varroa management and wintering preparation. The survey was conceived in the framework of the national platform on epidemiosurveillance in animal health at the request of the French Ministry of Agriculture and Food. An online questionnaire is sent each spring to beekeepers registered in the national list the previous year and who agreed to be reached by mail. The 2021-2022 survey took place from March 9th to May 2nd and was sent to 64 361 beekeepers registered at the end of 2021. The participation rate was of 32.4 % (20 858 respondents) namely the higher rate since the start of the survey (30.7, 19.1, 15.3 and 26.4 in 2018, 2019, 2020 and 2021 respectively). After consistency analysis with the national list of beekeepers and identification of duplicates and nonsense the final dataset was of 18 952 respondents. Winter colony mortality rate was weighted by the response rates of each bee operation category (less than ten colonies, ten to 49 and more than 50 colonies) in each French department. The national average mortality rate was 26.75% [25.9-27.6] for winter 2021-2022 considering all losses and 18.1% [17.4-18.8] considering only colony mortality. Winter mortality rate was lower the previous year 29.4% [28.3-30.4]. A similar trend was observed for each bee operation category. On a geographic level, a cluster of departments with rates higher than 30% was observed in Center East compared to 2021 where it was in South West. Results should be used taking into account bias of the survey (memorisation of respondents, measurement of mortality, selection of respondents…). This survey enables to have an image of French winter situation that could be further explored at the local level. Mortality results might change as analysis is under way. Analysis of other variables of the questionnaire will come.

Keywords: honeybee, winter mortality, national survey

USE OF PAN AND VANE TRAPS FOR MONITORING WILD BEES

Bevk, Danilo, Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
Rok Šturm, Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
Blaž Koderman, Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

Bees play an important role in natural and agricultural landscapes. Due to a lack of data on their population status and trends, there is a great need to develop an efficient and reliable monitoring scheme for bee populations. In our study, three differently coloured pan traps (blue, yellow, and white) and two differently vane traps (blue and yellow) were tested in five areas (two nature protected, two agricultural, and one urban) in Slovenia from April to September 2020. We had 10 sample sites in each area, that were sampled twice a month for 48 hours. All together 2899 wild bees were caught (211 species), of which 211 were bumblebees (17 species) and 2688 other wild bees (194 species). In terms of both the number of caught individuals and the number of species, the yellow-blue vane trap and the yellow pan trap were the most effective in all five areas. Bumblebees were almost exclusively caught in the blue vane traps, while other wild bees were caught in all types of traps, mostly in the blue vane traps and the yellow pan traps. We also compared the bee community composition of the studied areas. According to nMDS analyses communities in agricultural areas are more similar to each other than to other areas. We also compared bycatch (all non-Apoidea insects) in different traps and observed that vane traps have significantly less bycatch in comparison to pan traps. With further optimization, we estimate that trapping with the combination of pan and vane traps has the potential to establish reliable monitoring of wild bees.

Keywords: monitoring, wild bees, traps

REMOTE MONITORING OF COMMERCIAL BUMBLEBEES ENABLES REAL TIME ASSESSMENT OF COLONY STATUS, ACTIVITY AND POLLINATION EFFICACY

Clouston, George, BeeHero Ltd, Tel Aviv, Israel

The use of commercially reared bumblebee colonies for crop pollination started in Europe in the mid 1980's and is now a global industry. The pollination of greenhouse crops such as tomatoes, is largely dependent on managed pollination services. Today's high-tech greenhouses are equipped with sensors for monitoring light, temperature, humidity, and for controlling climate and crop inputs such as irrigation and fertiliser. However, pollination management is yet to adopt technology, despite its critical importance for crop yield and quality. Colony status and activity is assessed by human observation, counting bees at hive entrances, and visual inspection of flowers for signs of bee visitation. This approach is expensive, prone to error and generates very low resolution data. This study conducted trials of different sensors and monitoring techniques to remotely track bumblebee colonies pollinating tomatoes in greenhouse/poly tunnel conditions. The paper demonstrates how a combination of sensing and analytical techniques enable real time, continuous monitoring of colony status, activity and pollination efficacy:-

i. Colony Status (Brood Temperature and Hive Weight Monitoring): Brood temperature stability is used as an indicator of colony status and development, as well as identifying specific events such as heat stress. Scales track the rate of sugar consumption, indicating colony size. These measures enable remote and timely decisions for colony replacement.

ii. Hive Activity (Hive Entrance Audio Monitoring): Hive entrance microphones trained to detect the acoustic flight signature of bumblebees can quantify bee traffic. As well as providing a real time measure of colony activity, these data help to assess the impact of different climatic conditions, time of day and hive positioning on activity.

iii. Pollination Efficacy (In Crop Audio Monitoring): Detecting the unique acoustic signature of bumblebee buzz pollination, arrays of microphones placed in the crop can track foraging activity and pollination progress. As well as analysing pollination patterns, it will aid decisions on the density and placement of hives within a crop.

This study presents a foundation for the technology enabled management of bumblebee pollination services, that will help improve efficiencies and optimise yields, whilst enhancing bee welfare.

Keywords: Bumblebees, Pollination, Monitoring

APIS MELLIFERA COLONIES AS A BIOINDICATORS FOR THE ENVIRONMENTAL PRESENCE OF ANTIBIOTIC-RESISTANT BACTERIA

Cilia, Giovanni, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Resci, Ilaria, Department of Veterinary Sciences, University of Bologna, Bologna, Italy
Piva, Silvia, Department of Veterinary Sciences, University of Bologna, Bologna, Italy
Mondo, Elisabetta, Department of Veterinary Sciences, University of Bologna, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Nanetti, Antonio, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Antibiotic resistance is an increasing upset of public health relevance. Guidelines and recommendations have been set down about the proper use of antibiotics both for medical and husbandry purposes. Nonetheless, it is essential to assess the environmental occurrence of that phenomenon. Honey bees are highly-performing bioindicators for their morphological and behavioural characteristics, e.g., high mobility around the hive, frequent foraging flights, wide foraging area (approx. 1.5 km from the hive), hair and bristle coverage intercepting pollen and tiny particles like atmospheric particulate matter and microorganisms. *A. mellifera* is widely used as an environmental bioindicator, mainly in the detection of pollutants and pesticides. This study is part of the project “BeeNet – monitoring the environment through bees and biodiversity”. It was conducted in the Emilia-Romagna region (Italy) on 33 *A. mellifera* colonies to assess their possible use as bioindicators of environmentally-present antibiotic-resistant bacteria. Ten forager bees were collected from each investigated colony and microbiological cultures of samples from both the surface of the bee bodies and their gut microbiota were performed. Each sample was streaked onto a chromogenic non-selective medium and incubated aerobically at 37 °C. The cultures allowed the isolation of 300 different bacterial strains, which were identified using MALDI-TOF.High heterogeneity of plant, animal, and human bacteria was detected. In the latter category, a range of emerging opportunistic pathogens of high epidemiological and health relevance was found. The antibiotic resistance of the isolated bacteria was assessed using the Kirby-Bauer method by testing 18 of the most commonly used antibiotics in Italy. Excluding intrinsic resistance for each bacterial species, the highest antibiotic resistance values were found for amoxicillin (65%), vancomycin (63%), penicillin (62%), erythromycin (60%), aztreonam (59%), cephalothin (50%), and cefotaxime (45%). In addition, 37% of the considered strains showed multiple antibiotic resistance. The results above support the use of *Apis mellifera* colonies as bioindicators in the environmental assessment of antibiotic-resistant bacteria.

Keywords: bioindicator; antibiotic resistance; environmental bacteria

BEE POLLEN RECOGNITION AND QUANTITY ESTIMATION

Kampel Martin, Thomas Ruttner, Vienna University of Technology, Computer Vision Lab, Austria

Honeybees play an import role for the pollination of many plant species, and therefore impact our lives indirectly or even directly, due to honey production and the resulting products dependence on honey. Due to the increasing deaths in honeybee colonies around the globe the observation of the well-being of beehives is an important factor for keeping a healthy bee colony. Visual computing solutions might a valuable contribution to image based analysis of bee colonies. Technological advances in the field of computer vision, like the use of convolutional neural networks (CNN) for recognition algorithms, have enabled the option to provide possibly more helpful beehive observation tools for beekeepers. In this paper we want to recognize pollen bearings bees and its pollen baskets from images acquired at the entrance of the bee hive. We use a dataset created with a camera prototype to record bees entering and exiting a beehive entrance. A dataset with nearly 40.000 images of bees has been produced, although the majority are images of non-pollen bearing bees. Nevertheless, with nearly 2.000 images of pollen bearing bees, this dataset is large enough in order to test a deep learning approach, although with about 4 convolutional layers the presented models are shallower compared to the very deep CNNs. 100 bee images, which have not been part of the training dataset, are used for testing, with a 50%:50% proportion of pollen and non-pollen images. Sliding window models with the frame size of 50×50, 60×60, and 70×70 are tested, all of them having a separation of 10 pixels to each frame. For the frame size of 60x60 we achieve an F1-score of 92% demonstrating strong results for the given problem.

REMOTE MONITORING AND DETECTION OF INVASIVE SPECIES OF HORNET (*VESPA MANDARINIA*) USING ACOUSTIC TOOLS

Evans, Huw, Beehero Ltd, Tel Aviv, Israel

Invasive species are among the leading threats to native ecosystems, habitats, and wildlife, and can also have a negative impact on economy and livelihoods. An invasive pest of major concern in North West America is *Vespa mandarinia* or Asian Giant Hornet (AGH). The first confirmed sightings of AGH were in Washington State (USA) and British Columbia (Canada) in 2019. This wasp is a predator of honeybees and other pollinating insects and if it becomes established, could have a damaging impact on not only the environment but also the economy and public health. While it is not clear how the species entered the North American continent, the ability to detect its presence as well as monitor its spread is fundamental to any measures aimed at eradicating it or controlling it. Building on previous research on a similar species in Europe, *Vespa velutina*, the paper describes how acoustic based tools were used to develop a system that can remotely detect the unique flight signature of the AGH. Working in collaboration with the USDA, audio sensors were placed at the entrance of an established AGH nest thus generating large quantities of high resolution audio recordings of AGH flight activity, which were used to produce a detection algorithm. The data collected also offered insights into nest activity, such as commencement and cessation of daily flight and overall traffic which correlated well with the nest's population and manual observation. Furthermore, acoustic recordings were taken from hornets of measured sizes and weights when constrained within a flight tent. The resulting differences in acoustic signatures then enabled profiling of the nest demographic. This year a network of audio sensors is being deployed in apiaries at strategic locations across Washington State such as remote areas surrounding previous sightings, but also near ports/airports where AGH may enter the country. This AGH Sentinel Network will provide early detection of hornet presence, prompting nest location and eradication. It will also inform longer term strategies for eradication and containment, by improving knowledge of AGH behaviour and delineating areas of activity to help define how and where resources should be focused for maximum effect.

Keywords: Invasive species, Vespa mandarinia, acoustic monitoring

DEVELOPMENT OF IN-FIELD ACOUSTIC SENSORS FOR MONITORING POLLINATOR VISITATION RATES

Evans, Huw, Beehero Ltd, Tel Aviv, Israel

Significant proportion of crops grown are dependent on insect pollination by both domesticated and wild bees. Agricultural intensification is proposed as a means to counteract the detrimental effects of increased chemical use and farmland expansion into natural habitats. Pollination is one input in agricultural production that has potential for growth but at the same time is being threatened by environmental practices. In order to optimise the pollination process it is fundamental that there is a way to monitor and measure the presence, density and diversity of pollinators, which is in part reflected in the final crop yield. Large scale pollinator studies along gradients are rare and very resource intensive and most research quantifies the contribution of pollinators at a limited scale such as small areas of field or plant scale. While informative these approaches do not fully account for crops' capacity to compensate for pollination deficits and also for variation within fields. Based on technology originally used to monitor in-hive acoustics, a simple acoustic sensor that can be placed along a pollinator gradient to collect data on flying bees has been developed. The sensor collects and analyses the acoustic data which is compared to the acoustic signatures of target bee species which in turn were generated by correlating video evidence with sound recordings. To date field trials have been run in oil seed rape (OSR) fields pollinated by managed honey bees and leaf cutter bees. Regular measurements taken throughout the pollination cycle give a measure of pollinator activity throughout all the days and across all the rows monitored. The results were then compared to the predictive models used, based on meteorological conditions, showing that real time pollinator detection gives a more accurate representation of visitation. In another study the sensors were deployed in an onion seed production setting, however, in contrast to OSR, onions are far less attractive to honey bees and to obtain the resolution of activity desired we have developed a method of continuous recording of flying bees. Based on these data we have been able to create a simple pollinator visitation quantification tool for farmers and seed growers.

Keywords: In-field pollination, Acoustic monitoring, IoT

TRAP NESTS AS TOOLS FOR RESEARCH AND MONITORING OF STEM-NESTING BEES IN IRISH FARMLAND

Hodge, Simon, School of Agriculture and Food Sciences, University College Dublin, Dublin, Ireland
Bottero, Irene, School of Natural Sciences, Trinity College, Dublin, Ireland
Maher, Stephanie, Teagasc, Johnstown Castle, Wexford, Ireland
Stout, Jane, School of Natural Sciences, Trinity College, Dublin, Ireland

The well-documented declines in insect necessitate the evaluation and development of methods for long-term monitoring and applied field research. Additionally, recent amendments to government-funded agri-environment schemes mean that initiatives aimed to conserve pollinator abundance and diversity on farmland will be compensated based on success rather than the action per se. Accordingly, this study evaluated the use of trap nests ("bee hotels") as tools for monitoring above-ground cavity nesting Hymenoptera within Irish agricultural landscapes. The trap nests consisted of 110mm diameter plastic pipes each containing 100 cardboard nest tubes of five different diameters: 4, 5, 6, 8, 10 mm. Three trap nests were set out at eight apple orchards and eight oilseed rape sites at the start of the flowering season and left in place for five months. Sealed nest tubes occurred at 15 of the 16 sites, and in 77% of the 48 nests. However, only 7% of the 4800 individual nest tubes were sealed, and, after the occupants had been allowed to develop and emerge, only 4% of tubes actually produced cavity-nesting Hymenoptera. Three cavity nesting bee species (*Hylaeus communis, Osmia bicornis, Megachile versicolor*) and two solitary wasp species (*Ancistrocerus trifasciatus, A. parietinus*) emerged from nest tubes. There were significant differences among species in terms of emergence date and the diameter of nest tubes from which they emerged. This latter finding indicates that future studies, monitoring programs, conservation efforts or agri-environment schemes would benefit from providing cavities of varying sizes to optimize the diversity of species that may use the nests. Additionally, there may be potential to use occupied nests in local or national conservation translocation schemes. The disadvantages of using trap nests for monitoring include the extended time frame of data collection and frequent low occupancy rates of tubes. However, possibly in conjunction with other survey methods such as pan traps and transects, the results suggest that trap nests offer a valuable tool for fundamental ecological research of stem-nesting Hymenoptera and evaluation of agri-environment scheme success.

Keywords: Bee hotels, Ireland, Solitary bees

MONITORING AND ANALYSING HIVE SOUNDS AS A POTENTIAL TOOL FOR THE DETECTION OF THE QUEEN'S ABSENCE IN COLONIES OF *APIS MELLIFERA* L.

Kanelis Dimitrios[1], Ziogas Michail[2], Siozios Konstantinos[2], Liolios Vasilios[1], Rodopoulou Maria-Anna[1], Siskos Stylianos[2], Laopoulos Theodoros[2], Tananaki Chrysoula[1]

1 Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki, Thermi, Thessaloniki, Greece
2 Section of Electronics and Electronic Computers, Department of Physics, Faculty of Sciences, Aristotle University of Thessaloniki, University Campus, Thessaloniki, Greece

Honeybee has the most important contribution to the pollination among the other insects, playing an important role in the prosperity of ecosystems around the world. Several factors can affect them and contribute to the loss of bees and the total extinction of the colonies. Thus, the need for intensive monitoring of the activity of the whole colony is considered urgent, in order to understand the problems and the causes of bee mortality. Recording the sounds of the colony and creating an automated system which will be able to recognize and distinguish them, could provide useful information to the beekeepers for the remote monitoring of their colonies. The main purpose of a bee monitoring system is to identify the condition of the colony through the analysis of the acoustic position. The examined phenomena in the present study were the presence and absence of the queen (queenless colonies) and the workerlaid male eggs, while at the same time the environmental conditions were recorded. According to the results, when bees are in a queen-right colony, the frequency of their sounds is around 250Hz, while the harmonic sounds have small amplitude. On the other hand, in the case queenless colonies, there is large amplitude of intensity at low frequencies and the bees become noisier, with the frequency dropping to 240Hz. The bee colony, five days after the return of the queen, seems to return to its normal sounds.

Keywords: Colony sounds, bee-hive monitoring, queenless colony

A CITIZEN SCIENCE-BASED WILD BEE MONITORING APPROACH- HOW VOLUNTEERS WITHOUT TAXONOMIC KNOWLEDGE SURVEY CAVITY-NESTING WILD BEES

Lindermann, Lara, Thuenen Institute of Biodiversity, Brunswick, Germany
Dieker, Petra, Thuenen Institute of Biodiversity, Brunswick, Germany

Wild bees are important pollinators of numerous wild and cultivated plants. The occurrence and abundance of wild bees are mainly influenced by the presence of food and nesting resources within a radius of a few hundred meters. In order to be able to relate changes in abundance and diversity of cavity-nesting wild bees to heterogeneity of agricultural landscapes, and thus to advice policy-makers, there is a need to survey wild bees at long-term. To tackle this issue, we designed a wild bee monitoring scheme. One of the main objectives of the monitoring is to integrate volunteers in data collection and in species identification.

Nesting aids are used as a non-lethal and citizen science-based sampling method to survey cavity-nesting taxa in agricultural landscapes. Due to the simple handling and the possibility to follow the development of wild bees, they enjoy great popularity with volunteers. Taking monthly photos of each nesting board from April to September, volunteers without taxonomic expertise collect unbiased data of occupation rate and species. In order to ensure that this approach allows non-disruptive monitoring of local populations, we compared occupation rate, diversity and development of the taxa between controlled and uncontrolled nesting aids. There was no significant difference with regard to the parameters mentioned.

Furthermore, we studied whether volunteers can additionally be involved in species identification. After conducting identification courses during monitoring season, twelve participants attempted to identify cavities of 50 nesting aids, relating to 4,203 cavities. In total, the identification success rate reached 92.4 %. 2.8 % of the cavities contained taxa that could not be classified and 4.8 % were not correctly identified by volunteers. In principle, nesting aid inhabitants of larger cavity diameters and those with clearly recognisable characteristics for identification were correctly identified more often.

The monitoring approach presented here allows volunteers to participate without taxonomic knowledge. Thus, it represents a way of responding to the great willingness in society, including farmers, to get involved in wild bee conservation. Moreover, this non-lethal sampling approach enables to raise the awareness about the underlying relationship between wild bee diversity and heterogeneity of the surrounding agricultural landscape.

Keywords: wild bees, monitoring, volunteers

AN APPLICATION OF EDNA FOR NON-LETHAL DETECTION AND MONITORING OF CAVITY NESTING WILD BEES

Sickel, Wiebke, Thünen Institute of Biodiversity, Braunschweig, Germany
Kulow, Josephine, Thünen Institute of Biodiversity, Braunschweig, Germany
Dieker, Petra, Thünen Institute of Biodiversity, Braunschweig, Germany

Biodiversity is declining at an alarming rate worldwide. Therefore, large-scale biodiversity monitoring is urgently needed to understand changes and their drivers. Especially for wild bees, the classical taxonomic identification of species represents a huge challenge, as it is time- and labor-intensive, requires taxonomic expertise and often relies on lethal sampling. By applying DNA-based methods, especially DNA metabarcoding, monitoring activities could achieve a larger spatial coverage and sampling density could increase. We here present a DNA-based methodological approach for wild bee monitoring to identify cavity nesting wild bees, wasps and parasitoids. To develop this approach, we sampled empty nest tubes of wild bees and wasps. We used high-throughput amplicon sequencing of the COI gene for species identification. We successfully detected Hymenoptera from as little as one brood cell. We detected species mixtures in parasitized nests and nests of solitary wasps. In the latter case, we detected Arachnida, Lepidoptera and Coleoptera, which are probably remains of larval provisions. Despite the limited eDNA quality due to suboptimal storage conditions, we were able to identify >75% of the morphologically identified species and five additional Hymenoptera species. This approach holds great potential for a large-scale and non-lethal wild bee monitoring program, where DNA traces are used for species detections. The eDNA approach further provides additional information on parasitism rates and food webs. By incorporating plant genetic markers, we will moreover be able to identify the composition of pollen provisions of wild bees and thereby gain inside about spatio-temporal use of resources provided by the surrounding landscape.

Keywords: Biodiversity monitoring, eDNA Metabarcoding, non-lethal sampling

BEES AND VINES, APICULTURE AND VITICULTURE

Malagnini, Valeria, Centro Trasferimento Tecnologico, Fondazione E. Mach - Via E. Mach 1, San Michele all'Adige (TN), Italy
Fontana, Paolo, Centro Trasferimento Tecnologico, Fondazione E. Mach - Via E. Mach 1, San Michele all'Adige (TN), Italy
Zanotelli, Livia, Centro Trasferimento Tecnologico, Fondazione E. Mach - Via E. Mach 1, San Michele all'Adige (TN), Italy
Bertola, Naike , Azienda Agricola Mille1di Naike Bertola - Via Pratello 26, Padenghe sul Garda (BS) Italy
Bertola, Nathan, Azienda Agricola Pratello di Vincenzo Bertola - Via Pratello 26, Padenghe sul Garda (BS) Italy
Zanzotti, Roberto, Centro Trasferimento Tecnologico, Fondazione E. Mach - Via E. Mach 1, San Michele all'Adige (TN), Italy

Intensive agriculture is considered one of the most important factors that affect honey bees and wild bees, due to pesticide applications and landscape simplification. Moreover the frequent soli tillage reduce the floral resources and nest sites for wild bees. The commercial grape vine (*Vitis vinifera* L.) is self-pollinated and wind pollinated, thus pollination by insects only plays a minor role for grape yield, but vineyards, and in particular organic vineyards, can provide a good habitat for honeybee and wild bees. The presence of floral resources in the inter-rows of the vineyards and the reduction of pesticides in organic vineyards, positively affect honey bees as well as wild bee and solitary bees diversity and abundance.

As part of the Chaos project, we monitored the presence of pesticides in pollen collected by honey bees during two subsequent seasons. The pollen, collected monthly, was analyzed also from a botanical point of view. Pan traps were monthly positioned for monitoring Apoidea in two transepts located in vineyards with a different grass management. Bee hotels were also installed in the vineyards.

The data obtained from the pollen analysis revealed how the sowing used for green manure was used by bees for the supply of pollen and nectar for honey bees and wild bees.

Keywords: Honey bees, Wild bees, Vineyard

DIFFERENTIAL EFFECTS OF CLIMATE CHANGE AND PESTICIDE USE ON CROP POLLINATION SERVICES PROVIDED BY WILD AND MANAGED BEES

MacQueen, Sarah Anne, School of Agriculture and Food Science, University College Dublin, Dublin, Ireland

As providers of pollination services, both wild and managed bees are critical in ensuring ecosystem functioning and food security for the future. It is well known that the weather affects bees' activity, causing concern that climate change will have a detrimental effect on the pollination services that they provide. Pesticide use in agricultural crops additionally affects bees' activity, even at sublethal doses, and may therefore have additional consequences for pollination services. Different bee species have different thermoregulatory capabilities and different pollination behaviours, and the pollination services they provide may therefore be differently affected by climate change and pesticide use. Effects of climate change such as phenological and range shifts have been reasonably well studied. However, this is not the case for the changes in individual bee activity that may occur on a seasonal or daily scale due to the effects of climate change and pesticide use. In this work we measure the effects of climate change on crop pollination services by comparing modelled pollination service levels under current climate conditions and 2080 climate conditions predicted by two Representative Concentration Pathways, RCP 4.5 and RCP 8.5. We use a newly developed mechanistic model of physiological, behavioural, and environmental heating and cooling mechanisms to predict the thorax temperature of bumblebees and honeybees as a function of the climate conditions and foraging behaviours. This approach allows us to predict whether bumblebees and honeybees will stay within its thermal limits when foraging on a particular crop in a given set of climate conditions, and whether the pollination services they provide are affected by any behavioural changes necessary to keep within those limits. We use these predictions to provide insights into the effects of climate change and pesticide use on bumblebee and honeybee activity levels, and the resulting pollination services.

Keywords: mechanistic model, pollination services, climate change

THE INTERPLAY OF BEE STRESSORS, BEEKEEPING MANAGEMENT, AND CLIMATE ON HONEY BEE COLONY LOSS IN LATIN AMERICA

Sibaja Leyton, Malena, UMR EGCE, Université Paris-Saclay - CNRS - IRD / icipe, Gif-sur-Yvette / Nairobi, France / Kenya.
Requier, Fabrice, UMR EGCE, Université Paris-Saclay - CNRS – IRD, Gif-sur-Yvette, France.
Antúnez, Karina, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Montevideo, Uruguay.
And "Colony Loss" SOLATINA's working group.

Over the last decades and on a global scale, beekeepers have registered significant colony losses of honey bees *Apis mellifera*, encouraging the development of monitoring programs and bee research. Beekeeping management, biotic stressors, and environmental pressures were identified as risk factors affecting these losses in Europe and United States. Unfortunately, several regions of the world are lacking such important data, in particular in South Hemisphere. As part of a large-scale citizen science program aiming at monitoring program honey bee colony loss in Latin America, we analyzed whether beekeeping management, symptoms of bee pathologies and pesticides exposure (regrouped as "bee stressors"), and climate interplay in the mortality risk of honey bees. A total of 1758 responses were collected between 2016 and 2018 from 15 Latin-American countries. The data present an extended climate gradient never consider to our knowledge. We found an increase of colony loss with the accumulation of symptoms of bee pathologies and pesticide exposure while beekeeping management can mitigate or even reverse this adverse effect. For a high presence of bee stressors, hobbyist beekeepers can lose 70% more beehives than professional beekeepers. Interestingly, we found that this management-based mitigating effect on colony loss is dependent on the climate. Beekeeping management reduces colony loss in temperate climates but not in tropical climates. These results suggest that beekeeping management is critical for mitigating colony loss in Latin America but management recommendations need to consider the climate. We will discuss such interesting results with the main objective to orientate climate-dependent management recommendations in order to support beekeeping and colony survival in Latin America.

Keywords: *Apis mellifera*; Colony mortality; Large-scale monitoring

PARSING THE EFFECTS OF ANTHROPOGENIC STRESSORS ON POLLINATOR NUTRITIONAL RESOURCES THROUGH DATA INTEGRATION

Quinlan, Gabriela, Department of Entomology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, United States of America

Multiple anthropogenic stressors constrain the availability and quality of nutritional resources (flowers) for pollinators. Honey bees are one of the most important pollinator species, and as a managed, generalist with a broad foraging range, honey bees can serve as bioindicators of anthropogenic stressors on pollinator nutritional resources. That is, by monitoring honey bee resource accumulation (honey), we can draw inference on the quantity of nutritional resources in a given landscape. We can also use these data to monitor broadscale, long-term patterns to infer key drivers of nutritional resource availability. Our objective was to determine how anthropogenic stressors, such as climate change and extreme weather, soil health, land use and land use change, and pesticides influence pollinator nutritional resource availability and to parse the relative importance of each of these factors. Using hierarchical partitioning a 50-year data set on U.S. honey production, we were able to parse the spatial and temporal drivers of pollinator nutritional resources. We found that spatial factors such as climate and soil productivity were approximately ten times more important than temporal factors such as land use change and extreme weather in predicting honey production. We find similar results when using random forest models to examine hive scale data from across the Northcentral U.S.. Wherein, broadscale climactic conditions characterize colony weight gain (honey accumulation), and these effects are moderated by land use, specifically the area of grassy-herbaceous land. Together, these results suggest that there are broad regional patterns that constrain the landscape-level availability of nutritional resources for pollinators in the US, but these effects are fine-tuned by other factors such as land use and weather. It is important to parse the relative importance of these multiple anthropogenic stressors for targeted conservation efforts.

Keywords: climate change, landscape, hive scales

A FIRST APPROACH TO DESIGN A NEW BIODIVERSITY INDICATOR BASED ON WILD BEES FOR RURAL DEVELOPMENT PLAN

Zenga, Emanuele Luigi, CREA Research Centre for Agriculture and Environment, Bologna, Italy
d'Agostino, Marco, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Giovanetti, Manuela, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Galloni, Marta, University of Bologna, Department of Biological, Geological and Environmental Sciences, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Quaranta, Marino, CREA Research Centre for Agriculture and Environment, Bologna, Italy

This research was part of the LIFE 4 POLLINATORS project "Involving people to protect wild bees and other pollinators in the Mediterranean" (LIFE18 GIE/IT/000755), in which the Research Centre for Agriculture and Environment (CREA) of Bologna, Italy, leads a pilot project aimed at experimental application of a new biodiversity indicator for Rural Development Plans (RDP). The main goal is to identify taxa and/or eco-functional traits suitable to become components of an indicator. Through a 3-years monitoring, the project is collecting data once per month from five farms that apply different agricultural practices, along an elevation gradient. The study area is located in Emilia-Romagna region, characterized by widespread and intensive farming at low elevations and by fragmented cultivated areas, surrounded by natural landscape, on the pre-Appennine hills and mountains. Environmental and landscape data were retrieved by land-use and topographical cartography, while agricultural management data were obtained through farmers' interviews. We describe the wild bee communities based on their biological diversity and eco-functional traits (e.g. sociality, lecty, nesting habits). A correlation matrix is used to summarize our data and show the correlation pattern between bee diversity and environmental and agricultural variables. A preliminary analysis on the first-year data seems to indicate that environment and landscape composition affects bee abundance, enhancing species diversity at higher altitudes. Farms in natural and complex landscapes host a higher number of oligolectic and solitary species. Bees with a larger distribution range in Italy and ground excavating bees are related to agricultural areas. A higher abundance of *Hylaeus* spp. is associated to the presence of ponds in the sampling sites. Halictidae are more represented in agricultural and homogeneous landscapes. Megachilidae are more abundant in natural and fragmented contexts. The analysis highlights a positive correlation between the abundance of *Andrena* spp. and total bee diversity. This is a first approach to the development of an indicator able to assess the effects of Common Agricultural Policy measures on the wild bee community.

Keywords: eco-functional traits, Mediterranean, pollinators.

THE INFLUENCES OF ILLUMINATION REGIME ON EGG-LAYING RHYTHMS OF HONEY BEE QUEENS

Shpigler, Hagai Y, Department of Entomology, The Volcani Institute, Rishon LeZion, Israel
Yaniv, Almog, Department of Ecology Evolution and Behavior, The Hebrew University of Jerusalem, Jerusalem, Israel
Gernat, Tim, Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana IL, USA
Robinson, Gene E, Carl R. Woese Institute for Genomic Biology, The University of Illinois at Urbana-Champaign, Urbana IL, USA
Bloch, Guy, Department of Ecology Evolution and Behavior, The Hebrew University of Jerusalem, Jerusalem, Israel

Honey bee queens are extremely fecund, commonly laying more than a thousand eggs in a single day. It has proven challenging to study the temporal organization of egg-laying behavior because queens are typically active around the clock in the dark cavity of a densely populated nest. To contend with this challenge, we developed two novel methods allowing detailed monitoring of queen activity and egg-laying. We first adopted a high-resolution, continuous, tracking system allowing track the position of barcode-tagged queens in observation hives with colonies foraging outside. We found that the queen is active ~96% of the day with typically no diurnal rhythm. Next, we developed a new laboratory procedure to monitor egg-laying at single egg resolution under different light regimes. We found that under constant darkness (DD) and temperature conditions, queens laid eggs with no circadian rhythms. Queen fecundity was severely reduced under constant light (LL). Under a 12:12 illumination regime, queen fecundity was comparable to under constant darkness, with a higher number of eggs during the light phase. These daily rhythms in egg-laying continued when these queens were released to DD conditions, suggesting that egg-laying rhythms are influenced by endogenous circadian clocks. These results suggest that honey bee queens are active and lay eggs around the clock with no diurnal rhythms. Light has complex influences on these behaviors, but more studies are needed to determine whether these effects reflect the influence of light directly on the queen or indirectly by affecting the workers.

Keywords: Queen, Egg-laying, Circadian rhythm

DEEPWINGS©: A MACHINE LEARNING TOOL FOR IDENTIFICATION OF HONEY BEE SUBSPECIES

Yadró, Carlos, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Rodrigues, Pedro J., Research Center in Digitalization and Intelligent Robotics (CeDRI), Instituto Politécnico de Bragança, Bragança, Portugal
Adam Tofilski, Department of Zoology and Animal Welfare, University of Agriculture in Krakow, Krakow, Poland
Elen, Dylan, School of Natural Sciences, Bangor University, Bangor, United Kingdom
McCormack, Grace Patricia, Department of Zoology, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland
Henriques, Dora, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal

DeepWings© is a software that uses Machine Learning for fully automated identification of *Apis mellifera* subspecies based on wing geometric morphometrics (WGM). Here, we examined the performance of DeepWings© under realistic conditions by processing 14,782 wing images with varying quality and produced by different operators. These images represented 2,593 colonies covering the native ranges of *A. m. iberiensis* (Portugal, Spain and historical introduction in the Azores), *A. m. mellifera* (Belgium, France, Ireland, Poland, Russia, Sweden, Switzerland, UK) and *A. m. carnica* (Croatia, Hungary, Romania). The classification probability obtained for the colonies was contrasted with the endemic subspecies distribution. Additionally, the association between WGM classification and that inferred from microsatellites and SNPs was evaluated for 1,214 colonies. As much as 94.4% of the wings were accepted and classified by DeepWings©. In the Iberian honey bee native range, 92,6% of the colonies were classified as A. m. iberiensis with a median probability of 91.88 (IQR = 22.52). In the Azores, 85.7% of colonies were classified as *A. m. iberiensis*, with a median probability of 84.16 (32.40). In the Dark honey bee native range, 41.1 % of the colonies were classified as *A. m mellifera* with a median probability of 99.36 (8.02). The low percentage of colonies matching the native subspecies was mainly due to the low values registered in Avignon (20.0%), Poland (32.9%), and Wales (41.2%). In contrast, most of the colonies analyzed in other locations of the native range of *A. m. mellifera* matched this subspecies: Belgium (100.0%), Groix (63.9%), Ouessant (72.7%), Ireland (78.0%), Russia (96.2%), Sweden (84.2%) and Switzerland (55.6%). In the colonies from Croatia, Hungary, and Romania, 88.0% of the samples were classified as *A. m. carnica*, with a median probability of 98.49 (6.76). The association between WGM and molecular data was highly significant but not very strong (Spearman $r = 0.31$, $p < 0.0001$). A good agreement between morphological and molecular methods was registered in samples originating from highly conserved M-lineage populations whereas in populations with historical records of foreign queen importations the agreement was weaker. In general, DeepWings© showed good performance when tested under realistic conditions. It is a valuable tool that can be used not only for honey bee breeding and conservation but also for research purposes.

Keywords: Wing Geometric Morphometrics, *Apis mellifera* subspecies classification, honey bee conservation

SEMI-NATURAL HABITATS PROMOTE WINTER SURVIVAL OF WILD-LIVING HONEYBEES IN AN AGRICULTURAL LANDSCAPE

Rutschmann, Benjamin*, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
Kohl, Patrick L.*, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
Machado, Alejandro, Asociación Ridimoas, Ourense, Spain
Steffan-Dewenter, Ingolf, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany

* these authors contributed equally

The diversity of endemic honeybee subspecies and ecotypes is at risk in Europe because modern apiculture promotes only a small number of honeybee strains. A crucial step for the conservation of honeybee diversity is the assessment of the status of remaining wild populations and their limiting factors. Here we present a two-year census of native, wild-living honeybees inhabiting power poles in an intensive agricultural landscape in Galicia, NW Spain. The autumn colony densities were at least 0.22 and 0.17 colonies/km^2 and winter survival rates were 59 % and 26 % for the years 2019 (N = 29) and 2020 (N = 23), respectively. Both the initial occurrence and the subsequent winter survival of the colonies were positively correlated with increasing proportions of wood- and shrubland in the surroundings in both study years. These observations highlight the importance of semi-natural habitats for the conservation of wild-living honeybees.

Keywords: wild-living honeybees, power poles as nest sites, semi-natural habitats

MONITORING BEES

POSTERS

PROTECTING BEEHIVES FROM OTHER INSECTS WITH COMPUTERVISION & DEEP LEARNING

De Clercq Jean-Jacques, Dept. Research Cluster Big Data Analytics, Ghent University, Ghent, Belgium

Where actual methods for protecting bees from all kind of enemies use biochemical products (AFTER-THE-FACT or just-to-late approach), we have opted for a mechanical solution (PREVENTIVE or just-in-time) by using the most modern techniques available. Computer Vision and Deep Learning allow to protect the beehive and obtain a BIO-label for the honey collection. Time has come to change completely our way of thinking and look for more ecological and less detrimental solutions (misuse or overuse of formic-oxalic acid, amitraz, coumaphos and derivatives creating drug resistance).

Our method is based on a 16 channel 8x8mm acrylic passageway with an high-res camera on top that detects the difference between bees and all other unwanted insects (wild bees, hornets, beetles, varroa mites etc.) and captures the non-bees in the passageway by closing the front/back of the channel with electronic shutters used in digital cameras. At the same time the beekeeper is warned with an SMS-message of the channel# the unwanted insect. Trapped insects are freed by manually inverting the polarity of the shutters. The camera is connected to a Raspeberry3B+, fed with a battery and solar panel, which works with a trained mobilenet program that detects bees and non-bees. The training happened on a DELL cluster with 8 servers running on mesosphere using 12.000+ images and resulting after 83 training/validation runs (horizontal flipping & random rotation) into an hitrate of 94%. We used a pretrained model from Google who visually differentiates between cats & dogs, adapting the model with transfer learning & Early Stopping to bees/non-bees so it would become futureproof for all other upcoming enemies. Two software pre-filters were used for insect length and color. Labeling the photos for supervised learning resulted in a lot of human labor done by students. Software used: Tensorflow (Google), Keras framework (Google), Mobilenet V2 (Google) + Intel Neural Computing Stick with OpenVino library connected to the RPI3B+ for performance reasons. Finally, Tesseract OCR-software was used to detect the printed channel entrance numbers 1-16. Accuracy and Cross Entropy graphs show that both Training and Validation curves nearly overlap each other. Accuracy is close to 95% and Cross Entropy hits the 0.2 border.

Keywords: beehive security, beehive health, beehive monitoring

BEEHIVE ACOUSTIC MONITORING: CHALLENGES AND OPPORTUNITIES

Abdollahi, Mahsa, INRS-EMT, University of Quebec, Montréal, Canada
Henry, Evan, Nectar Technologies Inc, Montréal, Canada
Giovenazzo, Pierre, Biology Department, Laval University, Quebec, Canada
Falk, Tiago H., INRS-EMT, University of Quebec, Montréal, Canada

To ensure the health and wellbeing of the bees, hives have been traditionally monitored manually, a process that can be labor intensive and time-consuming for the beekeepers and disruptive for the bees. Moreover, the increased honeybee mortality rates seen worldwide over the last decade have called for more continuous and automated colony monitoring system. To this end, recent advances in sensing hardware have enabled the development of instrumented hives equipped with e.g., temperature, humidity, and audio monitoring sensors. These data, when coupled with machine learning algorithms, have enabled the development of automated bee monitoring tools, such as queen bee detection, swarming prediction, or even hive opening alarm systems. As bees are known to communicate within the colony via vibration and specific sounds, acoustic monitoring of hives can play a crucial role in automated beehive monitoring. Acoustic hive monitoring, however, can be extremely challenging, as audio signals can be corrupted by different environmental factors (e.g., rain), thus compromising system accuracy.

In this study, our goal was two-fold: (1) search the literature on beehive acoustic monitoring and implement several methods described in the literature, and (2) collect audio data from multiple hives over a period of three months to describe the challenges seen with acoustic beehive monitoring, validate the performance of existing systems, and further improve accuracy with noise-robust signal processing. More specifically, we instrumented 10 hives (one was left empty for reference) with microphones, as well as internal temperature and humidity sensors. Data was recorded over the months of August, September and October, 2021 in Montréal, Québec, Canada. Here, we report on the existing systems and their sensitivity to environmental factors, such as rain, nearby train whistling noises, as well as beekeepers talking near the hives. To build automated tools that are robust to such factors, we report preliminary results with a new signal processing tool called the modulation spectrogram, which measures the temporal dynamics of the beehive sound spectra. It was observed that signals of interest and noise become more separable in this representation, thus opening doors for more reliable beehive monitoring solutions.

Keywords: Honeybee, Bee acoustics, Beehive monitoring

IN-HIVE DATA FROM BEENET: CATEGORIES OF DATA OBTAINED FROM THE MONITORED APIARIES

Albertazzi, Sergio, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Caringi, Valeria, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Dettori, Amanda, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bogo, Gherardo, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Medrzycki, Piotr, CREA Research Centre for Agriculture and Environment, Bologna, Italy

In the context of the project “BeeNet – monitoring the environment through bees and biodiversity”, a large number of parameters are collected from field stations. A BeeNet apiary counts 5 stationary colonies identified by unique codes reported in a label applied on the hive. The beekeeper manages the hives focusing on the honey production (not swarm, royal jelly or pollen production) to have the most possible homogeneous “management stress”. Forager bees are sampled four times a year (March, June, September, and November) from the first three hives of each monitoring station to investigate pathogens for which a notification to the public Health Authorities is not compulsory. Beebread is collected as pool of all the 5 hives during the spring samplings (March and June) and destined to multi-residual analyses and the assessment of protein content. Technicians operate a visual colony assessment to estimate adult bee and brood quantity at every control for each hive. Continuously, beekeepers report to the project coordination each operation inside hive and unpredicted event which might influence individual colonies or entire apiary. Moreover, one third of BeeNet apiaries are provided with high-tech instruments, including automatic balance, multiple internal thermal sensor (set among frames) and the sensors of external temperature and light spectrum. These are installed on the same hives where pathogen analysis is carried out. All the data are recorded every 15 minutes and shared in cloud with researchers and the beekeeper twice a day. Also, the results of pathogen and chemical analyses of bees and beebread respectively are shared with the apiary’s owner.

We present, as an example, the entire data set produced in the first year of the project by a BeeNet apiary randomly chosen among those in the Emilia-Romagna region (central Italy). A discussion about the usefulness of the collected data and the way to elaborate it in the framework of the entire project is presented.

OUT-HIVE DATA FROM BEENET: CARTOGRAPHIC ANALYSES OF APIARY SURROUNDINGS AS AN EXAMPLE OF AGRICULTURAL LANDSCAPE DESCRIPTION

Albertazzi, Sergio, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Guerra, Irene, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Dettori, Amanda, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Giovanetti, Manuela, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Medrzycki, Piotr, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Cartographic analysis is a powerful tool to evaluate landscape, that can be applied also in bee studies. Recently, satellite well-defined images and land cover cartography (Corine Land Cover Project – CLC- for Europe) are upgraded with higher frequency compared to the past decades. This reduces gaps between available land description and actual in-field situations.

However, the level of interaction between bees and landscape is so punctual, specific and variable over time, that a detailed knowledge about each patch of landscape is often necessary. The best landscape description would include all botanical species and crop details. To our knowledge, similar maps do not exist, and the cartographic analysis must accept some simplification. When maps report botanical species (or their aggregation in specific habitats), usually they describe only a specific portion of the landscape (etc. protected areas or semi-natural zones). From the agricultural viewpoint, the problem of inaccurate land description increases in case of plots identified by field management and not by crop species. The category “arable land” or “mixed agricultural areas” are the most problematic examples of this lack of precise information. Indeed, the list of crops which can appear in these areas, concerns species completely different for bee’s interaction as alfalfa, wheat, maize.

Not only the legend, but also the map accuracy could be a challenge: CLC often simplifies landscape in wide homogeneous areas where only the most relevant crop/habitat is reported. Local maps can partially improve the accuracy level, but they are not managed under a unique administrative framework. They are usually available only for some regions and updated at different time schedule.

As an example, we present and describe the several cartographic sources used to describe the surroundings of one of the 370 BeeNet monitoring stations.

HONEYBEE SUBSPECIES MANAGED BY POLISH BEEKEEPERS- A 40 YEAR LONG STUDY SHOWS DECREASING POPULARITY OF *APIS MELLIFERA MELLIFERA*

Bieńkowska Małgorzata, Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland
Splitt Aleksandra, Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland
Węgrzynowicz Paweł, Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland

In some regions of Poland, particular honeybee subspecies are considered native, i.e. *Apis mellifera mellifera* (“dark bee”) in the north-east and *A. mellifera carnica* in the Island Beskids. Additionally, imported *A. mellifera caucasica* and ‘Buckfast bee’, *A. mellifera* are reported across Poland. The popularity of pure breed of the “dark bee” started declining with the appearance of imported subspecies and was placed under special legislation as early as in the 1970s. A survey was conducted annually from 1980 to 2018. The long-term survey was designed to provide information on which honeybee subspecies are kept by the Polish beekeepers. Our analysis revealed that throughout the four decades, there has been a rise in the beekeepers’ awareness as to which subspecies/types of honeybees they manage. Until 2000, the Polish beekeepers maintained up to four different subspecies per apiary, whereas in the last 15 years a shift to more homogenous share with the strong preference towards *A. mellifera carnica* has been observed. The popularity of indigenous *A. mellifera mellifera* has drastically declined over time: its presence dropped from about 40% to below 10% of apiaries. The management efforts towards conservation of the European “dark bee” seems to be ineffective and new solutions should be considered to sufficiently restore this native subspecies in Poland.

Keywords: apiary management, beekeeping, dark bee

POTENTIAL ECONOMIC BENEFITS DERIVING FROM INTRODUCING AROMATIC PLANTS IN MIXED BEEKEEPING-CITRUS FARMING SYSTEMS IN SICILY

Severini Simone1, Biagini Luigi[1], Dipasquale Daniele[1], Danieli Pier Paolo[1]

1 Depart. of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy

Adequate pollen nutrition is one of the key conditions for honeybee management. Therefore, it is crucial to provide an adequate amount of pollen as well as to enlarge the period in which the bees can find it. This is particularly the case when only a few pollen sources are available, as in other Mediterranean countries. Within the frame of the PLANT-B project, a European project granted by PRIMA Foundation (grant n. 1812), the analysis assesses the potential economic implications of implementing strategies to ameliorate the availability of pollen for the bees by introducing aromatic plants in mixed beekeeping-citrus farming systems. The research consists of a protocol of introduction applied on five citrus farms that relate to as much as beekeeping farms. After this, a questionnaire was submitted to 10 stakeholders belonging to the categories of local beekeepers, farmers, traders, and honey processors. The questionnaire asked questions related to the potential implications of introducing aromatic plants on the level of the price of honey, honey value chain economic performance, marketing strategy, and penetration of honey in foreign markets. Results suggest that the proposed approach could increase honey prices up to 25% of the current level. Because of this, the strategy is expected in most cases to improve the economic performance of the value chain. Furthermore, this can be a useful tool to enhance the marketing strategies by mentioning the innovative and eco-friendly nature of the PLANT-B strategy. Finally, results show limited optimism regarding the possibility of increasing the penetration of honey in foreign markets.

Keywords: Mixed farming-beekeeping systems, Aromatic plants, Economic assessment

EXTRAORDINARY ABILITY OF BEES TO REMOVE HEAVY METALS FROM NECTAR AND EXCRETE THE CONTAMINANTS FROM THEIR BODIES

Borsuk Grzegorz[1], Sulborska Aneta[2], Stawiarz Ernest[2], Olszewski Krzysztof[1], Wiącek Dariusz[3], Ramzi Noor[4], Nawrocka Agnieszka[3], Jędryczka Małgorzata[4]

1 Institute of Biological Basis of Animal Production; Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950, Lublin, Poland
2 Department of Botany, University of Life Sciences, Akademicka 15, 20-950, Lublin, Poland
3 Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland,
4 Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland

We examined the residues of 13 elements in soil, plant parts, nectar, bee heads, thorax, and abdomens, feces from bee guts, and bee products sampled from two Polish cities (Lublin and Poznań). Our findings indicated that bees have an extraordinary ability to remove metals from nectar when converting nectar into honey. Compared to nectar, honey contained 40-fold lower Fe, 26-fold lower Zn, and 8-fold lower Cu and Cd levels, indicating removal of these elements via nectar processing, during which water is evaporated and complex sugars are decomposed into simple ones. The amount of Pb remained unchanged; however, it can also be regarded as a 4-fold decrease due to water evaporation from honey, compared to nectar. Some portion of the ingested Fe, Cu, and Zn was used by bees, and the excess amounts were excreted in feces. All analyzed elements were present as biocomplexes transported from the alimentary tract through the abdomen to the thorax and head. Elements transferred in the alimentary tract were partially immobilized/metabolized in the bee fat body, and their residues were excreted with feces from the gut. We postulate that honey is not a good indicator of environmental pollution, as a high amount of elements is removed by bees from their bodies.

Keywords: heavy metals, contamination products, urban beekeeping

WHICH GLUE IS THE BEST FOR BEE TAGGING? LABORATORY TEST ON *OSMIA BICORNIS*

Splitt, Aleksandra, (1) Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland, (2) Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland
Borański, Mikołaj, Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland
Jachuła, Jacek, Apiculture Division in Puławy, The National Institute of Horticultural Research, Skierniewice, Poland

We conducted a test on the solitary, cavity-nesting bee *Osmia bicornis* (syn. *O. rufa*) aiming to determine the impact of the glue used for applying radio frequency identification (RFID) tags on this bee species. The experimental set up evaluated the effects on bees' longevity and fitness in the groups tagged with:

- a (CA) synthetic cyanoacrylic glue,
- a (N) nitro paint – where the nitrocellulose resin was a basic binder,
- a (S) shellac (E 904) - a type of natural resin - glue made of 50% organic shellac and 50% grain alcohol,
- and the (C) control group (without tagging).

In each group, 20 females and 20 males of *O. bicornis* were employed. The RFID tags (Hitachi Chemical Co. Ltd 2016; size of 2.5 × 2.5 × 0.4 mm, 5.4 mg in weight) were placed on bee's thorax, between the wings and glued with a drop of a chosen adhesive. Each group of tested females and males was kept in separate 1m3 cages. The bees had constant access to food in the form of sugar syrup (33% w/v) refilled on a daily basis. Each cage was inspected daily and the amount of taken syrup, along with the number of dead bees, were noted. The longevity part of the experiment lasted until the natural death of bees. To study the effects of glues on bees' fitness (fat body and flight muscle mass), 5 healthy bees from each group were captured and placed in Eppendorf type tubes after 7, 14 and 21 days of the study. The results of this study suggest that males are more sensitive and susceptible to the negative effects of glue. After 25 days, 90% of the females in the (C) group were alive, almost 70% in the (S) group and 60% in the (N) group, whereas just under 17% in the (CA) group (which performed the worst). This was a preliminary test to a field study with usage of RFID tags for *O. bicornis* monitoring.

This study was possible through implementation of the research project No. 2019/33/N/NZ8/02864 financed by the National Science Centre, Poland.

Keywords: *Osmia rufa*, cage study, longevity

ADAPTING MONITORING METHODS TO ENLARGE STAKEHOLDERS' PARTICIPATION IN POLLINATOR PROTECTION INITIATIVES

Bortolotti, Laura; Bogo, Gherardo; Giovanetti, Manuela; Zenga, Emanuele Luigi, d'Agostino, Marco; CREA Research Centre for Agriculture and Environment, Bologna, Italy
Traveset, Anna; Beltran, Rafel; Mediterranean Institute of Advanced Studies (CSIC-UIB), Mallorca, Balearic Islands
Petanidou, Theodora; Department of Geography, University of the Aegean, Mytilene, Greece
Navarro, Luis, University of Vigo, Vigo, Spain
Dante, Giovanna; Bitonto, Fortunato Fulvio; Galloni, Marta; Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy

Pollinators are very important due to their role in the maintenance of environmental biodiversity and crop production. However, they are often neglected by the general public and the diverse stakeholders operating in environmental and agricultural sectors. Particularly in Mediterranean countries, scientific knowledge at the species level is often inadequate regarding the risks pollinators face due to environmental changes, intensive agriculture, and pollution. Therefore, assessing their conservation status and the possible further decline of their populations involving the whole of society is crucial. The transnational project "LIFE 4 Pollinators - *Involving people to protect wild bees and other pollinators in the Mediterranean*" (LIFE18/GIE/IT000755) aims to improve the knowledge and conservation of pollinators and entomophilous plants across the Mediterranean, leading to a progressive change in behavior and practices of different stakeholder categories. To achieve this goal, activities have been set up at three levels: scientific monitoring (SM), simplified scientific monitoring (SSM), and citizen science monitoring (CS). At all levels, recording pollinators and their interactions with flowering plants will be conducted. However, they are designed to match different expertise of people and the final aims. SM is carried out by experts once a month at five farms in Emilia-Romagna region (Italy), focusing on bee collection and identification at species level. SSM is carried out at 12 sites in Italy and one site in each target area (Galicia, Balearic Islands, and Lesvos); pollinator insects are identified on the wing as morpho-groups, except bees that are identified to genus. Finally, CS involves the general public and schools and considers all pollinators at family or order level, recorded by photos (no insects collected); the general public can participate by submitting pollinator pictures through a dedicated webtool in the project website. We will describe in detail the adapted monitoring protocols, evaluate the acquired data and their use. Moreover, we will explain how the project may contribute to: i) improving basic knowledge of native Mediterranean wild pollinators and entomophilous plants, ii) encouraging behavior change towards pollinators, iii) sustaining environmental governance by transferring data to concerned authorities, and iv) favoring availability and sharing of knowledge across the Mediterranean area.

Keywords: LIFE 4 POLLINATORS project, pollinator monitoring, citizen science

TRANSFORMING THE FUTURE OF WORK FOR BEEKEEPERS USING DATA SCIENCE

Fisher-Maltese, PhD, Carley (PI), College of Education and Human Development, George Mason University, Fairfax, USA
Gring-Pemble, PhD, Lisa (CoPI), School of Business, George Mason University, Fairfax, USA
Bouga, PhD, Maria, Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, Athens, Greece
Zhang, Xiaolu (GRA), College of Education and Human Development, George Mason University, Fairfax, USA

The world faces an emerging and worsening crisis around pollinators. Due to colony collapse disorder, invasive mites (*Varroa destructor*), use of pesticides, and the rise of monoculture farming, honey bees are dying at unprecedented rates. The beekeeping workforce is facing a crisis. Traditional approaches to beekeeping have been unable to stem the tide of colony collapse. The results are devastating in terms of food security since bees pollinate one in every three bites of food that humans consume. The decline in pollinators also threatens economic stability. U.S. honey production since 1987 has declined 78 million pounds and costs of crop pollination have increased dramatically. The future of work for beekeeping will involve mastering research-driven practices that respond to bee health in different landscapes, and that deploy sensors to monitor beehive health and behavior. This project involves a multidisciplinary approach to upskill and transform beekeeping, with special attention to the education pipeline, which is necessary to prepare students for transformed careers. This project is a joint effort between the George Mason University (GMU) College of Education and Human Development (CEHD), the GMU School of Business (which houses the George Mason University Honey Bee Initiative), and experts on beekeeping and data science. Technically, the project (a) deploys sensors in hives to generate data colony health as monitored by measurement, such as temperature, acoustic activity, weight, vibration, CO2, humidity, and solar radiation; (b) and develops a cloud computing capacity to integrate the hive sensor data with field data (e.g., on phenology) to scientifically describe pollinator and ecosystem health.

Keywords: Beekeeping, Data Science, Sensor-enabled Hives, Education

MONITORING COLONY HEALTH WITH A GRID OF TEMPERATURE SENSORS

Linton, Frank, colonymonitoring.com, Chevy Chase, United States

We monitored the health and activities of a honey bee colony remotely, based on data from an unobtrusive grid of temperature sensors. The grid, consisting of four rows of nine sensors, was placed over either the brood box or the honey super of an 8-frame Langstroth hive. Data was collected approximately every half hour for more than a year, starting in November of 2019 and continuing through December of 2020.

Regarding the winter cluster, we found first, that the size of the cluster varied, filling the hive when the exterior temperatures were 10C or above, and contracting to 20% of the hive volume when the temperatures were 0C or below. We found that temperatures above the center of the winter cluster increased from >10C to >20C as spring approached. The highest temperature at any one moment indicated the center of the cluster, while the overall temperature gradually increased as the cluster rose in the honey super. Meanwhile, the declining variance indicated the increasing presence of brood. Observing the location, size, and movement of the winter cluster and the development of brood gave us confidence that the colony was overwintering well.

We then placed the sensor grid atop the brood box (under the honey super). The temperatures reported in this location were about 35C, with little variance, wherever brood was present. We observed significant declines and expansions in brood volume as the colony was split twice and swarmed three times. We also noted the effect on brood volume of a Varroa mite treatment.

The hypothesis that we could detect precisely where the queen was laying was not supported, possibly because the sensors were located too far from the queen. The hypothesis that we could detect robbing was also not supported.

To our knowledge, no other colony monitoring technology system currently in use can monitor these colony characteristics so closely. With devices such as these, we expect to enable beekeepers to intervene early to address issues, while avoiding unnecessary inspections.

Keywords: temperature sensor grid, colony health, brood volume

THE BEENET PROJECT: PIVOTAL MONITORING OF BEES IN ITALIAN AGRO-ECOSYSTEMS

Giovanetti, Manuela; Albertazzi, Sergio; Bogo, Gherardo; Capano, Vittorio; Cargnus, Elena; Caringi, Valeria; Carpana, Emanuele; Cilia, Giovanni; Dettori, Amanda; Flaminio, Simone; Guerra, Irene; Medrzycki, Piotr; Nanetti, Antonio; Quaranta, Marino; Ranalli, Rosa; Tafi, Elena; Zavatta, Laura; Bortolotti, Laura
CREA Research Centre for Agriculture and Environment, Bologna, Italy

In Italy we are carrying out an outstanding project, which adopts honey- and wild bees as sentinels of the agro-ecosystem, through a network of fixed monitoring stations distributed throughout the country. The mind map of the project includes the conceptual frameworks, designed around each of the two central elements: the honeybee and the wild bees. Few elements may coincide, but the two differ substantially: the honeybees are a managed species, on which a large literature exists, the commitment of beekeepers is often driving priorities; the wild bees include common and rare species, mostly lacking literature on their biology and distribution. More differences can be identified in the type of samples and sampling protocols, sample transfer to laboratories, returning results to stakeholders. However, encompassing everything is the landscape scale: a national monitoring implies to be inclusive of bureaucratical fragmentation; a bee monitoring implies the interpretation of cartographic information at adequate levels of resolution. The employment of Corine Land Cover inventory allows a national landscape perspective, and foster replicability in other countries. However, both the complexity of farmlands and bee flight ranges require to include landscape analyses based on local inventories of soil use and vegetation-based descriptors. The BeeNet operational structure is the large network in the backstage, managing the monitoring that will deliver data. BeeNet accomplished the integration of 371 sites with 1855 honeybee colonies (in all Italian regions) and 26 fixed transects for the wild bees (in 11 regions). Honeybee monitoring provides data on health and colony growth, quality of food sources. Moreover, with the help of high-tech hives, provides data on inside/outside environmental parameters. Wild bees monitoring, by identifying specimens at species level, will provide data on diversity, distribution and feeding preferences of a large portion of the almost 1000 species. Moreover, by evaluating flora diversity at farmlands it will compare opposed agricultural practices (intensive vs. semi-natural). BeeNet ensures a constant commitment on two sides: on the one hand, the management of a network to secure a scientific data gathering; on the other hand, a constant engagement with various stakeholders as beekeepers, natural park managers, farmers and policy makers.

Keywords: honeybees, wild bees, BeeNet network

MONITORING OF DIVERSITY AND ABUNDANCE OF WILD BEES, BUMBLEBEES AND HONEY BEES (*APIS MELLIFERA* L.) IN NORTHERN SERBIA WITHIN NATIONAL PROJECT SERBIAN POLLINATOR ADVICE STRATEGY

Mudri-Stojnić, Sonja, Milić, Dubravka, Tot, Tamara, Radenković, Snežana, Department of Biology and Ecology, Faculty of sciences, Novi Sad, Serbia

Importance of pollinating insects, mainly bees, as well as their role in pollination of crops and wild plants, is widely known. Honey bee (*Apis mellifera* L.) which is treated as the best and most universal pollinator, is facing many negative impacts and decreasing numbers of their colonies during past years. The often neglected fact is that besides honey bee there are other species of insect pollinators in agricultural and natural habitats, among which most important are wild bees and bumblebees. Their contribution to pollination is not properly valuated mainly due to limited insight in their ecology. Honeybees need human management, i.e. beekeepers to survive, while native bees are inhabiting nature and persisting without external help.

For wild bee and bumblebee diversity, conservation of grassland and forest habitats, as nesting and foraging places, is of great importance, especially in areas with high percentage of agricultural monocultures. This paper presents diversity and abundance of three groups of insect pollinators: wild bees, bumblebees and honey bees (*Apis mellifera* L.) on selected localities of grassland and forest habitats of Northern Serbia, highly agricultural province of Vojvodina. Monitoring was conducted during spring and summer seasons in 2022 by transect walks and pan traps. Each of localities shows different diversity and abundance of three analyzed groups of insect pollinators. Also, differences in ratio of honey bees within the total number of pollinators is registered among localities. Diversity and abundance of wild bees, bumblebees and honey bees are shown in relation to different land use practices, presence or absence of mowing and/or grazing on investigated localities. Pan traps were generally less efficient in monitoring compared to transect walks, but in rare cases detected species neglected by transect walks. Besides forest habitats, fragmented grassland patches are recognized as rich in diversity of wild bees and bumblebees, thus very important for their conservation in Serbia.

Keywords: Bees, Richness, Land use

A MOBILE PHONE APPLICATION TO SURVEY AND MONITOR THE WILD COLONIES OF *APIS MELLIFERA*

Fontana Paolo, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Daniele Andreis, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy
Malagnini Valeria, Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige (TN), Italy

Although *Apis mellifera* have been reared by humans for millennia, it remains a wild animal as reported by ancient authors and modern beekeepers and researchers. Until a few decades ago, wild colonies of *Apis mellifera* were largely common. However, since the early 1980s there has been a rapid and underestimated rarefaction of the “wild” colonies due to a parasite, the fearsome *Varroa destructor* mite. The effect of the Varroa mite on unmanaged colonies was so strong that today in Europe most of the survived honey bees live in hives managed by beekeepers. Indeed, for many years it has even been thought that in Europe wild honey bees were disappeared. Rather surprisingly, there are no scientific studies on this phenomenon and the only available information deals with the number and distribution of honey bees colonies owned by beekeepers. In recent years, reports and interest in wild honey bee colonies have increased significantly and today we can say that even in many areas of Europe these colonies are still there. The app BeeWild intends to survey and monitor these wild colonies through a typical citizen science action.

Keywords: Wild honey bees, BeeWild, mobile app

MODELING BEE POPULATION DYNAMICS USING DYNAMICAL SYSTEMS METHODS AND CONTEXT-AWARE MACHINE LEARNING TOOLS

Mengara, Orson, INRS-EMT, University of Québec, Montréal, Canada
Falk, Tiago, INRS-EMT, University of Québec, Montréal, Canada
Giovenazzo, Pierre, Biology Department, Laval University, Québec, Canada
Henry, Evan, Nectar Technologies Inc, Montréal, Canada

Over the last few years, beehive instrumentation with Internet of Things (IoT) sensors has emerged as a powerful tool for automated bee monitoring. Sensors, such as internal hive temperatures, humidity levels, and acoustics have been proposed for e.g., automated queen bee detection, swarming detection, and bee presence detection, to name a few. In this study, we report a multi-year data collection in which 50 honeybee colonies were monitored over two summer seasons (summers 2020/2021) and two winterization periods (winters 2021/2022) using internal and external hive sensors, including internal temperature, internal humidity, 15 internal audio power and frequency band parameters, as well as external (environment) temperature, humidity, wind speeds and direction, rain amounts, and sun intensity. Hives were monitored 24/7 in two apiaries near Deschambault, Québec, Canada, and were inspected every two weeks (during the summer) to monitor the number of frames of bees, presence of queen bee, brood population, amount of honey produced, as well as Varroa destructor infestation levels. Moreover, during the winter months, beehives were stored in an environmentally controlled chamber and their survival rate, honey consumption, and hive strength were monitored in the subsequent spring season. With this data, we report on dynamical systems models and machine learning models, such as deep neural networks and Gaussian mixture models, trained to predict several measures of interest. From the summer data, for example, we predict (1) the strength of a hive at any point in time, given by an estimate of the number of full frames of bees, (2) the amount of honey produced at any point in time, and (3) the varroa infestation levels in spring and fall. Next, by comparing models trained on the summer and winter datasets, we report preliminary results on the prediction of honey consumption during the winter months, as well as colony mortality. It is hoped that these findings will provide beekeepers with new tools to better monitor their bees in a more automated and reliable manner.

Keywords: beehive monitoring, machine learning, dynamical systems

LONG-TERM IOT-ENABLED TEMPERATURE MONITORING WITHIN A BEEHIVE

Farrel, Franklin, Mechanical Engineering, GMU, Fairfax, USA
Gring-Pemble, Lisa, School of Business, GMU, Fairfax, USA
Fisher-Maltese, Carley, College of Education and Human Development, GMU, Fairfax, USA
Lofaro, Daniel, Electrical Engineering, GMU, Fairfax, USA
Peixoto, Nathalia, Electrical Engineering, GMU, Fairfax, USA

In order to track and predict the health of beehives there have been several sensor systems suggested and deployed in the last decade. Most sensor systems target one modality (either temperature, weight, or light) and have a single point (one sensor) that is used per hive. In order to advance the field and to provide a more consistent and detailed profile of variation within a hive throughout the different seasons of the year, we have designed and tested a long-term, multisensor modality solution that can be easily integrated in any apiary.

We propose an "internet-of-things"-enabled sensor array powered by solar panels. The array includes carbon dioxide sensors, temperature sensors, accelerometers, and force sensors (for weight). Our design includes Raspberry-Pi boards and can transmit real-time data over a wireless network. In this paper we present results on a four-month long experiment spanning winter and spring, with recordings from six temperature sensors that connect through a serial port, I2C. The digital sensors are MCP9808, by Microchip, and have accuracy of 0.5 degrees C. We mounted them on custom-designed two-layer printed circuit boards. The three top sensors are 8 cm apart (center-to-center distance) and the lower sensors are 10 cm below the top 3. Our deployed system logs data approximately every 6 continuously in ASCII-formatted files. We then make the recorded data available for 24 hour increments . The recorded data are time stamped accordingly and can be easily manipulated to plot six profiles for each day.

We have deployed one sensor array and tracked temperature variations as described above from January, 2022 through June, 2022. By plotting graphs from our recorded data we are easily able to show bees thermally managing their hive. For example in the January and February there is up to a 20 degree celsius difference between sensors.

In conclusion, we have designed and deployed a sensor array that provides temperature profiles inside the beehive uninterruptedly for months. We intend to expand this work to include multiple hives and other sensors, in order to more fully investigate the dynamics of bees throughout the year.

LOOKING FOR BIOMARKERS OF HEALTH STATUS IN THE HEMOLYMPH OF *APIS MELLIFERA*

Rudelli, Cecilia, Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
Cabbri, Riccardo, Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
Bellei, Elisa, Department of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia
Andreani, Giulia, Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
Isani, Gloria, Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

The demographic decline of honeybees (*Apis mellifera*) determines negative effects not only for agriculture and apiculture, but also for the ecosystems.

For this reason, it is mandatory to understand the determinants causing the decline and to establish objective criteria to evaluate their health status. Proteins in the hemolymph may represent a source of valuables biomarkers to assess the health status of the hive; however, they are still poorly investigated.

The aim of this study is to separate, to identify the most abundant proteins in the hemolymph, and to identify biomarkers of health status that could help beekeepers and veterinarians in the management and treatment of the hives.

Four apiaries were chosen for this research, characterized by different management and location (A= hill, B and D= plain, C= apiary subjected to nomadism). In each apiary, three hives were selected and bees were collected in June, July, August, September, and October. Then two µL of hemolymph were withdrawn from each bee and the total proteins were determined by the Bradford method. The proteins of the hemolymph were separated using the SDS-PAGE electrophoresis and identified using mass spectrometry. Colony-level traits (areas covered with bees, brood, sealed brood and pollen) were also estimated.

In the hemolymph of honeybees, 20 proteins were identified and among these, five proteins showed relevant variations. These are vitellogenin, apolipophorin, hexamerin, transferrin and prophenoloxidase. In particular, in October, the apiary C presented the worst general conditions and a lower population associated with lower concentrations of total proteins, apolipoforin and vitellogenin. The apiary D, on the contrary, had the best trophic and health conditions, associated with higher concentrations of all the aforementioned five proteins.

Thereby, it is possible to hypothesize that these proteins may be valuables biomarkers of health status, and it is worthy to continue their study.

Keywords: Honeybee, Health status, Proteomics

WILD BEE DATA FROM BEENET: MONITORING BEES IN ITALIAN AGRO-ECOSYSTEMS

Ranalli, Rosa, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Giovanetti, Manuela, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Flaminio, Simone, CREA Research Centre for Agriculture and Environment, Bologna, Italy – Laboratory of Zoology, University of Mons, Mons, Belgium
Zavatta, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Bortolotti, Laura, CREA Research Centre for Agriculture and Environment, Bologna, Italy
Quaranta, Marino, CREA Research Centre for Agriculture and Environment, Bologna, Italy

Wild bees have become increasingly popular, and other wild pollinators are topics of various projects and monitoring plans at the European level. Mediterranean area represents a biodiversity hotspot, and Italy includes more than 1100 wild bee species, however, basic information on the distribution and abundance of species is often lacking. "BeeNet – monitoring the environment through bees and biodiversity" (2019-2023) is a national monitoring project providing data on wild bees and entomophilous plants in Italian agro-ecosystems. The monitoring is carried out in 11 Italian regions, in which two sampling sites have been defined: one in an intensive agro-ecosystem and one in a semi-natural agro-ecosystem within a protected area. In each sampling site, activities take place monthly, walking a fixed transect of 200x2m, and collecting all wild bees present on the blooms using the sweep-net method. Each specimen is recorded noting the plant species on which it was captured, and at the same time is carried out a botanical report of all entomophilous species in anthesis. During the first year of sampling (2021), 4135 specimens were captured, including about 330 different species of wild bees. Among these, the most represented genera are *Andrena* (69 spp.) and *Lasioglossum* (49 spp.). It is interesting to note the presence of kleptoparasitic genera among which the most represented are: *Nomada* (19 spp.) and *Sphecodes* (12 spp.): data supporting greater ecosystem stability. The botanical species identified within the transects differ in species composition and especially in abundance between intensive and semi-natural agro-ecosystems, with higher biodiversity in southern Italian regions and islands. These results contribute to increase the knowledge about wild bees in Italy, especially about their abundance, distribution and interaction with plants

Keywords: fixed transects, taxonomic identification, botanical reports

ARTIFICIAL INTELLIGENCE TO COUNT AND DISCRIMINATE HONEY BEE ACTIVITY AT THE HIVE ENTRANCE

Soledad Sagastume, CIAPA-IRIAF, Marchamalo, Spain
Mariano Higes, CIAPA-IRIAF, Marchamalo, Spain
Raquel Martín-Hernández, CIAPA-IRIAF, Marchamalo, Spain

As a part of the European MEDIBEES project, the study of the behaviour of foraging bees under different levels of environmental stress is one of the most ambitious challenges, as it requires field trials to determine their activity and to be able to compare the data obtained in different Mediterranean countries in a reliable way. The most commonly used method up to now has been to manually count bees entering and leaving the hive during a certain time. Ideally, several measurements should be made at different times of the day during the whole period of beekeeping activity. However, this method is very time-consuming and therefore the number of measurements is often reduced, which can lead to inaccurate data on bee activity. On the other hand, colonies in very warm environments maintain their optimal temperature conditions thanks to bees that remain static at the entrance, beating their wings to increase the airflow inside the hive. At CIAPA (Centro de Investigación Apícola y Agroambiental de Marchamalo), we have tested the computer tool KATUA® Bees, developed by a Spanish company which, by means of artificial intelligence, has managed to count with more than 96% reliability both the bees that enter and leave the colony and also those that remain static at the entrance of the hive. This tool works on high quality videos of about 2 minutes previously recorded from the entrance of the hive and can be programmed to collect several videos, with the added advantage that data from several hives can be collected at the same time, allowing a more accurate comparison to be made. This new device represents a very important advance in the study of the vigour of bee colonies in real time. MEDIBEES: *Monitoring of the Mediterranean Honeybee Subspecies and their Resilience to Climate Change for the Improvement of Sustainable Agro-Ecosystems.* This project is part of the PRIMA program supported by the European Union

Keywords: Climate change adaptations, honeybee conservation, resistance to stressors

PESTICIDES IN POLLEN: RESULTS FROM THE LONG-TERM GERMAN BEE MONITORING PROJECT

Traynor, Kirsten, State Institute of Apiculture, University of Hohenheim, Stuttgart, Germany
Groth, Detlef, Department of Bioinformatics, University of Potsdam, Potsdam, Germany
Genersch, Elke, Institute of Bee Research, Hohen Neuendorf, Germany
DeBiMo Consortium, State Institute of Apiculture, University of Hohenheim, Stuttgart, Germany

The German Bee Monitoring Project (DeBiMo) was launched in autumn 2004/2005 to document winter losses and continues to monitor honey bee health year-round, with 10 colonies in each apiary monitored three times per year. Each year around 120 apiaries are monitored throughout Germany for mortality, colony strength, disease load (nosema, varroa, viruses), and the pesticide exposure through bee bread. Here we present the results of the pesticide analysis since 2009, documenting an increase in the amounts of fungicides detected over time and a significant reduction of insecticides in the last two years, in line with changes in agricultural use. Network analysis was used to determine which pesticides frequently co-occur. In comparison to other countries in North and South America, the risk from pesticide exposure in pollen to honey bee health is substantially lower, reflecting the precautionary principle used in pesticide regulations within Germany.

Keywords: pesticides, pollen, *Apis mellifera*

OPEN

ORAL PRESENTATIONS

Scientific Committee
(for abstracts do not fit a symposium theme)

STABLE CARBON (Δ13C) AND NITROGEN (Δ15N) ISOTOPE RATIO IN THE ASSESSMENT OF SAGE (*SALVIA OFFICINALIS* L.) AND WINTER SAVORY (*SATUREJA MONTANA* L.) HONEY AUTHENTICITY

Bilić Rajs, Blanka, Department of Food and Nutrition Research, Faculty of Food Technology Osijek, Osijek, Croatia
Flanjak, Ivana, Department of Food and Nutrition Research, Faculty of Food Technology Osijek, Osijek, Croatia
Milojković-Opsenica, Dušanka, Department of Analytical Chemistry, Faculty of Chemistry, Belgrade, Serbia
Tešić, Živoslav, Department of Analytical Chemistry, Faculty of Chemistry, Belgrade, Serbia
Primorac, Ljiljana, Department of Food and Nutrition Research, Faculty of Food Technology Osijek, Osijek, Croatia

Sage (*Salvia officinalis* L.) and winter savory (*Satureja montana* L.) are melliferous plants which are common for Mediterranean region of Croatia, so as for other parts of southern Europe (Spain, Italy, France). Both plants are used as spice/flavouring plants or medicinal herb. Also, they are honey bearing plants and these two honey types are appreciated and run up high market price. Sage and winter savory honey have been characterized in the last years through physicochemical parameters, pollen spectra and sensory analyses but due to increased incidence of honey adulteration new parameters for providing honey authenticity are needed.

In the last thirty years stable carbon isotope ratio ($\delta^{13}C$) method is used for detection of honey adulteration with sugar cane or corn syrups. Recently, researches have been focused on application of stable isotopes determination for honey botanical and geographical origin assessment. The data about stable isotopes in honey are scarce compared to other well-known parameters, and sage and winter savory honey are not characterized from this aspect yet.

This paper presents the results of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio in sage and winter savory honey from the Republic of Croatia and considers the possibility of using this parameter in assessment of honey authenticity. Average $\delta^{13}C_{honey}$ and $\delta^{13}C_{protein}$ values for winter savory honey were -27.93‰ and -26.65‰, while for sage honey they were -26.38‰ and -26.57‰, respectively. Both honey types had low average $\delta^{15}N$ values (-0.10‰ and 0.95‰) which are among the lowest values in comparison to literature for other honey types.

Keywords: Mediterranean unifloral honey, stable isotope ratio, authenticity

HONEY QUALITY AND SAFETY: A PRELIMINARY INVESTIGATION ON KINDS OF CITRUS HONEYS FROM THREE MEDITERRANEAN COUNTRIES

Lazzari Filippo[1], Manganello Federico[1], Shairra Souad A.[2], Kasiotis Konstantinos M.[3], Cresta Eleonora[1], Dipasquale Daniele[1], Danieli Pier Paolo[1]

1 Department of Agriculture and Forest Science (DAFNE), Tuscia University, Viterbo, Italy
2 Department of Biological Control, Plant Protection Research Institute, Agriculture Research Center, Egypt
3 Laboratory of Pesticides' Toxicology, Benaki Phytopathological Institute, Athens, Greece

Honey from *Citrus* spp. (CH) is a highly valuable monofloral hive product that, together with other animal food products, must meet the request of European consumers regarding health safeguarding established by the European Union regulatory frame regarding the food safety policies. The purpose of this research, as part of the Plant-B, an European Project funded by PRIMA Foundation (grant n. 1812), is to verify the quality and safety of citrus honey from the Mediterranean area through the assessment of its content of undesired trace elements such Pb, Cd, Hg, Cr and As. The study was performed on 41 samples of the 2020 beekeeping season of CH from project case studies in Italy (Sicily, Campania and Sardinia) and Greece (Peloponnesus) and from the market, and 40 samples from the same Italian and Greek regions and Egypt, collected during the season 2021. The CH samples were pretreated through a microwave oven and then analyzed by atomic absorption spectrometry (AAS) with graphite furnace atomization (Pb, Cd, Cr), or a hydride generator (Hg and As). A bibliographic search was also carried out which led to the creation of a dataset related to the mineral profile of citrus honeys from the Mediterranean basin area. The results obtained showed that Pb in honey samples was generally very low (0.013±0.011 mg/kg), and no citrus honey samples from both harvesting seasons exceeds the threshold of 0.1 mg Pb/kg set by the Regulation (EU) 2015/1005. As far as As, Cd, Cr, Hg, comparing the results obtained with the data listed in the literature dataset, the measured values fell below the maximum average values found in the bibliography. In particular, the Cr and Cd contents, which gave average values of 0.012 (±0.022) mg/kg and 0.002 (±0.003) mg/kg respectively, turned out to be clearly lower than the minimum average values found in the literature for CH products in the Mediterranean basin. Finally, As (0.0678±0.674 mg/kg) and Hg (0.14±0.16 mg/kg) were slightly below the average found in the bibliography (0.19 mg/kg for Hg and 0.31 mg/kg for As). Looking ahead, further investigations are in progress to verify whether the element analysis can have a potential in the traceability of Mediterranean citrus honey.

Keywords: Honey quality and safety, Atomic Absorption Spectrometry, trace elements

DEVELOPMENT OF LIFE CYCLE OF *VESPA VELUTINA* IN NW SPAIN

Diéguez-Antón, Ana, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Rodríguez-Flores, M. Shantal, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Escuredo, Olga, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Meno-Fariñas, Laura, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Rojo, Sergio, Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain
Seijo-Coello, M. Carmen Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain

The yellow-legged hornet *Vespa velutina* was detected first time in Galicia (Northwest of the Iberian Peninsula) at the end of 2012. Two insights were detected at the same time in the North and the South of this geographical area, probably coming from two different hornet populations. Since then, the invasion progressed exponentially and nowadays it is the major threat to the honey bee colonies within lower altitudes and near coastal margins. The ability of the species to adapt to new territories and the favourable climatic conditions in these areas are the attributable reasons for the invasion's success. Galicia has an Atlantic climate characterized by mild winters and summers, abundant rainfall, and low annual thermal oscillation. Most of the territory is covered by forests and shrubs favouring the hiding of *V. velutina* nests. Nowadays, more than 28000 nests are detected each year in the area. This work presents the result of the study of the characteristics of different kinds of nests collected in this geographical area. The nesting habitats of these hornets differ between types of nests, while embryo nests are mainly recorded in buildings, secondary nests are established in vegetation. Secondary nests (44), embryo nests (62), and re-location nests (4) were morphologically studied, and the population was estimated. At the same time, field observations were done since 2017 to identify the onset and decay of the biological cycle. First queens were detected in February being the cell-building rate in the early stages from 1 to 3 cells per day. In these embryo nests, it was observed workers and commonly males. About secondary nests, the largest one we studied had 11 combs, a perimeter of 162 cm and it was estimated a total population of over 30000 insects. The decline period was characterized by rearing new queens and males differentiable even at the pupal stage, the appearance of two eggs per cell, and irregular brood pattern. In recent years active nests have been observed in January suggesting that the biological cycle is more prolonged and differs from what was previously known. *V. velutina* demonstrated a great ability to quickly adapt its life cycle to new conditions.

Keywords: *Vespa velutina*, nest, life cycle.

This research was funded by INTERREG ATLANTIC AREA PROGRAM (European Regional Development Fund—ERDF, European Union): EAPA_800/2018—Atlantic-POSitive.

HONEY BEES AS BIOMONITORS OF ARSENIC SPECIES

Zarić, Nenad, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Braeuer, Simone, Institute of Chemistry, University of Graz, Graz, Austria
Goessler, Walter, Institute of Chemistry, University of Graz, Graz, Austria

Honey bees have been used as biomonitors of metal and metalloid pollution for a few decades. They represent pollution from all aspects of the environment, direct deposition of particulate matter from air on their hairy body, water they drink, elements from soil taken up by plants (pollen and nectar), or are deposited on honey bees through the resuspension of soil into the air.

Some of the elements monitored in honey bees can be very toxic. A good example of this is arsenic (As). The toxicity of As does not only depend on its concentration, but also on the chemical form in which it is present. Highest toxicity is reported for inorganic As, which is also a class I carcinogen. Typical organic forms of arsenic are in general much less toxic, with exceptions of methylarsonic acid (MA) and dimethylarsinic acid (DMA), which are classified as group 2B carcinogens.

Although honeybees are used for monitoring of metal pollution for a couple of decades, until now they have not been used as indicators for detection of different arsenic (As) species. The aim of our study was to determine As species present in honey bees, and to compare As species at four different locations in Serbia (Mesic, Pancevo, Belgrade and Kostolac).

Total As was determined after microwave-assisted digestion using inductively coupled plasma mass spectrometry (ICPMS). As species were determined after extraction with hot water at 90 °C, using high performance liquid chromatography (HPLC) coupled to ICPMS.

Inorganic As was the dominant species at all four locations. It accounted for 95% of all As at all locations, except for Pancevo, where it represented 84%, while the second most dominant As species was DMA with 13%. Pancevo is well known for its petrochemical and fertilizer manufacturing companies, as well as an oil refinery. Further studies are needed to determine the definite origin of As species in these bees.

Keywords: *Apis mellifera*, Arsenic, Element speciation

DETECTION OF BEESWAX ADULTERATION BY FTIR-ATR SPECTROSCOPY AND HTGC-MS METHODS

Kusyk Katarzyna, Laboratory for Testing the Quality of Bee Products, The National Institute of Horticultural Research, Skierniewice, Poland
Szczęsna Teresa, Laboratory for Testing the Quality of Bee Products, The National Institute of Horticultural Research, Skierniewice, Poland

The methods recommended for routine analytical testing the adulteration of beeswax such as HTGC-FID(MS) and/or FTIR-ATR require further validation (Svečnjak et al. 2021). Therefore, the aim of our research was to refine the FTIR-ATR method and check its suitability for testing beeswax adulteration with foreign hydrocarbons, e.g. paraffin. In the studies on the development of the FTIR-ATR method, samples of virgin wax and beeswax comb foundation produced from it were used (25 samples in total). These samples came from conventional and ecological apiaries, where only the internal rotation of the beeswax and comb foundation were used.

The research were performed using the Fourier Transform Infrared Spectroscopy coupled with Attenuated Total Reflectance accessory (FTIR-ATR) according to Svečnjak et al. (2019) in own modification. The modification of this method consisted in establishing the optimal measurement temperature and FTIR-ATR spectra of virgin beeswax samples. The results obtained by the FTIR-ATR method were compared with the results obtained for the same samples by the Gas Chromatography with Mass Spectrometric Detection (HTGC-MS) method according to Waś et al. (2014). Twenty six samples of beeswax and beeswax comb foundation suspected of being adulterated with substances of foreign origin were tested using both techniques.

The optimal measurement temperature during FTIR-ATR analysis was established on 65°C. The ratio of target peak area 1724 - 1780 cm^{-1} (with absorption maximum at 1738 cm^{-1}) to target peak area 2800 - 2866 cm^{-1} (with absorption maximum at 2853 cm^{-1}) was chosen to establish calibration curve for adulteration analysis. In samples where adulteration by paraffin or other foreign hydrocarbons was detected using the FTIR-ATR method (with a detection limit of 3%), the sum of the hydrocarbons content (C_{20} - C_{35}) and the sum of the content of hydrocarbons with an even number of carbon atoms in the molecule was above the maximum content of these compounds in virgin beeswax. With high adulteration, the presence of long-chain hydrocarbons was also found. The results received by the HTGC-MS method for samples suspected of adulteration confirmed the usefulness of the FTIR-ATR method for detection of beeswax adulteration with hydrocarbons of foreign origin, e.g. paraffin.

Keywords: Adulteration, Paraffin, Beeswax

OPEN

POSTERS

SYMBEEOSIS: TOWARDS REGENERATING APICULTURE UNDER SCIENTIFIC SUPERVISION

Fytros Evangelos, Symbeeosis Herbs and Bees IKE, Athens, Greece.
Bouga Maria, Laboratory of agricultural Zoology and Entomology, Agricultural University of Athens, Athens Greece.
Papachristoforou Alexandros, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Greece

Symbeeosis is a Greek company producing organic and functional food products and supplements, aiming in achieving new standards of regenerating apiculture. Honey, raw or enriched with bioactive ingredients (such as propolis and herbs) as well as other apicultural products are produced in company's organic apiaries, counting almost 1000 colonies, in collaboration with beekeepers who share the philosophy of respecting and protecting the ecosystem and who practice nomadic beekeeping. During all stages of production, the beekeepers are under scientific supervision from experts on bee biology, bee pathology, bee genetics and bee products. More specifically, the annual development of colonies is monitored constantly by bio-sensors and beekeepers during apiary check, use innovating voice applications to record their observations. Collected data are analysed and decision making on colony manipulations, follows experts' advices. Honey is never exhausted from each hive and always enough is left in the honeycombs to allow the bees to replace the harvested quantity without being detrimental to the health of the colony. Bio pest management is based on regular sampling and analysis in order to determine the level of infestation of bee pathogens and decide the optimum timing and method for their control. Selection and queen breeding is applied in all apiaries after colonies are thoroughly tested for their hygienic behaviour, honey productivity and quantities of propolis collected. Special care is taken in order to secure that local subspecies, adapted to the Greek environmental conditions, are selected. The genetic origin of the bees is determined using COI mitochondrial gene segment sequencing analysis. Furthermore, Symbeeosis runs training programs in order to encourage a new generation of beekeepers, introducing them to good beekeeping practices, so that, ultimately, the community of organic honey producers, as well as the production of superior organic honey, can be expanded.

Keywords: Organic honey, Regenerating Apiculture, Apiary management

HOW TO CHARACTERIZE AND DESCRIBE A GOOD PROPOLIS? COMPARISON OF VARIOUS EXTRACTS BY DIFFERENT ANALYTICAL METHODS BOTH SCIENTIFIC AND COMMERCIAL VIEW

Aytekin Ahmet Murat, VP in University of Mons Department of Zoology Belgium
Aytekin Seçil, Hacettepe University Doping Control Center Sıhhiye Ankara Turkey

Propolis, is the generic name for the resinous substance that is collected by bees from different plant sources -like poplar, birch, pine, alder, willow and palm or sometimes from wounds in several other plants. It is sometimes classified as an opotherapeutic medicine due to its complex chemical composition of different botanical origins of the resins collected by bees. The chemical composition of propolis is greatly influenced by several factors like the honeybee species or the botanical source. The extraction method of bioactive compounds with different techniques from raw propolis is also influence the resulted product. But how to analyse the extract changes many other things. Classifying any material is a human nature, which helps our species to summarise and compare things. Many laboratories and commercial companies have their own method of defining the best propolis extract. Which is better and what is best are phenomenal questions. Propolis can be used as a dietary supplement or a toothpaste or a wound care product. Each need to have different properties. That is the reason we compared the same propolis' polyphenol contents and antioxidant activities prepared with different methods. We also compare the extracts by GC-MS and LC-MC. Four types of extracts were prepared. The first type was prepared classically by %80 ethyl alcohol. The second and third types were extracted by sterile distilled water and kept as both sterilised (*PS*) and non-sterilized (*PN*) *forms*. The fourth one was prepared with full vacuumed and freeze-dried propolis (*LH*). The raw material origins were from South America to West Asia and Europe. The antioxidant activities of these extracts was evaluated with DPPH radical scavenging, ABTS radical cation scavenging and the chemical compositions were compared.

Keywords: *propolis, antioxidant, LC-MS, GC-MS*

NEW REGULATION (EU) 2018/848 ON ORGANIC PRODUCTION AND BEEKEEPING. A CRITICAL ANALYSIS

Pier Paolo Danieli*, Paolo Scarpino*, Simone Bergonzoli§

* Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia (Viterbo)
§ Council for Agricultural Research and Economics, Research Center for Engineering and Agro-Food Processing (CREA-IT)

Although consolidated over the years, interest in the organic farming sector is experiencing a decline expressed in the value of purchases of agri-food products, a decline that has also affected honey sales. Beekeeping is a sector of great interest for the European Union. In fact, in the Member States, 240 million euros were spent on national programs for the protection of bees, the development of beekeeping and support for honey production. On 1 January 2022, the new organic regulation came into force, Reg. (EU) 2018/848 of the European Parliament and of the European Council which, due to the COVID-19 pandemic, is becoming applicable one year late than expected. A regulation on a European basis offers the possibility of harmonizing the strategies and objectives to be achieved, thus representing an opportunity for the beekeeping sector. From these premises arises the practical interest to examine the articles relating to organic bee production according to the new regulation. The critical analysis, supported by the comparison with some Italian stakeholders in the sector and by the comparison with the versions of the same regulation in the language of some Member States, considered the main practical/applicative aspects for the future application of some articles of the new regulation. First, it clearly arises the will to consider beekeeping as a full zootechnical activity, albeit with its rearing peculiarities. However, some issues pose interpretative difficulties that prelude to as many potential difficulties of application, and possibly differences in different Member States. The work, conceived to understand the critical issues and stimulate a useful reflection on the risks of distinct interpretations within and between States, propose possible standpoints to get clear rules shared by the entire sector with the aim of giving a new impulse to the "organic" beekeeping sector.

Keywords: Organic beekeeping; EU legislation;

THE CURRENT SITUATION OF BEEKEEPING IN ALGERIA AND DEVELOPMENT CONSTRAINTS (RESULTS OF THE MEDIBEES-PRIMA PROJECT)

Noureddine Adjlane[1], Yamina Haider[1], Raquel Martín-Hernandez[2]

1 Département d'Agronomie, Université de Boumerdès, Algérie
2 Centro de Investigación Apícola y Agroambiental de Marchamalo, Espanha.

As part of the MEDIBEES project - Monitoring Mediterranean bee subspecies and their resilience to climate change for the sustainable improvement of agroecosystems - a survey was carried out between October and November 2021, with the aim of characterizing and understanding the beekeeping activity and its main problems in Algeria, compared to other countries of the Mediterranean basin. This survey was conducted simultaneously in Spain, Italy, Malta, Turkey, Portugal, Lebanon and Jordan, partner countries of the MEDIBEES consortium. The survey was disseminated in Algeria by e-mail, sent to all beekeeping associations and beekeepers.

In this presentation, we will only present the results obtained for Algeria. A total of 200 questionnaires were analyzed. Respondents have apiaries in 19 wilayas of the country, which shows good geographical coverage of the survey.

The results of this study show the development constraints of beekeeping in Algeria in recent years, drought, very high mortality and the presence of pathologies. COVID has had a negative influence on the performance of beekeepers. A very low honey yield has been obtained over the past two years. Among the positive points of the survey, the presence of beekeepers who practice good beekeeping (renewal of queens each year, selection at the level of apiaries, the practice of transhumance) as well as periodic screening for varroasis in colonies of bees. Beekeeping associations must be on the ground to organize the beekeeping sector and improve the current situation.

Keywords: honey bee, Algeria, development constraints, MEDIBEES;

ISOTOPIC SIGNATURE OF CITRUS HONEY SAMPLES PRODUCED IN THE MEDITERRANEAN BASIN

Danieli Pier Paolo[1], Lauteri Marco[2], Kasiotis Konstantinos M.[3], Shairra Souad A.[4], Lazzari Filippo[1], Dipasquale Daniele[1], Manganello Federico[1]

1 Department of Agriculture and Forest Sciences (DAFNE), Tuscia University, Viterbo, Italy
2 Istituto di Ricerca sugli Ecosistemi Terrestri, CNR, Porano (TR), Italy
3 Laboratory of Pesticides' Toxicology, Benaki Phytopathological Institute, Athens, Greece
4 Department of Biological Control, Plant Protection Research Institute, Agriculture Research Center, Egypt

The demands of the consumers regarding the origin and quality of food products, have led to the exigency to define quantitative traits that are measurable, replicable and verifiable. Since monofloral honey from *Citrus* spp. (CH) is a product sought after and appreciated by European consumers, it is necessary to have adequate methods for characterizing its geographical and/or botanical origin. This research, as part of the Plant-B, a European Project funded by PRIMA Foundation (grant n. 1812), aimed at studying the relationship between geographical origin and the nitrogen isotope signature of CH from some countries of the Mediterranean basin. The study was performed on 41 CH samples of the 2020 season from case studies in Italy (Sicily, Campania and Sardinia) and Greece (Peloponnesus) and from the market. Furthermore, we analysed 40 samples from the same Italian and Greek regions and Egypt, collected during the season 2021. All the CH samples were analyzed by means of Isotope Ratio Mass Spectrometry (IRMS) in order to evaluate the nitrogen isotope composition (δ^{15}N). In partial agreement with the literature, the results were rather heterogeneous. Referring to δ^{15}N of the Italian (4.4±1.4‰) and Greek (2.3±0.5‰) CH samples of the 2020 season, the variation ranges define distinctive values for the two countries ($p<0.001$). Overall, these evidences can be explained by the strict relationship of δ*15*N with the plant nutritional status, depending on soil characteristics as well as on differences in agronomic management. Similarly, significant differences ($p<0.05$) were obtained comparing Greek (3.5±1.3‰) and Italian (4.7±1.4‰) honeys from the 2021 season. On the other hand, no significant differences were observed between Egyptian (3.4±1.0‰) *vs.* Italian honeys, or *vs.* Greek ones. Preliminary outcomes suggest that δ^{15}N analysis can be helpful in evaluating the geographical origin of citrus honeys. This preliminary work is being further extended by including the analysis of other stable isotope species in order to increase the geographical origin resolution of the IRMS technique.

Keywords: Isotope Ratio Mass Spectrometry, citrus honey, geographical origin

RELATIONSHIPS BETWEEN BLACK LOCUST PHENOLOGY AND HONEY PRODUCTION IN CENTRAL ITALY

Danieli Pier Paolo[1], Carbonari Fausto[1], Lazzari Filippo[1], Manganello Federico[1], Cresta Eleonora[1]

1 Department of Agriculture and Forest Sciences (DAFNE), Tuscia University, Viterbo, Italy

Robinia pseudoacacia L. (Black Locust, BL) is a highly nectariferous plant species used by beekeepers for the production of acacia honey. Through phenology it is possible to study the flowering evolution, and related nectar flows, that strongly affect the monofloral honey productivity. Upon a detailed literature survey, it seems that poor efforts have been spent in describing such relationship. This research represents the basis for the development of predictive models of flowering intended for insurance policies aimed at compensating beekeepers damaged by extreme atmospheric events. In April-May 2021, 16 hot spot stations were identified in central Italy (Lazio and Umbria) where the blooms of BL nearby apiaries (1 Km) for the production of acacia honey were monitored. Regular surveys (every 4 days) were carried out to describe the phenological trend using the international BBCH system, taking into account the phenophases between emergence of the inflorescences (BBCH 51) and floral wilting (BBCH 69). Thanks to beekeepers, data was collected on the average production of acacia honey in the year 2021, and in previous years. Late (end of April 2021) frosts affected 9 out of 16 monitoring stations. Only 13 out of 16 stations reached the complete flowers' development, with an average duration of the full flowering (BBCH 65) of 10 ± 2 days). The production data show that only 6 apiaries managed to produce quantifiable amounts of acacia honey, with an average production of only 2.8 Kg/hive. The 2021 yields were unsatisfactory and the 62.9% loss compared to the 2011-2020 average confirms the negative trend recorded recently in Italy. Furthermore, a significant difference ($p<0.05$) emerged comparing the 2021 average honey yield of the stations where late frosts occurred (2.6 Kg/hive) with the stations in which frost damages have not been seen (5.7 Kg/hive). The low temperatures recorded in April had a negative effect on flower development and consequently on acacia honey production. However also in the stations where no late-frost damages were observed, the yield was far below the expectation of the beekeepers. Further investigations are needed to understand the complex interactions existing between the BL flowering evolution, the production of acacia honey, and other main factors that generate instability in such type of honey production.

Keywords: *Robinia pseudoacacia* L, Phenology, Acacia honey

HONEY BEES AS BIOMONITORS OF PLANT PATHOGENS, PESTICIDES, AND ANTIMICROBIAL RESISTANCE GENES

Guarna, M. Marta; Cunningham, Morgan M; Newman, Tara; Wu, Linzhi; Pernal, Steve; Tran, Lan; Deckers, Thomas. Agriculture and Agri-Food Canada, Beaverlodge, Canada
Rott, Michael E. Canadian Inspection Agency, Sidney, Canada
Bilodeau, Guillaume J., Canadian Inspection Agency, Ottawa, Canada
Griffiths, Jonathan S. Agriculture and Agri-Food Canada, Vineland, Canada
Bishop, Christine. Environment and Climate Change Canada, Delta, BC
Jabbari, Hosna. Department of Computer Science, University of Victoria, Victoria, BC
Jovana Raičević, Faculty of Biology, University of Belgrade, Belgrade, Serbia
Ortega Polo, Rodrigo; Zorz, Jacqueline; Kurtis, Clarke; Lansing, Lance. Agriculture and Agri-Food Canada, Lethbridge, Canada

The European honey bee (*Apis mellifera*) can be exposed to pathogens and agrochemicals during foraging; carrying them to the hive where they can be detected and quantified in hive materials: honey, pollen, and wax. Results from our and other laboratories showed the potential of using honey bees as environmental biomonitors. In particular for monitoring a) plant pathogens, b) agrochemicals, and c) antimicrobial resistance (AMR) genes (ARGs). We tested the potential of plant pathogen monitoring (a), by analyzing bees and bee-collected pollen, and we established the presence of plant viruses, fungi, and bacteria using high-throughput sequencing. We analyzed samples for the presence of agrochemicals (b) and identified insecticides, fungicides and herbicides in colonies positioned near and far from blueberry fields; we also studied temporal and spatial patterns of systemic insecticides in bees and flowers. In addition, we identified antimicrobial resistance genes (c) and studied the honey bee gut resistome, i.e. the collection of AMR genes in a microbiome, using metagenomics sequencing. AMR can result in the loss of antibiotic effectiveness and consequently the use of higher doses. This poses an increased risk of residues in honey, gut dysbiosis, and other negative health effects on honey bees, other pollinators, and the ecosystem. We analyzed bee gut microbiome samples from different sites in Ontario, Canada; by processing shotgun metagenomic sequencing results through the AMR++ pipeline producing short-read alignment resistome results classified against the MEGARes database. The same reads were also de novo assembled for ARGs screening against different databases. We found a high relative abundance of tetracycline resistance genes across samples, in accordance with the common use of oxytetracycline for prophylaxis and treatment of bacterial diseases in ON. Sulfonamide and aminoglycoside ARGs were also found consistently across samples. Monitoring AMR genes in apiaries and their surrounding environment can guide decisions towards limited and informed antibiotic use. Bee-based surveillance of ARGs, agrochemicals, and plant pathogens has the potential to be an effective tool in environmental monitoring programs; crucial for protecting human, agriculture, and overall ecosystem health.

Keywords: pathogens, agrochemicals, antimicrobial resistance

FUNCTIONAL PROPERTIES OF GALICIAN BEE POLLEN AND ITS RELATIONSHIP WITH BOTANICAL ORIGIN

Rodríguez-Flores, María Shantal; Rojo, Sergio; Diéguez-Antón, Ana; Meno-Fariñas, Laura, Seijo-Coello, María Carmen; Escuredo, Olga.
Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain

Pollen load gathering by honey bees is especially important in terms of pollination as workers bees visit hundreds of flowers commonly of same species, guaranteeing pollen transfer between plants both agricultural crop and wild plants. On the other hand, pollen has been shown to be a very complete food resource. Bee pollen contains substances essential for the development of the hive and it is considered a supplement with high nutritional value and many therapeutic applications. One of the characteristics of bee pollen is the bioactive or functional properties such as the antioxidant capacity. Galicia (NW of Iberian Peninsula) has around 200,000 hives mainly dedicated to honey due to the existence of the PGI "Mel de Galicia", but diversification and the consequences of climate change favorable for bee pollen harvest, promote this activity. Due to this increase in bee pollen harvesting, studies on botanical origin and the physical and chemical properties that can contribute to its valorisation are relevant. So that this work has the aim to investigate the total phenol content, total flavonoid content and antioxidant activity (RSA% and IC_{50}) of bee pollen samples collected in this geographical area. Samples were separated according to their botanical origin and 15 monofloral types were obtained: *Castanea, Rubus, Erica, Genista, Trifolium, Lythrum, Olea europaea, Quercus, Campanula, Foeniculum vulgare, Cistus, Brassica, Echium, Taraxacum officinale* and *Calluna vulgare*. Color was also measured since it provides a commercial value to the product. All the chemical parameters were measured in ethanolic pollen extracts. Each of them showed different properties and color depending on their botanical origin. Phenol content varied from 481.1 to 2987.8 mg/100g, flavonoid content varied from 69.6 to 1334.1 mg/100g. Finally, antioxidant capacity varied from 29.7 to 84.0 RSA %. The pollen types *Castanea* and *Lythrum* stood out for having a good combination in both phenolic content and antioxidant capacity.

Keywords: Bee pollen, Functional properties, Galicia, Spain

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TRANSFORMING THE FUTURE OF WORK FOR BEEKEEPERS AND RELATED CAREERS USING DATA SCIENCE: EDUCATING THE NEXT GENERATION

Fisher-Maltese, PhD, Carley (PI), College of Education and Human Development, George Mason University, Fairfax, USA
Gring-Pemble, PhD, Lisa (CoPI), School of Business, George Mason University, Fairfax, USA
Bouga, PhD, Maria, Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, Athens, Greece
Wilson, Regan (GRA), College of Education and Human Development, George Mason University, Fairfax, USA

The US and the globe face an emerging and worsening crisis around pollinators (US Pollinator Report, 2015). Traditional approaches to beekeeping have been unable to stem the tide of colony collapse, which has placed stress on the beekeeping workforce. While summer losses have held steady at 20.5%, winter loss figures for 2020 were up 8.9% over the average winter loss (2006-2019). The loss of honeybees impacts both food security and national security. Given this context, the future of work for beekeeping will involve mastering research-driven practices (Kulhanek et al, 2017) that respond to bee health in different landscapes (Patel et al., 2020), and that deploy sensors (Catania & Vallone, 2020;). In this planning grant, we focus on a solution-related design and deploy sensor-supported urban beekeeping (Catania & Vallone, 2020).

To facilitate learning from sensor monitoring technology in bee hives in a K-12 setting, education experts are developing lessons for grades K-2, 3-5, 6-8, and 9-12 that correspond directly with four data points that can be read from sensors:

☐ Weight (indicates colony productivity and health)
☐ Temperature (indicates colony health, size, and location)
☐ Audio (indicates stress or effort levels of colony)
☐ External Camera (provides visuals of flight patterns and intruders)

This project provides an authentic, problem-based-learning (PBL) opportunity for students, in which they will learn about the awe-inspiring life science concepts around honey bees to help solve a real-world problem. It also will teach students critical computer science concepts necessary for data-driven beekeeping, which may be the bees' only hope for survival. The students will acquire critical skills necessary to eventually participate in a tech driven economy; the bees stand to gain from a better understanding of population decline.

Keywords: Education, Beekeeping, Data Science, Sensor-enabled Hives

DETERMINATION OF PHENOLIC COMPOUNDS IN MONOFLORAL HONEYS USING AN HPLC-DAD METHOD

Rodopoulou Maria-Anna, Tananaki Chrysoula, Kanelis Dimitrios, Liolios Vasilios, Laboratory of Apiculture-Sericulture, Aristotle University of Thessaloniki (AUTH), Thermi, Thessaloniki, Greece

The components honey contains, such as phenolic compounds, enzymes and vitamins, make it a good source of antioxidants and thus there is a great research interest on their determination. Also, recently, there has been an increase in demand and purchase value of monofloral honeys, due to their distinct organoleptic and physicochemical characteristics, giving beekeepers the opportunity to compete with low-priced honeys imported from abroad. Thanks to its Mediterranean climate, Greece offers the possibility of producing various monofloral honey types, many of which however, remain uninvestigated or have been little studied. Given also that the composition of phenolic compounds in honey depends mainly on its botanical origin, in this study we determined phenolic compounds in 15 monofloral honeys produced in Greece, [Thyme (*Thymus capitatus*), autumn pine and spring pine (*Pinus* sp.), chestnut (*Castanea sativa*), knotgrass (*Polygonum aviculare*), strawberry tree (*Arbutus unedo*), jerusalem thorn (*Paliurus spina-cristi*), fir (*Abies* sp.), oak (*Quercus* sp.), sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), erica (*Erica manipuliflora*), clover (*Trifolium* sp.), citrus (*Citrus* sp.) and judas tree (*Cercis siliquastrum*)]. Specifically, we investigated the phenolic acids homogenistic, protocatechuic, caffeic, syringic, p-coumaric, ellagic and the flavonoids catechin, epicatechin, rutin, quercitrin, quercetin and chrysin. For the isolation of the compounds, the liquid - liquid extraction in combination with lyophilization was used, while the detection and quantification was achieved using a High Performance Liquid Chromatography - Diode Array Detector system (HPLC-DAD). Strawberry tree honeys presented the highest average concentration of homogenistic acid (1942,0±1752,4 μg/100g) followed by erica, thyme and cotton, while protocatechuic acid seems to characterize the oak honey (151,6±100,8 μg/100g). Additionally, the erica honey showed the highest concentration of ellagic acid (668,0±585,9 μg/100g), epicatechin (69,4±96,1 μg/100g) and rutin (117,6±173,5 μg/100g), the spring pine honey of catechin (122,7±207,7 μg/100g) and the judas tree honey of p-coumaric acid (68,7±7,9 μg/100g). Finally, chrysin was found in certain honey types (jerusalem thorn, cotton, erica, sunflower and judas tree). The results showed the influence of plant sources on the phenolic compounds of the studied monofloral honeys, while the findings may be used as an additional tool for their promotion worldwide.

Keywords: phenolic compounds, monofloral honey, HPLC – DAD

INCENSE HONEY (*PITTOSPORUM UNDULATUM*) IN THE AZORES: BOTANICAL AUTHENTICATION BY USING REAL-TIME PCR APPROACH

Lopes, Ana R., Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301-857 Bragança, Portugal
Moura, Mónica B.M.V, REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
Grazina, Liliana, REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
Costa, Joana, REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
Amaral, Joana S., Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301-857 Bragança, Portugal
Pinto, M. Alice, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301-857 Bragança, Portugal
Mafra, Isabel, REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

Honey is a widely consumed food and much appreciated for its nutritional, organoleptic, and health properties. The honey produced in the Azores archipelago can be categorised as incense honey or multifloral honey. Incense honey classification should account with >30% from *Pittosporum undulatum* pollen grains and it is generally perceived as a unique and high-quality product and, consequently, susceptible to be adulterated through incorrect labelling or admixing with low-cost and low-quality honeys. Therefore, assessing the authenticity of such highly appreciated honey is a key issue for its valorisation. In this work, a real-time PCR method targeting the ITS region was proposed for the first time to detect *P. undulatum* species. To that end, the DNA extracted from the leaves of *P. undulatum* and from other endemic species (e.g. *Eucalyptus* spp., *Acacia* spp., *Trifolium* spp., *Castanea sativa* Mill., *Hydrangea macrophylla*, *Rhododendro indicum*, *Hedychium gardnerianum*, *Pericallis malvifolia*) of the Azores and mainland Portugal was used to test the cross-reactivity of the ITS primers by qualitative PCR, revealing full specificity for *P. undulatum*. Posteriorly, a real-time PCR approach was proposed, exhibiting high analytical performance (PCR efficiency = 97.4 ± 6.4%, R^2= 0.991 ± 0.003) and a limit of quantification of 0.01 pg of incense DNA. The method was successfully applied to the 22 honey samples, from which incense was detected in all 9 monofloral incense honeys and in 5 out of 10 multifloral samples from the Azores. Generally, the quantitative results for incense DNA were in good agreement with the melissopalynological data, showing that all samples, except two, were according to their labelled statements. Herein, a new, simple, cost-effective and reliable molecular approach was proposed to authenticate and valorise the Azores honey.

Keywords: Incense, honey, authentication

MACHINE LEARNING APPROACHES IN BEE RESEARCH

Dimitrijević, Sonja, Mihailo Pupin Institute, University of Belgrade, Belgrade, Serbia
Zogović, Nikola, Mihailo Pupin Institute, University of Belgrade, Belgrade, Serbia

Machine learning (ML), a prominent subfield of artificial intelligence, has brought many new opportunities in advanced bee data analysis in the last few years. Different bee research fields can benefit from ML in terms of efficient handling of data, automation of data processing and analysis, trends and patterns identification, a wide range of prospective applications, etc. Therefore, bee researchers need to be familiar with promising ML approaches and opportunities to choose future research directions wisely. To this end, a systematic review has been conducted to reveal bee research areas with a recent focus on ML, including their specific ML objectives, ML models applied, as well as model evaluation results. More specifically, 41 research studies, which were published in good-quality journals in 2020 and 2021, have been carefully selected, analyzed, and discussed considering the review objective. The results have shown that almost half of the studies address the area of bee monitoring (18 studies). The other research areas reported in the selected studies are bee products properties (10 studies), the toxicity of plant protection products (3 studies), climate change impact (3 studies), bee flora (3 studies), biodiversity (2 studies), genetics and genomics (1 study), and microbiota (1 study). Compared to the area of bee products' properties, bee monitoring is characterized by a greater variety of ML application objectives/cases (e.g., identification or prediction of various sets of beehive conditions). As for the reported ML models, supervised learning, particularly classification, has been predominantly applied. The models have been based on different algorithms including deep learning algorithms (such as Convolutional Neural Network), Random Forest, Support Vector Machine, K-Nearest Neighbors, etc. Thereby, many studies have comparatively used a couple of models. The reported evaluation results are mainly promising. However, most of the studies suffer from limitations related to the size and/or diversity of datasets (e.g., data from several beehives instead of data from several apiaries). Although machine learning is still in its early infancy in bee research, many opportunities are evident from the recent literature. It should not be long before existing and future research directions rely more on ML.

Keywords: Machine learning, Bee research, Systematic review

VALORISATION OF BEEHIVE BY-PRODUCTS OBTAINED FROM AN APIARY AT CENTRAL SPAIN

López-Pérez, J.A., González-Porto, A.V., García-Villarrubia, C., Higes Pascual M., Sagastume de Andrés, S., Martín Hernández, R. Centro de Investigaciones Apícolas y Agroambientales-CIAPA, IRIAF, Marchamalo, Guadalajara, SPAIN

A pre-eliminary study of beehive by-products has been carried out. The aim of this study is to find out its potential as a local source of organic matter to be used as a soil improver and an organic source for soil biodisinfestation able to reduce soil phytopagens. The content of these by-products are mainly wax, pollen, honey, propolis, feces, remains of dead bees or of possible intruders that appear in the bottom of the beehive. This composed by-products were generated as result of the beekeeping activity in an apiary located in Central Spain (Guadalajara, Castilla-La Mancha), placed in the facilities of the apicultural and agro-environmental research center located in Marchamalo (CIAPA, IRIAF). The material were collected throughout the year 2021 and were kept frozen for analysis. The by-products were crushed to homogenize the residue and set it ready for analysis. Among the parameters analysed, dry weight, organic matter, water activity, pH, electric conductivity, and N content, it is noteworthy the high C/N ratio obtained. Results were compared with previous results of pollen, and honey samples in order to establish the contribution of these elements to final composition of these by-products. Further analyses are in progress to fully characterize the by-product.

This project is part of the PRIMA program supported by the European Union

Keywords: local organic residues, physic-chemical properties, soil improver

MASS SPECTROMETRY-BASED CHARACTERISATION OF SELECTED BEE PRODUCTS

Matuszewska Eliza, Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland
Matysiak Jan, Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland

Due to nutritional and bioactive compounds, such as proteins, amino acids and essential macro-, and microelements, bee products benefit the human body. However, because of their natural origin, they may contain toxic inorganic contaminants, including heavy metals. Moreover, a strong allergic reaction may occur after exposition to these products. Thus, to improve the safety of the therapeutic use of bee products, it is necessary to know all bioactive components contained in them and clarify both beneficial and adverse effects occurring in the body under their influence.

Therefore, this study aimed to characterise selected bee products, focusing on their composition and biological properties. The performed experiments allowed for the characterisation of the protein-peptide composition of royal jelly, bee pollen and bee larvae and the determination of the content of chemical elements in royal jelly, bee pollen and propolis.

Analysis of the protein-peptide composition of royal jelly resulted in the identification of 86 proteins taxonomically classified to *Apis* spp. In bee pollen, we identified 63 proteins of *Apis* spp. and almost 500 proteins classified to *Viridiplantae* (green plants) clade. In honeybee larvae, 287 *Apis* proteins were identified. In all examined products we found hypothetical proteins, which are particularly interesting, as their existence in bee products has not previously been experimentally confirmed.

Elemental studies on bee pollen, propolis and royal jelly have shown that the concentrations of individual elements, including macro-, microelements, and contaminants in bee products, differ depending on the product type. Differences were also noted between propolis samples collected in two consecutive years. Bees and their products are considered sensitive indicators of anthropogenic environmental pollution. Thus, the obtained results may indicate the degree of emission of factors leading to environmental degradation. In addition, the knowledge of mineral contamination of bee products allows for estimating the safety of their oral intake.

The research led to identifying new proteins contained in royal jelly, bee pollen and bee larvae. Also, for the first time, such a broad spectrum of chemical elements present in bee products originating from an apiary located in the Greater Poland region was determined.

Keywords: Proteomics, Multi-elemental Analysis, Selected Bee Products

COMPARISON OF COMPONENTS AND PHARMACOLOGICAL ACTIVITIES OF PROPOLIS SOLUTIONS PRODUCED BY DIFFERENT METHODS

Unal, H. Hüseyin[1], Hos, Aysegul[2], Sait, Ahmet[3], Omurtak Gulden Z.[2]
1 Department Of Pharmacology&Toxicology, Pendik Veterinary Control Institute, Istanbul, Turkiye
2 School of Pharmacy, İstanbul Medipol University, Istanbul, Turkiye
3 Department of Virology, Pendik Veterinary Control Institute, Istanbul, Turkiye

Propolis is a miraculous bee product that honeybees collect from nature, carry to the hive, and use to protect both their own health and the health of the colony. Propolis is a natural bee product that honeybees produce by mixing their own enzymes with resin-containing components collected from the stems, leaves, shoots, and buds of plants. Propolis generally has a complex structure containing more than 300 compounds. It is not correct to use propolis taken from the hive as raw. It is separated from wax and foreign materials and its components are extracted with different solvents. We use water, olive oil, and ethanol were used as solvents while preparing propolis solutions. It was determined that there were significant changes in the amounts and ratios of the components with pharmacological effects. As seen in the analysis, it contains many valuable pharmacologically effective components such as resin, bee wax, essential aromatic oils, pollen, amino acids, minerals, carbohydrates, phenolic substances, flavonoids, and terpenes. Different endemic plants and medicinal aromatic plants in the Anatolian geography are very valuable sources of propolis for honeybees. They have flavonoids, phenolic, and essential aromatic compounds, which are very strong pharmacological effects on health. The use of propolis as apitherapy applications within the framework of traditional complementary therapy applications for public health and animal health is increasing to support treatment. It is known that propolis has antibacterial, antiviral, antifungal, antioxidant, anticarcinogenic, anti-inflammatory, and immunostimulant properties and has important effects on health. The protein, carbohydrate, lipid, and vitamin-mineral content of propolis is quite low. For this reason, the use of propolis on its pharmacological effects is more valuable than evaluating it as a nutritional element. The highest resolution was obtained with ethanol. On the other hand, it could be detected very low levels of propolis components and a few flavonoids when we used water and olive oil as solvents

Keywords: comparison, pharmacological, activities, propolis, solutions, different methods

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