EurBee 10

# 10th CONGRESS OF APIDOLOGY

16.-19.09.2024 Tallinn, ESTONIA

# Abstract book

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# Welcome

It is our sincere pleasure to welcome you on the EurBee 10 Congress in Tallinn, Estonia! The Congress is organized by the Estonian University of Life Sciences with assistance by Publicon OÜ.

EurBee is the event, where old and new friends get together to exchange the knowledge of novel scientific findings, associated with honeybees and other pollinators.
We encourage young researchers to meet the leading scientists on their field. Establishing networking and creating new connections is extremely important for sustainable bee research.

The City of Tallinn is the capital of Estonia. Tallinn's Hanseatic old town and nowadays modern architecture is a great mixture for every taste. We recommend you to discover the great Estonian flavors and the interesting culture that Tallinn offers you in abundance on every corner.

Looking further, Estonian nature with its forests, bogs and swamps is unique in the world – all the EurBee guests have the opportunity to experience its magic!

*Experience magic – experience Estonia!*

Sincerely Yours,

Risto Raimets
President of EurBee 10

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# Programme overview

# Monday, 16 September

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| | 4 Pollinators and pollination ecology in natural and agricultural landscapes |
| | 10 Bee diversity, conservation and interaction among species |
| | 9 Beekeeping issues |
| 10:45-11:00 | Coffee break |
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| | 10 Bee diversity, conservation and interaction among species |
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| | 11 Bee nutrition |
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| | 8 Evolution and population genetics of bees |
| | 3 Impacts of climate change and other factors in a changing environment |
| 10:45-11:00 | Coffee break |
| 11:00-12:00 | 6 Ecotoxicology, pesticides, pollutants |
| | 8 Evolution and population genetics of bees |
| | 3 Impacts of climate change and other factors in a changing environment |
| 12:00-13:00 | Lunch |
| 13:00-14:00 | Plenary session, Simone Tosi |
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| 16:00-17:30 | 6 Ecotoxicology, pesticides, pollutants |
| | 13 Open topic |
| | 5 Novel technologies and methodologies in bee research |
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## 1-2 Bee immunity and diseases. Poster presentations

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3 Impacts of climate change and other factors in a changing environment. Poster presentations

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13 Open Topic. Poster presentations

# Keynote speakers

Moderators:
Risto Raimets
Dalial Freitak
Reet Karise

# Dalial Freitak

Dalial Freitak is an insect physiologist focusing on social insect immunity and behaviour. Specializing in understanding how insect immunity functions on the molecular level, but also how interactions between the hosts and parasites take place, her work has brought a groundbreaking new understanding to the field of immune priming in insects. The discovery has paved the way to revolutionize the animal health sector worldwide and to the development of the first vaccination for honeybees.

In addition to her academic career, Dalial Freitak is a founder, co-owner and scientific leader in the start-up company Dalan Animal Health., Inc. The company has won several prizes, including the Times magazine Top 200 mention for 2023 and has brought to the North American market the first vaccine against American Foulbrood in honeybees.

Originally from Estonia, Dalial Freitak graduated with a Master's degree in Animal Ecology from University in Tartu. She obtained her PhD working at the Max-Planck Institute for Chemical Ecology in Jena Germany. After that, she has been working in the University of Giessen, Germany and the Universities of Helsinki and Jyväskylä in Finland.

Currently, Dalial Freitak’s research focuses on trans-generational immune mechanisms in honeybees and self-medication behaviour in ants. She works at Karl Franzen University of Graz with different honey bee pathogens, such as American and European Foulbrood, as well as chalkbrood. Her main ambition is to understand how insects adapt to the stressors in the environment and how to develop applications to safeguard insect health.

## HONEYBEE VACCINATION – A NEW FRONTIER IN ANIMAL HEALTH SECTOR

Insects lack antibodies, the carriers of immunological memory that vertebrate mothers pass on to their offspring. However, our research has shown that an insect mother exposed to pathogens can prime her offspring's immune system. Until now, it has remained a mystery how insects achieve specific trans-generational immune priming without antibody-based immunity. Here, we reveal how honeybees accomplish this through an egg-yolk protein called vitellogenin, which binds to bacterial fragments and transfers them to the eggs. Our findings demonstrate that this natural form of "vaccination" occurs against various diseases and that honeybee queens, when orally exposed to pathogen fragments, can boost the immunity of their offspring. Honeybees face numerous diseases at every stage of their lives, many of which are highly infectious and harmful. Current treatments are not always achieving desired results and can lead to significant losses of honeybees. Moving forward, it is crucial to prioritize honeybee health as seriously as we do that of other livestock. The development of the first vaccine to prevent the spread of AFB marks a significant step towards this goal. This conditionally licensed vaccine was introduced in the U.S. in December 2022 and is currently being used in thousands of hives. The aim is to slow down and ultimately prevent the spread of this highly contagious diseases, while also encouraging a rethinking of bee disease management strategies.

# Olli Loukola

Olli Loukola is a behavioural ecologist deeply fascinated by animal intelligence and social learning. Specialising in bumblebee cognition, his recent work challenges the conventional belief that insects have only basic learning abilities. Instead, he has uncovered evidence of complex cognition in bumblebees, showcasing their ability to adapt and innovate in response to new challenges.

Collaborating with a dedicated team, Olli Loukola explores various aspects of bumblebee cognition, from spontaneous learning to complex cooperation. Their findings highlight the impressive cognitive potential of bumblebees, showcasing their capacity to innovate in the face of new challenges.

With a PhD from the University of Oulu, focusing on social information use in passerine birds, his academic journey continues at the University of Oulu. He has also conducted research at Queen Mary University of London.

Currently, Olli Loukola's research centres on the buff-tailed bumblebee (*Bombus terrestris*), aiming to unravel the intricacies of their cognitive processes. Through interdisciplinary approaches, he aims to deepen our understanding of animal cognition and its ecological and evolutionary implications.

## COMPLEX COGNITION OF BUMBLEBEES: SPONTANEOUS LEARNING TO COOPERATION

Bumblebees (Bombus terrestris) demonstrate remarkable cognitive abilities that challenge our understanding of insect intelligence. This talk will present findings from two recent studies on bumblebee cognition. The first study explored cooperative behaviour in bumblebees through tasks requiring them to cooperate with a partner – push a block and touch a door simultaneously with their partner– to access a reward. Results indicated that bees' cooperative actions are socially influenced, with bees showing signs of active coordination, such as facing their partners during critical moments. The second study investigated bumblebees' spontaneous tool use. Bees were trained to associate a blue ring with a reward by moving a ball. In a novel setting, bees successfully moved the ball to a new location and used it to reach the reward, demonstrating cognitive flexibility and insight learning. These studies highlight the sophisticated nature of bumblebee cognition, showcasing their ability to engage in socially influenced cooperation and solve novel problems spontaneously. This talk will provide insights into the complex cognitive processes underlying bumblebee behaviour, offering a fresh perspective on insect intelligence.

# Stefan Mandl

Stefan Mandl is the founder of the company “Bienenhof Mandl”, which is located in Traiskirchen near Vienna. The 40 employees are caring for 15.000 bee colonies all over Vienna and Lower Austria.

It started 27 years ago with one colony of bees and little knowledge, since then we have been able to at least increase the number of colonies. We have succeeded in setting up an organically run beekeeping operation that is able to ensure widespread pollination. There is close cooperation with farmers whose crops require insect pollination, e.g. B. rapeseed, sunflower, oil pumpkin or seed propagation.

During the season, our bees pollinate around four billion flowers a day which has a significant positive impact on our ecosystem. We produce honey, bee pollen, propolis, beeswax and most importantly queens and bee colonies for sale. Recently, we added breeding bumble bee colonies to our list, to give them to interested garden owners. With this comprehensive range of products, it is possible to offer around 40 families a secure livelihood, to help Austrian beekeepers with bee colonies after lossy winters and to ensure nationwide pollination. We select our honey bees for Varroa tolerance to make them more resistant to this parasite and to offer a solution to the mass colony losses caused by the mite.

## BEE HEALTH: BREEDING FOR VARROA RESISTANCE

Bienenhof Mandl (bienen.at) is engaged in breeding for varroa resistance across its 15,000 bee colonies, focusing on the six historic Austrian Carnica lines: Wintersbach, Troiseck, Peschetz, Bukovsek, C1, and Putz.

Performance testing is conducted annually on approximately 10% to 15% of the hives by our team of 40 employees. The queens in these test colonies are in their first production year and are daughters of selected ancestors. They are mated either at our isolated mountain mating stations or through artificial insemination.

Our systematic testing includes the following assessments: honey yield measurement, varroa mite counts in spring, summer, and autumn (using icing sugar three times), pin test, monitoring for swarming, evaluating gentleness, observing hive occupancy, and finally, SMR (suppressed mite reproduction) counting during winter.

The estimation of breeding values is managed by the Bee Institute Hohen Neuendorf, Berlin, and the results are published on beebreed.eu. On this platform, we are listed as the "Carnica Austrian Professional Beekeepers" ("Carnica Österreichische Berufsimker") under AT-2 Bienenhof Mandl, collaborating with around 50 other professional breeders.

In total, we test approximately 2,000 colonies each year, achieving promising results in varroa resistance. Last year, 37.7% of the test colonies required no varroa treatment.

Importantly, this resistance has not led to a significant reduction in other desirable qualities of the honeybees.

While the selection process is still ongoing, the promising results so far indicate that breeding for genetic resistance to Varroa is a crucial step towards developing colonies that can cope with the mite without the need for human intervention.

# Tia-Lynn Ashman

Distinguished Professor of Biological Sciences, University of Pittsburgh, Pittsburgh PA. She received her Bachelor's degree from the University of California at San Diego, and PhD from the University of California at Davis, and was Postdoctoral Scholar at McGill University.

Her work focuses on the plant-pollinator interaction in native habitats and explores how other biotic associates (floral microbes and pollen viruses) impact and/or benefit from the association, and how it contributes to biodiversity maintenance. Her work mines the interrelationship between ecology and evolution and spans scales of single interacting populations to diffuse interactions within whole communities.

Dr. Ashman has published 203 publications and has an H index of 66. She is the recipient of a Humboldt Award from the Alexander von Humboldt Foundation, the Distinguished Naturalist Award from American Society of Naturalists, Helmholtz International Fellow Award and a Provost's Award for Excellence in Doctoral Mentoring from University of Pittsburgh. She has served on the Editorial boards of several prominent journals including The American Naturalist, Ecology, and New Phytologist, as well as on councils of American Society of Naturalists, and Society for the Study of Evolution. She is deeply committed to outreach and expanding diversity within science.

## CONSEQUENCES OF SHARING POLLINATORS IN THE ANTHROPOCENE

Native flowering plants most often exist in multispecies communities where they share pollinators. Anthropogenic activities are increasingly being recognized as forces altering the structure of plant-pollinator communities via both species gains and losses, potentially changing the functional aspects of the of these communities. The quantity and quality of pollen transferred within and between plant species is well recognized to determine reproduction, population abundances, and mediate selection on floral traits and mating systems, but also may also be critical to pathogen transmission. I will draw from a diversity of studies in my lab to illustrate how community-level patterns in pollinator sharing result in variation in pollen transfer among plants. I further will discuss how plant traits, pollinator traits, changes in plant and pollinator communities, and surrounding landscapes can affect pollen transfer. Finally, I explore the ways these features cascade to impact plant reproduction via the quantity and quality of pollen transferred, fitness lost through improper (heterospecific) pollen transfer and potentially via pollen-associated viruses transferred among plants. Appreciating this panoramic view of pollinator-mediated interactions in plant communities will help efforts to conserve natural biodiversity as well as promotes sustainable agricultural practices.

# Pilar De la Rúa

Pilar De la Rúa is a Professor of Zoology at the Faculty of Veterinary Medicine of the University of Murcia (Spain). Her main interest is to understand how different factors influence the conservation of pollinators biodiversity, with a special interest in honey bees and bumblebees. She is particularly interested in how the development of certain beekeeping activities (introduction of subspecies, transhumance, etc.) affects the genetic diversity and health of the honey bee. In recent years, she has also conducted research in the field of parasitology and landscape ecotoxicology to understand how different bee species are exposed to and affected by the spread of pathogens and the use of pesticides in the landscape.

She was the first woman president of the European Association of Apidology (2012-2014) and the main organiser of the EURBEE6 congress held in Murcia in September 2014. Since 2021 she has been Director of the Office for the Transfer of Research Results at the University of Murcia.

Profiles:
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## CONSERVATION GENETICS OF BEE POPULATIONS WITH A FOCUS ON HONEY BEES, STINGLESS BEES AND BUMBLEBEES

Bee species, such as honey bees and stingless bees, have been used by humans for centuries as food providers. Additionally, in recent decades, due to their primary role as pollinators, some bee species are being managed for crop pollination. Due to these crucial functions, the decline in bee populations has sparked significant concern regarding its impact on ecosystem functioning, which could affect different trophic levels and threaten human food security. Conservationists and policy makers are therefore seeking effective strategies to conserve and restore bee populations in all habitats. To aid and support these efforts, researchers use molecular methods, based on the analysis of genetic and genomic diversity, which have become essential tools for developing effective conservation strategies.

In this presentation, I will describe how we have been using molecular tools to characterize bee populations in order to enhance conservation efforts. First, I will present our studies on honey bee populations in island ecosystems and examine the genetic consequences of introducing non-native subspecies. In addition, I will discuss phylogeographic studies on stingless bees to understand how geography and management have influenced the population structure of these pollinators in Mesoamerican ecosystems. Finally, I will address how commercial bumblebee management is altering the genetic diversity and structure of these vital crop pollinators in an important hotspot of bumblebee diversity, the Iberian Peninsula, which is also one of the areas with most intensive use of managed bumblebees for pollination.

The primary objective of these conservation genetics studies is to use the acquired information to improve managed bee breeding programs and to enhance conservation efforts of locally adapted managed and wild bee populations in unique habitats.

# Simone Tosi

Simone Tosi is a biologist interested in the health and behaviour of social and solitary bees. Investigating the multifaceted impacts of anthropic and environmental drivers, his team integrates traditional and novel laboratory, field, and in silico approaches using bees as model organisms.

He explores how multiple drivers, mainly pesticides and malnutrition, shape bee behaviours such as flight, navigation, locomotion, thermoregulation, reproduction, and phototaxis. Through biomonitoring surveys using bees, bioindicators of environmental health, Simone's team and collaborators further investigate environmental contamination and its impact on pollinators.

Simone's work further aims at refining Environmental Risk Assessments to better assist our society protect bees and the environment. Towards this goal, he has served as scientist expert for the European Food Safety Authority (EFSA) and other EU institutions.

His academic journey has led him to study bees in South Africa (University of Pretoria), USA (University of California San Diego), and France (ANSES Animal Health Laboratory). As Associate Professor at the University of Turin (Italy), he now coordinates the Bee Health and Behaviour Laboratory (www.beelab.unito.it) and teaches "Beekeeping", "Biodiversity and management of pollinators", and "Biomonitoring and biodiversity management".

## BEE HEALTH: THE INTRICATE IMPACTS OF PESTICIDES

Modern society increasingly needs to protect humans, animals, and the environment from the risks posed by pesticides. The official Risk Assessment processes used to identify harmful pesticides is however marginally aligned with recent scientific findings and relies on the basic, outdated evaluation of the lethal effects of individual pesticides on honey bees. This raise concerns over the limited understanding of the actual harm pesticides pose to bees, especially given their alarming health decline.

In this talk, I will draw on various studies to discuss the intricate relationship between bees and pesticides. I will first highlight the importance of biomonitoring studies using bees to reveal how multiple pesticides contaminate bees and the environment over time and space. Next, I will delineate the broad range of side-effects that pesticides cause to both managed and wild bees exposed to low, field-realistic levels. I will detail the variety of cognitive, behavioural, and physiological effects posed by individual or combined exposure to multiple stressors, such as malnutrition. I will finally discuss the risk implications of current pesticide uses, further exploring the critical yet understudied process of pesticide "Emergency Authorisations". This commonly used process permits using highly toxic pesticides regardless of risk assessment outcomes, raising concerns on the broad human, animal, and environmental implications and about the enduring state of emergency that acts in derogation of the EU Regulation.

This overview aims to enhance the understanding of bee health and facilitate the uptake of research into policies and practices, towards a greater sustainability of our society and the entire environment.

# 1-2 Bee immunity and diseases

## Oral presentations

Moderators:
Dalial Freitak
Fabio Manfredini

# JUDICIOUS ANTIMICROBIAL THERAPY OF THE ENIGMATIC EUROPEAN FOULBROOD DISEASE

Sarah C. Wood[1], Thanuri L.K. Edirithilake[1], Midhun S. Jose[1], Marie Blanchemanche[1], Mya Desmarais[1], Maria Janser[1], Harmony Woodrow[1], Erin Baril[1], Fatima Masood[1], Marcelo P. Camilli[1], Marina C. Bezerra da Silva[1], Oleksii Obshta[1], Emilio E. Tellarini Prieto[1], M. Fahim Raza[1], Igor Moshynskyy[1], Belarmino Lopes Neto[1], Ivanna V. Kozii[2], Ryan Merkl[1], Elemir Simko[1]

[1]University of Saskatchewan, Western College of Veterinary Medicine, Canada
[2]Prairie Diagnostic Services Inc., Canada

## Abstract

European foulbrood (EFB) is a mysterious and unpredictable brood disease of honey bees caused by larval infection with the bacterium *Melissococcus plutonius*. In North America, the antimicrobial oxytetracycline is widely used as part of an integrated pest management approach to control this disease. By comparison, antibiotic use in beekeeping is not permitted in the European Union. To promote judicious use of antimicrobials for EFB in North America, we developed an in vitro, larval infection and treatment model of EFB, which allows us to investigate antimicrobial alternatives, such as propolis and probiotics, for EFB control. Additionally, we established an in vitro, adult bee infection and antibiotic treatment protocol to allow investigation of the role of adult bees as a reservoir of *M. plutonius*. Using our larval model of EFB, we found that neither propolis nor *Lactobacillus* sp. probiotics prevented clinical signs of EFB in vitro; however, we did observe mild improvement in larval survival from EFB. In contrast, we found that oxytetracycline treatment of both honey bee larvae and adult bees significantly improved their survival and clinical outcomes from EFB, as well as significantly reduced, but did not eradicate, their enteric colonization with *M. plutonius*. Taken together, these results suggest that oxytetracycline is more effective than propolis or probiotics for treatment of larvae with EFB; however, treated larvae may continue to shed *M. plutonius* as adult bees within a colony.

# SOCIAL ENCAPSULATION OF PARASITE EGGS BY HONEY BEE COLONIES

Francesca M. Grech[1], Aura K. Palonen[1], Andrew F. Brown[1], Geoffrey R. Williams[2], Peter Neumann[1], Anna Papach[1]

[1]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[2]Department of Entomology & Plant Pathology, Auburn University, Auburn, AL, USA

## Abstract

Encapsulation is a common defence mechanism exerted by a host insect in response to invasion by a parasitoid. Social insect colonies being superorganisms can display analogues of encapsulation to combat adult nest parasites; however, it has not been reported for encapsulation of parasite eggs. Here, we show that honey bee, Apis mellifera colonies use propolis to encapsulate small hive beetle, Aethina tumida eggs, thereby preventing the spread of larvae after hatching. Oviposition sites (tapped microscope slides with a cover slip in between) with and without beetle eggs were introduced into ten honey bee colonies of mixed European origin. After 24 h, the slides were removed so that the amount of propolis sealing the gap and number of eggs remaining could be quantified. In addition, the maximum egg laying depth and the length of honey bee proboscis were measured. The number of eggs before and after introduction into the colonies did not differ, even though the honey bee proboscis was significantly longer compared to the maximum egg laying depth. Instead, honey bees used significantly more propolis on slides with eggs compared to the controls, indicating that the honey bees were able to detect the eggs. Notably, colony origin was a significant predictor of propolis usage, suggesting genetic variation for this behaviour. Social encapsulation of parasite eggs instead of removing them appears to be an alternative and possibly more sustainable defence against parasite infections. This is the first report of the use of propolis against parasite eggs by a social insect.

# *PAENIBACILLUS MELISSOCOCCOIDES*: A NEW PATHOGEN OF HONEY BEE BROOD?

Benjamin Dainat[1], Jean-Daniel Charrière[1], Vincent Dietemann[1,2]

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

## Abstract

Because honey bee colonies contain many individuals in close physical contact, they are threatened by numerous pathogens that find fertile grounds to multiply and spread. Among these, the two globally distributed brood bacteria *Paenibacillus larvae* and *Melissococcus plutonius* are the causative agents of American (AFB) and European foulbrood (EFB), respectively. By affecting the larvae, these bacteria can lead to colony weakness and even losses. In Switzerland, high numbers of EFB cases were reported each year, leading to an increased research efforts into the epidemiology of EFB. During such research, an unknown bacterium was found in addition to *M. plutonius* in an apiary affected by EFB. This new species belonged to the genus *Paenibacillus* and was named *Paenibacillus melissococcoides*. As this bacterium was associated with a brood disease, we investigated whether it was present in larval jelly and in larvae, and tested, in vitro, whether it was pathogenic to the brood. We also screened EFB diseased and healthy honeybee colonies in Switzerland and sequence databases to determine how prevalent this bacterium is worldwide. To better understand the interaction between bacteria of the genus *Paenibacillus* and *A. mellifera*, we also compared the virulence of *P. melissococcoides* with that of two closely related *Paenibacillus* species, which were previously found to be associated with honey bees. In this presentation, we review what we have learned to date on the ecology, distribution and pathogenicity of this species and discuss whether *P. melissococcoides* is a new honey bee pathogen. The discovery of this new species brings new insights into EFB epidemiology and the suite of bacteria associated with it, as well as a better understanding of the ecology of *Paenibacillus* species when they encounter honey bees.

# DEFENSE MECHANISMS OF HONEY BEE COLONIES AGAINST THE BROOD PATHOGEN *MELISSOCOCCUS PLUTONIUS*

Vincent Dietemann[1,2], Alexis Beaurepaire[1,3], Jean-Daniel Charrière[1], Benjamin Dainat[1]

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[2]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland
[3]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

## Abstract

Honey bees are known to harbor a variety of pathogens. Our knowledge of the nature and diversity of defense mechanisms of honey bees against these pathogens is still fragmentary, hindering our ability to understand host-pathogen relationships and to develop effective control methods to mitigate their negative impact. The aim of our recent studies was to test the involvement of several social mechanisms in colony defense against European foulbrood (EFB), an emerging and economically important brood disease triggered by infection with the bacterium *Melissococcus plutonius*. We tested i) the role of communal feeding in reducing pathogen load, and in particular the bactericidal action of the nutritive jelly produced by nurse bees and fed to larvae, ii) the occurrence of transgenerational immune priming of larvae via previous exposure of the queen to *M. plutonius*, and iii) whether the different worker patrilines found within a colony are differentially affected by the pathogen, hinting at variations of immune defenses caused by polyandry. We found that i) queen and worker jellies, as well as jellies from different colonies, have different bactericidal properties, ii) experimental or natural exposure of queens to viable *M. plutonius* bacteria did not protect their offspring from infection under our exposure conditions, and iii) workers of different patrilines differed in their susceptibility to infections, possibly affecting colony resistance level. Our results improve our knowledge of defense mechanisms of honey bees against *M. plutonius* and open avenues for sustainable control methods of this pathogen via the harnessing and promoting of natural innate or acquired defenses.

# ASSESSMENT OF THE EFFICACY OF EXISTING AND NEW IN FIELD AND LABORATORY *TROPILAELAPS* SPP. DETECTION METHODS

Maggie Gill[1,2], Bajaree Chuttong[3], Paul Davies[4], Dan Etheridge[4], George Tonge[4], Victoria Tomkies[5], Giles E. Budge[6]

[1]Defra, United Kingdom
[2]Keele University, United Kingdom
[3]Chiang Mai University, Thailand
[4]APHA, United Kingdom
[5]Fera Ltd., United Kingdom
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## Abstract

*Tropilaelaps* mites are brood parasites of the Asian honeybee species *Apis dorsata*, *Apis breviligula* and *Apis laboriosa* and they have subsequently spread to colonies of *Apis mellifera* which have been introduced to Asia. Infestations of *A. mellifera* by *Tropilaelaps mercedesae* occur in regions well beyond the distribution of their native Asian honeybee hosts, and therefore the mite is regarded as an emerging global threat to *A. mellifera.* Increased global trade and changes in beekeeping practices provide transmission routes for *Tropilaelaps.*

Laboratory and field standard operating procedures (SOPs) for the detection of *Tropilaelaps* have been used in the UK for several years and are based on techniques outlined by Pettis *et al.* (2013). However, beekeepers frequently objected to the destructive nature of these techniques and there has been scepticism over the efficacy of detecting a mite incursion at potentially low levels.

Colonies *of A. mellifera* in Chiang Mai, Thailand were used to trail existing SOPs for *Tropilaelaps* detection. Methods involving brood uncapping, floor debris sampling, comb bumping and sticky floor inserts were assessed alongside additional detection methods not previously used in SOPs which utilised an alcohol wash, $CO_2$ sampling and icing sugar roll of 300 adult honeybees.

Results showed that existing SOP methods of comb bumping and floor debris sampling were not reliable for the detection of *Tropilaelaps*. Adaptation was required to improve the reliability of brood uncapping to an acceptable level of mite detection and sticky floor inserts reliably detected *Tropilaelaps*. in colonies. Of the additional methods trialled the icing sugar roll was the only method used that reliably detect the presence of mites.

The results from this study have been used to inform government policy decision making in the UK and implement changes to monitoring and detection methods, and has also highlighted the importance of 'real life' trials of detection methods for exotic pests.

# TRACKING THE EVOLUTION OF VIRAL TITERS IN COLONY SUB-POPULATIONS THROUGHOUT THE SEASON AND IN RESPONSE TO VARROA TREATMENT STRATEGIES

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## Abstract

*Varroa destructor* vectors numerous economically important honey bee viruses; however, the full details of the varroa-virus-honey bee dynamic remain to be elucidated. For example, varroa preferentially parasitize drone larvae and adults, yet the role played by drones with respect to viruses is currently unknown. Additionally, the impact of type and timing of varroa treatments on viral titers has not been determined. To address these questions, we ran field studies in southern Ontario, Canada tracking viral titers in colony sub-populations across the season and subjected to varying varroa treatment regimes. From May to October, we sampled worker and drone purple-eyed pupae from cells that were or were not infested with varroa, as well as adult workers and drones. Conditions in the field were masked so as to conduct the sampling blind. Additionally, samples from were anonymized to remove treatment information before being sent to an independent diagnostic laboratory for quantification of DWV-A and DWV-B. Our results highlight the role of drones in the varroa-virus-honey bee system, and underscore the importance of appropriate varroa treatment and timing.

# POPULATION-WIDE MODELLING REVEALS PROSPECTS OF MARKER-ASSISTED SELECTION FOR PARASITIC MITE RESISTANCE IN HONEY BEES

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## Abstract

Implementation of marker-assisted selection (MAS) in modern beekeeping would improve sustainability, especially in breeding programs aiming for resilience against the parasitic mite *Varroa destructor*. Genomically selecting honey bee colonies for natural resistance traits, such as brood-intrinsic suppression of varroa mite reproduction, reduces the use of chemical acaricides while respecting local adaptation. In 2019, eight genomic variants associated with varroa non-reproduction in drone brood (= drone brood resistance or DBR) were discovered in a single colony from the Amsterdam Water Dune population in the Netherlands. As these eight Single Nucleotide Polymorphisms (SNPs) were found in only one ssp. *mellifera* -derived colony, they could not be used directly on a population-overarching scale in the northern part of Belgium (Flanders), with different genetics and preferred subspecies (ssp. *carnica*), environmental conditions and beekeeping practices. To determine whether these eight SNPs remained associated with the DBR trait on a Flemish colony-broad scope, we performed population-wide modelling through sampling of various *A. mellifera carnica* colonies, DBR scoring of varroa-infested drone brood and variant genotyping with TaqMan assays. Novel eight-variant modelling was performed on the obtained population-wide data set and the classification performance of the eight SNPs was evaluated. Besides, we constructed a reduced three-variant model retaining only three out of eight SNPs and found that this model classified 76% of the phenotyped drones correctly.

In a follow-up study, we determined the allele frequencies of all eight genetic variants in more than 360 *Apis mellifera* colonies across the European continent and found that variant type allele frequencies of these variants are primarily related to the *A. mellifera* subspecies or phylogenetic honey bee lineage. Our results show that population-specific genetic markers should always be evaluated in a new population prior to using them in MAS programs.

# THE EFFECT OF OXALIC ACID TREATMENT AGAINST VARROA MITES ON HONEY BEES (*APIS MELLIFERA*)

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## Abstract

The honey bee (*Apis mellifera*) populations are declining, mainly due to the effects of various factors on bees. *Varroa destructor* mites are responsible for the most serious problems with honey bee health worldwide. The mites transmit viruses that are mostly lethal to honey bee colonies. There are several treatments against varroosis; however, natural acaricides such as oxalic acid (OA) have become widespread among beekeepers around the world. These miticides are effective against *Varroa mites*; on the other hand, they also influence honey bees. The aim of this research was to determine the effect of oxalic acid on honey bees (*Apis mellifera*), mainly on the immunity of adult bees and honey bee brood. Methods of molecular biology were used to study protein components involved in the immune responses of honey bee brood. In adult bees, antimicrobial peptides (AmPs) were quantified by ELISA and LC-MS methods, and the antimicrobial activity of hemolymph was determined by diffusion tests. The results of this study showed that long-term exposure to OA resulted in induced humoral immune responses in adult bees. The antimicrobial activity of hemolymph was higher after OA treatment, which correlates with higher levels of antimicrobial peptides in hemolymph. In honey bee brood, relative expression of AmPs defensin and hymenoptaecin were increased after OA treatment. Similar responses of bee immune components were reported after exposure to pesticides. Abaecin, apidaecin, defensin, and hymenoptaecin are peptides produced in responses to pathogens. Our results open a question, why is the immune system activated by OA? Further studies are necessary to reveal whether immune activation is a positive or negative effect of OA treatment.

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# VARROA CONTROL STRATEGIES OF DIFFERENT BEEKEEPER GROUPS AND CORRESPONDING HONEY BEE COLONY MORTALITY IN CENTRAL EUROPE

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## Abstract

Control of the honey bee parasite *Varroa destructor* is still a challenge for veterinary services and beekeepers. Beekeepers usually use more than one treatment per season, but there is a lack of information which treatment combinations they apply and how these affect colony survival. This study aimed to establish the most frequently used treatment strategies in 4 Central European countries, also in context of the size of beekeeping operations. In addition, we determined "the best treatment strategy" (BTS) which was the control strategy that resulted in the lowest winter loss rate. We analyzed winter colony losses from 2 seasons (2019/20 to 2020/21) in Austria, Czechia, Poland and Slovakia. Data was taken from COLOSS surveys of beekeepers on honey bee colony losses. We included data from 8,655 respondents about 171,756 overwintering colonies. Three categories of beekeeping operations were established: small-scale beekeepers (SSB) with ←25 colonies, medium-scale beekeepers (MSB) with 26-79 colonies and large-scale beekeepers (LSB) with →80 colonies. We merged treatment methods into 5 categories based on the drug's active substance or the type of treatment (Biotechnical methods, Organic acids, Essential Oils, Acaricides, Other methods) and pooled 12 months into 4 seasons (Spring, Summer, Autumn, Winter). We used the Classification Tree, part of the Orange Data Mining software to determine BTS. Differences in treatment strategies were observed between countries. Austria differed from other countries, as beekeepers avoided Acaricides. Preferences in Czechia, Poland and Slovakia were quite similar, however, only Slovak beekeepers used Essential Oils more often. BTS was observed in LSB in every country, resulting in a loss rate below 7% of colonies. In each country, either Organic acids or Biotechnical methods were used by beekeepers to reach BTS.

# UNRAVELLING FUNCTIONS OF NITRIC OXIDE IN HONEY BEE HEALTH AND IMMUNITY

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## Abstract

In insect physiology, nitric oxide (NO) serves multiple essential functions. It is a crucial component of molecular mechanisms during physiological processes and also participates in the insect immunity and stress responses. In the context of honeybee immunity, NO is known for its dual role as both a signalling and an effector molecule toxic to microorganisms. However, the mechanisms of concrete molecular processes and NO functions in bees remain unclear and poorly characterised.

According to recent research in the field of honey bee immunity, NO plays a role in the encapsulation process and also participates in the activation of bee hemocytes during foreign material recognition and their immune response to injury. The goal of our project is to further characterise the role of NO in bee immunity, unravel the mechanisms and regulation of NO production and its biological activity at the molecular level, as well as investigate NO's role in bee defences to pathogen infection. NO is primarily produced by specialised enzymes known as nitric oxide synthases (NOS), found in invertebrates with one active isoform. Our research aims to investigate modulations of *NOS* gene expression and enzyme activity of NOS protein during bee development. In our experiment, bees were collected from frames upon emergence and transferred to individual laboratory cages, where they were kept for different time intervals. Preliminary data from qPCR analysis of *NOS* gene expression demonstrate variations in its expressed levels in different body parts of bees, spanning from nurse bees to several-day-old adults.

The purpose of this research is to provide a deeper understanding of NO functions in honeybee defences to pathogen infections, which requires detailed knowledge of the metabolism of NO under physiological conditions. Moreover, the study also provides the groundwork for possible applications in improving bee immunity and health.

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# THE EFFECT OF SYMPATRIC MANAGED AND WILD BEES' FORAGING PATTERNS AND IMMUNITY ON THE INTER-TAXA VIRUS SHARING

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## Abstract

Viruses are considered a major factor underlying global bee declines. Shared floral resources are hypothesized to serve as a main hub for inter-specific virus sharing. Therefore, foraging patterns on different flowers are anticipated to affect virus sharing across taxa. However, differences in immune defences among bee taxa and their possible effects on viral profiles in co-foraging bees are unknown. We surveyed five sites in a Mediterranean agroecosystem in central Israel 5 to 7 times throughout the blooming season, recording bee-flower visits and collecting honey bees (*Apis mellifera*) and three dominant co-foraging wild bee genera - *Andrena*, *Eucera*, and *Hylaeus*. Following the extraction of RNA from individual bees, we sequenced genus-based pooled samples to describe their viromes. We found shared bee viruses (Lake Sinai Viruses) only between *Eucera* and honey bees. These two Exhibited the highest overlap in foraged plant species, mostly legumes and composites, thus supporting the hypothesis that shared floral resources contribute to inter-genus virus transmission pathways. Using individual-based RT-PCR, we found that mean virus prevalence throughout the season was more than twice greater in honey bees than in *Eucera*. Since honey bees, unlike the studied wild bee taxa, are social and live in large colonies, exposure from nestmates may contribute to this high prevalence. The other studied genera did share floral resources, thus, we were curious to know if internal factors, such immune function, affected the differences in the virome of those four co-foraging bee genera.
Therefore, we evaluated siRNA profiles across the four genera as well as *Bombus imaptiens* specimens from Pennsylvania, since siRNA is a key component of anti-viral responses. The profiles were most similar between honey bees, *Eucera* and *Bombus*, all members of the Apidae family. Thus, similarities in foraging preferences and immune responses may underlie the similar viromes found in honey bees and *Eucera*.

# THE CORE VIROME QUEST – BACTERIOPHAGES IN A LARGE EUROPEAN SAMPLE SET OF *APIS MELLIFERA*

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## Abstract

Bacteriophages (or phages, i.e. bacteria-infecting viruses) are known to shape their ecosystems by exerting high selection pressures on their hosts and by their involvement in horizontal gene transfer. Bacteria-dense environments, like animal guts, exhibit a high abundance of phages, presenting an additional layer to the complexity of microbiome interactions. *Apis mellifera* houses a stable set of gut bacteria consisting of only 5 genera. While more and more studies demonstrate the effect of these core bacteria on the bee, the viruses infecting these bacteria are much less investigated.

In our EU-funded study (part of the ITN Viroinf, grant No 955974), we used *A. mellifera* gut samples in the context of the also EU-funded B-GOOD project (grant No 817622) from 8 European countries and 8 hives per country during spring, summer and autumn 2020. Each sampling included a pool of 10 bees whose guts were separated into midgut, ileum and rectum. The total of 450 samples underwent virus-like particle enrichment, DNA/RNA extraction and shotgun Illumina sequencing, followed by de novo metagenome assembly and data analysis with specialised software tools.

We found that phage diversity differs between countries and gut parts but much less between seasons, indicating a temporal stability. Additionally, we identified 97 distinct phage genomes present in all 8 countries, representing approximately a quarter of the total phage abundance. They form 18 putatively novel orders within the *Caudoviricetes* class, the majority of them have the predicted ability to integrate into their host's genome and 32 of them contain potential metabolic genes used by their host. All 5 core bacteria genera are among the predicted hosts of 86 of these phages.

We therefore believe that these shared phages are part of a core honey bee gut virome and thereby play a crucial role in its microbiome – with implications for honey bee health.

# THE EFFECT OF JUVENILE HORMONE ON THE ACTIVITY OF VIRUSES IN BUMBLE BEES

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## Abstract

Bees are essential for pollinating agricultural crops; however, viral diseases like the Israeli acute paralysis virus (IAPV) can risk their health and inflict significant economic losses. Viruses depend entirely on host cell resources for reproduction. Juvenile hormone (JH) regulates bees' adaptation to environmental changes, influencing gene expression, physiology, and behavior. JH also enhances metabolism and reproductive functions while suppressing immune responses. We hypothesized that elevated JH levels create conditions that favor viral replication. To test this hypothesis, three experiments were conducted to observe IAPV replication in the brain, ovaries and fat body of queen bumblebees both before and after hibernation. The first experiment assessed virus replication in queens across four groups: pre- and post-hibernation, each with a live or non-viable virus. It was found that post-hibernation queens, who naturally exhibit higher JH levels, had increased virus replication in all tested tissues. In the second experiment, pre-hibernation queens with naturally low JH levels were treated with either JH or a solvent (DMF), along with either a live or non-viable virus. Results showed that queens treated with JH and a live virus displayed significantly higher virus transcript levels compared to those treated with the solvent, confirming that JH enhances viral replication. The third experiment tested whether artificially increasing JH levels in post-hibernation queens, already high in JH, affected virus replication. Queens were again split into four groups in the same manner. No further increase in virus replication was noted in the JH-treated group, indicating a "ceiling effect" where additional JH does not boost viral activity. These experiments suggest that IAPV may synchronize its replication with the bee's life cycle, regulated by JH levels. This synchronization allows the virus to propagate through generations without compromising the host's viability, ensuring effective transmission and survival.

# HONEY BEE HEALTH: EXPOSURE INDEX GENERATED FROM LARGE DATASETS CORRELATES PESTICIDES BUT NOT PATHOGENS WITH THE AGRICULTURAL ENVIRONMENT

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## Abstract

Declines in insect pollinators have been linked to a range of causative factors such as disease, loss of habitats, the quality and availability of food, and exposure to pesticides. We analysed two extensive datasets generated from pesticides and infectious and parasitic agents (IPAs) screenings. Within the PoshBee framework (www.poshbee.eu), the sentinel bees were deployed in a network of 128 sites in eight European countries adjacent to either oilseed rape fields or apple orchards during focal crop bloom. We measured the field exposure of three sentinel managed bee species (*Apis mellifera*, *Bombus terrestris* and *Osmia bicornis*) to 11 IPAs (6 RNA viruses, 2 bacteria, and 3 microsporidia). Pesticides were screened in two bee species (*Apis mellifera* and *Bombus terrestris*), in beebread (pollen-nectar stores), in pollen collected with traps and directly on flowers and in regurgitated nectar by honey bees and bumble bees. We developed a new index to summarise key aspects of complex pesticide exposure data and to understand the links between pesticide exposure. We found that matrices collected from apple orchards generally contained a higher number of pesticides (7.6 pesticides per site) than matrices from sites collected from oilseed rape crops (3.5 pesticides), with fungicides being highly represented in apple crops. Concerning IPA, we described differences among bee species in IPA profiles - richness, diversity, detection frequencies, loads and their change upon field exposure, and exposure risk- with no clear patterns related to the country or focal crop. We also report positive correlations of IPA loads supporting the potential IPA transmission among sentinels. Our results show that for a complete assessment of pollinator exposure to stressors, it is necessary to consider several exposure routes and multiple species of bees across different agricultural systems.

# INSIGHTS INTO THE IMMUNE RESPONSE OF HONEY BEES (*APIS MELLIFERA*)

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## Abstract

In the temperate climate 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 and is characterised by different physiological parameters (e.g., higher protein and vitellogenin levels in the hemolymph of winter bees). Our previous research revealed differences within the immune response of 10-day-old honeybee workers from winter and summer populations after immune stimulation by bacteria and its inducibility within 24 hours. Winter bees exhibited a more intense immune response, including higher expression of antimicrobial genes and antimicrobial activity and a significant decrease in vitellogenin gene expression and its concentration in the hemolymph.

This research, built on previous studies, is focused on immune system activation, and signalling pathways. 10-day-old worker bees were exposed to oral application of selected immune inducers (lipopolysaccharides, bacteria). After 24 hours, the immune response and activation of signaling pathways were measured at the level of relative gene expression. Activation of humoral immunity was confirmed by detecting humoral immunity components (e.g., antimicrobial peptides) and their gene expression. The results confirmed the activation of Toll and Imd signaling pathways after oral application of lipopolysaccharides. This research was supported by projects QK1910286 and QL24010241 provided by the National Agency for Agricultural Research of the Czech Republic.

# API-VECTORING OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* BY *BOMBUS TERRESTRIS* FOR THRIPS CONTROL IN STRAWBERRY TUNNELS

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## Abstract

Thrips infestations are becoming an increasing problem in tunnel production of strawberries, particularly early in the season, and the growers have limited control options against these pests. Application of the entomopathogenic fungus *Beauveria bassiana* as a microbial control agent in the tunnels represents a promising tool against the pest, but it remains a challenge to target the thrips directly. We investigate the potential of vectoring *B. bassiana* (BotaniGard WP) to strawberry flowers by bumblebees (*Bombus terrestris*), which are already used in strawberry tunnels for pollination. Api-vectoring of microbial control agents is used against grey mold in strawberry production using the 'Flying Doctors' hive design, while few attempts for insect pest control with this method have been made. We evaluate the ability of commercial bumblebees to vector *B. bassiana* to strawberry flowers and study the establishment and persistence of the fungus. We also assess the susceptibility of thrips to *B. bassiana* in the flowers and evaluate the consequences for the bumblebees after *B. bassiana* exposure, both individually and in groups, as well as potential contamination of the hives by *B. bassiana*. Finally, we characterize the bumblebee microbiota after exposure to BotaniGard to identify potential indicators of bee health. The project aims to establish foundations for more widespread use of api-vectoring of microbial control agents beyond control of plant pathogens and to qualify risk assessment when using microbial control agents for insect pest control.

# CHRONIC AND ACUTE BEE PARALYSIS VIRUS INTERACTIONS IN ARTIFICIALLY INOCULATED ADULT HONEY BEES

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## Abstract

While acute bee paralysis virus (ABPV) and chronic bee paralysis virus (CBPV) belong to different taxonomic families, infections can cause similar trembling and paralysis symptoms in adult honey bees even though these neurological signs appear at different times post-inoculation. Outbreaks of either virus can cause drastic mortality rates and the collapse of colonies. However, paralysis viruses can also be tolerated by bees without triggering clinical signs. The factors involved in the onset of clinical signs remain unknown. Since both viruses can trigger similar symptoms and both invade neural tissue, interactions between CBPV and ABPV are plausible, and co-infection may contribute to either virus outbreak.

Here, we co-inoculated emergent honey bees with both virus at different concentrations and followed their *in vitro* survival for 21 days. To mimic natural routes of infection, the varroa-transmitted ABPV was injected while topical applications were performed for CBPV. We quantified ABPV and CBPV loads in individual bees, either alive or dead, at different times post-inoculation. For co-inoculated bees that died after 9 dpi, at medium doses, significantly higher CBPV loads were found in co-inoculated bees than in bees inoculated with CBPV alone. In parallel, we observed that co-inoculated bees died faster between 8 and 13 days post-inoculation than expected for independent effects. These data suggest conditional synergism between the two viruses, regarding both survival and virus replication.

# GAME OF DRONES: THE KEY FOR TRYPANOSOMATIDS' DISPERSAL IN APIS MELLIFERA?

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## Abstract

Regarding honey bee epidemiology and parasitology research, drones are commonly left out due to their lower population and participation in colony duties. However, their movement between colonies during the mating season makes them potential sources for pathogen dispersal. To provide new information on trypanosomatids' intra and inter-colony transmission dynamics, this work addressed whether *Crithidia mellificae* can infect honey bee males and their transmission potential. We detected the trypanosomatid in both castes, at low parasitic loads, in 4/5 colonies screened in natural conditions in the field. Under experimental conditions, drones and workers were inoculated (strain ATCC 30254), kept separately and caged with non-inoculated individuals of the opposite sex, and tested for the presence of the parasite by qPCR after 16 days. Not all workers were positive for infection and transmitted the parasite to a small percentage of the drones caged with them. In contrast, all inoculated drones were positive, transmitting *Crithidia* to most of the workers kept with them. Moreover, horizontally infected drones developed similar trypanosomatid loads to those that received *Crithidia* with the inoculum, but this did not happen to the workers. For the first time, these results suggest that there would be differences in susceptibility and transmission potential related to the host sex and that honeybee drones could play a key role as superspreaders in trypanosomatids transmission dynamics.

# EFFECT OF THE INFECTION LEVEL OF *VARROA DESTRUCTOR* AND RNA VIRUSES IN BEE POPULATION AND COLONY LOSSES

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## Abstract

*Varroa destructor* is the main sanitary problem for honey bees worldwide. This parasite does not only directly affect the bees at the individual and colony level, but it also transmits RNA viruses. Acaricides are applied periodically to control the mite infestation. However, the definition of an economic threshold level has not been established for the treatment application and consequently, it is usually applied according to the calendar. The objective of this study was to analyze the association between the infection levels of *V. destructor* pretreatment with the adult and brood population and the colony losses in later times. An apiary with 37 standardized colonies was settled in Colonia (Uruguay) in autumn 2023. The infection level of *V. destructor* was analyzed in winter and colonies were divided into four groups according to their infection levels with the mite: G1 (<2%, N=7), G2 (between 2 and 4%, N=13) and G3 (between 4 and 8%, N=17). Mite treatments were immediately applied. In spring, adult and brood populations were estimated and nurse bees were sampled to analyze the infection levels with phoretic mite, and the titers of the Acute bee paralysis virus, Deformed wing virus and Sacbrood bee virus (by qPCR). Colonies with more than 4% of *V. destructor* had lower adult and brood populations in the spring and higher colony losses than colonies with lower infection levels with the mite (pretreatment). Virus dynamics in these groups of colonies will be shown. These results show evidence that in the area of this study, infection levels with the mite should not achieve 4% in order to avoid economic losses.

# DEFORMED WING VIRUS AFFECT SURVIVAL OF A SOLITARY BEE OSMIA BICORNIS IN LABORATORY CONDITIONS

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## Abstract

Deformed wing virus (DWV) is a globally distributed pathogen of honeybees. So far, the presence of this and other pathogenic honeybee viruses in other bee species has been confirmed, but there is little data on whether and how these viruses affect the health of solitary bees. The aim of this study was to establish a methodology to compare the survival of *Osmia bicornis* after exposure to DWV genotype A (DWV-A) and genotype B (DWV-B) and check how the virus titer in their bodies changes over time.

Experiments took place at the Institute of Botany of the Jagiellonian University from April to May 2023 and 2024. In the first laboratory experiment, two-day-old male and female red mason bees were fed food experimentally spiked with $10^7$ virus copies (genome equivalents), and then their lifespan was monitored. Post inoculation, they were housed by sex in cages of 30-60 individuals with or without access to sugar solution. To check how the virus titer changed post-inoculation, we collected 4 infected and 4 control individuals every 4 days. Viral titre was determined by qPCR following transcription of viral RNA into cDNA. We statistically analyzed red mason bee survival using Cox proportional hazards regression with cage as a random factor, and we analysed viral titre using a linear model with the fixed factors time and treatment.

The survival of starved bees infected with DWV-B and control female mason bees did not differ (Cox; Exp = 1.563; p = 0.05; nDWV = 191; nC = 175), but males infected with DWV-B had significantly lower survival compared to control (Cox; Exp = 2.129; p<0.001; nDWV = 169; nC = 157). The viral titre of DWV-A differed between control and infected individuals for 4 days, after which all bees were virus-free.

*Financed by PRELUDIUM 22 (MO-2023/49/N/NZ8/04233*).

# COULD HIVE DEBRIS SAMPLES AND QPCR EASE THE INVESTIGATION OF FACTORS INFLUENCING AMERICAN FOULBROOD SPORE LOADS?

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## Abstract

American foulbrood (AFB) is a serious disease caused by the spore-forming bacterium Paenibacillus larvae which can cause honeybee colony death within one season. Determining the number of P. larvae spores present in hives is useful for users for assessing and mitigating AFB risks and can be performed in various bee matrices, including hive debris. The quantitative polymerase chain reaction (qPCR) method is a valuable tool for defining the P. larvae load in beehive matrices. We aimed to analyse hive debris from several Estonian apiaries, using qPCR methodology, and to find correlations between P. larvae spore number and characteristics of the sampling sites, such as apiary dimensions, closeness to other apiaries, and number of apiaries and hives within a 7 km radius. Of the analysed samples, 52.5% were positive for P. larvae, and we observed high variability in spore loads. Most samples showed a low infection rate, with the number of P. larvae spores below 10 CFU/g. A positive correlation was found between P. larvae spore levels and the number of surrounding apiaries and apiary location. Applying P. larvae-targeted qPCR to hive debris could allow the estimation of AFB risk in areas with high apiary density.

# 1-2 Bee immunity and diseases

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## Poster presentations

# ALCOHOL EXTRACT OF GYPSY MUSHROOM INHIBITS THE DEVELOPMENT OF DEFORMED WING VIRUS INFECTION IN WESTERN HONEY BEE

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## Abstract

Deformed Wing Virus (DWV), transmitted by the parasitic mite Varroa destructor, stands out as a prominent cause of decline in Western honeybee (*Apis mellifera*) colonies. Despite this fact, the range of applicable antiviral interventions remains limited, primarily to indirect control by reducing the number of Varroa mites in honeybee colonies. In this study, we investigated the potential antiviral effects of the gypsy mushroom (*Cortinarius caperatus*) against DWV. Our results demonstrate that the alcohol extract of *C. caperatus* effectively prevented DWV infection in both cage experiments and field applications on honeybee colonies, without shortening the lifespan of honey bees. Furthermore, the inhibition of DWV development in *C. caperatus*-treated honeybees in cage experiments was accompanied by significant changes in the gene expression of Tep7, Bap1, and Vago. Importantly, no residues of *C. caperatus* were detected in honey harvested from colonies supplemented with the mushroom extract for winter feeding. These findings suggest that the alcohol extract of *C. caperatus* holds promise as an effective tool to limit the development of DWV infection in honeybees.

## APIARY VS. LABORATORY. CHANGES IN HONEYBEE IMMUNE PARAMETERS

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### Abstract

The honeybee *Apis mellifera* L. is a key pollinator in the world and plays an important role in maintaining biodiversity. Recently, a decrease in the population of honeybee colonies has been observed, which may be due to the increasingly frequent occurrence and spread of honeybee diseases, including nosemosis. Nosemosis causes anatomical and physiological changes in the honeybee intestine, which in turn lead to malnutrition and energy stress in honeybees.

Our experiments examined changes in two parameters of the immune system, i.e. lysozyme-like and phenoloxidase (PO) activities, in 1-day-old, 19-day-old, and 28-day-old healthy and *Vairimorpha ceranae*-infected honeybees kept in the apiary and in the laboratory. The experiments were conducted in May, June, September 2023. Lysozyme-like activity in hemolymph was checked on agarose plates with *Micrococcus lysodeikticus* by observing peptidoglycan degradation zones. Moreover, PO activity was determined in the hemolymph using the spectrophotometric method with L-DOPA as a substrate. The results were subjected to statistical analysis performed using Python version 3.11.

It was shown that the lysozyme-like and PO activities in the 19-day-old and 28-day-old healthy and infected honeybees kept in the apiary, were significantly higher than in the honeybees kept in the laboratory. Moreover, it was also found that, under the influence of nosemosis, the lysozyme-like and PO activities increased, compared to the healthy honeybees.

In our work, we present the first results showing the complex correlation between the conditions of beekeeping honeybees and the analyzed parameters of the immune system. The results contribute to a better understanding of the mechanisms of honeybee immunity and a more accurate interpretation of the results obtained in subsequent laboratory experiments, taking into account the effects of these experiments that may potentially appear in the apiary.

*The work was financially supported by grant number 2021/41/N/NZ6/00735 from the National Science Centre (Krakow, Poland).*

# BIOFILM-RELATED GENES AS POTENTIAL VIRULENCE FACTORS OF LOTMARIA PASSIM: A CRISPR-CAS9 SCREENING

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## Abstract

*Lotmaria passim* is a trypanosomatid parasite of honey bees. It is highly highly prevalent in the honey bee colonies worldwide and significantly reduces honey bee lifespan under laboratory conditions. During its cycle*, L. passim* transitions from a planktonic stage, the promastigote, to an adherent form, the haptomonad, during which it shows an active secretion of extracellular polymeric substances (EPS) involved in the formation of biofilm. EPS matrices have been extensively characterized in fungi and bacterial communities in which they can act as virulence factors. However, its role in *L. passim* is still unknown. Here, we aimed to investigate the role of EPS in the L. passim lifestyle and their potential role in virulence in honey bees. First, we identified EPS-related genes in the genome of *L. passim* from homologous genes in bacteria. Next, we performed a RNA-seq analysis of promastigote and haptomonad-like biofilms to select those genes differentially expressed between parasite forms. Then, we performed a loss-of-function assay using CRISPR/Cas9 in a genetically modified *L. passim* strain and studied the role of these genes in growth dynamics, biofilm transformation and EPS production of *L. passim*. We further explored whether knocked-out *L. passim* cells lines had a different virulence following experimental infections of honey bees. We found that deleted L. passim EPS-related genes from the EPS biosynthetic route have affected their growth rate and biofilm production, indicating the essentiality of the genes in the differentiation and cell reprograming of the parasite. We discuss the implication of these genes and this phenotype modulation in the L. passim lifestyle between its two stages and in its virulence to Apis mellifera. This data will set the ground for further investigations to unveil the molecular basis of parasite differentiation into multicellular biofilms.

# CHALLENGES IN VARROOSIS CONTROL: PRELIMINARY INVESTIGATION OF AMITRAZ RESISTANCE IN VARROA DESTRUCTOR IN PORTUGAL

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## Abstract

Varroosis is a disease caused by the ectoparasitic mite *Varroa destructor*, identified as one of the most significant global threats to the honey bee (*Apis mellifera*). The most effective control of this mite is through synthetic or organic acaricides. However, the excessive and repeated use of synthetic acaricides has led to the development of resistance. Amitraz is a synthetic pesticide commonly used in the control of *V. destructor*, but resistance to this compound has been observed. Previous studies observed a substitution of asparagine by serine at position 87 (N87S) of the Octβ2R gene, associated with amitraz resistance in France, and a substitution of tyrosine by histidine at position 215 (Y215H), associated with amitraz resistance in the USA. Building upon this knowledge, we aim to implement the first screening in Portugal for mutations associated with *V. destructor* resistance to amitraz. Unlike several European countries and the USA, Portugal lacks information regarding gene variation implicated in *V. destructor* resistance to amitraz, as well as allelic frequencies and their geographical distribution. To investigate the resistance mechanism, primers were developed to amplify the two known target regions of amitraz in *V. destructor*. DNA was extracted from individual female varroa mites using a commercial extraction kit, and the obtained DNA was PCR-amplified with the developed primers, followed by Sanger sequencing. With the knowledge obtained, we hope to assist beekeepers in selecting the most suitable acaricide to manage *V. destructor* in their apiaries and gain a deeper understanding of amitraz resistance in Portugal.

*Project "MITE- Varroa e vírus transmitidos: MonItorização de muTações e dEsenvolvimento de ferramentas moleculares inovadoras" is funded by National Beekeeping Programme 2023-2027. FCT provided financial support by national funds (FCT/MCTES) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021).*

# CONSIDERATIONS WHEN SAMPLING HONEYBEE (*APIS MELLIFERA*) COLONIES FOR VOLATILE ORGANIC COMPOUNDS ASSOCIATED WITH HONEY BEE DISEASES, PESTS AND ENVIRONMENTAL CONTAMINATION

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## Abstract

It has long been established that odour and pheromones in the form of volatile and semi volatile organic compounds (VOCs) play an important role in honeybee colonies. Honeybee diseases such as American foulbrood and pests such as Varroa destructor, Small Hive Beetle and *Tropilaelaps* cause significant colony losses globally each year. Diseases and pests produce specific VOCs and cause behavioural changes within a colony, therefore altering the colony VOC profile. Due to their wide-ranging foraging activity honeybees collect VOCs from the local environment in the form of nectar, propolis, water and electrostatic particles. Hive samplers collect VOCs indiscriminately, and therefore analysis yields valuable data related to both the colony and the local environment.

Honeybee colonies located at the University of Maryland, USA, were sampled using a variety of samplers (PSP, MonoTraps and Tenax TD) positioned at different location within colonies and for different time periods. GCxGC-TOFMS was used to determine the most effective sampler, position in hive and duration of sampling to detect VOCs associated with either diseases, pests or environmental contamination.

The number and peak area of VOCs detected associated with honeybees increased with duration of sampling but decreased for VOCs associated with environmental contamination. The peak area of honeybee and environmental VOCs detected varied with the type of sampler used. MonoTraps consistently detected the smallest peak areas, with PSPs and Tenax TD being comparable for honeybee VOCs and Tenax TD detecting the largest peak areas for environmental VOCs.

These results have demonstrated the type of sampler and location in hives that have the lowest burden on colonies and beekeeper to effectively detect specific VOCs. Further research will be undertaken to identify honeybee disease and pest biomarkers which could be used for early detection and treatment and to also ascertain the level and distribution of environmental contamination to improve decontamination.

# CONVENTIONAL CONTROL OF VARROA DESTRUCTOR AGAINST AN INNOVATIVE METHOD: A COMPARATIVE TEST

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## Abstract

Varroa mite is one of the biggest challenges in beekeeping almost all over the world. It is due to the ectoparasitic mite *Varroa destructor*, which attacks brood and adult bees. It was first observed in 1904 in Southeast Asia parasitizing the Indian honey bee Apis cerana. Its transfer to the bee *Apis mellifera* was also the cause of its spread to all areas of the world, especially after 1960. The mite reproduces inside the brood cells, where it is very difficult to be treated. For this reason, beekeepers prefer to treat it when there is little or no brood. In the past, beekeepers dealt with the mite with synthetic chemical pesticides (acaricides). Nowadays, the organic oxalic acid is one of the most widespread pesticides worldwide for dealing with the mite, since the shift of European consumers to organic products. This study utilizes a comparison control group design with two groups of bee colonies; one group dealing with the mite in a conventional way, compared with another, in which mites are treated in an innovative way. In the latter, the bee queen is trapped in a cage and when the colony is left without any brood, a treatment with oxalic acid is performed. The experiment is conducted on 20 beehives (2 groups of 10 beehives), which are equal in population, have sister bee-queens and equal infestation by V. destructor. The measurements carried out concern the size of the Colony (brood, population and weight), as well as their honey production. The expected results of the experiment are encouraging, as it seems that healthy and productive Colonies can be maintained without the burden of chemical pesticides.

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# DOES POLLINATION NETWORK STRUCTURE EXPLAIN PATHOGEN SPREAD THROUGH BEE COMMUNITIES?

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## Abstract

Pollinators are paramount in managed and unmanaged ecosystems since most of the angiosperm plants and crops rely on animal vectors for their reproduction. However, such crucial ecosystem service is threatened by a worldwide decline of pollinators. Factors behind this reduction in the abundance and the diversity of pollinators are manifold, but the interspecific transmission of pathogens between managed and wild pollinators seems to be prominent. In this context, most investigations have focused on specific taxa, while the spread of pathogens at community level has been rarely assessed.

Bees exchange pathogens when they share floral resources, and hence, the structure of plant-pollinator networks could shed light on the dynamics of pathogen spread through bee communities. Here, we investigated the prevalence of a widespread pathogen (*Vairimorpha ceranae*) in nine pollination networks sampled from winter to late spring in the Southeast of the Iberian Peninsula. We applied a network approach to discern whether direct interactions and/or total effects of species in networks (i.e. direct plus indirect interactions) explain pathogen spread in bee communities.

Results showed that the prevalence of the pathogen in honey bees, main host of the pathogen in the studied ecosystem, was not a good predictor of the pathogen spread through bee communities. Furthermore, we have observed a temporal mismatch between the pathogen dynamics in managed and wild bees. Pollination networks with more plant species and more diverse interactions had lower pathogen prevalence. However, total effects (i.e. direct and indirect interactions) failed to explain pathogen dispersal across pollination networks. These outcomes show the importance of assessing species interactions and accounting for their temporal dynamics when studying pathogen spread at the community level.

# EFFECT OF DIET AND CASTE ON THE GUT MICROBIOTA OF BOMBUS PAULOENSIS AND BOMBUS BELLICOSUS

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## Abstract

*Bombus* spp. are among the most important wild pollinators in natural and agricultural habitats. These insects are suffering dramatic declines in their populations. Changes in land use, habitat destruction, nutritional stress and pathogens infections have been associated with those declines. As in other species, gut microbiota might play an important role in the maintenance of health. In this study, we examined whether the diet and castes (queen and worker) influenced gut microbiota and the development of pathogen, under laboratory conditions. Ten fertilized queens of *Bombus pauloensis* and ten of *Bombus bellicosus* were collected and transferred to the laboratory. They were caged and fed with two different diets *ad libitum*: monofloral pollen (*Eucalyptus grandis*) or polyfloral pollen. After the microcolonies were developed, at 5 days, queens and workers from both groups were sampled, guts were extracted, DNA was obtained and subjected to 16S rRNA gene sequencing to evaluate the gut microbiota. DNA was also subjected to PCR to evaluate the presence of *Nosema ceranae*, *Nosema apis*, *Nosema bombi* and *Lotmaria passim*.

The gut microbiota of both *Bombus* species was mainly composed of Snodgrasella spp., *Gilliamella* spp., Bifidobacterium spp. and *Lactobacillus* spp. Pollen diet affected the composition of gut microbiota, monofloral pollen increased the richness in both *Bombus* species, and increased Gilliamella sp abundance.

Regarding the caste, queens of *B. bellicosus* had more diverse gut microbiota than workers, while no difference was observed between queens and workers in *B. pauloensis*. On the other hand, no pathogens were detected in any sample. These results evidenced that nutrition influences the abundance of key components of gut microbiota in B. pauloensis and *B. bellicosus* and contribute to the understanding of the impact of agriculture intensification and bee`s health.

# EFFECT OF LiCl ON SURVIVAL, HYPOPHARYNGEAL GLANDS, OVARIAN DEVELOPMENT AND HEMOLYMPH COMPONENTS OF TELLIAN HONEY BEE WORKERS UNDER CONTROLLED CONDITIONS

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## Abstract

Lithium chloride (LiCl) is a salt that is well-known for its effect against varroa mite (*Varroa destructor*), but to date there are few investigations on its effect on the western honey bees (*Apis mellifera*). For this reason, this study investigates the impact of LiCl on the physiological functions of Tellian bees (*A. m. intermissa*) under controlled conditions. Emergent workers (0-24 hs) were caged (100/cage, in triple) and fed with lithiated syrup at a concentration of 25 mM (T), or sugar syrup as control (C). The titer of hemolymph components (proteins, lipids, and sugars), the development of hypopharyngeal glands, and the ovarian development of queenless bees were monitored at three different times (0, 7, and 14 days). The mortality rate was measured over a period of 21 days. The exposure to LiCl had no effect on the total protein titer in the hemolymph. However, the total lipids titer was almost double ($p<0.05$) at 7 days in treated workers compared to the control. Total sugars in the hemolymph of both C and T workers was saturated, resulting in no significant difference between the groups. No difference in the development of the hypopharyngeal glands between the treated workers and the control batch was observed. Differently, LiCl inhibited ($p<0.05$) the ovarian development of treated workers (2.2 ± 3.8% and 4.4 ± 7.6%, at 7 and 14 days respectively) in comparison to the controls (16.7 ± 5.8% and 15,6 ± 3.8%), and significantly increased their mortality rate. In fact, the survival rate of treated bees decreased from day 10 onwards, with a final survival rate of 70.0 ± 2.6% in treated bees compared to 90.0 ± 1.5% in the control ($p<0.001$). This preliminary study demonstrates a negative impact of LiCl on the survival and ovarian development of queenless Tellian bees.

**Keywords:** lithium chloride; hypopharyngeal glands development; ovarian development; hemolymph components; *Varroa destructor*, *Apis mellifera intermissa*

# ELEMENTAL COMPOSITION OF CHALKBROOD INFECTED LARVAE AND MUMMIES: IMPLICATIONS FOR HONEY BEE HEALTH

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## Abstract

The health of honey bees is influenced by various factors, including nutrition and disease incidence. Chalkbrood is a disease of honey bee brood caused by the fungal parasite *Ascosphaera apis*. In this study we examined the elemental composition of honey bee larvae and chalkbrood mummies to investigate possible links between nutrient availability and chalkbrood disease. The samples came from an urban, disease free apiary and rural apiary showing symptoms of chalkbrood. Sample were analyzed with inductively coupled plasma mass spectrometry after microwave assisted acid digestion. Chalkbrood mummies showed increased levels of macroelements (Na, Mg, P, S, K, Ca) and some microelements (Rb ad Sn). At the same time, the mummies had lower concentrations of B, As, Sr, Ag, Cd, Sb, Ba and Pb. Larvae from infected hives had lower levels of several elements (Al, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, Cs) compared to larvae from the disease-free apiary. The concentrations of elements with potential antifungal properties, B, Al, Zn and Ag were significantly lower in mummies, indicating a possible role of these elements in disease susceptibility. In addition, most elements that were found in lower concentrations in mummies and larvae from infected hives (Al, Cu, Zn, Ni, Cr, Mn, Co, Mo, V, Sr) have known antifungal properties, indicating a possible link between element deficiency and disease onset. Our results suggest that poor larval nutrition may contribute to susceptibility to chalkbrood or may be caused by the infection itself. Further research is needed to clarify the exact mechanisms underlying these relationships and their impact on honey bee health.

# EPIDEMIOLOGY OF *NOSEMA CERANAE* IN THE ABSENCE OF *VARROA* SPP. - LONG TERM SURVEY ON NEW CALEDONIAN HONEY BEE COLONIES

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## Abstract

The microsporidium *Nosema ceranae* is a worldwide spread pathogen that has been found in the tropical New Caledonian archipelago, located in the Pacific Ocean, where the mite *Varroa destructor* is still absent. In this work, three apiaries (Noumea and Vidoire in Grande Terre Island; Hnasse in Lifou Island) were surveyed for a year to detect the percentage of honey bees infected by the microsporidium along with the record of colony population and climatic conditions. The percentage of infected honey bees varied significantly among the apiaries and fluctuated over time, being Hnasse the location with higher number of infected honey bees in contrast to Vidoire, which maximum parasitism point was 20% during the study. The monthly mean precipitation and temperature were positively correlated with the *N. ceranae* level of infection in the colonies, whereas the humidity and the wind speed did not show any effects on it. The colony population fluctuated among the apiaries, being Hnasse the one with highest number of adults on average. However, colony population was not correlated to *N. ceranae* infection. Colony mortality only occurred in Hnasse, where one colony died after detecting 95% of *N. ceranae* infection. The rest of the colonies survived the whole study period registering few sampling points with equal or more than 20% of honey bees infected. Among these, one colony reached 40 % of infected honey bees and another 60 % by the end of the study, suggesting that the microsporidium parasitism at low levels does not lead to increased colony mortality.

# EVALUATION OF THE TRANSMISSION ROUTES OF AMERICAN FOULBROOD IN SLOVENIA BY GENOTYPING OF *PAENIBACILLUS LARVAE*

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## Abstract

The control of American foulbrood (AFB) in Slovenia is based on restrictions and time-consuming clinical examinations of all bee colonies within a 3-km radius around the AFB-positive apiary (AFB zone). If a new clinical case of AFB is detected within this zone, the restrictions are extended and the infected area is enlarged. In this study, we investigated whether the 3-km radius covers AFB transmission and all clinical outbreaks are caused by the same *P. larvae* clone.

A total of 202 *P. larvae* isolates from different AFB outbreaks across Slovenia detected in the period 2017–2020 were typed using whole-genome multilocus sequence typing (wgMLST). Based on epidemiological and genetic data, isolates differing in ← 35 alleles were categorized as clonal. The identified clusters were interpreted in conjunction with the associated spatio-temporal data.

Sixteen different clusters (clones) of *P. larvae* were found. Nine clusters were geographically limited, but seven also contained isolates from very distant locations (far outside the same or a nearby AFB zone). When calculating the geographical distances between neighboring apiaries with confirmed AFB for each cluster, we found that the defined 3-km radius covered well all AFB transmissions attributable to natural bee activity. However, the seven *P. larvae* clusters that contained isolates from geographically distant apiaries indicate AFB transmission due to beekeeper activity. Multiple *P. larvae* clones were also found within a single AFB zone, suggesting sporadic AFB cases unrelated to the primary detected case.

This study underlines the importance of unrecognized and unreported clinical AFB cases for the spread of AFB. It also underlines the importance of e.g. migratory beekeeping, exchange of beekeeping equipment or sale of bees for AFB transmission over large geographical distances. Therefore, the introduction of mandatory monitoring and early diagnosis of AFB based on laboratory testing of bee-related samples is highly recommended.

# FIRST DETECTION OF LESS KNOWN HONEY BEE VIRUSES IN APIARIES OF REPUBLIC OF KOSOVO

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## Abstract

The recent decline of honey bees (*Apis mellifera*) population worldwide represents an economic and environmental threat because of their role in pollination. Epidemiological surveys of honey bee pathogens are thus necessary to understand and prevent further losses of bee colonies. Recently, less known honey bee viruses were discovered to infect honey bees worldwide. However, our knowledge on their possible clinical signs, if any, and their interactions with the host and other pathogens is still limited. Following the current collaboration with the Veterinary Laboratory of the Republic of Kosovo, focused on the presence of honey bee pathogens, the occurrence and distribution of less known honey bee viruses such as *Apis mellifera* Filamentous Virus (AmFV), Apis Rhabdovirus-1 (ARV-1) and Apis Rhabdovirus-2 (ARV-2), Bee Macula-like Virus (BMLV), Lake Sinai Virus (LSV) and Aphid Lethal Paralysis Virus (ALPV) were investigated in the same apiaries previously tested. Adult honey bees were collected from eight apiaries from eight Kosovar municipalities during a passive surveillance program for honey bee diseases. DNA and RNA were extracted pooling five bee specimens per apiary and then amplified by PCR and RT-PCR; viral presence was further confirmed by Sanger sequencing. Among the known viruses, DWV and BQCV were the most prevalent, detected in all the apiaries analysed, followed by ABPV (7/8 apiaries), SBV (2/8 apiaries), and CBPV (1/8 apiaries). Of the less known viruses investigated, AmFV was detected in six out of eight apiaries, ARV-1 and ARV-2 (always in co-infection) and BMLV were detected in five apiaries, while LSV only in one apiary. No samples tested positive to ALPV. These results represent the first detection of less known and never investigated viruses in honey bees from the Republic of Kosovo and update the current epidemiological data about these viruses in this country and in Europe.

# FURTHER INVESTIGATION OF REGULAR DORSAL DIMPLES ON *VARROA DESTRUCTOR*, A DEVASTATING PARASITIC MITE OF HONEY BEES

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## Abstract

Mites of *Varroa destructor* (Anderson and Trueman) parasitize multiple life stages of *Apis mellifera* L. In an earlier study (Apidologie 40:151-162), evidence suggested that regular dorsal dimples (RDD) on the dorsal shield are developmental faults, rather than damage symptoms, in 10.6% of adult female mites. RDD exist as indentations at a maximum of two predictable, symmetrical locations per mite, situated directly above a set of obliquely-aligned muscles running dorso-ventrally within each side of the mite's body. These muscles appear to slowly expand and contract - typically independently from the set of muscles on the body's other lateral side - allowing female mites to ultimately achieve their wide, crab-like shape by adulthood. Recently, we focused on examination of debris nested within RDD on the dorsal surface, plus characterization of the internal musculature below RDD, in resin-embedded specimens. Non-dimpled adult females (controls) were compared to mites having one or two RDD. The attachment points, paths, plus lengths and widths of muscles were tracked and measured from semi-thin anatomical sections stained with toluidine blue 0. Six dorsal-ventral muscles exist per lateral side per mite, but only the outer few muscles showed a significant increase in cross-sectional area, reflecting their relative position beneath the deepest portion of an RDD indentation. Results are consistent with the hypothesis that a mite's rapid sclerotization can fix a sustained, contracted set of the dorso-ventral muscles in place, thus manifesting formation of up to two RDD at an adult mite's dorsal shield, and potentially reducing the female's internal body cavity available for egg development. Indeed, research by others indicated that female mites with RDD may suffer reproductively. Accordingly, strategies that promote RDD formation among mites of *V. destructor* may hold promise for implementation as a non-chemical control to the beekeeping industry largely dependent on acaricides for *Varroa* control.

# IMPACT OF VARROA MITE INFESTATION ON HONEY BEES: INSIGHT FROM PROTEOMICS AND METABOLOMICS

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## Abstract

*Varroa* mites pose a significant threat to honey bee populations worldwide, impacting colony health and productivity. Parasitism by the *Varroa* mite is often associated with deformed wing virus (DWV) co-infection. In this study, we investigated the effect of *Varroa* mite infestation on short-living summer honey bees through comprehensive proteomics and metabolomics analyses, accompanied with the DWV loads evaluation. Our findings reveal profound alterations in the proteome and metabolome profiles of *Varroa* parasitised honey bees, indicative of systemic physiological perturbations. Specifically, we observed dysregulation in proteins responsible for nutrition, longevity, and hygienic behaviour. In *Varroa* parasitised bees, we detected an upregulation in proteins associated with energy metabolism, stress response, and immune function, which is consistent with our metabolomic data. Additionally, our analysis demonstrated a significant increase in DWV loads in *Varroa* parasitised honey bees, confirming the role of *Varroa* mites as vectors for viral transmission within honey bee colonies. These findings shed light on the multifaceted impact of *Varroa* mite infestation on honey bee health during the summer period.

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## INVESTIGATING THE HONEY BEE APOLIPOPHORINS

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### Abstract

Apolipophorins (ApoLp) are a group of insect proteins belonging to the large lipid transfer protein superfamily. They ensure the transport of lipids, carbohydrates, and other hydrophobic compounds by forming lipophorin particles. Lipophorins are assembled in the fat body and after their release to the hemolymph operate as reusable lipid shuttles between the fat body and target tissues such as flight muscles or epidermis. Our research focuses on the ApoLp I and its precursor, the ApoLp II/I. Based on shared functional domains with another pleiotropic protein, vitellogenin, we believe that these proteins may substitute vitellogenin in some of its functions and that their research in the context of immune responses and physiological adaptations may bring new insights into honey bee biology.

So far, in-house monoclonal antibodies have been deployed to determine the variation of ApoLp I in different contexts using the western blot technique. We observed that the ApoLp I level varies through the seasons and shows a reversed trend compared to vitellogenin. We also observed increased ApoLp I levels in bacteria-infected bees. These findings support our hypothesis that apolipophorins are multifunctional proteins playing a crucial role in honey bee physiology and immunity. This will be followed by tandem mass spectrometry-based techniques to determine interacting partners of ApoLp I and knock-down experiments to shed more light on its function.

*Our research is supported by the Ministry of Agriculture of the Czech Republic's projects QK1910286 and QL24010241.*

# INVESTIGATION OF DEFORMED WING VIRUS IMPACTS ON *APIS MELLIFERA* BROOD THROUGH ARTIFICIAL INOCULATION SIMULATING THE TRANSMISSION ROUTE BY *VARROA DESTRUCTOR*

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## Abstract

Deformed wing virus (DWV) and its vector, *Varroa destructor*, are among the most serious threats to honey bees worldwide. Their closely linked relationship significantly impacts honey bee health and colony survival with noteworthy implications for agriculture and ecosystem health. As the transmission route seems to influence the severity of the infection, trials of artificial injection were conducted mimicking the transmission through varroa. Two solutions with different viral loads were utilized ($10^2$ and $10^7$ DWV copies) to study after the injection the effects on mortality, clinical symptoms, and viral load progression in honey bees . In each experiment replicate, one group was also inoculated with an inactivated virus solution using UV irradiation. Our research highlights the negative effect of viral load on the development stages of bees. In each case, mortality was directly related to the level of viral load with which individuals in each group were infected. Nymphs that did not receive any treatment exhibited a constant load, while a progressive increase was observed in the other groups. The day after the injection, a dark coloration was observed concentrated or diffused in the bodies of nymphs in all groups except the control. The phenomenon was more frequent and severe in the group injected with $10^7$ viral copies and became more intense over time. In conclusion, it appears that the impact of the DWV on brood is related to the viral load introduced through direct injection into the hemolymph. It is also assumed that the injury from varroa feeding or the introduction of the solution as foreign substance may result in adverse effects. This underscores the significance of early mite control and maintaining virus levels within safe thresholds to support optimal brood development and colony viability.

# LAKE SINAI VIRUS IN AZOREAN HONEY BEES: UNDERSTANDING THE IMPACT OF *VARROA DESTRUCTOR* ON PREVALENCE, LOADS, AND STRAIN DISTRIBUTION

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## Abstract

Lake Sinai virus (LSV) is a pathogen affecting honey bees worldwide. It was first discovered in 2009 in Lake Sinai, USA, and manifests as a multi-strain virus. The Azores archipelago comprises islands with and without *Varroa destructor*, offering a unique setting for studying viral epidemiology. Building upon insights from the heterogeneous distribution of Deformed wing virus (DWV) strains, which was modulated by varroa's invasion, this study sought to evaluate prevalence, load, and diversity of LSV in the Azores, and explore potential changes in the viral landscape attributable to the mite's presence. In July/August of 2014/15 and 2020, 494 colonies were sampled across the archipelago. These were screened for LSV using RT-qPCR with a primer pair that allowed detection of at least four strains (LSV-1,-2,-3, and -4). Positive samples were further examined by high-throughput sequencing (HTS). The impact of varroa on prevalence and loads was evaluated using general linear mixed models in the framework of Bayesian analysis. LSV was detected on all islands (prevalence range: 7.7%–89.9%), with varroa's presence significantly increasing prevalence (mean increase: 19.5±9.5, Probability of increase=97.6%). HTS identified two known strains (LSV-2, LSV-3) and one novel strain (LSV-9). Phylogenetic reconstruction revealed a strong geographic structure in which LSV-2 was found dominating all varroa-infested islands (Flores, Faial, and Pico) and one varroa-free island (São Jorge). LSV-3 and LSV-9 dominated varroa-free islands, Graciosa and Terceira, and São Miguel and Santa Maria, respectively. Viral loads varied greatly among islands, from 4.77 log10 copies/bee to 8.71 log10 copies/bee. Varroa's presence affected LSV-2 loads, with a mean increase of 2.5±0.7 log10 copies/bee (Pr=100%). Our findings highlight mite-driven evolutionary changes in LSV in the Azores and identify a novel strain dominating the easternmost islands, coinciding with the unique refuge of DWV-C existing in this part of the archipelago.

# MAPPING CHRONIC BEE PARALYSIS VIRUS (CBPV) IN HONEY BEE COLONIES

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## Abstract

Chronic bee paralysis virus (CBPV) is responsible for an infectious disease affecting *Apis mellifera*. Symptoms primarily manifest in adult worker bees, characterized by a swollen, dark, shiny, and hairless abdomen, accompanied by widespread tremors, ataxia, and flight inability. Direct contact and trophallaxis are the main transmission routes of the virus. The clinical signs manifestations are often associated with adverse weather conditions and hive overcrowding, typically occurring in spring during colony development. Recent observations in various Countries have noted an increase in cases and a loss of seasonality in disease incidence.

This study aimed to examine the evolution of CBPV in Italy, analysing data collected from 2009 to 2023 across three discontinuous nationwide monitoring projects focused on non-migratory apiaries. Besides, the CBPV's spatial distribution evolution was studied, identifying high-risk areas for the virus spread.

Results indicate a notable increase in virus spread, with prevalence rising from 4.3% during 2009-2010 to 84.7% during 2021-2023 monitoring years. Outbreaks of CBPV were irregular across investigated seasons, with spring and autumn identified as the most susceptible periods. Over the years, the risk of CBPV infection has increased at the provincial level, starting from initially very low-risk values that have evolved into high-risk areas with highly heterogeneous values. The CBPV sequence analysis of *RNA-dependent RNA-polymerase (RdRp)* gene within the three projects did not differ from 2009 to 2023 and showed higher similarity with other isolated Italian CBPV strains.

This study offers epidemiological insights into the aetiology of this emerging disease. Understanding CBPV distribution is crucial for predicting its future spread, shedding light on propagation dynamics and factors affecting honey bees and other pollinators and environments.

# MICROORGANISMS ON HONEY BEE DRONE LARVAE AND VARROA MITES – IMPLICATIONS FOR HIVE HEALTH AND FOOD SAFETY

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## Abstract

Varroa mites are detrimental to hive communities as they can cause infectious disease outbreaks by weakening honey bees' immune system and acting as viral vectors. Varroa mites prefer honey bee drone larvae due to their longer larval phase than worker bees. Therefore, honey bee drone larvae are usually removed from the hive to reduce mite infestation. Honey bee drone larvae are also generating more interest as a novel food rich in protein, fats, vitamins, and minerals. Thus, it is important to identify potentially harmful microbial contamination on these larvae. Furthermore, honey bee drone larvae microbiota could be indicative of the overall microbial status of the hive.

During this study, culturable bacterial and fungal microorganisms distributed on honey bee drone larvae and Varroa mites were isolated and investigated using microbiological and molecular biology methods. The molecular analysis of morphologically distinct microorganisms allowed us to detect *Candida* spp. and *Metschnikowia* spp. yeasts as well as bacteria from *Staphylococcus*, *Hafnia*, *Bacillus,* and *Micrococcus* genera. The biocontrol properties of identified microorganisms and the antimicrobial efficiency of plant-origin essential oils (EOs) against honey bee contaminants were analyzed by focusing on the prevention of honey bee diseases. Using the disk diffusion method, it was identified that oregano, lemongrass, and lemon balm EOs displayed the greatest antimicrobial potential. Yeast *Candida* sp. was more resistant to the effect of EOs than bacteria, of which *Staphylococcus* sp. was the most sensitive.

Data on honeybee drone larvae-associated microorganisms is important for the safety of insect-based food production and comprehensive analysis of natural, human, and environmentally friendly antimicrobials is relevant for improving overall hive health.

# MOLECULAR DETECTION OF VIRAL PATHOGENS IN BEE PRODUCTS

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## Abstract

Several pathogens affecting honey bees have already been identified in bee products, including honey, pollen, bee bread and royal jelly. A total of 29 pollen samples and 15 royal jelly samples were collected from different regions of Bulgaria. Total RNA was extracted from each sample and used for reverse transcription polymerase chain reaction (RT-PCR) analysis. The objective of the present study was to investigate the incidence of the most widely distributed honey bee viruses, including Deformed wing virus (DWV), Sacbrood virus (SBV), Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV) and Chronic bee paralysis virus (CBPV) in pollen and royal jelly. DWV and SBV were identified in the pollen samples. DWV, BQCV, ABPV and IAPV were found in the royal jelly samples. Our results demonstrate that pollen and royal jelly are suitable sources for honey bee RNA viruses. This is the first instance of the molecular detection of viral pathogens in pollen and royal jelly in the Balkan region.

Keywords: honey bee viruses; pollen; RNA; RT-PCR

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# MONITORING OF DISEASES AND PARASITES IN MANAGED HONEY BEES IN EUROPE FOR THE H2020 PROJECT B-GOOD

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## Abstract

Managed *Apis mellifera* colonies from 14 European countries were sampled three times per year (spring, summer, autumn) as part of the Horizon 2020 project B-GOOD (Giving Beekeeping Guidance by cOmputatiOnal-assisted Decision making). Sampled bees were examined at two National Reference Laboratories for Bee Diseases, for the presence of 11 important pathogens.

During three years (2020, 2021, 2022), eight colonies from eight research institute apiaries (Belgium, Switzerland, Germany, France, United Kingdom, Netherlands, Portugal, Romania) were sampled. In five countries (Switzerland, Germany, Finland, Italy, Netherlands), three colonies from eight beekeepers were sampled in 2021 and 2022. Additionally, in 2022, bees from three colonies of 56 beekeepers from 11 countries (Belgium, Switzerland, Germany, France, Greece, Italy, Latvia, Netherlands, Poland, Portugal, Sweden) were collected. Over the three years, 1580 samples from 358 colonies were examined. All samples were tested for the viruses DWV-A, DWV-B, BQCV and SBV and the level of infestation with *Varroa destructor* was determined. All spring and summer samples were also analysed for *Nosema apis* and *Nosema ceranae*. Additionally, spring and autumn samples were tested for ABPV and CBPV and autumn samples were tested for foulbrood (*Paenibacillus larvae* and *Melissococcus plutonius*).

The results give an overview of the honey bee health situation in Europe for 2020-2022. Differences in the levels of parasites and pathogens were observed between different countries and seasons. The most prevalent viruses were BQCV, DWV-B and SBV, although with variable viral loads in the different countries. *Nosema apis* seems nearly vanished in Europe, while *Nosema ceranae* was frequently detected. We found only few cases of foulbrood. The frequencies of different viral loads were used to suggest a threshold for each virus above which occurrence of clinical symptoms is more likely. The relationship between presence and intensity of pathogen infection and the death of colonies during winter was studied.

# NOSEMA (APIS AND CERANAEE) AS HONEY BEE WELFARE INDICATOR

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## Abstract

It is known that Animal Welfare in farming leads to healthier, resilient animals with higher productivity and fewer treatments. In beekeeping, as attempt to mitigate hive depopulation and losses, effects efforts are underway to define and apply it practically. Honey Bee Welfare (HBW) strives for equilibrium within a semi-natural environment and a managed agroecosystem framework. Biosecurity Measures (BMs) and Good Beekeeping Practices (GBPs) serve as crucial guidelines, while Animal-Based Measures (ABM) provide observable indicators of their success.

Welfare addresses stressors comprehensively, serving as a valuable tool for understanding and preventing hive depopulation and losses, and other multifactorial conditions prevalent in the modern world.

Nosema ceranae and Nosema apis alongside with other potential indicators like Wax moth, American and European Foulbrood, are compelling for future research as they reflect the quality of beekeeping practices and of environmental impact in consideration of bee health, management, pesticide exposure, and nutrition. Nosemosis, especially classic (Nosema apis), presents gastrointestinal and hypofaringeal disorders, leading to springtime losses with weakness and lack of brood. Nosema ceranae, (gastrointestinal nosemosis), associates with winter losses, emerging in suboptimal conditions and facilitating virus spread among apiaries.

Diagnosis involves methods like culture, PCR, ELISA-TEST, and Immunochromatography, with spore count/bee categorized from LOW to MEDIUM to HIGH; but this aspect is not practical for Welfare assessment. On the other side as indicator of prevalence it is possible to promptly assess Nosema through direct observation and sampling (diarrhoea in case of N. apis, general weakness) and empirical method (sampling of worker bees and observation under the microscope on field) even if it may be less precise. Nosema spp. Prevalence may serve as a low-stress indicator for honeybee general welfare, aligning with One Health and One Welfare approaches. Further research is needed to enhance diagnostic techniques and understand Nosema's role in bee welfare.

# *NOSEMA CERANAE* DETECTION IN YELLOW-LEGGED HORNET, *VESPA VELUTINA NIGRITHORAX*, IN NORWEST OF SPAIN

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## Abstract

The *Vespa velutina nigrithorax* is an invasive hornet from Asia that is causing significant concern for its impact on the European beekeeping sector. Its high adaptability and reproductive capacity have enabled the registration of over 50,000 nests in Galicia (northwest Spain) in 2023. It has been estimated that more than 20,000 individuals may emerge from each colony. This hornet consumes large quantities of honey bees in apiaries to satisfy the protein needs of its larvae. It has been demonstrated that this hornet can contain various pollinator pathogens after consuming its preys. The microsporidium *Nosema* provides a useful illustration of this. *N. ceranae* is a pathogen that causes a disease called nosemosis, which infects adult honey bees and contributes to colony depopulation. The presence of *N. ceranae* in *V. velutina* could have an impact on the dynamics of native host pathogens and contribute to the spread of this parasite. Therefore, there is a need for further knowledge on the presence of *N. ceranae* in *V. velutina*.

A total of 62 individuals of *V. velutina* were collected from 11 apiaries located in different municipalities of Galicia to analyze the presence of *N. ceranae*. After DNA extraction, *N. ceranae* was determined in the *V. velutina* specimens by PCR amplification. *N. ceranae* was detected in five of the 11 apiaries (Culleredo, San Sadurniño, Gondomar, Fonsagrada, and Sergude). In these apiaries, nine hornets were found to be positive for *N. ceranae*. The results of this study provide information on the presence of *N. ceranae* in *V. velutina*, which is likely to have been acquired from honey bee consumption. This study suggests that *V. velutina* may contribute to the spread of this pathogen, which could represent a potential long-term risk for apiaries.

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# OCCURRENCE OF THE ABPV-KBV-IAPV COMPLEX IN ITALIAN APIARIES

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## Abstract

Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), and Israeli acute paralysis virus (IAPV) are a complex of viruses generally persisting as latent infections in managed honey bee colonies. However, severe infection can lead to colony mortality, especially in association with high *Varroa destructor* infestations. Despite their potential lethality, the incidence of these viruses is often less monitored. Thus, a comprehensive picture of their distribution and prevalence is lacking in many countries. To fill this gap, honey bee samples from 370 apiaries located in all Italian regions were collected from June 2021 to March 2022 and analysed by molecular method to assess the prevalence and abundance of ABPV, KBV, and IAPV in Italy. This activity was carried out within the Italian project "BeeNet – monitoring the environment through bees and biodiversity". While IAPV was never detected, ABPV and KBV were found with a prevalence of 16.45% and 0.67%, respectively. Most ABPV infections occurred in late summer-autumn and were concentrated in the regions at the northern and southern extremes of Italy. The sporadic detection of KBV did not allow the definition of significant seasonal or geographical trends. Different climatic conditions, *Varroa* infestation dynamics, and virus genetic variances may have influenced the spread of infections leading to differences among regions and seasons.

# PREVALENCE AND DISTRIBUTION OF PEST AND PATHOGENS IN LATIN AMERICA: A PRELIMINARY REPORT

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## Abstract

Latin America is a wide region with diverse geography and climates that exhibits a vast biological diversity, and is characterized by being a food-producing region. Currently, over 8 million *Apis mellifera* colonies are producing around 70,000 tons of honey. However, LA-managed honey bees are under threat. A high percentage of annual colony losses have been recently reported, with pests and pathogens being one of the main causes. As eusocial insects, honey bees are particularly susceptible to infection by various groups, including wasps, beetles, mites, bacteria, fungi, and viruses. Reliable information on the health status of is a key input to design health policies, to prevent the entry of new parasites (or their variants) into a country, and to avoid colony losses. In this study, we aimed to summarize and update the scientific information regarding the presence and prevalence of the most important honey bee pests and pathogens in Latin America.

We found that Varroa destructor, *Nosema* spp., Paenibacillus larvae, and different RNA viruses are widely distributed in the region. However, the information provided by different countries, based on scientific publications, official reports, or postgraduate theses, is uneven. Some countries provide comprehensive information on the parasite/pathogen species, circulating variants, infection levels, and/or prevalence at national or regional levels. A second group of countries offers limited information and mainly have studies in collaboration with scientists from other countries.

Finally, a third group of countries lacks information. We explored and discussed the potential reasons explaining these differences, including beekeeping practices, productivity, the number of beekeepers, resources dedicated to science and technology, and socioeconomic indicators, among others. This review highlights the necessity to support countries with limited information regarding honey bee pathogens, to develop consensus protocols of monitoring, and to define mitigation methods at a regional scale.

# RESISTANCE OF VARROA DESTRUCTOR TO PYRETHROIDS: A PRELIMINARY ANALYSIS IN PORTUGAL

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## Abstract

Infestations of *Varroa destructor* are closely associated with colony collapse in the honey bee (*Apis mellifera*). Currently, three classes of acaricidal compounds are utilized to combat these infestations: pyrethroids (fluvalinate and flumethrin), organophosphates (coumaphos) and formamidines (amitraz). The first cases of *V. destructor* resistance to pyrethroids were reported in Italy, followed by numerous instances of resistant populations worldwide. The molecular mechanism of *V. destructor* resistance to pyrethroids is well understood and primarily linked to an amino acid change at position 925, where a leucine (L) is typically found. At this position, three alleles have been described that confer resistance to pyrethroids, where leucine is substituted by valine (L925V), isoleucine (L925I), or methionine (L925M). A novel mutation at position 918 was described in a population from Spain, where the amino acid methionine, normally found at position 918, was replaced by leucine (M918L). In Portugal, gene variation associated with pyrethroid resistance remains unknown. To investigate this mechanism in *V. destructor* populations from Portuguese apiaries, DNA was extracted from individual mites. This DNA was PCR-amplified using the primers 273-IF_VD (5′-AAGCCGCCATTGTTACCAGA-3′) and 1973-IR_VD (5′-CTGTTGTTACCGTGGAGCA-3′) and subjected to Sanger sequencing. The expected outcome is that mutations are found in Portugal at both positions 925 and 918, due to its geographical proximity to Spain. These results will contribute to the Portuguese beekeeping sector by facilitating the development of strategies for handling *V. destructor* resistance.

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## RNA VIRUS SPILLOVER FROM MANAGED HONEY BEES TO OTHER POLLINATORS

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### Abstract

Virus spillover from managed Western honey bees (*Apis mellifera,* HB) might contribute to declines of other wild pollinators. Indeed, RNA viruses associated with HB have repeatedly been found in many other species. However, disease impact relies on viral replication, which has received less attention in the field. Here we provide evidence that despite frequent detection rates of HB-associated viruses in other flower-visiting pollinating species in the field, viral replication is not often observed. The prevalence and titers of Deformed Wing Virus genotype A (DWV-A), DWV genotype B (DWV-B), and Black Queen Cell Virus (BQCV) were determined for field-sampled pollinators in Switzerland (total N=1105). BQCV and DWV-B were detected in 57% and 48% of all samples, respectively, whereas DWV-A was less frequently detected (4% of all samples). BQCV was detected in 86% of HB surveyed, compared to 51% of other bees, 35% of syrphids, and 6% of butterflies/moths. The detection rate for DWV-B was lower than for BQCV in HB (64%), hoverflies (24%) and butterflies/moths (<1%), but similar in other bees (49%). Intermediate strand analyses were performed on individuals with moderate to high viral loads of either BQCV (N=31) or DWV-B (N=25) in species for which viral replication has not been reported yet. Replication was not detectable for BQCV in any sample. Furthermore, replication of DWV-B was detected only in three bumble bee species not previously reported (*Bombus hypnorum*, n=2*; B. subterraneus,* n=1; *B. sylvarum,* n=1). Despite the frequent detection and moderate to high viral loads of HB-associated viruses in other flower-visiting pollinators, our findings emphasize the necessity of considering viral replication to fully comprehend transmission dynamics and the risk of disease posed to various pollinator populations.

## STABILITY OF DWV-B AND BQCV UNDER HEAT AND LIGHT MAY HELP EXPLAIN THEIR PREVALENCE ACROSS BEE SPECIES

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### Abstract

Honey bees (*Apis mellifera*) are widely considered to be the reservoir host of a number of pathogens, among which the most prevalent are +ss RNA viruses such as deformed wing virus (DWV) and black queen cell virus (BQCV). Many other bee species have more recently been found to harbour these same viruses, albeit at a much lower prevalence than in honey bees. There is strong support for the idea that these viruses spill over from honey bees to wild bee species, especially bumble bees (*Bombus* spp.), presumably having been picked up by a wild bee from a flower previously visited by an infected honey bee i.e. transmission *per os*. If so, a question arises as to how stable viruses are on flowers, when exposed to environmental challenges (heat, light) that might lead to their rapid degradation. We undertook a series of experiments in which we exposed virus (DWV and BQCV) to either heat or light, then tested its integrity and viability. We found that both DWV and BQCV could withstand several days of elevate temperature (35°C). DWV was also able to withstand 1 hour of sunlight, though appeared to decrease in viability after 10 hours of sunlight. Our data suggest that, though these viruses degrade after extensive exposure to heat or light, they can nevertheless survive for one or more hours on a flower when deposited by an infected honey bee, allowing them to be picked up and to infect a wild bee subsequently visiting the same flower. It is therefore not surprising that wild bee species are often found to be infected with so-called 'honey bee' viruses like DWV and BQCV.

# TAXONOMIC NOTES ON PARASITIC MITES ON HONEYBEE IN KOREA

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## Abstract

The honey bee mite surveys in Korea, began in 1984, with additional investigations conducted in 1993 and 1995. Throughout this series of surveys, *Varroa destructor* (Anderson & Trueman), *Varroa underwoodi* (Delfinado-Baker & Aggarwal), and *Tropilaelaps mercedesae* (Derfinado & Baker) were recorded. However, *V. underwoodi* was not observed again after the initial record (Woo, 1992), and no further survey has been conducted. As the *Varroa* mites became one of the major factor of honeybee decline recently in Korea, a year-long survey was conducted in collaboration with beekeepers in 2023. A total of 480 samples were collected from 45 bee hives in 8 regions using the sugar powder shaking method. *V. destructor* dominanted in most regions, while *Tropilaelaps* was found only in Gwangju city. The records of *V. underwoodi* will be discussed based on a literature review and re-examination of the deposited specimens, with the taxonomic review of the parasitic mites on honey bee in Korea.

# THE IMPACT OF PESTICIDES AND HEAT STRESS ON HEMOLYMPH CELLULAR RESPONSE IN HONEYBEES

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## Abstract

Honeybees play an important role in plant pollination and biodiversity sustainability. The alarming increase in colony losses is a multifactorial problem caused by both biotic and abiotic stressors, including pesticide exposure, inadequate nutrition, pathogens, and climate change. Climate change poses a threat to honeybee health through rising temperatures, shifting seasons, extreme weather, and reduced forage. Foragers come in contact with pesticides through oral and contact exposures to contaminated nectar, pollen, or water. The impacts of individual stressors on honeybee immune response are well described, however, our knowledge of their cumulative effects is still very limited.

In our study, we focused on the synergistic effects of heat stress and pesticide exposure on honeybee immune response and physiological changes. We collected one-day-old honeybees and housed them in experimental cages for 18 days. Cages were divided into 3 main groups: the control, the acute heat stress group, and the group that was exposed to acute heat stress while being fed with sublethal doses of pesticides throughout the experiment. One day before sampling, half of the cages from each group underwent mechanical immune stimuli. On the last day of the experiment, we collected hemolymph samples to evaluate the total and differential hemocyte count. Throughout the experiment, we also measured food consumption and mortality. We hypothesized that heat stress and exposure to sublethal doses of pesticides reduce total hemocyte count and change the proportion of hemocyte types present in hemolymph.

As climate change continues to worsen, it is important to understand how honeybees react when they experience higher temperatures along with other stressors. The study will shed light on the physiological and immune responses of honeybees under conditions mirroring real-world stress scenarios.

# THE OCCURRENCE OF TRYPANOSOMATIDS IN ITALIAN HONEY BEE (*APIS MELLIFERA L.*) COLONIES: A TWO-YEAR NATIONWIDE MOLECULAR SURVEY

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## Abstract

Trypanosomatids are unicellular parasites with fecal-oral transmission that infest the intestines of bees. Three species have been identified in managed honey bees (*Apis mellifera* L.): *Lotmaria passim*, *Crithidia mellificae*, and *C. bombi.* Among honey bee pathogens, the trypanosomatids are often neglected and little is known about both their epidemiology and their actual distribution. Within the Italian project "BeeNet – monitoring the environment through bees and biodiversity", a two-year survey was carried out on trypanosomatids occurrence in managed honey bee colonies throughout Italy. A total of 8146 honey bee colonies were investigated in all Italian regions in four different months (March, June, September, and November) in the years 2021-2023 and were analysed with molecular methods to assess the prevalence and abundance of the three trypanosomatids. None of the samples analyzed resulted positive for *C. mellificae* or *C. bombi*, while *L. passim* was detected with a low prevalence (2.29 and 1.22% in the first and the second monitoring year, respectively). No specific significant seasonal or geographical trends of prevalence and abundance of this pathogen emerged. Only a prevalence peak in November of the second monitoring year and a higher national prevalence in the first year concentrated mainly in northern Italy were observed. The national average abundance of *L. passim* ($7.80\times10^3$ copies per bee) was below the value at which a significant increase in mortality of experimentally infected bees was observed. However, scant information is available on symptomatic trypanosomatid infections in bees and their infection thresholds. This study represents the first comprehensive investigation of the occurrence of trypanosomatids within managed honey bees in Italy. It would be advisable to persist with monitoring projects of these neglected gut parasites to deepen the knowledge of their effects on bees and their interactions with other biotic and abiotic factors.

# THE ROLE OF INNATE IMMUNITY FOR VIRUS TOLERANCE OF GOTLAND HONEYBEES

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## Abstract

Pathogens lead to numerous pressures on the health of honeybees (*Apis mellifera*) worldwide. Honeybees, being social insects, have a complex immune system to cope with pathogens, with both innate immunity at the individual level and social immunity at the colony level. The honeybee population from the island of Gotland, Sweden, are well-known for their independent resistance to *Varroa destructor* mite infestation and tolerance to virus infections, in particular Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV). However, the immune mechanisms underlying the virus tolerance characteristic in this population are still unclear. Here, we investigated the innate immunity of Gotland honeybees towards virus infections to determine possible genetic variation of the expression of different immune genes. The Gotland honeybees and mite-susceptible honeybees were collected at different time points after oral inoculation with virus infections to detect the function of innate immunity in the competition between honeybees and viruses. The Gotland honeybees survived better than non-selected mite-susceptible honeybees. Several genes related to the production of antimicrobial peptides along with the genes involved in RNA interference (RNAi) pathways were tested using qRT-PCR. The results of this study will be discussed. Comprehensively investigating innate immune strategies, their relative importance for bee health and their costs and benefits, will benefit breeding efforts to safeguard honey bees and the important pollination services they provide for human well-being.

# THE VARROA DESTRUCTOR MITE IN LOCAL BEE COLONIES APIS MELLIFERA INTERMISSA IN ALGERIA: POPULATION DYNAMICS AND INFLUENCE OF CLIMATIC CONDITIONS

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## Abstract

Varroa has existed in Algeria since 1984. This mite has caused a lot of damage in the country's apiaries, despite treatments carried out by beekeepers. However, few studies have been carried out on the development of this parasitosis on the local Algerian bee. This study aims to determine population trends of Varroa destructor in local untreated bee colonies in the semi-arid zone of Algeria. The results have important implications for the establishment of control measures and their rational application, because treatments are often used without being preceded by a parasitological examination. This study was carried out in an apiary in the Djelfa region. The 20 colonies were treated once with Apivar® in the summer of 2014 to bring the Varroa populations to the same level. The population dynamics of the mite was studied using a low and estimated number of Varroa for all colonies. The study was conducted over 12 months (May 2021-May 2023). This involves monitoring the evolution of the dynamics of bee and varroa populations through regular counting which is carried out each month at colony level. We showed in this study that the Varroa population increases in the absence of treatment, high temperatures and the reduction in the number of brood cells did not have a negative influence on the development of the mite in the colonies. In the absence of treatment, the number of varroa cases well exceeds 3000 after one year of treatment.

The parasite population approximately follows the development of its host. These two factors are influenced by seasonal variations. Our results show that the summer period is an opportunity for the beekeeper to treat and eliminate as many varroas as possible due to the reduction in brood.

# UPDATE OF SMALL HIVE BEETLE BIOLOGY AND CONTROL

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## Abstract

Small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae; SHB) is a free-flying generalist associated with bee nests, which has become a widespread invasive species. However, there are still major gaps in our knowledge of this parasite often hampering respective mitigation. Here, we update SHB biology and control using observations in the region of Calabria (Italy) since 2014. SHB are locally established, but were successfully contained despite previous local spread in the Americas and Australia. Illegal transport of managed *Apis mellifera* hives lead to three long-range transports, but SHB could be eradicated confirming earlier cases. This underlines the importance of timely eradication/containment measures and of the ban on bee exports and migratory beekeeping. It seems as if flights of adult SHB, hosts other than managed honey bees and alternative food sources (flowers, fruits) played minor roles only for local dispersal of this invasive species. The presence of few non-managed honey bee colonies may have also contributed to successful containment in this specific area. Further, the surprisingly low SHB infestation levels of honey bee colonies in this region and the minimal damage to local beekeeping require dedicated research. In conclusion, timely and consequent eradication/containment measures were evidently successful, thereby supporting that stakeholder engagement was a key factor underlying this success story. We recommend following this example and impose the same measures for future SHB introductions.

# VARROA DESTRUCTOR AND NOSEMA CERANAE SHAPE THE HONEY BEE GUT MICROBIOTA

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## Abstract

The Azores archipelago consists of nine islands with endemic bee populations belonging to the African lineage, although there is also genetic evidence of the introduction of European bees. These introductions have in all likelihood introduced pathogens like *Varroa destructor* (V) and *Nosema ceranae* (N). However, the restricted movement of bee colonies between islands has limited their spread throughout the archipelago, so that we can find islands with different combinations of these pathogens, i.e. positive (+) or negative (-) to *V. destructor* and/or to *N. ceranae*. In this work, we studied the microbiota of bees belonging to 4 islands with this different pathogen scenario to determine whether the presence of any of them has influenced the presence and/or abundance of any of the main bacterial taxa that make up the intestinal microbiota of bees. Therefore, 15 bees from 10 colonies of Pico (V+, N+), Flores (V+, N-), Terceira (V-, N+) and Santa Maria (V-, N-) were selected and pooled and the complete bacterial 16S rRNA gene was sequenced using PacBio Sequel II sequencing (HiFi/CCS mode). Preliminary results showed that bacterial taxa characteristic of the honey bee gut were found in almost all samples, with *Lactobacillus*, *Bartonella*, *Bifidobacterium*, and *Snodgrassella* being the most abundant. In the samples from Terceira, *Bifidobacterium* and *Bartonella* were less abundant than in the other islands, while *Arsenophonus* was more abundant. *Arsenophonus* was also found, although at lower abundance, on Flores and Pico and was virtually absent on Santa Maria, which interestingly is the island free of *V. destructor* and *N. ceranae*. These results provide a first characterisation of the gut microbiota of bees in geographically isolated areas such as the Azores and how pathogens such as *V. destructor* and *N. ceranae* may have influenced the gut microbiota of honey bees.

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# WINTER COLONY LOSS RATE IN THREE TYPES OF BEEKEEPING IN POLAND

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## Abstract

Poland participates in the Colony Loss Monitoring Group, a core project of COLOSS Association. In years 2017-2022, our scientific team conducted a citizen science questionnaire survey among Polish beekeepers to investigate winter colony losses of honey bees. Respondents were asked about the number of colonies before and after winter, a suspected reason for the colony losses, and various beekeeping practices. The colony losses were classified into losses due to natural disasters and management-related losses, which could, in turn, be associated with the presence of a dead colony or a living colony but with an unsolvable queen problem. We developed a mixed-effect binary logistic regression model to investigate the influence of management-related factors on the winter colony loss rate. In total, data from 2169 beekeepers including 77 867 overwintering colonies were collected from 5 seasons. The colony losses were analyzed separately in three types of beekeeping (small-scale beekeeping [←25 colonies, SSB], medium-scale beekeeping [26-80 colonies, MSB] and large-scale beekeeping [>80 colonies, LSB]) and linked with potential explanatory variables, such as migration of colonies, proportion of queens replaced every season, replacing brood combs, Varroa monitoring and Varroa treatment. The majority of beekeeping was SSB (56.9%) and they also suffered from significantly higher winter colony loss rate (14.8%; CI 95%: 13.2%, 16.7%) compared to MSB (11.4%; CI 95%: 10.4%, 12.5%) and LSB (11.6%; CI 95%: 10.4%, 12.8%). The main type of losses in all types of beekeeping was this associated with the presence of a dead colony. Monitoring and treatment of *Varroa destructor* was equally often practiced in all types of beekeeping. Despite that, the overall winter colony loss rate was the highest in SSB. This may suggest that Varroa control in SSB is ineffective and an extensive education programme in bee diseases is needed to reduce winter colony losses in Poland.

# 3 Impacts of climate change and other factors in a changing environment

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## Oral presentations

Moderator:
Laura Bortolotti

# SHIFTING SANDS OF METACOMMUNITY OCCUPANCY: OCCURENCE OF WILD BEES OVER 100 YEARS IS LINKED TO CLIMATIC NICHES

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## Abstract

Our study presents a long-term perspective on wild bee diversity in Linz, Austria, utilizing a unique historical dataset spanning from 1910 to 2021 which includes 17,500 specimen records from museum collections. Through an analysis of temporal distributions across eight sampling sites, we examine the metacommunity dynamics of wild bees to elucidate patterns of species extinction and colonization. Our findings reveal a clear correlation between species turnover and both human population changes and climatic warming. Notably, a sharp increase in the human population mid-20th century coincides with significant local extinctions of many specialized bee species which outsets a continuous decrease in local species diversity. Concurrently, recent temperature increases correlate with the immigration of thermophilic species, whereas climatic generalists have demonstrated resilience over the observation period. Our study underscores the influence of species climatic traits on regional bee occupancy and identifies two pivotal ecological transformations: human-induced changes in landscape structure and rising annual mean temperatures. These insights highlight the complex interplay between environmental changes and biodiversity, offering a crucial understanding of how species metacommunities adapt to or decline under shifting ecological conditions.

# CHROMOSOME-SCALE GENOMIC ANALYSIS INSIGHTS INTO LOCAL ADAPTATION AND FUTURE CLIMATE-INDUCED VULNERABILITY OF TWO BUMBLEBEE SPECIES: *BOMBUS LAPIDARIUS* AND *BOMBUS NIVEATUS*

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## Abstract

Bumblebees, ubiquitous, cold-adapted, and primitively eusocial insects, play crucial roles as pollinators in agricultural and natural ecosystems. Nonetheless, numerous bumblebee species are experiencing global declines, attributable to many factors, including anthropogenic habitat degradation, exposure to agricultural chemicals, and the effects of climate change. Notably, projections of future climate scenarios suggest an impending alteration in the spatial distribution patterns of bumblebee populations, wherein certain species may face declines while others could potentially expand their ranges. In this study, we present the comprehensive assembly and annotation of chromosome-scale genomes for two bumblebee species, Bombus lapidarius and Bombus niveatus, to elucidate species-specific genomic adaptations to environmental stressors. These genomes, encompassing over 23,000 protein-coding genes, exhibit sizes of 244.44 Mb (with a scaffold N50 of 9.45 Mb) for B. lapidarius and 259.84 Mb (with a scaffold N50 of 10.94 Mb) for B. niveatus, consistent with genomic characteristics observed in related bumblebee species. Comparative analysis of gene families reveals distinct expansions in each species: B. lapidarius demonstrates enrichment in genes associated with synaptic organization, while B. niveatus displays expansions in gene families implicated in cellular growth, aging, and responses to environmental stressors, notably featuring SCAN domains, WD-repeats, and Ras-related proteins. Furthermore, genome-wide screening identifies positive selection signals acting on genes involved in environmental stress response pathways, such as dip2, yme1l, and spg7 in B. lapidarius and myd88, mybbp1A, and rhau in B. niveatus. These findings underscore the adaptive evolutionary trajectories of bumblebees in response to changing environmental conditions, providing valuable insights into strategies for their conservation and management.

# BROOD PAUSE IN WINTER PROLONGS LIFESPAN OF WINTER BEES AND DECREASES PARASITE LOAD IN HONEY BEE COLONIES

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## Abstract

In temperate climates, honey bee colonies used to cease breeding in autumn and queens resumed to lay eggs in early spring. Recently there has been an increasing number of colonies breeding throughout the winter: Since 2016, the brood activity of 60 standardized colonies were checked every year in late November. The average temperature of the second half of November correlated with the brood activity of the tested colonies.

To induce a brood pause, we caged queens in October for 0 (n = 38), 74 (n = 38) or 117 (n = 28) days over four winters to prevent egg laying and analysed the effects on queen survival, worker physiology, longevity and colony health. We found that caging had no negative effects on queen survival or overall colony strength. However, the lifespan of winter bees in breeding control colonies was significantly shorter.

At the age of five months, workers from caged colonies showed similar gene expression profiles as young worker bees. In contrast, bees from brood caring control colonies exhibited profiles characteristic of older, forager-like workers, suggesting a link between brood care and accelerated aging. We demonstrated that brood activity in winter lead to an increase in *Varroa destructor* infestation. Strikingly, we were able to reduce the mite infestation by caging the queens. In summary, the elevated winter temperatures linked to climate change correlated with heightened breeding activity within honey bee colonies. This phenomenon contributed to increased mortality among winter bees and a rise in infestation levels by the brood parasite *V. destructor*. Whether winter caging can also influence the reproduction rate of *V. destructor* in the following spring still needs to be elucidated.

## STRESS-RELATED ENZYME ACTIVITY IN *APIS MELLIFERA IBERIENSIS*

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### Abstract

Climate change is a powerful source of stress for bees as it involves an increase in temperature and changes in precipitation patterns that directly influence relative humidity. Both variables are highly correlated and, when acting in combination, may represent a new independent stressor. Due to the action of these abiotic stressors, alterations in the expression of enzymes vital for bees can occur, which can result in a reduction or increase in their activity. In this work we present a protocol to determine the effect of temperature and relative humidity on the enzyme activity of acetylcholinesterase (AcE) and peroxidase (POD). The heads were separated from the bodies (thorax and abdomen) and they were analyzed separately. The *Apis mellifera iberiensis* were conducted to the following conditions: 35ºC/15% RH; 35ºC/25% RH; 35ºC/50% RH; 35ºC/75% RH; 40ºC/75% RH; 45ºC/25% RH; and 45ºC/75% RH.

These effects have been studied as acute (2h) and chronic (2 days) stressors. Both factors, together with the exposure time, influenced the enzyme activity in some of our assays. For example, bees exposed for two days showed an increase in AcE concentration, in contrast to those exposed for only two hours. In another case, the bee heads showed a higher concentration of AcE at 35°C than at 45°C at the same RH (25%). On the other hand, no differences were observed between bodies and heads in the peroxidase enzyme (POD) assay at the same conditions as before, that is to say, bees exposed to 35ºC and 25% RH and bees exposed to 45ºC and 25% RH.

# SURVIVAL TO THERMAL STRESS IN MEDITERRANEAN SUBSPECIES OF *APIS MELLIFERA*

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## Abstract

Heat stress in bees produces a series of adverse effects on bees, affecting their growth, development and reproduction. It can also condition their ability to forage for food, thus affecting their pollinating activity. The climate change scenario makes it likely that increasingly hotter environments will affect the life expectancy of bees. On the other hand, environmental humidity may be another stressor in isolation and may also act synergistically with temperature.

To understand the effect of temperature and humidity on the survival of *Apis mellifera* subspecies, bees were exposed to six different conditions: 35°C, 40°C and 45°C at 75% relative humidity (RH), and 35°C combining 15, 30 and 50% RH. During the trial, the bees were kept on food ad libitum and the mean survival (in days) was recorded. This trial was conducted with 4 subspecies of Mediterranean honey bees (*Apis mellifera iberiensis*, *Apis mellifera ligustica*, *Apis mellifera anatoliaca* and *Apis mellifera syriaca*) in order to determine if there are differences between them.

Our results showed a clear negative effect of heat on the survival rate of the bees, as in all cases the bees survived less than half of the days at 40°C versus 35°C, and in no case were they able to survive 24 hours at 45°C. The effect of humidity was not relevant for the subspecies studied. Differences between species were observed, suggesting that each subspecies has developed different heat adaptation mechanisms. It is noteworthy that *A. m. iberiensis* was the subspecies that showed the longest life expectancy compared to the rest of the subspecies studied.

*These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.*

## BODY WATER LOSS AS AN ADAPTIVE CAPACITY OF HONEY BEES TO CLIMATE CHANGE

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### Abstract

Environmental temperature and relative humidity are important factors affecting honey bee welfare. The two variables are closely related and it has been observed that in bees, low RH levels combined with high temperature can exacerbate heat stress, while high humidity can reduce the severity of heat stress. Therefore, bees are able to regulate both temperature and humidity inside the hive by evaporating water from the nectar and regurgitating droplets of liquid to restore favourable conditions. One way to study this dehydration capacity is to calculate the body water loss (BWL) per bee before and after exposure to specific temperature and humidity conditions. In this work, 9 different conditions combining 35, 40 and 45°C temperature with 10, 25 and 50% RH were analysed. The same experiment has been carried out in Spain, Italy and Jordan with their corresponding subspecies (i.e. *Apis mellifera iberiensis*, *Apis mellifera ligustica* and *Apis mellifera syriaca*), and the results showed the protective effect of high RH under high temperature conditions. On the other hand, differences in dehydration capacity between subspecies have been found, which point to a different capacity to adapt to hot environments.

*These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.*

# TOLERANCE TO HEAT AND COLD OF *APIS MELLIFERA IBERIENSIS* AND *APIS MELLIFERA SYRIACA*

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## Abstract

The temperature range between the coldest temperature (critical thermal minimum, CTmin) and the warmest temperature (critical thermal maximum, CTmax) at which an organism can maintain muscle control is called thermotolerance. Both characteristics seem to be different among honeybee subspecies, which today are trying to adapt to new and increasingly extreme climatic environments caused by climate change. In bees, both thermal tolerance and the degree of plasticity or adaptation play key roles in determining the geographical distribution of species.

In this paper we present the results of exposing *Apis mellifera iberiensis* from Spain and *Apis mellifera syriaca* from Jordan to two different temperature ramps: from 30 to 70ºC (heat study), and from 35 to 0ºC (cold study). Our results showed differences in heat and cold tolerance between subspecies, as well as sex- and age-related differences within both subspecies. In the heat test, Iberian bees suffered heat stress up to 40ºC, and both *A. mellifera iberiensis* and *A. mellifera syriaca* individuals showed hyperthermia up to 45ºC, where mortality was detected in some groups. Regarding cold tolerance, both subspecies showed different effects: while no signs of cold stress or hypothermia were detected in *A. mellifera syriaca*, all of them appeared in *A. mellifera iberiensis*. In the latter, mortality of foragers occurred below 6ºC. Further experiments are currently being carried out at MEDIBEES to understand the molecular basis of the differences found.

*These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.*

# 3 Impacts of climate change and other factors in a changing environment

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Poster presentations

# ADVANCING HONEY BEE NUTRITION AND METABOLISM STUDIES THROUGH CALORIMETRY: INSIGHTS INTO ENERGY UTILIZATION AND COLONY HEALTH

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## Abstract

Calorimetry is a fundamental technique for quantifying heat exchange in chemical and biological systems. It offers innovative possibilities for assessing nutrient utilisation in honey bees and for elucidating metabolic processes in honey bee colonies. We applied this method in worker bees to explore the effects of different feeding and nutrition on honey bee energy utilisation, and in different pollen to evaluate the gross energy content (GE).

A comprehensive assessment of the GE of worker bees using the calorimeter system with adiabatic oxygen method was carried out as part of the quality assessment, alongside gross energy measurements in nutritional studies. The use of these calorimetric instruments in honeybee biology and nutrition is a challenging endeavour that promises advances in beekeeping practices. Thus, calorimetry helps to determine the energy value of worker bees facing malnutritional, thermal and other stressful environment conditions. In addition, the knowledge gained from calorimetry studies can contribute to wider conservation efforts aimed at protecting pollinators and maintaining key ecosystem services that are vital for agriculture and biodiversity conservation.

In summary, the versatility of calorimetry methodology enables a holistic study of honey bee nutrition and metabolism, allowing the refinement of feeding strategies to protect bees from fluctuating environmental conditions. Through interdisciplinary collaboration and the initiation of new research projects, calorimetry remains central to improving our understanding of honey bee biology and promoting resilient and sustainable beekeeping practices in the midst of a changing climate.

# ASSESSMENT OF THE EXPOSURE OF HONEYBEE COLONIES IN POLAND TO PESTICIDE RESIDUES - STUDIES OF WINTER BEE DEBRIS

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## Abstract

As a part of the monitoring programme of the health status and losses of bee colonies in domestic apiaries (which has been in operation in Poland for 10 years) monitoring of pesticide residues in the hive environment and in bees is also carried out, including studies of post-winter bee debris.

In 2023, 37 samples of post-winter bee debris collected during spring monitoring visits to supervised apiaries were subjected to toxicological analysis. The analysis showed that 97% of the samples contained pesticide residues at concentrations above the limits of quantification of the test method. The percentage of bee samples containing pesticide residues is very high, however, mainly due to residues of amitraz metabolites (DMF, DMPF). A total of 18 pesticide residues were determined in samples of dead bees: DMPF, DMF and mainly pendimethalin, azoxystrobin, prosulfocarb, tau-fluvalinate, chlorpyrifos, cypermethrin and difenoconazole. Residues of up to 5 pesticides were determined simultaneously in the samples of bees.

Due to the very high percentage of samples containing pesticide residues, winter bee colony samples are a particularly interesting group of monitoring samples. Such a statistic is mainly due to the widespread presence of amitraz metabolites in the colony debris samples and is therefore a natural consequence of autumn varroa treatment. Very high concentrations of DMF and DMPF, even reaching the $LD_{50}$ dose, may indicate that the timing of varroa treatments was too late. Climate changes and a prolonged warm autumn are causing veterinary drug treatments to be delayed. Treatment on the winter generation of bees characterised by slowed metabolism of xenobiotics, combined with several months of exposure to amitraz metabolites, and infestation by *Varroa* and *Vairimorpha*, is a highly probable cause of many of the analysed cases of winter bee losses.

# DEHYDRATION AS A POSSIBLE ADAPTIVE TRAIT IN *APIS MELLIFERA SYRICA*

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## Abstract

The European honey bee *Apis mellifera* has a large number of subspecies, all adapted to their environment. Among these, *A. m. syriaca* is a subspecies native to some countries of Wester Asia as Jordan and Lebanon. Although these two countries share a Mediterranean climate, relative humidity follows different patterns in the two countries, being very high and constant in the Lebanese cost and lower and seasonal in the central Jordan. This provides an opportunity to study the adaptation of the same subspecies to different environments keeping in mind that, the bees are able to evaporate nectar water and regurgitating liquid droplets to regulate both temperature and humidity inside of the hive. To determine if they have developed different adaptations, newly emerged worker bees of syriaca subspecies from both countries were exposed to different temperature and humidity conditions in order to calculate the body water loss in each case. Nine different conditions were tested, combining 35, 40 and 45°C temperature with 10, 25 and 50% Relative Humidity. Our results show differences between bees from the two countries: while Lebanese bees seem to have a protective effect of heat stress in environments with higher humidity, Jordanian bees seem to follow a different pattern. On the other hand, Lebanese bees showed higher dehydration percentages compared to Jordanian bees, indicating a higher adaptability to heat environments. Future genetic studies will provide more information about this possible adaptation.

*These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union*

# INFLUENCE OF HIVE CONSTRUCTION MATERIALS ON TERMOREGULATION AND BIOLOGICAL PARAMETERS OF *APIS MELLIFERA* LINNAEUS, 1758 IN A TEMPERATE SUBCONTINENTAL CLIMATE AREA

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## Abstract

The Po Valley is the geographical area that extend along the idiographic basin of the Po River. Due to its position and morphological characteristics, the Po Valley has a temperate subcontinental climate with long, cold winters and hot, humid summers. In recent years, however, the climate has changed, with dry winters, hot and dry summers and rainfall, mostly thunderstorms, concentrated in spring. This atypical weather trend poses a serious problem for beekeeping. Extreme events such as cloudbursts, unseasonal cold spells and prolonged periods of no rain can affect flowering and therefore the bee's possibility to forage; climate change may affect colony homeostasis affecting the thermoregulatory abilities of bees in different seasons. A possible solution could be to replace the wood in hives with other materials to facilitate inside-hives insulation to help bee-thermoregulation. In the present research, from 2022 to the beginning of 2024, we compared the effects on thermoregulation and general well-being of honeybee colonies in aluminium and cork hives with the classic wooden hives. Colonies were located in Montanaso Lombardo (Lombardy, Italy) and were provided with data loggers to detect inside-hive temperatures. Inspections were carried out at least fortnightly from spring to late summer to evaluate biological parameters (brood, queen presence or replacement, worker mortality), and productivity (honey produced, pollen collected). Statistical analysis showed a significant effect (p<001) of the materials on the daily temperature oscillation in the different monitoring periods, with less fluctuation inside aluminium-cork hive. However, this variability did not result in any significant effects on the biological parameters considered. In conclusion in the geographical area considered aluminium and cork hives seem to play similar role in the survival of the colonies. However, considering that differences in thermoregulation were detected, we need to evaluate in different climatic conditions, the biological responses in the colonies could be different.

# SAFFLOSPERMIDINE ISOLATED FROM SUNFLOWER BEE POLLEN ON CELLULAR TYROSINASE INHIBITION

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## Abstract

Climate change has been the inevitable situation worldwide for decades. Due to ozone layer decreasing in the atmosphere, we, especially people living in the equator and tropical zones, have to face the problem of UV radiation overexposure. Although melanin is synthesized to protect our skin, excessive melanin production can occur and cause undesirable hyperpigmentation issues such as freckles, melisma, skin cancer eventually. Since melanogenesis in the skin is catalyzed by tyrosinase acting as a key enzyme in this pathway, searching novel melanogenesis or depigmentation agents has been involved mostly in tyrosinase inhibiting activity. Although several compounds have been reported, their skin-related adverse effects and cytotoxicity concerns are still found. Thus, a new natural compound with tyrosinase inhibitory activity and less harmful effects is still necessary. A compound from natural products has long been the main source for this purpose. Previously, we reported that successfully purified spermidine derivatives (safflospermidine) from *Helianthus annuus* L. (sunflower) bee pollen harvested by *Apis mellifera* in our lab could perform very interesting in vitro tyrosinase inhibition. Here, we continue our work to investigate the potential of the safflospermidine on tyrosinase inhibition at the cellular level by using B16F10 mouse melanoma cells. It was found that safflospermidine at the concentrations lower than 1,000 micrograms/mL has no cytotoxicity to the cells. For melanin content assay, safflospermidine can reduce both extracellular and intracellular melanin content with the dose dependent manner. Safflospermidine at 62.5 micrograms/mL can significantly reduce the cellular tyrosinase at 25.71%. Hence, safflospermidine is promising for future experiments which will eventually lead to apply it in depigmentation products.

# THE BETTER-B PROJECT AIMS TO RESTORE THE RESILIENCE OF HONEY BEES

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## Abstract

The overall aim of this 4-year BETTER-B project is to improve the resilience of beekeeping to abiotic stresses such as climate change, habitat loss and hazardous chemicals. Honey bee colonies are often poorly adapted to cope with these stresses, in no small part due to modern beekeeping practices. The key to resilient beekeeping is to harness the power of nature to restore harmony and balance inside the honey bee colony and between the colony and the environment, both of which have been disturbed by human activities. We believe that the path to harmony and balance is shown by Darwinian colonies: abandoned colonies and feral colonies that have survived in the wild. However, such colonies usually lack many favourable characteristics that are important in modern beekeeping. The solution here is to understand the processes and mechanisms that apply in nature and to adapt modern beekeeping practices and decision making accordingly, and when appropriate using the benefits of advanced technologies. This is what BETTER-B stands for. The implementation of this new approach in apicultural management will be done in close collaboration with the actors involved. The restoration of harmony and balance must take place on three levels: the environment, the honey bee and beekeeping practices.

# THE HEAT IS ON: IMPACT OF HEATWAVES ON CRITICAL THERMAL MAXIMA IN LARVAE AND ADULT OF SOLITARY BEE *OSMIA BICORNIS*

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## Abstract

Extreme temperature events, such as heatwaves, are increasing in frequency, magnitude, and duration due to climate change. These events push ectotherms, which lack significant mechanisms to regulate body temperature, toward their upper critical thermal limits ($CT_{max}$), impacting physiology, individual survival, and population persistence. Climate-induced changes are one of the main drives of pollination decline. In this study, we investigate how current and predicted temperature extremes approach the upper thermal tolerance capacities in the solitary bee *Osmia bicornis*, one of the most common and important pollinator in Central Europe. Specifically, we examine how the rate of temperature increase affects $CT_{max}$ in larvae, and how heatwaves experienced during the larval stage affect heat tolerance capacities of adult bees. We found that in larvae, $CT_{max}$ significantly increased with the rate of temperature ramping, with the fastest ramping rates resulting in the highest achieved $CT_{max}$. Additionally, $CT_{max}$ varied among life stages, with adults exhibiting higher $CT_{max}$ than larvae. Interestingly, adult bees exposed to different heatwaves during their larval stage did not exhibit significant shifts in $CT_{max}$. However, thermal plasticity may be indirectly mediated by changes in body mass, as heatwaves were associated with a reduction in body mass, and smaller individuals attained higher $CT_{max}$. These results suggest that bees may have limited capacity to enhance heat tolerance in response to prior heat exposure. Understanding the mechanisms underlying thermal tolerance in bees is crucial for predicting their response to ongoing climate change and developing effective conservation strategies.

# THE IMPORTANCE AND SPATIAL DISTRIBUTION OF THE MAIN NATIVE AND ALIEN BEE PASTURES WITHIN HUNGARY

Viktor Szigeti[1,2], Tamás Ádám Tőkés[3], Ildikó Arany[1], Zsolt Molnár[4], Zsolt Poroszka Magyar[5], Anikó Kovács-Hostyánszki[1,2]

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## Abstract

The flowering landscapes, called also ‘bee pastures’, are under pressure and altered due to human-induced environmental changes. Simultaneously, detailed knowledge is lacking about the condition of these habitats. Throughout the whole season, bee pastures are constituted by several different plants, including crops, native and an increasing amount of (invasive) alien species. Understanding the role of both components has an interdisciplinary importance for ecology, conservation and economy. Although beekeepers are excellent knowledge holders in this context, their perspectives are understudied and rarely acknowledged during decision making. Hence, investigating their ecological knowledge about condition of bee pastures can also help to reveal ecology of flowering landscapes, honey bees and wild pollinators. Therefore, we asked >400 beekeepers, by questionnaires, about their opinions on 17 types of bee pastures, within Hungary. The three most favored bee pastures were an alien tree species planted in large areas (black locust), and two mass-flowering crops (sunflower and oilseed rape). Many other plant species and habitats are also important periodically, regionally, and for migratory beekeeping. Recent generations of beekeepers are facing to lose the native bee pastures, gradually replaced by agricultural and alien mass flowering plants. In parallel, invasive alien plants are also raising conflict between stakeholders of apiculture, nature conservation, crop production and forestry. Respondents of our questionnaire considered that the eradication of invasive alien plants is the most problematic factor about the future of bee pastures, causing serious economic problems without alternative foraging resources. Flowering landscapes may become even more vulnerable to the upcoming climate and environmental changes. Therefore, ‘to sow the seeds of bee pastures’, more discussion among stakeholders, collaborative researches, and long-term monitoring are needed, using also the interdisciplinary approaches of sociology and ecology.

# 4 Pollinators and pollination ecology in natural and agricultural landscapes

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## Oral presentations

**Moderators:**
Tsipe Aavik
Reet Karise

# EXPLORING HONEYBEE FORAGING PATTERNS AND MELISSOPALYNOLOGY AS INDICATORS OF ENVIRONMENTAL DIVERSITY: A COMPARATIVE STUDY IN ITALY AND BOLIVIA

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## Abstract

Honeybees, patrol areas ranging from a few metres to kilometres, depending on the availability of plants. However, independently from the plant monitored, honey samples often contain a diverse range of pollen, influenced by various factors such as plant abundance, anther location and surrounding vegetation. As a result, the composition of pollen in honey can be considered a unique environmental fingerprint, reflecting the botanical diversity of the landscape. In this study, we explore the complex relationship between honeybee foraging patterns and pollen in honey focussing on understanding environmental diversity and its implications for beekeepers. To do so the research was based mainly on honey collection in two geographical regions: Northern Italy and Bolivia. These areas exhibit stark differences in climate, vegetation, and baseline knowledge. Melissopalynological analysis applied to monofloral honey-samples collected from various locations in Italy over 10 years were associated to field research, providing insights into the diversity and abundance of plant species visited by honeybees in the area. In contrast, the study in Bolivia (inserted in the project SANAPI coordinated by ASPEM financed by AICS) began with a comprehensive review of existing literature of the local vegetation and floral resources available for honeybees, followed by melissopalinological analysis on honey-samples collected from different geographical region of the country. By comparing and contrasting the findings from these two distinct regions, this research aims to elucidate the relationship between honeybee foraging behavior and environmental diversity. Furthermore, the melissopalynological analysis provides valuable insights into the unique pollen signatures of each environment, highlighting the importance of honeybees as bioindicators of ecosystem health and biodiversity. Overall, this comparative study highlights the importance of honeybees and melissopalynology in monitoring environmental change and assessing the suitability of landscapes for beekeeping, contributing to the understanding of ecosystem dynamics and conservation efforts on a global scale.

# PROMOTING WILD POLLINATORS IN AGRICULTURAL LANDSCAPES - WILD BEE DIVERSITY, RESOURCE-USE AND YIELD IN A LATE MASS-FLOWERING CROP

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## Abstract

Facing a loss of biodiversity due to agricultural intensification, questions arise on how to implement farming practices that promote farmland biodiversity and ecosystem services, such as pollination. Organic farming is one of the most popular agri-environment measures in Europe. Pollinator-dependent flowering crops like sunflowers provide the opportunity to study both, the effects of organic farming on pollinators and pollination services. Here, we aim to identify local and landscape management effects that influence wild pollinators, pollination services, and sunflower yields. Furthermore, we studied the use of flower resources and effects of honeybees on wild pollinators.

Our study was conducted in 2022 in Northern Bavaria, Germany. During sunflower bloom, we conducted pollinator surveys in 14 conventional and 15 organic sunflower fields using transect walks. We assessed abundance, species richness and plant-pollinator interactions of four major functional groups (bumblebees, other wild bees, hoverflies and honeybees). In addition, we conducted pollinator exclosure experiments and determined the yield per plant for open and netted sunflowers.

In our study, both, honeybees and bumblebees positively affected sunflower yield. We found that bumblebee abundance was higher in conventional fields, but generally increased with organic farming in the landscape. Other wild bees however did not respond to landscape effects and benefitted from increased weed cover and richness. We further show that resource use differed between functional groups: bumblebees and honeybees both foraged almost exclusively on the sunflower itself, while other wild bees mainly foraged on weeds growing beneath the sunflower. We conclude that farmers benefit from increased bumblebee abundances and therefore, organic farming in the landscape. However, other wild pollinators rely on weeds as food resources. Thus, we appeal to famers to tolerate a certain level of weed growth or, as a concrete measure, consider undersowing diverse flowering plants in the sunflowers.

# NOTES ON CALCULATING NECTAR AND POLLEN RESOURCES FOR POLLINATORS AT DIFFERENT LEVELS: SPECIES, HABITAT AND LANDSCAPE

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## Abstract

Pollinator-oriented landscape management requires recognition of the most valuable, in terms of nectar and pollen production, habitats. Here we propose a method to assess nectar and pollen production at species, habitat and landscape levels. Briefly, seven main steps are to be done:
(1) selection of habitats for detailed studies (analysis of satellite images and orthophotomaps, criterium: habitat coverage and frequency within a landscape),
(2) phytosociological relevés (list of plant species with their cover),
(3) selection of species to assess nectar sugar and/or pollen production (based on frequency and cover),
(4) recording flowering phenology and abundance,
(5) species-level quantification of sugar and/or pollen mass,
(6) habitat-level quantification of sugar and/or pollen mass (based on the species-level production, cover, abundance and phenology),
(7) landscape-level quantification of sugar and/or pollen mass (based on habitat coverage within a landscape).

The method allows to show the spatial and temporal distribution of pollinator food resources within a landscape. It can be also useful in designating habitats that are of key importance to safeguard the continuity of nectar and pollen supplies to undertake conservation efforts.

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# LONG TERM BUMBELBEE MONITORING DEMONSTRATES THE BENEFITS OF ORGANIC AND ENIROMENTALLY FRIENDLY FARMING PRACTICES

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## Abstract

The integral part of European Union policy has been agri-environmental schemes following the implementation of Regulation 2078/92 across the EU member states. Crucial part of these schemes are meant to motivate farmers to produce food in environmentally friendly way in return to compensatory payments. Together with the interventions there is rising need to measure the effect of these costly interventions as well as understand how agriculture impacts biodiversity in general. Although various studies address the issue, long term studies are still scarce.

Here we demonstrate one long-term study on bumblebees targeting field edges in agricultural landscapes. In particular, we have been measuring bumblebee numbers and species richness on 66 sites in Estonia since 2009 whereas 1/3 of the sites are organic certified farms, 1/3 are implementing environmentally friendly farming practices and 1/3 are conventional farms for control. Although some of the sites have been replaced over time due to change in farming practices, there has been constantly the same methodology and field protocol. Standard transect counts are used and all the sites have the same transect length (500 meters). All sites are visited three times over the season when the weather is favourable largely by the same team over the years.

Total of 46 817 bumblebees from 21 species have been counted since 2005, whereas 37% of the individuals are from organic farms, 38% from environmentally friendly farming system and 25% from control sites demonstrating the positive effects of environmentally friendly and organic farming to surrounding biodiversity. The sample sites are distributed over various landscapes and results clearly demonstrate that bumblebees prefers smaller fields, higher forest cover and wider flower-rich field edges. Wider analyses of the dataset still lies ahead whereas various spatio-temporal questions can be addressed both in species as well as community level.

# MANAGEMENT METHODS TO OPTIMIZE COMMERCIAL BUMBLE BEE (*BOMBUS IMPATIENS*) COLONIES' PERFORMANCE DURING THE LOWBUSH BLUEBERRY (*VACCINIUM ANGUSTIFOLIUM*) POLLINATION

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## Abstract

The most economically important managed bumble bee species in North America is *Bombus impatiens*. It is very efficient to pollinate the lowbush blueberry (*Vaccinium angustifolium*), and many thousands of managed colonies are used each year to increase production. The methods to manage these commercial colonies are not well known and must be explored to maximize their performances during blueberry pollination. This study's goal is to test different colony management methods that may increase foraging activity and sustain stronger and healthier colonies. We hypothesized that the colony preparation and timing of their arrival in blueberry fields as well as their protection against direct sunlight will affect the workers' activity, and the colonies' strength and health. Three groups of *B. impatiens* colonies were placed in blueberry fields during peak flowering period. One group (N=64) was received from a supplier one week before peak flowering and placed directly in blueberry fields, the second group (N=64) was received two weeks before peak flowering and placed directly in blueberry fields, and the third group (N=64) was received two weeks before peak flowering and placed in a farm field before placement in blueberry fields to promote colony development. Once in blueberry fields, half the colonies were protected against direct sunlight, while the other half was not. We recorded the number of workers entering and exiting the colonies and the quantity of pollen retrieved to assess the foraging activity. We analyzed the growth and composition of the colonies and studied the presence of pathogens to establish the strength and health of the colonies. Results showing how these methods influence the foraging activity of the workers, and the growth and health of the colonies will be presented. Our work will help optimize the management of commercial bumble bee colonies during pollination services, and thus increase lowbush blueberry yields.

# COMMERCIAL POLLINATOR DENSITY AND LOWBUSH BLUEBERRY YIELD IN QUÉBEC, CANADA: AN AGRO-ECONOMIC STUDY

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## Abstract

Lowbush blueberry (*Vaccinium angustifolium* Ait.) production has the highest export value among all fruit crops in Canada. Since lowbush blueberry flowers require pollination by insects, blueberry farms use commercial pollinators to ensure productivity of their fields. The objective of our project was to assess the effects of the density of commercial pollinators on lowbush blueberry yield using historical data, from 2015 to 2021, and a variety of production contexts. A total of 562 blueberry fields were included in this study, representing approximately 3,500 ha and 12% of the total Québec crop production surface. Historical data on field size, yield, pollinator density (honeybees; *Apis mellifera*, bumblebees; *Bombus impatiens*, and alfalfa leafcutter bees; *Megachile rotundata*), year of establishment, and management type were collected for each field. Our study also included historical data on the strength of honeybee hives placed in the fields, the landscape structure surrounding each blueberry farm, and several meteorological variables affecting yield. Yield increased significantly with hive density, up to ~4-5 hives/ha when accounting for hive strength. Yield also increased with a bumblebee density of more than 4 colonies/ha, and with a leafcutter bee density of more than 5 gallons/ha. The diversity of commercial pollinators placed in the fields also increased yield. Best predictive variables of lowbush blueberry yield were the honeybee density, the year, the snow cover period during winter, the field age, the management types, and the precipitations during the fruit growth period. This study clarified the relationship between the various commercial pollinators and lowbush blueberry yield, while demonstrating the influence of several other parameters on field productivity. All available commercial pollinators contributed to increased yields, especially when used in complementary ways. Our results highlight that blueberry growers in Quebec could boost their yields by increasing their pollinator density and diversity.

# OPTIMIZATION OF LOWBUSH BLUEBERRY POLLINATION AND EVALUATION OF HONEY BEE'S AND BUMBLE BEE'S HEALTH

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## Abstract

The province of Quebec is the main producer of lowbush blueberries (*Vaccinium angustifolium*) in Canada. The rental of honey bee (*Apis mellifera*) hives for pollination accounts for 44.2% of Quebec beekeepers' income. However, optimal colony densities for blueberry pollination remain unclear, and commercial pollination has been associated with detrimental effects on honey bee health. This research aims to optimize blueberry pollination by comparing two honey bee densities (2.5 hives/ha and 5 hives/ha) and one density of the commercial bumble bee *Bombus impatiens* (1.5 multi-hives/ha). Twelve blueberry fields in the Saguenay-Lac-Saint-Jean region were selected for the study. Pollination quality variables, foraging activity, and the impact on pollinator health were measured during the summers of 2022 and 2023. Preliminary findings indicate a significant reduction in honey bee colony strength post-pollination, with further reductions observed at doubled colony densities. However, neither honey bee pollination nor increased colony density had a significant effect on the parasitic load of *Nosema* spp. or viruses such as DWV A and B, ABPV, IAPV, KBV, CBPV and BQCV. Conversely, *Varroa destructor* and *Ascosphaera apis* infestation rates increased one month after pollination, particularly with doubled colony densities. Honey bee colonies utilized for pollination exhibited 23 active pesticide components in beebread and nectar before pollination, decreasing to five components post-pollination. These results suggest that the reduction in colony strength may be attributed to nutritional factors rather than pathogen or pesticide presence. In the case of common eastern bumble bee (*B. impatiens*) colonies, no significant effects of commercial pollination on the parasitic load of *Nosema* spp. and *Crithidia* spp. were observed. These findings will help to improve management of honey bee and bumble bee colonies used for blueberry pollination and will enhance our comprehension of their population dynamics.

# POLLINATION DEFICITS IN PEAR ORCHARDS IN NORTHERN ITALY: THE ROLE OF POLLINATOR COMMUNITY, LANDSCAPE CONTEXT AND PESTICIDE RISK

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## Abstract

Pear (*Pyrus communis* L.) is an important entomophilous crop for Europe and Italy is one of the main pear producer. Most pear varieties are self-incompatible and strongly dependent on insect pollinators for fruit set. However, the frequently harsh conditions during bloom and the low sugar content of nectar compared to other co-flowering fruit trees often lead to low pollinator visitation rates. The aim of this study was to assess pollination deficits in pear orchards in the Emilia-Romagna region (Italy), and to analyze the effects of pollinator community, landscape context and pesticide loads on pollination levels and yield. To achieve these objectives, pollination treatments (*pollinator exclusion*, *open pollination* and *supplementary pollination*) alongside pollinator surveys during peak bloom were performed in 16 orchards (cv. *Abate Fetel*). Notwithstanding the use of synthetic growth regulators to increase fruit retention, our results confirm the high dependence of fruit set on pollination. Our results also show high levels of pollination deficit (mean: 21%, range: 0%-64%). Seed production was negatively correlated to pollination deficit. The most abundant flower visitors were honeybees, followed by dipterans, hoverflies and bumblebees, but they were not significantly correlated to pollination deficits. Chemical analysis of pear flowers revealed high levels of pesticide residues. All flower samples were contaminated with at least four different pesticides, mostly fungicides. Our results indicate insufficient pollination services in pear orchards and highlight the need to reduce pesticide pressure on pollinators.

# IMPROVING AVOCADO POLLINATION BY INTEGRATING INTER-ROW NATURAL AND SOWN ANNUAL PLANTS

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## Abstract

Avocado is pollinated mainly by honeybees. However, the bees tend to prefer competitive blooming outside the orchard over the avocado flowers, causing a pollination deficit. Wild pollinators may contribute significantly to avocado pollination, but little is known about management tools to enhance their activity or honeybees activity within orchards. In the current study, we evaluate the possibility of increasing pollinators populations in the orchard, to improve avocado pollination and productivity by augmenting diverse plant resources.

We tested the effect of (1) spontaneously growing vegetation in five orchards, and (2) sown blooming cover crops and cereals in distinct richness levels, in additional orchard, on various parameters related to pollination services and orchard productivity. In study (1), a significant positive correlation was found between the number of honeybees visits to avocado flowers and inter row vegetation cover. An interesting temporal effect was found in both daily and monthly levels: a significant inverse correlation was found between the number of visits to avocado flowers by honeybees versus wild pollinators along the blooming period - honeybees were dominant in the beginning of the season and wild pollinators become dominant toward its end. We see the same trend throughout the day - honeybees are dominant in the morning and wild pollinators are dominant toward noon time. In study (2), more pollinators were visiting avocado flowers in the treated (sown) transects, including honeybees compared to control. The highest fruit-set in the avocado trees was found in the richer cover crops treatment.

Honeybees and the various wild pollinators exhibit a complementary activity pattern, enhancing the stability of pollination throughout the flowering season. These results provide an initial support for the contribution of orchard inter row rich vegetation to provide potential habitat to wild pollinators and increase avocado pollination by wild, as well as commercial pollinators.

# OSMIA BEES AS POLLINATORS OF ALMOND ORCHARDS: CURRENT WORK AND INSIGHTS FROM STUDIES IN THE BALEARIC ISLANDS (SPAIN)

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## Abstract

Given the ongoing decline of honeybee colonies over the past years, solitary bees, particularly *Osmia* bees (Megachilidae), are emerging as a promising alternative for crop pollination. Here, we will make a comprehensive review of the studies carried out by our research group on these solitary bees in the Balearic Islands, along with an update on our current project, aimed at creating an *Osmia* bank. Our first studies within mason bees focused on nesting ecology in handmade trap nests, as well as the effect of different overwintering protocols in survival, emergence time and longevity. Then, we investigated how the release and establishment of *Osmia bicornis* (syn. *O. rufa*) populations could enhance fruit set in almond orchards. The results showed a 25% increase in fruit set. Based on these findings, our current research aims to create an *Osmia* cocoon bank as a supplementary pollination strategy for almond crops. To archive this goal, we have installed 37 BioDar® trap nests across 18 almond orchards throughout Mallorca. We want to investigate how both internal (i.e. presence of honey bees, plant coverage, use of pesticides, …) and external factors (i.e. landscape traits) can influence the abundance and diversity of mason bees. In addition to the nest traps, we are customizing the "Pollinator Habitat Assessment Form and Guide" to characterize *Osmia*'s habitats within agricultural landscape and evaluate the potential correlation with their abundance and diversity. This new multivariable analysis seeks to provide valuable information about the ecological dynamics of wild *Osmia* bee populations in almond orchards, contributing to the development of new strategies for crop pollination.

# HIVE ORIENTATION AND COLONY STRENGTH AFFECT HONEY BEE COLONY ACTIVITY DURING ALMOND POLLINATION

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## Abstract

Managed honey bees are virtually exclusive pollinators of almonds in commercial agricultural contexts such as that of California's Central Valley where 80% of the world's almonds are produced. Pollination costs are estimated to account for up to 20% of the growers' operating budget while beekeepers themselves face numerous challenges in ensuring adequate supply of the pollinators. Streamlining almond pollination by utilising honey bee colonies in the most efficient way aims to support both the growers and beekeepers. This study examines whether hive entrance orientation and hive strength influence the pollinating activity of honey bees in almond orchards. Working in conjunction with USDA we assessed the various hive metrics across three genetic lines of bee stock (Pol-line, Russian and unselected stock). Twenty-four colonies of honey bees, twelve in each group, were situated with their entrances facing east and west cardinal points. Bee out counts and hive weight data were recorded continuously while colony strength was assessed on three occasions throughout the course of the study. East-facing hives started flight 44.2 min earlier than west-facing hives. The hive direction did not affect the timing of the cessation of foraging activity. The hive strength played a significant role: hives assessed as weak (←3.0 FOB) commenced foraging activity 45 min later than strong hives (>3.0 FOB) and ceased foraging activity 38.3 min earlier. Our study showed that, during almond pollination, both hive entrance exposure and hive strength have quantifiable effects on colony foraging behaviour and that these effects combine to regulate the overall foraging activity of the pollinating colonies.

# IMPACT OF GAMMA IRRADIATION ON POLLEN QUALITY AND COLONY DEVELOPMENT IN *BOMBUS TERRESTRIS*

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## Abstract

The bumble bee *Bombus terrestris* plays a central role in greenhouse pollination services. Colonies, founded by single queens, contain tens to hundreds of bees. The colonies are reared year-round in isolated facilities fed with sugar water and pollen. The pollen is collected in the fields by honey bees, and therefore, it is a potential health risk to the bees as the pollen is contaminated by pathogens like viruses. Gamma irradiation is employed to sterilize pollen, mitigating the risk to bee colonies. This study aims to assess the impact of gamma irradiation on pollen quality over time and its consequent effects on Bombus colony development. Microcolonies, each housing three worker bees, were divided into three treatment groups: untreated pollen, pollen irradiated at 10 kGy, and pollen irradiated at 20 kGy, sourced from the same origin. Pollen cakes, with 90% pollen and 10% sugar water, were provided weekly to the colonies. Each experimental group included 10microcolonies, received 100 grams of 60% sugar water and five grams of fresh pollen cake per week. After one week, food consumption and egg laying were measured. After five weeks, colonies were frozen, and egg, larval, pupal, and emerging male bee were counted and analyzed. The experiment was repeated three times: one month, four months, and ten months post-irradiation treatment. Results revealed that in the first trial after one month there were no significant differences between the treatments. However, after four and ten months colonies exposed to 20 Kgray irradiation consumed more pollen but showed reduced egg laying compared to control colonies. Male bee size and numbers followed a similar trend. These findings suggest that gamma irradiation may adversely affect pollen quality and subsequent Bombus colony development, with potential implications for time-dependent decay of irradiated pollen compared to the control treatment.

# ADAPTING GREENHOUSE POLLINATION PRACTICES TO NORDIC CLIMATE CONDITIONS

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## Abstract

While greenhouse and grow tunnel cultivation is rapidly increasing in Finland, honeybee pollination of these protected crops is currently in its infancy. Current practice for indoor pollination relies solely on imported bumblebees.

To address the increasing need of pollination services in closed spaces, and to decrease the risks associated with the use of imported bumblebees, the Domestic Pollinators for Commercial Horticulture project is developing new pollination practices for protected crops using local honeybees. Pollination tests in strawberry greenhouses and grow tunnels show that honeybees are viable pollinators in greenhouses and grow tunnels. Honeybees may even provide certain benefits compared to bumblebees, rather than just serving as a replacement.

The main challenge is to adapt both beekeeping management practices and greenhouse set-up to the local nordic climate conditions. At the time greenhouse pollination starts in spring, honeybee colonies are normally still in wintering mode in most of Finland, so new management protocols are necessary to get the colonies ready for pollination at the time crops start to flower. Other challenges include hampered bee navigation capabilities under greenhouse plastic, and bee loss to cold outside conditions. It is essential to include commercial growers and their insights when developing new pollination methods, because successful a pollination service relies on the cooperation between beekeepers and growers.

Based on the results and experience of our tests thus far, we are confident that new methods for managing honey bee colonies and greenhouse spaces can be developed to allow the use of honey bees for providing pollination services in commercial greenhouses and grow tunnels in Finnish climate conditions. This will broaden the range of pollination services available to growers while opening up new opportunities to beekeepers.

# MULTIFUNCTIONALITY OF FLOWERING COVER CROPS: SUPPORTING POLLINATORS WHILE ENHANCING WEED CONTROL AND SOIL QUALITY

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## Abstract

Flowering cover crops are an important asset for the support of wild and managed pollinator populations in agricultural landscapes. These crops can also perform different ecosystem functions, including weed control and soil quality enhancement. While the contribution of individual cover crop species or mixtures to each of these functions has been extensively investigated, experiments testing all these ecosystem functions simultaneously to select the best species or mixture overall are rare. In this study, we evaluated the performance of six flowering summer cover crop species in terms of support to pollinators, biomass production, weed suppression and soil fertility enhancement potential. Tested species included buckwheat (*Fagopyrum esculentum* Moench.), white mustard (*Sinapis alba* L.), berseem clover (*Trifolium alexandrinum* L.), blue tansy (*Phacelia tanacetifolia* Benth.), fenugreek (*Trigonella foenum-graecum* L.) and common vetch (*Vicia sativa* L.). Field work was carried out in four sites in Northern Italy during the summer months of 2020 and 2021. Buckwheat was identified as the overall best-performing species, by virtue of its high biomass production, ability to control weed growth, and abundant and long-lasting flowering that could support honeybees and hoverflies during a low-resource period without promoting competition. Buckwheat was, however, less promising regarding soil enhancement potential and support to wild bees, highlighting the need to continue searching for complementary cover crop species to be used alongside it in a mixture. Our results could improve cover crop selection schemes, suggesting the necessity for a comprehensive approach aimed at enhancing multiple ecosystem functions in agroecosystems.

# EFFECTS OF FLOWER PLANTINGS ON BEES EXPOSURE TO PESTICIDES AND POLLINATION SERVICE IN APPLE ORCHARDS

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## Abstract

Flowers in agroecological infrastructures can provide a more diverse and less pesticides-contaminated diet for bees compared to crops. Therefore, flower plantings could reduce bees' exposure to pesticides. In France, public policies envisage promoting these agroecological infrastructures to mitigate pesticide risk to bees.

We carried out field experiments during two years in the Provence Alpes Côte d'Azur region of France to explore how flower plantings affect 1) bees' exposure to pesticides and 2) the pollination service by insects in apple orchards. Six flower plantings of mustard, phacelia and faba beans (3400±1100 square meter each in average) were sown within apple orchards. Pesticides exposure was analysed in three bee species: *Apis mellifera*, *Bombus terrestris* and *Osmia cornuta* through samples of pollen, nectar and bees. The pollination service was evaluated at varying distances from the flower plantings. We assessed the abundance and diversity of flower-visiting insects using transects within the flower plantings, in adjacent apple rows and in apple rows further away.

Results from the first year suggest that pesticides were omnipresent in pollen and bees from the orchards as well as from the flower plantings. No pesticide residues were detected in the nectar. There was a trend towards better fruit set in apple trees close to the flower plantings compared to those further away. In general, we observed a low diversity of flower-visiting insects in the orchards, and honey bees were overwhelmingly dominant. However, wild bees were observed more frequently on apple flowers close to flower plantings than on those further away. Finally, these results suggests that flower plantings may attract a diversity of insects and don't adversely affect apple pollination service. However, these preliminary results are not conclusive on a reduction in bees' exposure to pesticides in flower plantings.

# 4 Pollinators and pollination ecology in natural and agricultural landscapes

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## Poster presentations

# APICULTURAL IMPORTANCE OF *PHACELIA TANACETIFOLIA* AND ITS POLLINATOR VISITATION IN HUNGARY

Edit Zajácz, Kata Donkó, Anita Sisa, Tünde Kiss

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## Abstract

Lacy phacelia (*Phacelia tanacetifolia* Benth.) is a popular plant among bee pastures in Hungary, as it can provide a good source of nectar and pollen for honeybees. It is cultivated on thousands of hectares in the western parts of Hungary, mainly for seed production. However, it can be successfully cultivated throughout the country, except for landscapes with extreme soil fertility. In addition to its economic role, phacelia is also of great importance to beekeeping, as it can provide a significant amount of honey for beekeepers. Several cultivars of phacelia are grown in Hungary, and currently, there is no information available on their nectar production and insect visitation.

Our research goal was to determine the yield and sugar concentration of nectar and calculate the sugar value. Furthermore, in 2016 pollinator visitation of phacelia varieties was also observed in Mezőcsát, in the north-eastern part of Hungary. For a period of four days, the 24-hour nectar production of 6 phacelia cultivars was investigated, with pollinators counted for 10 minutes every hour between 8:00 a.m. and 5:00 p.m.

Based on the results, the amount of nectar varied between 0.1-3.7 mg/flower, while the sugar concentration of the nectar was 9.0-63.0% (with an average of 25%), resulting in a calculated sugar value that varied between 0.02-0.83 mg sugar/flower. The insects visited the flowers throughout the entire day, with the proportion of honeybees accounting for 63%, bumblebees 5%, and other pollinators 32%. The majority of honeybees visited phacelia flowers with the highest sugar value (0.15 mg sugar/flower, 938 honey bees/4 days*)*, although this was not consistently observed in the case of bumblebees and other pollinators.

# APPLICATION OF WIDE SCALE ACOUSTIC BASED IN-FIELD POLLINATOR SENSING NETWORK

Jeffrey Shaw, Huw Evans

BeeHero, USA

## Abstract

Significant proportion of crops grown are dependent on insect pollination by both managed and wild bees yet these pollinators continue to face population pressures due to a myriad of stressors such as agricultural practices, climate change and diseases. Monitoring and measuring the presence, density and diversity of pollinators as well as their distribution in the crop offers insights into effectiveness of the pollination and how it correlates to crops yields, as well as further understanding of the dynamics of pollination communities in an agricultural context. Based on technology originally used to monitor in-hive acoustics, a simple acoustic sensor placed in the crop is connected to hundreds of others via a newly developed mesh technology allowing spatial and temporal information on bee visits, ambient temperatures, humidities and light levels to be monitored continuously over large areas. These data are supported by measurements from the hives, namely bee out counts and hive weight data and together are send to the cloud for analysis and interpretation. Here we present the results of the application of this new technology to a wide variety of crop types: almonds, onion seeds, apples, blueberries, carrots, watermelons, sunflowers, radishes and oil seed rape, showing how it can be used to accurately map the pollinator activity in the field at any given time. Lastly we discuss the potential for the use of the in-field mesh sensing for monitoring pollinators in contexts other than agricultural settings.

# DEVELOPMENT OF REGIONALLY OPTIMIZED FLOWER MIXTURES FOR SUPPORTING THE WILD BEE POLLINATORS IN SERBIA

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## Abstract

Intensive agricultural practices have led to landscape simplification, resulting in a significant reduction of essential floral resources for various pollinator groups. Among the mitigation measures, EU and national strategies promote the establishment of sown flower strips, tailored to attract and sustain pollinator populations in various local and regional contexts. Within the EcoStack project (H2020), we aimed to develop regionally specific flower mixtures suitable for supporting the wild bee populations in large agricultural areas of Serbia. In the study conducted during 2021–2022, we tested a total of 32 flower species (24–25 per year; randomised block design of four square metre plots per sown species), of which 26 were established successfully (variably between the years). We carried out multiple timed sampling of bees from May through July, and recorded 20 bee genera (15–19 per year). The three most attractive plants (based on the frequency of wild bee visitation) were: *Cynara scolymus*>*Phacelia tanacetifolia*>*Coriandrum sativum* in 2021, and *Phacelia tanacetifolia*>*Coriandrum sativum*>*Daucus carota* in 2022. Other prospective plants were: *Pimpinella anisum*, *Centaurea cyanus*, *Sinapis alba*, *Onobrychis viciifolia* and *Calendula officinalis*. *Phacelia tanacetifolia* attracted the highest number of visiting bee genera in both years (9–12). Four bee genera (*Lasioglossum*, *Apis*, *Andrena*, *Hylaeus*) contributed each with more than 10% of all visits in at least one year (totalling 73–77%). The analysis of bee-plant visitation network parameters yielded similar results in both years. The networks were relatively simple, with low connectance and low specialisation. Among the bee genera *Andrena*, *Halictus* and *Seladonia* showed the lowest specificity in both years, while *Lasioglossum* and *Apis* only in 2021, whereas among the plants *Phacelia tanacetifolia* had the lowest specificity in both years. Starting with these initial findings, we are now extending the analyses of further plant combinations and of interactions at bee species level.

# EXPLORING POLLINATOR DIVERSITY OF *PRUNUS AMYGDALUS*: INSIGHTS FROM PAN TRAP SAMPLING IN DIFFERENT HABITATS

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## Abstract

*Prunus amygdalus,* commonly referred to as the almond tree, is predominantly dependent on insect pollinators for fruit set. However, during its blooming season in late winter to early spring, insect activity tends to be limited. This phenomenon may be attributed to factors such as sparse vegetation, suboptimal soil temperature, and unfavorable weather conditions.

The aim of the study is to sample potential pollinators and visiting insects of *P. amygdalus* during its flowering period in two different habitats (Plot 1 and 2) using white, blue, and yellow colored pan traps. To prevent bias in sampling, pan traps were placed both at ground level and 75 cm above ground level in sets of three for 24 hours, then collected the next day, and specimens were transferred to falcon tubes containing 70% alcohol. Sampling was conducted on March 12th, 16th, and 20th, 2024, in Ankara, Turkey. The collected insect specimens were prepared as museum material using insect pins appropriate for their size. Diagnoses were made using different keys.

Our investigation explored diversity across habitats, pan trap colors, and heights. Plot 2 showed significantly greater insect species diversity compared to Plot 1, supported by Shannon diversity indices ($H_{\text{plot 1}}$=0.9192, $H_{\text{plot 2}}$=1.5339). Comparing color groups within Plot 1 and Plot 2, white exhibited slightly higher diversity ($H$=-0.467) than blue ($H$=-0.446) and yellow ($H$=-0.459). Ground-level traps displayed higher diversity ($H$=-0.706) than elevated traps ($H$=-0.497).

In conclusion, our study reveals that white pan traps exhibit superior efficiency, likely attributable to the prevalence of white flowers during the sampling period. Furthermore, the comparatively heightened insect diversity in Plot 2, in contrast to Plot 1, is hypothesized to be explicable by the phenomenon of edge effect.

# FARMING FOR BEES: ENHANCING NATIVE BEES AND POLLINATION THROUGH SUSTAINABLE AGRICULTURE GRADIENTS

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## Abstract

Industrial agriculture has standardized landscapes and agricultural practices, resulting in the decline of pollinators and compromising the sustainability of production systems heavily reliant on inputs. *Cucurbita maxima*, a crop entirely reliant on pollinators, is cultivated on farms located peri-urban areas that still employ various management strategies and are situated in diverse landscapes. The aim was to assess the impact of different agricultural practices and landscape features, utilizing territorialized environmental sustainability indices (SAT) at both regional (SATR) and local (SATL) scales, on the abundance of native and exotic bees, as well as their influence on the pollination process of the model crop (*C. maxima*). SAT indices were developed following the methodology outlined by Mazzei et al. 2024, involving the creation of land cover maps and semi-structured interviews with farmers to characterize their practices. Bees were sampled on *C. maxima* flowers for 2 hours over 3 days per season (number of seasons = 2, number of farms = 22). The indices generated gradients of sustainability and modelled the abundance per flower of each bee morpho-species: Eucerini, Halictidae (native), and *A. mellifera* (exotic) using Zero-Inflated Models, with crossed random effects (farm and season). Higher SAT indices were associated with increased abundance and reduced probability of absence of native bees, but increased probability of absence of exotic bees. SATL and SATR had distinct effects on the bee community, with high SATL linked to decreased *A. mellifera* (probability of absence) and increased Halictidae and Eucerini (probability of absence and abundance), while SATR was associated with an increase in Eucerini bees (presence and abundance). Moreover, in farms where Eucerini bee abundance was high, more seeds with embryos were observed in the squash, suggesting enhanced pollination effectiveness. Sustainability can promote the conservation of native bees in peri-urban areas improving squash pollination and potentially benefiting other pollinator-dependent crops.

# HOW NECTAR RESOURCES AFFECT POLLINATOR ASSEMBLGES

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## Abstract

Pollinators provide a key ecosystem service which is ecologically and economically important to the United Kingdom. Widespread global declines have been reported in both pollinator species richness and abundance, which have been linked to similar declines in wild plant species richness and abundance. These declines have been attributed to factors such as landscape change, habitat loss, pests and diseases, pesticides and climate change. The UK government is addressing these declines through schemes such as the Nature Recovery Network and the National Pollinator Strategy.

This study investigated a broad spectrum of floral nectar resource available to UK pollinators, and identified nectar characteristics that affect pollinator foraging choices. The selection of flowers during foraging of pollinators from the orders Hymenoptera, Diptera and Lepidoptera was most significantly related to the percentage and volume of glucose present in the nectar offered by studied flowers, and to a lesser extent the percentage and volume of fructose and sucrose.

As nectar quality and quantity are both a plant species characteristic and primary drivers of pollinator forage choice the findings from this study will support policy makers in decision making on schemes to improve pollinator diversity and abundance. In particular it offers an insight into the nectar feeding preferences of a wide range of pollinators at an order, genera and species level, with many of these species being uncommon and understudied.

# IMPACT OF THE AGROENVIRONMENT ON WILD BEES AND SPONTANEOUS PLANT COMMUNITIES

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## Abstract

Pollinators are essential for maintaining ecosystems, and three-quarters of the world's major food crops require animal pollination for fruit and seed production. However, in recent decades, we have been witnessing a steady decline in these crucial insects worldwide, resulting in a deficit in agricultural production. While agriculture is closely linked to pollinators, it is also one of the causes of their decline. For this reason, in Italy, the BeeNet project was initiated to assess the health status of Italian agricultural ecosystems through monitoring of honey bees and wild bees. This study presents data from the project's first year, 2021, on wild bees in four regions (from North to South: Veneto, Emilia-Romagna, Campania, and Puglia), comparing two different agricultural ecosystems: one intensive and the other semi-natural. Once a month, from early spring to autumn, in all the regions and in both the ecosystems, we sampled bees along a transect (200 × 2 meters) walked in the morning and afternoon. Additionally, on the same days, we recorded all entomophilous plant species flowering along the transect. We found that intensive agro-ecosystems had lower biodiversity, both in terms of species richness and in abundance of individuals, and a bee community skewed towards generalist species. This outcome suggests that the use of more impactful agricultural practices and environmental homogeneity strongly, and negatively, affect these insects and the wild plants they rely on to survive. Therefore, measures need to be implemented in these environments to protect pollinators as required by the European Community, through targeted strategies such as the new CAP 2023-2027.

# PLANT-POLLINATOR NETWORKS IN FRAGMENTED GRASSLAND SYSTEMS

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## Abstract

The loss of area and connectivity of semi-natural habitats in Europe is threatening the functioning and resilience of plant-pollinator networks in these biodiversity hotspots. FuncNet is a Biodiversa+ project between Estonia, Belgium, Germany, Sweden and the Czech Republic for gaining knowledge about the effects of land use change on functional connectivity on plant-pollinator networks. As a part of this study, we aim to assess the structure of plant-pollinator networks in response to the fragmentation of semi-natural grasslands.

As the area and connectivity of semi-natural grasslands has been decreasing over the past century in Europe, other marginal landscape elements, such as road and ditch verges, power-line clear-cuts, can be vital for supporting insect-pollinated grassland plants and pollinators. However, mismatches in plant-pollinator interactions in response to landscape change have not been studied thoroughly before and are vital to understand in order to maintain important ecosystem functions.

We aim to assess plant-pollinator networks in well-connected and fragmented landscape systems through observations, while also mapping the pollinator diversity and plant species composition within each fragment. In addition, pollen samples are being collected from pollinators for metabarcoding analyses to evaluate the floral availability for pollinators. The results will go towards giving recommendations to stakeholders for implementing biodiversity-friendly landscape management practises.

# POLLEN ANALYSES OF HONEY FROM FINLAND IN 2008-2023

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## Abstract

The present study reports results of qualitative melissopalynological analyses of Finnish honey between the years 2008–2023 and changes in their pollen content from the period 2000-2023. *Rubus* spp*., Salix* spp.

and *Trifolium* spp. were present in most of the samples, and these pollen types were also found in the highest proportions. In addition, the presence of pollen grains from Apiaceae groups and *Filipedula species* is typical for Finnish honey samples. Annual variation in the relative amounts of the most numerous pollen types is presented and on the basis of the pollen spectra of the honey samples, some regions of forage plants for bees can be identified. In the period between 2000 and 2023, it seems that the proportions Rosaceae pollen types have been increasing. Reasons for this will be discussed.

# POLLINATORS ENHANCE THE PRODUCTION OF A SUPERIOR STRAWBERRY – A GLOBALREVIEW AND META-ANALYSIS

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## Abstract

Strawberry (*Fragaria x ananasa* Duch.) is the most economically important soft fruit worldwide. While self- and wind-pollination is possible for strawberry, without biotic pollination (animal pollinators, including artificial pollination by humans) rate of strawberry flowers rarely exceeds 60% and thus fruit production is decreased. At a time of widely recognized decline of pollinators and increasing global demand for balanced food, we need a comprehensive understanding of the worldwide valuation of these ecosystem services. In this paper, we use a transparent and systematic review process to detect gaps in the available literature. By applying multilevel metanalytic models, we quantified the contribution of biotic pollinators and different pollinator types to strawberry fruits. Our review showed that research on strawberry pollination is clearly concentrated in European countries, with limited information for Asia – the largest strawberry producer. Additionally, we detected disproportions across research topics, stressing the need for further research on pollinator preferences between strawberry cultivars and the occurrence of managed and wild bee communities. The overall estimate of pollination benefit to strawberry crops is on average a 25% reduction in fruit weight for plants not receiving a biotic pollination treatment. This benefit is similar regardless of the pollinating species. The mean amount globally (2011–2020) receivable by producers from purchasers due to biotic pollination is $5.36 billion per year. Moreover, we demonstrated that strawberry plants that are dependent on self- or wind-pollination set on average 43% fewer seeds (fertilized achenes) than biotically pollinated plants. Such a huge reduction in seed set can cause fruit deformation and reduce the commercial value of strawberries. Together, these findings indicate the critical role of biotic pollination in strawberry production and quality. To ensure pollination services in strawberry farms, growers should take action to sustain healthy pollinator communities and utilize them with the optimal cultivar.

## PRESSURE OF THE VINEYARD LANDSCAPE ON THE BEENET HONEY BEE COLONIES

Manuela Giovanetti[1], Irene Guerra[1], Sergio Albertazzi[1], Giuseppe Arena[1], Michela Boi[1], Vittorio Capano[1], Valeria Caringi[1], Roberto Colombo[1], Francesca Corvucci[1], Amanda Dettori[1,2], Francesca Vittoria Grillenzoni[1], Giulia Lora[1], Piotr Medrzycki[1], Giorgia Serra[1], Gherardo Bogo[1], Laura Bortolotti[1]

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### Abstract

The BeeNet project counts on a large network of 360 apiaries all over Italy and aims to investigate the quality of the agroecosystem by employing honey bees as on-site sentinels. Honeybees are pollinators of many native and cultivated plants. The Italian agroecosystem mostly reflects a territorial heterogeneity, especially in hilly areas where olive groves, orchards, vineyards, and meadows are intermingled. A network of apiaries on the entire territory allows an overview of the current state of the agroecosystem and its crops.

By employing the Corine Land Cover Inventory, we selected BeeNet apiaries with vineyards in their surroundings (a buffer area of 1,5 km of radius, the average flight distance of a honey bee forager). Out of this selection, we grouped apiaries according to the percentage of included vineyards (A= 1-20% or B= >20%), and analysed colony growth and development (estimates of the number of individuals, brood, and pollen stock). We also verified the presence, in the beebread, of active ingredients and estimated the toxicity load. Finally, in a subsample of apiaries, we also identified vine pollen in bee bread and estimated frequency and abundance.

The first interesting result is that 2/3 of all apiaries in the network had more than 1% of vineyards in their surroundings, sustaining a view of the Italian agroecosystem as mostly heterogeneous. Pollen identification confirms the presence of vine pollen in numerous apiaries, which future studies may investigate for tastiness. Brood showed a reduction in B buffers, as well as pollen stock, sustaining the need to reinforce food sources for pollinators in the vineyard inter-rows. Agrochemicals were also more abundant in B buffers, but the toxicity did not show differences between A and B and low toxic compounds were the most abundant, possibly a sign of improvement in treatment selection and distribution in our country.

# QUANTIFYING FLORAL RESOURCES AND HABITAT QUALITY OF POLLINATORS IN AGRICULTURAL LANDSCAPES USING FIELD DATA AND UAV REMOTE SENSING

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## Abstract

Plant-pollinator interactions are crucial for the ecosystem, but the robustness of their interactions highly depends on the quantity and the quality of floral resources available. In agricultural landscapes, where diversity and quality of floral resources are often in degraded conditions for pollinator populations, the need for quantitative assessment of habitat quality is significant. Most of the studies focus on quantity, but not on quality, and often at small scales because of the difficulty of collecting enough data on the field. To help understand the distribution of floral resources in agricultural landscapes, we estimated the spatial heterogeneity of nectar and pollen in the different agroecological regions of Wallonia. We assessed floral nutritional quality by measuring chemical components (carbohydrates, lipids and proteins) of the pollen and nectar of the main entomophilous plant species. We then combined multi-temporal field flower data and UAV remote sensing to train a deep learning network capable of counting the precise number of flowers in each type of habitat. This method upscaled the quantification of floral resources in ecosystems, enhancing both the efficiency and accuracy of measurements. Using these results and the phenology of each observed species, we can directly quantify the nutritional potential each landscape offered to pollinators through time, a critical factor in designing effective conservation strategies.

# STUDYING THE FACTORS AFFECTING THE PREVALENCE OF PATHOGENS IN BUMBLEBEES

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## Abstract

Bumblebees play a crucial role as pollinators in both agricultural and natural ecosystems, contributing significantly to the reproductive success of many plant species. Their unique foraging behaviour and ability to efficiently transfer pollen make them essential for the production of various crops and the maintenance of biodiversity in wild plant populations. However, emerging evidence of the decline of many bumblebee species at local and regional scales raises concerns about reduced agricultural productivity and sustainability in natural ecosystems. Although there is no single factor identified as the origin of bee declines, there is evidence suggesting that it could be a multi-causal phenomenon, with pathogens playing a key role in this problem together with exposure to pesticides, habitat loss, introduction of non-native bees, and climate change. Several pathogens are related to the decline of bumblebee species, but there are important knowledge gaps about their distribution and prevalence in bumblebee populations. If we aim to avoid the severe consequences of pathogen infection in bumblebee populations, we fist need to establish a comprehensive monitoring scheme to detect emergence of new and existing pathogens.

In this study, 11 sampling sites were established on a South-North gradient along Spain, where 20-30 wild *Bombus terrestris* workers per site were collected. Bumblebees were individually analysed to study the prevalence of the main bumblebee pathogens in this species throughout the country. Furthermore, we analysed the degree of hybridization and introgression from commercial subspecies with endemic ones, using a genomic approach, and analysed the possible relationship between the prevalence of pathogens and the degree of introgressive hybridization in each specimen. The results of this research enhance our understanding of the mechanisms influencing pathogen prevalence in bumblebee populations. A better understanding of the epidemiology of bumblebee diseases is essential to improve our ability to mitigate bumblebee declines.

# TESTING A POLLINATOR ASSESSMENT PROTOCOL WITH *OSMIA* SPP.

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## Abstract

Nowadays, there has been a general decline in the abundance and diversity of pollinating insects. Moreover, honeybee (*Apis mellifera)* stocks are not able to supply pollinator demands at the agricultural market. Therefore, alterative wild pollinators are emerging to supply that demand. This study is part of a project to create an Osmia spp. (Hymenoptera: Megachilidae) bank to improve pollinator function. The abundance and diversity of *Osmia* will be obtained with nesting boxes placed previously in the field. Here we present an adaptation of an existing north-american protocol (The Xerces Society for invertebrate Conservation, Lee *et al.*, 2015) for pollinator habitat assessment in almond orchard of the Balearic Islands. This protocol allows to obtain a score related to (i) landscape, (ii) feeding habitats, (iii) nesting habitats and (iv) agronomic management that will be correlated with abundance and diversity of Osmia. In this way, an adapted guide could be created to assess whether a habitat would be favourable for this study of *Osmia.* For the (i) landscape, we considered a 500 m radius for characterizing the landscape. The mapping was carried out with QGIS software using the SIOSE AR+ (2017) and CLC Backbone (2018) rasters. For the (ii) feeding habitat, the number of pollinator-friendly plants and vegetation cover was estimated to study the feeding habitat. For the (iii) nesting habitat, we study the importance of habitats according to the different nesting sites of *Osmia*. Ground habitats were deemphasised, and importance was given to aerial cavities. For the (iv) agronomic management we developed a questionnaire for farmers to study crop characteristics and agronomic management has been developed. Although we have not yet obtained results, we expect that the conclusions of this study will be useful to create a protocol to improve pollinator conservation into a management plan adapted to *Osmia* in Balearic Islands.

# THE DREAM PROJECT: POLLINATOR-FRIENDLY ACTIONS FOR MULTIFUNCTIONAL BIODIVERSITY ENHANCEMENT IN APPLE AGROECOSYSTEMS OF NORTHERN ITALY

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## Abstract

DREAM (Diversified orchards for REsilient and sustAinable Mediterranean farming systems) is a PRIMA project aiming at developing a sustainable fruit production approach for Mediterranean agroecosystems in climate change scenarios. One of the prime objectives of the project is to enhance functional arthropod biodiversity in the agroecosystem, thus improving the related ecosystem services. As pollination is pivotal for flowering crops such as fruit trees, a particular focus is placed on pollinator conservation. A number of pollinator-friendly agroecosystem management practices are however thought to also benefit arthropod biodiversity in general, by providing a variety of microhabitats, microclimates, and alternative food sources. This means that, in some contexts, enhancing pollinator communities might also have positive effects on biological control and other services.

The DREAM project field activities are being carried out in Italy, Spain and Morocco. In Northern Italy, we are investigating the effect of pollinator-friendly practices on arthropod pollinators, predators, and herbivores in 3 experimental apple orchard pairs near the city of Bologna. Each pair includes a conventional orchard with a single apple variety (Pink Lady®) and a pollinator-friendly orchard with 9 apple varieties flowering at different times, as well as a mix of flowering cover crops in the interrow. Pollinators are sampled using pan traps, transects for apple flowers and quadrats for interrow vegetation. Other arthropod guilds are sampled using a variety of methods depending on the microhabitat (pitfall traps for soil, sweep netting for herbaceous vegetation and visual sampling and mechanical knock-down for trees). The project will contribute to the understanding of the strengths and weaknesses of these management practices for multifunctional biodiversity conservation in Mediterranean orchard agroecosystems.

# 5 Novel technologies and methodologies in bee research

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## Oral presentations

Moderator:
Sjef van der Steen

# THE USE COMPLEMENTARY SEX DETERMINING GENE DIVERSITY FOR STUDYING THE GENETIC STRUCTURE OF HONEY BEE COLONIES

Robert Mroczek[1], Joanna Niedbalska-Tarnowska[1], Ajda Moskric[2], Agnieszka Laszkiewicz[1], Kinga Adamczyk[1], Malgorzata Cebrat[1]

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## Abstract

Polyandry plays a significant role in shaping genetic diversity within bee colonies. The complementary sex determining gene (*csd*) gene, responsible for sex determination, displays considerable intraspecific polymorphism, making it a promising target for investigating patrilines. We have studied the potential of the *csd* gene as a marker for genetic inquiries into honey bee colonies, comparing its effectiveness with standard microsatellite markers. Worker brood samples from five colonies underwent genotyping using both *csd* and microsatellite markers. The findings reveal that *csd* alleles exhibit greater variability than microsatellite markers, thereby offering superior genotyping resolution. Each colony harbored a greater number of distinct *csd* alleles compared to microsatellite markers, highlighting the potential of *csd* for comprehensive genetic analyses. Despite challenges in *csd* amplification efficiency, a two-step nested-PCR protocol proved successful. Intriguingly, *csd* genotyping alone identified more patrilines than a set of five microsatellite markers, underscoring its efficacy. The combination of *csd* and microsatellite genotyping enhances the precision of genetic investigations in honey bee colonies, providing valuable insights into genetic diversity, reproductive success, and social dynamics. The *csd* gene emerges as a promising tool for advancing genetic studies in honey bee populations.

# PLANT-POLLINATOR INTERACTIONS UP CLOSE: A NEW MONITORING TOOL

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## Abstract

Conservation efforts counteracting the current decline of pollinators can only be effective when based on unbiased information about target taxa. Classic monitoring techniques, such as transects and pan-traps, have intrinsic downsides (e.g. time-consuming and indiscriminately lethal, respectively) that might limit researchers' access to crucial information. In recent years, advanced video-monitoring started to be implemented in pollinator studies, ensuring continuous monitoring while sensibly cutting down the time operators spend on field. Many of these solutions, however, include expensive components and/or are too bulky to be useful in remote study sites.

We present a new, relatively inexpensive and portable device running an ad hoc, free and open-source software that allows to capture pollinator activity in several contexts (e.g. on flowers, at nest entrance). The software relies on motion detection, so that moments of complete stillness are not recorded, saving video-elaboration time, memory and battery.

The tool has been used, along with transects, to monitor plant-bee interactions in high mountain sites. To assess its efficacy, Precision and Negative Predictive Value (NPV) were computed. Monitored time cut automatically by the software and number of records obtained, compared to that of records collected via transects, were also considered.

The device ran for a total of 257 hours, of which more than 50% were cut by the motion-detecting algorithm, and records from the device accounted for 79% of all records collected during samplings. Although precision resulted to be only 0.185 due to several instances of false positive triggers (due to wind interference), no false negatives occurred (NPV = 1).

The proposed tool was proved to be useful in bee monitoring, providing a huge amount of data without increasing time dedicated to direct observation, and still it shows room for improvement. Future work should focus on detection optimization and automatic recognition (e.g. via Machine Learning).

# INSIGNIA-EU. THE EU PREPARATORY ACTION FOR MONITORING OF ENVIRONMENTAL POLLUTION USING HONEYBEES

Jozef van der Steen[1], Andreia Quaresma[2], Hans Baveco[3], Robert Brodschneider[4], Valters Brusbardis[5], Norman Carreck[6], Leonidas Charistos[7], Ellen Danneels[8], Amadeo Fernández-Alba[9], Dirk de Graaf[8], Kristina Gratzer[4], Alison Gray[10], Fani Hatjina[7], Konstantinos Kasiotis[11], Ole Kilpinen[12], Maria J. Bueno[9], Alice Pinto[2], Marco Pietropaoli[13], Maria Murcia[9], Ivo Roessink[3], Evangelia Tzanetou[11], Flemming Vejsnæs[12]

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## Abstract

INSIGNIA-EU is the first pan-European action that has provided the baselines for the use of honey bees as environmental monitoring tools. In this citizen scientist beekeeper project, performed in 2023, honey bee colonies were used to biomonitor environmental pollution in 315 apiaries in the 27 EU countries.

Non-polar pesticides were monitored with APIStrips every two weeks; 202 compounds were detected in the 5,524 APIStrips. Azoxystrobin, boscalid, and acetamiprid were the most detected pesticides. The median number per APIStrip was four in agricultural-, two in urban- and two in natural areas. Honey was sampled at four-week intervals, giving 1,164 samples. Polar pesticides found in honey were glyphosate, AMPA, phosphonic acid, or N-acetyl-glyphosate. Microplastics were sampled at four-week intervals; 52,099 synthetic polymer fibers and 7,244 synthetic polymer fragments and films were detected and analysed. Polyester, polypropylene, and polyacrylonite were the most detected microplastics. Metals were sampled at four-week intervals, and greater amounts of metals were found in southern Europe, due to natural soil sources. The analysis detected point emission sources. After four weeks of in-hive exposure for 1,216 silicone bands, all 20 target VOCs were detected. Isoprene was followed by hexane and benzene. Of 35 target PAH

compounds, 34 were detected, the dominant compounds being naphthalene, methylnaphthalenes and pyrene. Significant exceedance of the average values indicated locally increased emissions. The 2,490 bee-collected pollen samples, showed 501 genera. Pollen of *Trifolium*, *Plantago*, *Brassica*, *Rubus*, and *Castanea* were most abundant. Pollen diversity was higher in urban and natural- than in agricultural areas. The Mediterranean area has the most differentiated bee-collected pollen in Europe. Pollen diversity and the distribution of environmental pollution in the European Union throughout the bee season are visualized in spatially explicit models.

*The study has already generated eight scientific publications. INSIGNIA-EU is an EU-funded project (N° 09.200200/2021.864096/SER/ENV.D.2.".).*

# ADVANCED IOT SENSORS IMPROVE UNDERSTANDING OF HONEY BEE FORAGING BEHAVIOUR IN APPLE ORCHARDS

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## Abstract

Most apple cultivars are self-incompatible, requiring pollen transfer from a “polliniser” cultivar to set fruit. Managed pollinators (predominantly honey bees) are used by many apple growers and thus understanding their foraging behaviour in orchards is essential for apple production.

Apple farmers often grow different apple varieties, as well as other tree fruits such as cherry or pear. Thus there is a mixed floral resource that will vary in attractiveness to bees in terms of quality and quantity. Managing pollination to match bee activity with bloom progression across this type of mixed orchard planting is therefore difficult. Also in recent years there has been a shift to more intensive hedgerow production systems. The pollination needs of this type of growing system are less well understood, and traditional hive densities and placement strategies may no longer be applicable.

In our 2024 study we deployed state of the art IoT sensors in a high density trellised apple orchard to remotely monitor honey bee activity in real time both at the hives and on the trees.
Sensors deployed included:
- Hive entrance bee counters, to detect bee trips made by the colonies
- Hive scales, to track nectar flow
- Weather stations
- In Field Sensors using audio techniques to detect bee activity in the trees. In Field Sensors were distributed throughout 5 orchard blocks planted with 2 different varieties.

The results show bee activity spatially across the orchards, movement between varieties as bloom progresses and comparative activity on polliniser trees. Orchard bee activity is correlated with hive activity, bloom progression, and fruit set after pollination. These data will help inform pollination management strategies, including the timing of hive deployment and removal, optimum densities, and placement strategies, as well as the selection of polliniser varieties.

# HOW TO CHANGE A HONEY BEE'S COLOR: KNOCKING OUT PIGMENT SYNTHESIS-RELATED GENES USING CRISPR/CAS9 AND THE EFFECTS ON PHYSIOLOGY

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## Abstract

The cuticle represents the outermost layer of an insect. It is a hardened structure of chitin and other compounds like sclerotins, creating a tight barrier towards the environment. Different pigments are incorporated into the cuticle during development. They define the color of the insect. The functions of pigmentation are diverse, including adaptation to temperature, humidity, UV and pathogens, and sometimes contributing to sexual selection. While pigmentation has been studied in some insects including the fruit fly *Drosophila*, knowledge about pigment synthesis in honey bees (*Apis mellifera)* is scarce. We investigated the function of *ebony* and *tan*, two genes required in pigment synthesis, in the Western honey bee (*Apis mellifera carnica*) using CRISPR/Cas9-mediated gene knockout. While *ebony* drives the production of yellow sclerotin from dopamine, *tan* catalyzes the reverse reaction. Lab-reared honey bee mutants differed significantly from wildtype controls in their cuticle coloration. Homozygous mutation of *ebony* led to very dark bees with pale hair and dark wing venation. Knocking out *tan* made the emerging bees brighter and more yellowish than wildtype bees, while the pigmentation difference was less prominent compared to the creation of *ebony* mutants. RNA-sequencing indicates differential gene expression in head, thorax and abdomen between mutants and wild types. Notably, some of the differentially expressed genes were related to metabolism and cuticular hydrocarbon synthesis. Our study demonstrates that *ebony* and *tan* are important genes driving honey bee pigmentation and cuticle-related transcriptomic processes.

# BE-eRNA: USING NEXT-GENERATION SEQUENCING TO CHARACTERIZE THE COMMUNITY OF HONEY BEE VIRUSES FROM HIVE ENVIRONMENTAL SAMPLES

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## Abstract

Recent advances in next-generation sequencing have led to the development of novel molecular tools that can be used to monitor emergent threats to biodiversity. The western honey bee (*Apis mellifera* L.) plays a critical role in agricultural pollination services globally, yet is threatened by many pests and pathogens, particularly RNA viruses. Here, we demonstrate that high-throughput sequencing of samples from the hive environment can serve as an "early warning" biomonitoring tool to rapidly detect new threats to honey bee health. Specifically, we investigated the utility of environmental RNA (eRNA) collected from hive substrates to characterize the virus communities of honey bee colonies. We swabbed the following substrates from test hives: hive entrances, hive tools, hive bottom boards, sugar feeders and honey. RNA was extracted from all swabs, libraries were prepared for each RNA sample, and RNA-seq was performed on an Illumina NovaSeq instrument. We found that we were able to characterize the virus community within honey bee colonies from these eRNA samples. Certain hive substrates yielded more viral RNA than did others and we recommend sampling from these substrates for standardized monitoring. In the future, we will conduct a ring-test among beekeepers in the United States to validate this method as a scalable tool to monitor for emergent viruses. This study demonstrates the untapped potential of eRNA as a tool for global viral monitoring in honey bee colonies.

# HONEY DNA METAGENOMIC ANALYSIS TO IDENTIFY HONEY COMPOSITION AND MONITOR HONEYBEE PATHOGENS

Kairi Raime

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## Abstract

Honey contains DNA traces from various organisms, including plants, bacteria, fungi, arthropods, viruses, and mammals, from the environment of foraging honeybees or honey production. Our aim was to develop DNA-based methods to identify the biological composition, authenticity, and origin of honey and to track honeybee pathogens and pests using honey DNA.

Developed DNA untargeted metagenomic analysis describes the taxonomical composition of the honey based on the millions of short sequencing reads. The composition of plants in honey DNA reflects the honeybees' foraging preferences as well as the foraging and hive environment. In addition to pollen plants, nectar and honeydew plants were also identified. Monofloral honey was shown to contain more DNA sequences from the corresponding plant. The honey DNA metagenomic analysis that is not targeted to only a few specific genomic regions and organism groups also enabled the identification of important honeybee pathogens and parasites (including bacteria, fungi, and arthropods) and could be used as a monitoring method.

We analyzed more than 550 honey samples from Estonia and other countries. The whole DNA taxonomical profile of honey contains hundreds of species from various organism groups and is unique for every honey sample. However, there are similarities between authentic honey or honey from the same geographical regions and differences between authentic and inauthentic honey or honey samples from different geographic origins. The comparison of DNA taxonomical profiles enables to describe also the authenticity and origin of the honey.

Honey DNA untargeted metagenomic analysis gives a broad, high-resolution picture of honey biological composition, honeybees' foraging preferences, and honey production environment.

# THE POTENTIAL OF AME-711 CONTINUOUS HONEY BEE CELL LINE IN GENOTOXICOLOGY RESEARCH

Uros Glavinic, Marko Ristanic, Milan Rajkovic, Stefan Jelisic, Nina Dominikovic, Jevrosima Stevanovic, Zoran Stanimirovic

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## Abstract

Genotoxicity testing is one of the most important steps in evaluation process of different chemicals, drugs, supplements and all other substances that need to be tested before their introduction in experimental or commercial use. This testing should provide information whether a compound has the potential to be genotoxic, or has potential genoprotective effect for an organism. The Comet test is important tool to estimate DNA damage at the single cell level. Evaluation of cytotoxic and genotoxic properties of compounds intended to be used in beekeeping was carried out using several cell systems (primary or continuous cell lines of other species), but no genotoxic or genoprotective investigation has ever been done on a continuous honey bee cell line. The AmE-711 cell line, established from *Apis mellifera* embryonic tissue is the first continuous cell line from this insect species. The aim of this study was to test if this cell line enables above mentioned toxicology testing. We used AmE-711 cells in alkaline Comet test to evaluate genotoxic/genoprotective effect of thymol (one of the most popular substance used in beekeeping) and water extract of mushroom *Agaricus bisporus* (extract which showed great potential in bee health improvement). Results showed that thymol has a genotoxic effects in concentrations of 100 and 1000 µg/mL revealed through DNA damage in AmE-711 honey bee cells. On the other hand *A. bisporus* extract did not demonstrate genotoxic effects in any of tested concentrations but showed promising genoprotective properties. AmE-711 cells from negative (non-threated) control remained undamaged, while $H_2O_2$ induced expected damage of the cells in positive control. These results proved that AmE-711 cell line may serve as the adequate cell culture system for genotoxicity investigations in bee research.

# COST-EFFECTIVE WHOLE GENOME SEQUENCING TO IDENTIFY LOCAL OUTBREAKS OF EUROPEAN FOULBROOD

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[2]Fera Science Ltd, United Kingdom

## Abstract

European foulbrood (EFB) is a major bacterial disease infecting honey bees internationally, caused by the Gram-positive bacteria *Melissococcus plutonius*. Currently a four-gene multi-locus sequence typing (MLST) scheme is used to assign a sequence type to each outbreak site in England and Wales, allowing us to monitor disease spread within a sequence type. However, 72% of cases are made up of three sequence types spread with large geographical ranges, making outbreaks from these common MLST types difficult to track. We developed a whole genome sequencing pipeline to provide more specific genetic information from outbreak sites to provide greater clarity about possible transmission events.

The whole genome sequencing pipeline developed was designed to be rapid and cost-effective, and to fit into existing national surveillance systems that confirm disease presence using Lateral Flow Devices (LFDs). Samples of LFD buffer bottles were collected from outbreak sites across England and Wales were processed through a pipeline which included host depletion, DNA extraction, Oxford Nanopore sequencing, and a single nucleotide polymorphism (SNP) analysis. Data provided clear outbreak clusters within single MLST sequence types that were geographically linked, and potential transmission events were identified. In addition, the sequencing data were interrogated for virulence genes, antimicrobial resistance genes, and traces of other bacteria associated with *M. plutonius* infection.

Our results demonstrate the power of whole genome sequencing as a tool to monitor disease movements, and the low-cost per sample means this technology is affordable to investigate local- and national-scale outbreaks to follow transmission events. Our methods represent an important tool to improve our understanding of EFB disease epidemiology and improve disease control. Finally, the method has the potential to identify genes that are important in disease progression and control, as well as investigate the microbes associated with this damaging disease.

# COMPUTER VISION MONITORING OF KIWIBERRY POLLINATION

Joris Mattheijssens, Filip Debersaques, Jan Verwaeren

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## Abstract

Kiwiberries (Actinidia arguta) are delicious fruits, growing on plants that suffer from almost no pests and can thrive in a wide range of weather conditions. The dioecious nature of A. arguta requires plantations to have both male and female plants, usually in a ratio of 1:4 to 1:8. We study pollination behaviour in an experimental plantation with variety of cultivars, having asynchronous flowering periods. The main pollinators are honeybees (Apis mellifera) in beehives and various species of wild bumblebees (Bombus spp.). We monitored the visits of both genera to a male and a female plant with partly overlapping flowering periods, using timelapse cameras. Insects were detected on the images using a chain of deep learning and computer vision techniques. We made timelines of the visits per plant gender and insect genus, and coupled this to weather data. We found that B. spp. are much more active than A. mellifera, especially during less optimal weather conditions. As a consequence, most of the useful pollination work, i.e. visiting both male and female flowers on the same day, is done by bumblebees. Furthermore, we observed the tendency of B. spp. to crack open flowers before anthesis. When this happens of female flowers, this often damages the fruit. However, when it happens to male flowers, it can prolong the overlap in flowering periods, which is beneficial for the pollination.

# 5 Novel technologies and methodologies in bee research

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## Poster presentations

# A COMPREHENSIVE MANUALLY CURATED RESOURCE OF CIRCULAR RNA IN HONEYBEE

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## Abstract

Circular RNAs (circRNAs) are a class of non-coding RNA that form a closed, continuous loop structure without termination at 5′-caps and 3′ poly(A) tails generated by backsplicing of exons, introns, or a combination of both. Studies have revealed cell-type, tissue-type, and developmental-stage specific expression patterns of circRNAs in the eukaryotic transcriptome, revealing their regulatory functions in gene expression. With advances in high-throughput sequencing technology and bioinformatics, a number of circRNAs have been identified in animals and plants. The presence of circRNAs has been detected and studied in the fruit fly, silkworm, and honeybee. In the honeybee, circRNAs are differentially expressed in different castes, suggesting that they are involved in caste-specific gene regulation. The role of circRNAs has also been hypothesised for honeybee immunity and response to pathogens. In the current study, we collected and manually curated honeybee circRNAs from large-scale circRNA identification studies in honeybees. We annotated circRNA transcripts and identified potential interactions of circRNAs with miRNAs. The circRNA sequences were obtained from publicly available sources and aligned to the current honeybee genome assembly Amel_HAv3.1. Potential interactions of circRNAs with miRNAs were predicted using the miRanda software and the miRBase database. The database contains annotated circRNAs detected in different developmental stages, castes, and physiological conditions of honeybees. circRNAs represent the hotspots in current transcriptomic research field. The circRNAs in our database are named according to the nomenclature system based on the name of the host gene and the genomic position. The dedicated database for circRNAs in honeybees provides comprehensive information that helps to uncover the molecular mechanisms and biological function of circRNAs in the honeybee and to bridge the large gap between the overwhelming number of circRNAs and their biological functions.

# ACCURATE LABORATORY MEASUREMENT OF METABOLIC RATES IN BUMBLEBEES (*BOMBUS* SPP.) IN RESPONSE TO ENVIRONMENTAL STRESSORS

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## Abstract

Accurate measurement of metabolic rates is crucial for understanding the physiological responses of test organisms to environmental stressors, pivotal for assessing their adaptability and resilience in changing ecosystems. In laboratory conditions, a significant problem is the variability of test organisms, which can mask the small effects of different tested stressors. This study aimed to enhance the precision of these measurements by systematically minimizing environmental perturbations such as light intensity, pressure, vibrations, and pesticide exposure, which are known to influence physiological data.

Utilizing advanced respirometry techniques within a controlled laboratory setting, metabolic rates of bumblebees were measured under three distinct experimental conditions: a control group with baseline settings, a vibration treatment group where mechanical disturbances were introduced, and a light variation group where light intensity was periodically altered. The experimental setups were meticulously controlled: light intensity was standardized using programmable LED systems capable of simulating natural lighting conditions; pressure fluctuations were minimized with the aid of sealed chambers equipped with pressure-regulating devices; vibrations were isolated using anti-vibration setuptables, and pesticide exposure was carefully managed through the use of environmentalally control.led exposure chambers.

Preliminary results suggest that our rigorous control of environmental conditions substantially reduces confounding effects, thereby maintaining the integrity of metabolic rate measurements.

By establishing standardized protocols for controlling environmental perturbations, this study provides a replicable model for future research in entomology and environmental sciences. The implications of this research extend beyond academic boundaries, offering valuable insights for the development of sustainable agricultural practices and the conservation of pollinator populations facing environmental challenges.

# AN UNCONVENTIONAL POPULATION GENETIC ANALYSIS USING HONEY AS A SOURCE OF ENVIRONMENTAL DNA: APPLICATION IN CASTANEA SATIVA GENETICS

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University of Bologna, Italy

## Abstract

Honey bees are considered generalist foragers because they collect nectar and pollen for their nutrition based on the availability of flowers. The European chestnut (*Castanea sativa*) holds significant value in beekeeping due to its abundant nectar production. While chestnut trees blooms, honey bees can produce chestnut monofloral honey, a unique and highly appreciated honey type. The production area of chestnut honey corresponds to natural distribution of this common deciduous monoecious tree (with domestic and wild varieties), which covers southern Europe, including the entire Italian peninsula. Chestnut honey contains chestnut tree pollen which is a source of chestnut tree DNA. In this study, we obtained information on the genetic variability of *C. sativa* by analysing DNA extracted from 56 chestnut monofloral honey samples produced in Italy. DNA was analysed with 16 chestnut microsatellite markers commonly used to genotype *C. sativa* genetic resources. These microsatellites are molecular markers that can also provide the identification of chestnut varieties, which are mainly related to fruit and flour production. In each honey sample, the DNA fingerprinting was compatible with the presence of more than one chestnut varieties. The fingerprinting of some honey samples suggests the presence of wild chestnut trees often used for wood production. The results revealed a high level of genetic variability in the Italian chestnut tree population. The presence of multiple microsatellites per sample is due to the honey bee foraging activity that can visit several plants in an area of 3 km radius; on the other hand, the chestnut wild tree is more polliniferous than the domestic one and this can explain the high quantity of its pollen inside the honey samples. In summary, our study provided a novel overview of the genetic variability of chestnut accessions in Italy and insights into the honey bees' preference for certain chestnut tree varieties.

# EXPLOITING THE MITOGENOMES OF APIS MELLIFERA SUBSPECIES TO DEVELOP AN AUTHENTICATION TOOL TO VERIFY THE ENTOMOLOGICAL ORIGIN OF MEDITERRANEAN HONEYS

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## Abstract

Honey is highly susceptible to adulteration. Currently, the assessment of its geographical origin remains one of the most difficult tasks, which is typically performed by melyssopalynology. Recently, the attention has shifted towards indirect approaches such as the entomological origin based on geographical distribution patterns of honey bee subspecies. Although queens' trade has impacted the natural subspecies distribution, honeys produced with autochthonous bees or bearing a Protected Designation of Origin specifying the producing honey bee subspecies, offer a unique avenue for authentication.

In the MEDIBEES project, we aim to develop a DNA-metabarcoding approach to authenticate honey's entomological origin focusing on mitochondrial lineages A, M, C, and O. To achieve this goal, the DNA from 1251 honey bees representing 16 subspecies (*A.m. sahariensis, A.m. intermissa, A.m. siciliana, A.m. ruttneri, A.m. iberiensis, A.m. ligustica, A.m. macedonica, A.m. adami, A.m. cecropia, A.m. cypria, A.m. caucasia, A.m. meda, A.m. anatoliaca, A.m. syriaca, A.m. jemenitica, A.m. lamarcki*) was extracted and the whole genome sequenced. From those, 740 mitogenomes were assembled using the MitoZ software. The quality of the assembled mitogenome was assessed by aligning all the sequences using MEGA and 348 samples were deleted. Finally, a phylogenetic analysis was conducted to eliminate non-local subspecies, resulting in a total of 326 mitogenomes. This dataset was used for calculating the fixation index (FST) pairwise values, and a sliding window of 400bp was used to identify single nucleotide polymorphisms that effectively differentiate (FST>0.98) the four lineages, enabling the identification of promising regions for primer design. In this study, three regions were identified that discriminate the four maternal lineages while showing an appropriate length for metabarcoding, namely in the COI, ND1 gene, and CYTB genes.

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# EXPLORING THE ANTIVIRAL POTENTIAL OF *APIS MELLIFERA* VENOM AND ITS KEY COMPOUNDS: AN *IN VITRO* PROTEOMIC ANALYSIS

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## Abstract

Honeybee (*Apis mellifera*) venom (HBV) contains many bioactive elements, particularly allergens such as melittin, apamin and tertiapin. Known for its anti-inflammatory, antibacterial and antiviral properties, HBV is used for various conditions. Despite its widespread use, the precise mechanisms underlying the bioactivity of HBV remain unclear. Therefore, this study aimed to investigate the effect of HBV and its selected compounds on inhibiting SARS-CoV-2 infection.

hACE2 cells (human ACE2-expressing Hek293 cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with non-cytotoxic concentrations of either whole HBV or its selected components: melittin, apamin or tertiapin. SARS-CoV-2 Spike pseudotyped lentiviral particles (SARS-CoV-2 PsVs) were then introduced into the cells. Control samples consisted of cells cultured in DMEM only. After 30 hours of incubation with HBV and SARS-CoV-2 PsVs, cell lysis was performed, followed by a luciferase reporter assessment of viral entry. Uninfected cells were included on the assay plate to measure background signals.

In addition, proteomic analyses of the samples were performed using nano liquid chromatography-matrix assisted laser desorption/ionisation-time of flight-tandem mass spectrometry (nanoLC-MALDI-TOF/TOF MS/MS). This step was performed after cell lysis, followed by trypsin digestion and protein purification using solid-phase extraction micropipette tips.

The results showed that both whole HBV and its selected components at specific concentrations effectively suppressed SARS-CoV-2 activity in hACE2 cells. A comparative analysis of the protein-peptide profiles of the hACE2 cells used in the experiments revealed significant changes in the protein composition of the cells following incubation with HBV or its selected allergens.

Both HBV and its compounds exhibit antiviral properties against SARS-CoV-2. The effect of HBV on infected hACE2 cells was evidenced by detectable changes in the proteomic profiles within the samples. These findings promise to further our understanding of the mechanisms of bee venom in living organisms.

# IDENTIFICATION OF FINNISH HONEY BASED ON MINOR ORGANIC COMPOUNDS AND ELEMENTAL COMPOSITION

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## Abstract

Food frauds have a long history starting from ancient Rome. Nowadays this is a growing phenomenon, especially in Europa. Honey is one of the most common sources of food frauds with olive oil, milk, wines and fruit juices. Simple analytical tools are needed to recognize the fraudulent adulteration's.

In this study we had ca. 60 finish honey samples from the Åland Islands to Lapland and 10 samples from abroad. Mostly the samples were from year 2023 but from four producers we got honeys also from years 2021 and 2022. Organic compounds were studied with nuclear magnetic resonance (NMR) spectroscopy and elemental composition with total reflection X-ray fluorescence (TXRF) spectroscopy.

According to $^1$H NMR measurements honey is mostly fructose, glucose, water, other sugars and several hundreds of other organic compounds on mg/kg level. Based on TXRF measurements most common elements are potassium (average value 850 mg/kg), chloride (140 mg/kg), calcium (50 mg/kg), phosphorus (40 mg/kg), sulfur (10 mg/kg) and several other elements on mg/kg level (like rubidium, manganese, strontium, iron) or on ug/kg level (e.g., zinc, chromium).

Interestingly, the combined results from organic minor compounds even after visual comparison of $^1$H NMR spectra and amounts of the elements, the data was characteristic for different producers and the year when honey was collected. Unexpectedly, honeys from Lapland were easily identified from other honeys based on their elemental composition, e.g., no detectable amount of sulfur.

As a conclusion, it seems that it is possible to trace down honeys from different area/producers based on amounts of minor organic compounds and elements, if spectral library with elemental composition is available.

*This study was a part of ELTUVA project (Ensuring food safety and developing analytical methods) partially co-funded by EU and regional council of Pohjois-Savo.*

# IMPLEMENTATION OF BEE WING GEOMETRIC MORPHOMETRICS: TOWARDS A MORE SUSTAINABLE METHOD

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## Abstract

Bee wing geometric morphometrics is a crucial tool for identifying species, subspecies, lineages, and hybrids. It also allows the study of wings' fluctuating asymmetry, an important indicator of environmental stress. Typically, insect wings are photographed under a microscope after being dissected and mounted on slides. In the photos, landmarks at the intersection of the veins are digitised and their coordinates are analysed to study wing shape and size. These operations require killing and dissecting the insect and are time-consuming. Moving towards increasingly less invasive and destructive entomological research practices, we tested the application of geometric morphometrics to non-dissected honey bees' wings in two subsequent trials. The first trial consisted of comparing the wing shape of sacrificed bees photographed under different conditions: 1) still attached to bees; 2) still attached to bees but placed inside a transparent plastic clamp to flatten them: 3) dissected and photographed with a mobile phone; and 4) dissected and photographed under a microscope. The results of the canonical variates analysis (CVA) and PERMANOVA showed no significant differences between conditions 2, 3, and 4. The similarity between photos taken with the mobile phone and those taken with the microscope was also highlighted. These findings open the way to non-invasive wing geometric morphometrics. The second trial involved live anaesthetised bees, whose wings photographed under conditions 2 and 3 were compared. In addition, before killing for wing dissection, the bees were placed in flight cages to ensure that they were still able to fly after manipulation. This approach would allow wing geometric morphometrics to be applied also to live bees collected in the field and then released and could be a useful tool for citizen science. Specimens preserved in entomological collections and museums could also be exploited without damaging them.

# INTELLIBEE - USING MACHINE LEARNING AND IOT WITH EARTH OBSERVATION DATA AS INNOVATIVE METHOD FOR PREDICTION OF SUITABLE BEE FORAGE AREAS

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## Abstract

Due to the lack of systematic and data-driven planning for suitable bee forage locations, fluctuations in honey yield and price cause differences in agricultural income from year to year. Rapidly changing climatic conditions were reported as important factors affecting production. Because of the stated, determination of optimal apiary locations throughout the year seems essential to sustain and increase the yield and efficiency. Intellibee project aims to develop an innovative solution, leveraging IoT sensors, Earth observation (EO), and in-situ data to support beekeepers in planning and managing their activities using a data-driven approach. By integrating these datasets, beekeepers are offered a user-friendly platform accessible from computers or mobile phones. Several notable achievements include successful development of a methodology for predicting and classifying optimal bee forage locations using EO, IoT, and in-situ data. This approach has significantly enhanced the ability to identify prime areas for bee foraging. Additionally, a comparative analysis was conducted between XGBoost and Graph Neural Network (GNN) models. That demonstrated the superior performance of the GNN model, which achieved an accuracy of over 93% (Cohen's Kappa 0.89), with high precision, recall, and F1-scores across the classes, showcasing its efficacy in predictive analytics. Furthermore, to ensure the practical relevance of the developed methodology, various validation activities were carried out, including expert evaluations and field validations with 12 beekeepers throughout Croatia. These activities confirmed the robustness and applicability of the methodology in real-world scenarios. This methodology represents a promising advancement in the field of beekeeping by harnessing the advantages of EO data, machine learning, and IoT sensors. Its ability to automatically identify suitable bee forage locations based on parameters such as humidity, temperature, precipitation, weight, and vegetation health provides beekeepers with valuable insights to optimize their operations and mitigate risks, enhancing decision-making processes and improving the productivity and sustainability of apiculture.

# METHODOLOGY FOR ESTIMATING HONEY YIELD IN SLOVENIA

Anja Pavlin, Janez Prešern

Agricultural Institute of Slovenia

## Abstract

Slovenia is one of the smallest countries in EU that lies at the intersection of the Alps, Dinaric Alps, Pannonian Basin, and Adriatic Sea Basin, which is reflected in its diverse landforms, biodiversity, climate, and weather. These diverse ecological characteristics, together with beekeeping practices, play an important role in honey yield. Currently, the national honey yield is acquired by averaging data from the hives in the observational network and multiplying the average by number of registered production colonies. However, the data from the observational network is used as a guidance for the migratory beekeepers who choose certain location only if there is a promise of honey flow. With migratory beekeeping on the rise, the traditional calculation is obsolete.

Therefore, to obtain a more accurate estimation of the honey yield, we propose different approach, which is based on i) data on hive migrations and stationary hives obtained from the register of veterinary administration, ii) data on honey yield from hives in the observation network and iii) data on resources from Slovenian forestry services and agricultural subsidy agency for crops.

In the next step, we will augment our dataset with weather and phenological data to obtain more realistic evaluation of honey crops for selected areas such as the Vipava Valley, which is famous for its acacia forage. The final data will be of spatio-temporal nature, for which models for estimation of yield will be tested.

The obtained model for estimating honey yield with a better understanding of the relationship between bees and the environment could potentially help beekeepers manage bees with respect to location and its climate.

# SPLENDID BEE COUNTER: OPEN BEECOUNTER FOCUSSED ON LONG TERM MONITORING AND COUNTING VALIDATION

David Atauri

Universidad Rey Juan Carlos, Spain

## Abstract

Bee counters are devices that count the number of bees entering and leaving the hive. The information they provide is very valuable for research, highlighting the daily mortality of bees.
The counters began to be developed a few years ago but despite the great interest they aroused, they did not become popular due to two main problems: On one hand their validation was very complex and it was not easy to know the accuracy and reliability of the measurements made; on the other hand, many designs were sensitive to dirt and in some of them the weakest bees died in the tunnels blocking them, which required constant and impractical maintenance of the device.
Splendid Bee Counter is an open hardwere, open software, and open data bee counter project whose main objectives are to allow validation of sampling and to allow long periods of monitoring without maintenance.
We have already made a prototype that meets both objectives and with which we want to create a network of monitored apiaries whose open data can be used by the scientific community for research in bee biology, beekeeping, climate change or any other discipline.

# 6 Ecotoxicology, pesticides, pollutants

## Oral presentations

Moderator:
Jens Pistorius

# TOXICOLOGICAL COMPARISON OF COMMERCIAL INSECTICIDE FORMULATIONS ON FIVE HONEYBEE GENOTYPES

Bhanu Yadav[1], Abdulrahim T. Alkassab[2], Jens Pistorius[2], Sergio Angeli[1,3]

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## Abstract

This research aims to assess the toxicological response of different western honeybee, *Apis mellifera* L. genotypes after exposure to insecticides. The commercial formulations tested include Danadim Progress (a.i. Dimethoate), Sivanto Prime (a.i. Flupyradifurone), Karate Zeon (a.i. Lambda-cyhalothrin), and Minecto One (a.i. Cyantraniliprole), which belong to various chemical classes, each with unique modes of action. Both acute and chronic oral toxicity tests were performed following OECD guidelines on selected pure honeybee genotypes such as *A. m. mellifera*, *A. m. carnica*, *A. m. ligustica, A. m.* Buckfast, and a local genotype from queens maintained at Julius Kühn-Institut, Braunschweig. The comparative trials were performed during the spring and summer of 2023, exposing adult caged bees to different concentrations of each insecticide. The results from the toxicity tests varied significantly across honeybee genotypes. The acute oral $LD_{50}$ values of Danadim Progress, Sivanto Prime, Karate Zeon, and Minecto One demonstrated variation in toxicity among the genotypes by 7-fold, 2-fold, 1.7-fold, and 8.6-fold, respectively. The $LC_{50}$ and $LDD_{50}$ values in the chronic oral toxicity tests for tested insecticides showed variations ranging from 1.6 and 8.4-fold between different genotypes. Mellifera genotype showed significantly low sensitivity to Karate Zeon after acute and chronic exposure. Ligustica exhibited high sensitivity to Danadim Progress and low sensitivity to Minecto One after acute exposure. Carnica was found to have high sensitivity to Sivanto Prime and Minecto One after chronic exposure and low sensitivity to Danadim Progress after both acute and chronic exposure. Buckfast and local genotype showed moderate sensitivity to tested insecticides. Further analysis of the behavioral abnormalities will be presented. These results provided insights regarding possible within-species variation of the obtained toxicity results, which should be considered in the eco-toxicological studies and, hence, risk assessment.

# FROM *IN SILICO* TO *IN VIVO*: UNDERSTANDING HONEYBEE FORAGING UNDER PESTICIDE EXPOSURE

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## Abstract

Honeybees (*Apis mellifera*) hold significant economic and ecological value as pollinators for global agriculture and natural ecosystems. For colony survival, foragers are dispatched to gather essential resources, which makes honeybee foraging vital for colony viability.

In a previous study, we demonstrated that field-realistic sub-lethal doses of the neonicotinoid imidacloprid inhibited honeybee foraging, particularly pollen foraging. A plausible mechanism underlying this was then identified by a mechanistic simulation model (BEEHAVE). The simulations indicated that foraging trip durations of pollen foragers were significantly increased by imidacloprid, subsequently decreasing the number of foraging trips per day, thus reducing foraging at the colony level. A possible explanation for the observed effect is that the cognitive functions of pollen foragers are impaired. An indication of this would also be drifting, where foragers return to colonies other than their own.

To test this hypothesis, we conducted a field experiment. We aimed to investigate if the detrimental effects at the individual level, such as increased foraging trip durations and the occurrence of drifting, could be observed. More than 200 foragers and newly hatched bees per colony were marked and monitored during the study (21 July – 25 August 2023) near Pforzheim in southwestern Germany. We used four control hives with a daily in-hive feeding rate of 500 g sugar solution and four treatment hives with a daily sublethal dose of 200 µg imidacloprid/kg sugar solution fed in-hive. The feeding lasted for 10 days (28 July – 06 August 2023).

We found that the predicted detrimental effects at the individual level could be observed under imidacloprid exposure. Foraging trip duration was increased and drifting indeed occurred in the treatment hives. This suggests that imidacloprid affects the cognitive functions of foragers, causing them to spend more time completing their pollen-foraging trips and have difficulties in returning to their colony.

# LABORATORY TOXICITY OF MOSQUITO CONTROL PRODUCTS TO HONEY BEES (*APIS MELLIFERA*)

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## Abstract

Effective mosquito control is essential to Florida residents, as the state's environment and climate are ideally suited for mosquitos. Unfortunately, some mosquito adulticides and larvicides administered by pesticide applicators could have non-target impacts, including on honey bees (Apis mellifera). By elucidating the potential acute effects of mosquito adulticides and larvicides to honey bees, mosquito control personnel can make more informed decisions to minimize non-target impacts of pesticide applications. In this study, we exposed honey bees to one of four mosquito adulticide active ingredients (chlorpyrifos, naled, prallethrin, sumithrin), their commercial formulations (Mosquito Mist®, Dibrom®, Duet®) or one larvicide (Bacillus thuringiensis israelensis - Bti) via oral and topical application to determine acute toxicity (LC50) to larvae and adult honey bees. Honey bee larvae reared in vitro were exposed to pesticides in their diet, on day three after grafting. Groups of 20, newly emerged (one day old) adult bees were exposed to pesticides in cages (n = 5 cages per concentration) through sucrose solution (oral and contact). We hypothesize that the formulated products will be more toxic than the active ingredients alone and the sensitivity between adults and larvae will be different. Our initial results show a high toxicity of naled, for adult honey bees (LC50 = 0.29 ng/bee) after an acute oral exposure and larvae (LC50 = 23 ng/larvae) after acute exposure. The toxicity of prallethrin and sumithrin to adult honey bees are LC50 = 457 ng/bee and LC50 = 1320 ng/bee, respectively. The resulting data will be used to inform on the toxicity of mosquito control products and best management practices for mosquito control programs and apiculture in the future.

**Keywords**: mosquito control products; honey bees; toxicity tests; $LC_{50}$

# THE EFFECT OF LITHIUM CHLORIDE TREATMENT ON HONEY BEE BEHAVIOR

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## Abstract

Lithium chloride is a promising treatment for varroa mites in colonies. Former studies tested its effect on bees' survival; however, its impact on bees' behavior is unknown. In the current study, we tested the effect of LiCl treatment on three fundamental behaviors: the queen egg laying, workers' brood care behavior, and aggression toward other bees, all in lab assays. The bees were placed in cages and fed ad libitum with pollen pest, water, and sugar candy or with sugar candy spike with LiCl at a concentration of 50nM. For the egg-laying assay, we used special cages that allow egg laying for over ten days under lab conditions. Each cage contained 50 workers and a single egg-laying queen. The egg-laying rate was similar in both experimental groups, suggesting that LiCl has no effect on the queen egg-laying. We used Petri dishes with 10-day-old workers in each plate for nursing behavior. On day seven, we introduced a four-day-old queen larva to the bees, and we tracked the bees' responses for five minutes. We found no difference in the number of nursing bees or the intensity of the behavior between the groups. In our last experiment, we also used ten bees in a petri dish and tested the effect of LiCl treatment on their aggression toward an intruder bee for five minutes. We found that the number of aggressive bees and the intensity of the aggression of the lithium-treated bees were significantly lower than that of the control bees. This finding is consistent with data on other animals that show that lithium treatment reduces aggressive behavior. Our findings suggest that LiCl treatment has behavioral effect in bees but not on all behaviors. Further studies are needed to test the effect of lithium treatment on the behavior of full colonies.

# INTERPRETING THE IMPACT OF ORAL, DIRECT CONTACT, INDIRECT CONTACT VIA TREATED SURFACE, AND CHRONIC DIETARY UPTAKE WITHIN ONE MODELLING FRAMEWORK

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## Abstract

Recently, it was shown that acute oral, acute contact and chronic tests for bees can be interpreted within one consistent framework: the BeeGUTS framework. Since all tests can be interpreted within one framework results from one test can be extrapolated to any other test. What was missing were indirect exposure tests. In these tests organisms are put on treated glass plates (or leaves) and take up the compound through their legs and/or cuticle. In such test, the exposure is expressed in g/surface area of the treated medium (glass plate or leave) and the actual dose for the bee is unknown. This research aimed at including the indirect exposure test in the BeeGUTS framework, which would allow extrapolation from the acute oral, acute contact or chronic tests to the indirect exposure test.

An indirect exposure test with bees exposed to Imidacloprid was carried out and was used to translate the exposure from ng/cm2 sprayed surface to an actual dose (in ng/bee) by using the BeeGUTS framework. The model was used to calculate the exposure from observed effects using known parameter values instead of calculating the parameters from observed effects and a known exposure. When assuming that 1) toxicity is a combination of dose, kinetics and intrinsic sensitivity of organisms, independent of the test and 2) the concentration in a glass plate test is constant over time and the dose linear with the exposure concentration, this resulted in almost perfect predictions of the effects in the indirect exposure test using the parameters of the chronic test as a starting point. This implies that we can now do one single test and predict the outcome of all other tests for Imidacloprid. However, the BeeGUST framework showed that the general principles apply to the majority of tested compounds.

# HONEYBEE GUT MICROBIOTA AS AN EMERGING ENDPOINT FOR PESTICIDE RISK ASSESSMENTS

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## Abstract

A recent roadmap for the integration of environmental microbiotas in risk assessments under the European Food Safety Authority (EFSA) remit has been published. Healthy honeybee gut microbiota has emerged as a promising avenue to protect bees against stressors. Honeybees exhibit a consistent core microbiota, and dysbiosis, as part of a multiple stressor system, may be an indicator of adverse scenarios. We therefore investigated the honeybee gut microbiota of *Apis mellifera carnica* workers exposed to a single concentration of the insecticide flupyradifurone (FPF, 36ppm). The laboratory trials were carried out in accordance with official protocols (OECD Nº 245). The abdomen of each bee was separated from the thorax, and DNA extraction was performed individually. Full-length 16s rRNA amplicon metagenomic was sequenced through PacBio sequel II (HiFi/CCS mode). The absolute abundance of four bacterial genera constituting the core honeybee microbiota unveiled a *Lactobacillus*-dominated gut in both treated and non-treated bees. Treated bees exhibited a twofold increase in the bacterial load of *Snodgrassella,* contrasting with a 50% reduction in the *Bifidobacterium* load and the complete absence of *Gilliamella* as compared to the untreated bees. Our findings revealed that FPF disrupted the honeybee gut microbiota. We have developed a new approach, overlooked in risk assessments studies so far, to assess the impact of pesticides bee health until now. Thus, we propose its use as a novel endpoint in pesticide risk assessments. Current risk assessments are performed in a tiered approach, *i. e.*, moving from laboratory assays (first screening) to semi field and field studies, and require no sublethal effect assessments. We therefore advocate for the inclusion of honeybee gut microbiota dysbiosis as a sublethal effect in the first screening step of risk assessments, and as a key parameter to assess pollinator's health.

# PESTICIDOVIGILANCE - TEN YEARS OF THE HONEYBEE POISONING DIAGNOSTIC PROGRAM IN POLAND

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## Abstract

An innovative system for diagnosing bee poisoning cases has been in place since 2014. In the 10 years that this system has been in operation, more than 500 bee poisoning incidents have been diagnosed. Each sample represented an apiary where the beekeepers had observed a sudden and massive bee lose and there was a suspicion of misuse of plant protection products (PPPs) in the surrounding agricultural crops plantations.

Pesticide residues were found in 96% of the samples tested and only in single cases bees were free from pesticide residues. In four out of five diagnosed cases, bee analysis showed the presence of residues of highly toxic pesticides. The percentage of confirmed bee poisonings is therefore very high. At most, residues of up to 25 different pesticides were determined simultaneously in a sample.

Insecticides account for almost half of the substances detected, often several at the same time. Various substances from the group of fungicides, herbicides, acaricides and residues of the use of varroacides were also very frequently detected in diagnosed samples of dead bees. However, the three main substances most frequently causing bee poisoning in Poland were chlorpyrifos, clothianidin and dimethoate.

With the withdrawal from use of PPPs containing chlorpyrifos or dimethoate, there has been noticed a significant reduction in the number of recorded bee poisoning incidents. In 2023, not a single case of bee poisoning with dimethoate has been reported anymore. This confirms the effectiveness and correctness of the bee poisoning diagnosis and registration system run in Poland.

The system not only provides individual assistance to affected beekeepers, but also makes it possible to assess and characterise in detail the phenomenon of bee poisoning in Poland and to confirm its causes.

# INTEGRATION OF MULTI-OMICS ANALYSES OF THE GUT-BRAIN AXIS WITH IN-HIVE BEHAVIOURAL RESPONSES OF HONEYBEE WORKERS (*APIS MELLIFERA*) AFTER PESTICIDE EXPOSURE

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## Abstract

Honeybee colonies, operating as superorganisms, rely on effective communication and coordination, which can be disrupted by pesticide exposure. By investigating the gut-brain axis of workers in tandem with in-hive behaviour monitoring enables us to associate the structural and functional impacts of pesticides on the gut microbiome with changes in brain function and behavioural phenotypes. In this study, we conducted an in-hive feeding experiment using SIVANTO® Prime (Flupyradifurone), Cantus® (Boscalid), and Click Pro® (Terbuthylazine and Mesotrione). Pesticide-spiked sucrose solutions with environmentally relevant concentrations were offered to hives acutely for one day, with subsequent monitoring of mortality and in-hive behaviour among age-matched workers over eight consecutive days. In total, we documented 16 different behavioural phenotypes, which were grouped into five larger categories (social, individual, brood care, colony maintenance, and foraging behaviour). The experimental setup was replicated thrice during the honeybee season. Despite no noteworthy impact on mortality, varied in-hive behaviours were observed. The insecticide SIVANTO® prime significantly decreased colony maintenance behaviours, but increased the proportion of allo-grooming in the first days after exposure. The fungicide Cantus® increased resting behaviour, while Click Pro® decreased brood care behaviours, driven mainly by a decrease in the feeding of larvae. Complementary multi-omics analyses will reveal structural and functional changes in the gut microbiome and brain of worker bee pre- and post-exposure, providing insights into associations between health and behavioural changes. This study elucidates the relationship between pesticide exposure, the gut microbiome, brain function, and behavioural phenotypes in nucleus colonies.

# A COMMON PESTICIDE IMPAIRS THE MATING BEHAVIOUR AND THE FERTILITY OF A SOLITARY BEE, *OSMIA BICORNIS*

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## Abstract

Mating behaviour and fertility can act as strong relevant selective forces on reproductive trends in male insects. Therefore, disorders on mating process may be a key driver of global bee population declines. Pesticide impacts on the mating behaviour and fertility have yet to be studied for the red mason bee, *Osmia bicornis* (L. Hymenoptera: Megachilidae). Here, we aim to fill this knowledge gap by testing the impact of a field-realistic exposure of a common sufoximine insecticide, sulfoxaflor (20 ppb). We therefore assessed the sublethal effects of chronic oral exposure to sulfoxaflor on key behavioural (e.g., consumption, daily activity, mating behaviour and success) and reproductive physiological (i.e., sperm quantity) fitness traits. Our results showed that exposure to the pesticide increased food consumption, altered daily behavioural activity, mating behaviour (e.g., mating displays, number of copulations, mating duration) and success, as well as reduced sperm quantity, as compared to controls. As *O. bicornis* males are polygamous, reduced sperm quantity inevitably means fewer females can successfully be mated. Further, our data suggest that field-realistic exposure to sulfoxaflor causes mating to become a risky, long, and unsuccessful process which most likely will yield in reduced male fertility, reproduction success, and fitness at both individual and population level. Given the common use of our tested pesticide, its detrimental sublethal effects raise global concerns. In fact, while some countries acknowledged its impacts, most have posed no use restrictions. Refined risk assessments that investigate sublethal effects appear crucial to better estimate and reduce pesticide impacts on pollinating insects and the environment. Further laboratory and field research are urgently required to better understand the impact of stressors on the mating behaviour of bees and other insect pollinators.

# INTERACTIONS BETWEEN *VAIRIMORPHA (NOSEMA) CERANAE* MICROSPORES AND A "BEE-SAFE" INSECTICIDE FLUPYRADIFURONE IN THE SOLITARY BEE *OSMIA BICORNIS*

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## Abstract

Pesticides and pathogens have been identified as two major drivers of bee declines. However, their interaction is poorly investigated and mostly limited to the western honey bee *Apis mellifera*. In this study, we assessed the combined effects of *Vairimorpha (Nosema) ceranae*, a common and widespread honey bee pathogen, and flupyradifurone, a new neurotoxic insecticide considered "safe to bees", on the solitary bee *Osmia bicornis*. Newly emerged females of *O. bicornis* were orally infected with 100,000 spores of *V. ceranae* and later exposed *ad libitum* to flupyradifurone at a field-realistic concentration of 4.38 mg/L. We tested four treatments: control (C), flupyradifurone (F), *V. ceranae* (V), and *V. ceranae* + flupyradifurone (V+F). Our study shows that *V. ceranae* is able to replicate in *O. bicornis*. Spore levels increased up to 10-fold in V+F bees. Six days after the ingestion of the spores, bees showed high levels of cell damage and alteration of the midgut structure. Phototactic response was impaired in bees exposed to V and F singly but, surprisingly, not in combination. Food consumption was lowered in V and V+F but not in F bees. The two stressors had a synergistic effect on survival rate. In comparison to *A. mellifera*, *O. bicornis* was more susceptible to the two stressors, even though spore replication was higher in *A. mellifera*. In the current scenario where pathogen spillover between managed and wild bees is common and pesticides are still largely used, our findings have important implication for elucidating the decline of bee populations and underscore the necessity of investigating the interaction between pesticides and pathogens in solitary bees.

# HOW IS BUMBLEBEE FLIGHT PERFORMANCE AFFECTED BY PESTICIDE EXPOSURE?

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## Abstract

Pesticides are important for human food production. However, they also pose a risk to our environment when non-target species are exposed to them. Species such as bumblebees are important insect pollinators, yet can be exposed to cocktails of pesticides when foraging in treated crops. Farmers frequently use the neonicotinoid insecticide acetamiprid, which has been suggested to be much less toxic to bee species than recently banned alternative neonicotinoids. However, simultaneous exposure to other compounds, such as the azole fungicide tebuconazole may disrupt physiological detoxification mechanisms. Hence, it is possible that exposure to this compound may exacerbate the effect of acetamiprid.

We therefore hypothesize that the flight precision of bumblebees is affected by insecticide (acetamiprid) exposure and that exposure to tebuconazole increases this effect. In ongoing experiments, we are testing this by challenging bumblebees to fly through a narrow gap. It has previously been shown that bees turn and fly at an angle through a gap that is smaller than their wingspan. Our experiments exploit this precise flight manoeuvre to compare non-exposed bumblebees with bumblebees exposed to acetamiprid, tebuconazole or a mixture of the two when crossing the narrow gap. We will change the background behind the gap to alter the contrast of the gap in front, allowing us to look at the effect pesticides have on contrast sensitivity during flight.

We expect exposed bumblebees to reject crossing the gap more often than non-exposed bumblebees and to fly with less precision resulting in more collisions. We also expect to find bumblebees are more strongly affected when exposed to a cocktail of pesticides as compared to single components. Our study will help us to understand better what kind of risk pesticides pose on non-target species.

# AZOXYSTROBIN HIDES THE RESPIRATORY FAILURE OF LOW DOSE SULFOXAFLOR IN BUMBLE BEES

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## Abstract

Pollinators are exposed to various agricultural pesticides throughout their lifespan. Many pesticide combinations have not been thoroughly tested, resulting in limited understanding of their interactions. Here we explore the effects of sulfoxaflor, a novel sulfoximine insecticide, and azoxystrobin, a widely used strobilurin fungicide, on bumble bee *Bombus terrestris* survival and physiological functions. We assessed the impacts of acute exposure via oral and contact routes, both individually and in combination, with the explicit objective of elucidating potential synergistic or antagonistic interactions between sulfoxaflor and azoxystrobin.

Our results revealed varied responses to oral and contact exposure routes, where oral treatment produces rapid but temporary effects, while contact exposure leads to more enduring physiological changes. In case of oral exposure the combined effect is rather towards being additive while in contact treatment it is synergistic within 6 h and clearly antagonistic thereafter. This unexpected finding suggests a complex interplay between these pesticides, potentially involving mechanisms of metabolic modulation and neurotoxicity. While this interaction may confer temporary resilience to beneficial fauna, facilitating recovery from pesticide toxicity, it also raises concerns regarding the potential development of resistance in pest populations. These insights underscore the importance of comprehensive risk assessment protocols in pesticide management, emphasizing the need for balanced approaches that prioritize both pest control efficacy and pollinator preservation.

# ASSESSING THE IMPACT OF BOSCALID AND COPPER-BASED COMPOUNDS ON POLLINATORS

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## Abstract

Fungicides and heavy metals are essential tools in agriculture, but recent research has focused on their potential adverse effects on non-target organisms such as pollinators. Simultaneous exposure to multiple chemicals may also result in synergistic, additive, or antagonistic effects with unpredictable consequences. The present research aimed to evaluate the effects of two fungicides widely used in agriculture (Boscalid and copper) on different pollinator species. Thirty-four articles evaluating the effects of these fungicides on *Apis mellifera*, *Apis cerana cerana*, *Osmia lignaria*, *Osmia bicornis*, *Partamona helleri*, *Bombus huntii*, *Melipona quadrifasciata*, and Syrphid were considered. Residues of Boscalid after field applications caused adverse effects on flight performance, memory, learning and nest recognition abilities, as well as microbiota composition in different pollinator species (e.g. sublethal doses of 0.063 mg/mL in *Apis cerana*, and 1.6 kg/ha in *Osmia lignaria)*. Similarly, Copper-based compounds were found to exhibit acute toxicity to bees, influencing antioxidant enzyme activities at sublethal doses (e.g. 142.95 µg/ml of CuSO4 in *Partamona helleri* and 10 mg/L of CuCl2 in *Apis Mellifera*). Articles also highlighted the potential risks of long-term exposure to sublethal concentrations of chemicals on gut microbiota with impact on the microbial community composition, compromising honeybee immunity and indicating potential vulnerabilities within immune defence mechanisms. In a second phase the chronic effect of sublethal doses of copper chloride and Boscalid administered singularly and in combination was evaluated on *Osmia bicornis* in laboratories trials specifically set up to assess survival, microbiome, and biomarkers. Test were executed singularly on *Osmia* bees in test cages. In conclusion, shedding light on the effects of different phytosanitary products, such as Boscalid and copper-based compounds, in laboratory studies provides a foundational understanding crucial for subsequent investigations, informing agricultural practices and safeguarding non-target pollinators.

# A REVIEW OF NECTAR AND POLLEN SAMPLING METHODS FOR PESTICIDE RESIDUE ANALYSIS

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## Abstract

In the study of plant-animal interactions, nectar and pollen are frequently analyzed for their nutritional properties. They also serve as important matrices in understanding how pollinators are exposed to pesticide residues in both crops and wild plants. Given that nectar and pollen are vital food sources for pollinators, it is essential to gather data on the pesticide residues present in these matrices to understand the real-world risks that pollinators face. To determine the pesticide content in nectar or pollen, samples must be collected before they can be analyzed. Various methods are employed to collect nectar and pollen samples, but most involve a compromise in some aspect. The ideal collection method should be cost-effective, easy and quick to use in the field, suitable for all types of flowers, and yield samples that accurately represent the pesticide residues, while being non-destructive. In our systematic review we cover the existing literature on nectar and pollen sampling methods, aiming to find all potential sampling techniques, evaluate their strengths and weaknesses, identify knowledge gaps, and address some of these gaps. We find that while nectar sampling methods have been compared in the literature for various analysis purposes, there is a knowledge gap concerning sampling methods for pesticide residue analyses. Similarly, there are very few studies that compare pollen sampling methods for any analysis purpose. To address these issues, we will conduct a field study to compare the most common nectar and pollen sampling methods and their suitability for pesticide residue analysis specifically.

# SELENIUM IN HONEY BEES

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## Abstract

Honey bees are social insects divided into different sexes and castes. Female honey bees differentiate in two different castes, queens or worker bees. The queen, usually only one in the hive, is the reproductive female. Her role is to lay eggs and regulate the hive's activity with pheromones. The second female caste, that develops from fertilized eggs are non-reproductive workers. The third group is comprised of male bees called drones, originating from unfertilized eggs. It was proven that selenium (Se) is very important for fertility and sperm quality in many animals. For insects, Se could be beneficial for egg fertilization. The aim of this study was to assess Se content in workers, drones and unmated and mated queens. Individual worker bees, drones, unmated and three-month-old mated queens collected from the colony were analyzed after microwave assisted digestion using ICPMS. It was found that worker bees had significantly lower concentrations of Se compared to drones. Considering that the drones' main role is to produce sperm and mate with a queen, we assume that higher Se content is due to an active accumulation in their sperm. To further test this we have compared unmated and three-month-old mated queens. The unmated queens had Se concentrations comparable to worker honey bees. The mated queens had concentrations that were between drones and workers. This suggests that Se is most likely accumulated in drone sperm and transferred to queen bees during mating.

# WHAT ARE THE TRANSFER ROUTES OF FUNGICIDE RESIDUES FROM FIELD TO THE HIVE?

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## Abstract

Boscalid is the most often detected fungicide in bee products such as bee bread, beeswax or honey. In formulated crop protection products, boscalid is commonly combined with strobilurin fungicides like pyraclostrobin. Residue detection in bee products can be one factor to assess potential effects on honey bee health due to pesticides, as bees will come in contact with and consume potentially contaminated hive material. However, it is still not entirely clear if and in which ways pesticides might be diluted or accumulated by the transfer from plants to hive. We investigate the complete transfer pathway after in-field spray application from plants, to foraging and nursing bees, stored products, and finally to the larval food jelly and the larvae themselves. The product Pictor Active®, containing the active ingredients boscalid and pyraclostrobin, was used as a model fungicide. It was applied on oilseed rape in a German-wide field setting according to label instructions and good agricultural practice.

Residues of the active ingredients decreased logarithmically with time in plants, pollen baskets and honey sac samples collected throughout the exposure. We found a higher concentration of both compounds in stored pollen when compared with stored nectar, which occurs presumably due to a chemical attraction between the lipophilic physiochemical characters of pollen and the ingredients. Transfer routes show a decrease in concentrations from one stage to another, except for a possible elevation in stored products. Calculated risk quotient levels were at least seven times lower than recommended levels of concern for honey bees. These results provide a more detailed understanding of the pesticide transfer pathway from field to honey bee colonies with accumulation and dilution factors. On this basis, more products containing active ingredients with different physicochemical properties should be tested, for example there might be differences when testing polar substances.

# THE EXPOSURE AND EFFECTS OF PESTICIDE USE ON POLLINATORS IN AGRICULTURAL LANDSCAPES

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## Abstract

Pesticide use in agricultural landscapes creates environmental contamination that is heterogenous in space and time. Mobile organisms, such as bees, are exposed to multiple contamination sources when visiting patches that vary in the amount, timing and toxicity of pesticides used. Indeed, our recent biomonitoring of bee pesticide exposure demonstrates the presence of multiple compounds in bee pollen and nectar, but the effects of this landscape exposure on colony growth and development of any bee species remains unknown. We show that the many pesticides found in bumble bee-collected pollen are associated with reduced colony performance during crop bloom, especially in simplified landscapes with intensive agricultural practices. Our results from 316 *Bombus terrestris* colonies at 106 agricultural sites across eight European countries demonstrate regulatory system failure to sufficiently prevent pesticide-related impacts on non-target organisms, even for a eusocial pollinator species in which colony size may buffer against such impacts. We provide trait- and landscape-dependent information on the occurrence, concentration and effect of pesticides, which is necessary for more realistic and holistic risk assessment and essential information for tracking policy goals to reduce pesticide risk.

# PYRROLIZIDINE ALKALOIDS AND CLOPYRALID CONTAMINATION IN HONEY- CASE STUDY IN NORWAY

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## Abstract

Honeybee products may be contaminated by various natural and anthropogenic substances, which can pose a health risk to consumers. One such substance is pyrrolizidine alkaloids (PAs), which are toxic plant compounds produced by many plant species to protect against herbivores. The natural occurrence of toxin-producing plant species in honey production areas or their deliberate use as honeybee forage plants can lead to the contamination of honey with PAs. Clopyralid is the active ingredient in the herbicide 'Matrigon 72 SG', which is approved for weed control in oilseed rape in several countries. As a systemic substance, its application before flowering can result in significant residue levels in nectar and honey. We tested the content of PAs and clopyralid in Norwegian honey produced in 2023. Thirty honey samples were tested for the contamination of PA plant toxins. Thirteen samples were obtained directly from beekeepers in coastal areas of southern Norway where PA-producing plants are expected to be most prominent. Additionally, 17 samples were received from a major Norwegian wholesaler. Twenty-two honey samples underwent testing for clopyralid content. The samples included five collected from honeybee colonies placed next to rapeseed fields sprayed with “Matrigon 72 SG” before flowering, five collected from honeybee colonies placed next to organic rapeseed fields, and 12 random samples. PAs were detected in 6 out of 30 honey samples, but predominantly at very low levels (<12 µg/kg). One sample contained a level, which might be of concern for consumption, particularly in children. The EU has not yet established maximum levels for PAs in honey. Clopyralid was detected at levels exceeding both the existing EU Maximum Residue Level (MRL) (0.05 mg/kg) and the 2023 proposed EU MRL (0.15 mg/kg) in 7 out of 22 honey samples, including five honey samples produced close to clopyralid treated rapeseed fields.

# ANTIOXIDANT POTENTIAL OF BIOGENIC AMINES IN HONEY BEES

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## Abstract

Biogenic amines are organic nitrogen compounds that play a vital role in various physiological processes across animals, plants, and microorganisms. Biogenic amines such as dopamine, octopamine, and histamine function as neurotransmitters in insects. In honey bees (*Apis mellifera*), they have been demonstrated to be involved in processes like memory and learning, reproduction, social behavior, defense reactions, and cuticle melanization. Dopamine and tyramine have been reported to have antioxidant capacity, however, their potential role as antioxidants in honey bees has not been thoroughly investigated yet.

In our study, we developed and validated a method for accurately measuring neuroactive biogenic amines in honey bees. Our approach combines reverse-phase liquid chromatography with tandem mass spectrometry, resulting in enhanced sensitivity and specificity. This method was used to analyze honey bees treated by inductors of oxidative stress. Concretely, we focused on the antioxidant system activation, oxidative damage, and the levels of neuroactive biogenic amines in honey bees fed by paraquat and its modified form mitoparaquat targeting mitochondrial superoxide anion radical production.

The honey bees exposed to the oxidative agents exhibited activation of their antioxidant system. Concomitantly, we observed a significant drop in dopamine, octopamine, and tyramine levels, while histamine displayed an opposing trend. We did not detect oxidative protein damage or lipid damage.

Based on the results of our pilot study and the antioxidant properties of dopamine and octopamine molecules, we hypothesize that these biogenic amines may serve as an additional compensatory mechanism to counteract the oxidative stress induced by paraquat and mitoparaquat in honey bees. However, the depletion of biogenic amines could imply subsequent neurotoxicity related to paraquat.

# 6 Ecotoxicology, pesticides, pollutants

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## Poster presentations

# A MULTI-BIOMARKERS APPROACH TO EVALUATE THE TOXICOLOGICAL EFFECTS OF PLANT PROTECTION PRODUCTS IN HONEYBEE AS MODEL SPECIES FOR POLLINATOR INSECTS

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## Abstract

Pollinator species are crucial in maintaining biodiversity in natural and agricultural ecosystems. They provide services that are critical to human nutrition and global food security. Of the many causes of the pollinators' decline one of the most important is exposure to plant protection products widely used in agriculture. Many products are labelled as harmless to pollinators but this often refers only to the active ingredients and not to the commercial formulations. *A. mellifera* is a suitable model species to define the toxicological effects of PPPs on pollinators because it's one of the most studied insects, with its physiological function deeply investigated over the years. Since honeybee occupies the same ecological niche as many other species of pollinators, their loss caused by the use of PPPs suggests that other pollinator insects may experience a similar outcome. Besides the numerous data on insecticides, other classes of PPPs need to be investigated in laboratories and in particular in field studies. Our seven-year studies aimed to develop and apply a multi-biomarkers approach to assess the toxicological effects caused by PPPs in honeybees as a model species. We evaluated the neurotoxic effects, the effects on the detoxification process, the immune system and genotoxicity. We applied an Integrated Biological Index (IBRv2) to assess the toxicological status of this species. This approach allows us to evaluate the toxicity of commercial individual PPPs or mixtures of these, under laboratory conditions, and in agroecosystems during well-known PPP treatments. Laboratory experiments highlighted how exposure to multiple active principles causes different effects compared to single molecules. The fungicide Amistar®Xtra caused neurotoxic, genotoxic and immunotoxic effects. The most evident biomarker alterations in the field monitoring were shown in the hives sampled in the summer season suggesting that longer periods of exposure are needed to show toxicological effects on bees exposed to pesticides.

# ACETAMIPRID: I'M STILL STANDING

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## Abstract

Neonicotinoids are a class of pesticides that have been used for more than 30 years and occupy approximately one-quarter of the global insecticide market. These systemic insecticides remain in the vascular system of the plants for several days, protecting the crop from a variety of pests. Such protection occurs by blocking impulse signals on insects' central nervous system, thus limiting their activity (i.e., paralysis), and therefore causing death. Honey bees are also exposed to these pesticides, potentially harming, or impairing its normal behaviour. Over the years, several researchers have explored the potential negative effects of this class of pesticides and the European authorities (EFSA) have used this knowledge to aid the European Commission decision-making for a stronger legislation of these substances. Considering that acetamiprid is the only neonicotinoid that can be currently applied outdoors in Europe, several experiments have been conducted over the years to evaluate their possible negative effects. Exposure was evaluated by measuring acetamiprid concentrations on samples of flowers, fresh nectar and pollen, honey bees, beebread, and honey. While effects were evaluated by comparing exposed colonies with control ones, by measuring bee mortality, colony strength, nest size, resources, activity and even foraging ranges. We aim at showing if this substance is indeed safe for honey bees and what experiments are needed to improve our risk assessment of this substance.

# AGROCHEMICALS IN BEEBREAD: AN INDICATOR OF LANDSCAPE MANAGEMENT

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## Abstract

Pesticides are recognised as one of the major causes of the current pollinators' decline, along to habitat loss and degradation. These factors are intricately connected to agricultural practices and landscapes, resulting in profound adverse effects on ecosystems. Our study evaluated the status of agroecosystems within the Emilia-Romagna region (Northern Italy), as part of the national monitoring project BeeNet. We examined pesticide residues in 100 beebread samples collected from 25 BeeNet stations during March and June of both 2021 and 2022. Our analysis focused on assessing the diversity and concentration of these chemicals, their toxicity-weighted concentrations (TWC) to honey bees, and their correlation with land use composition. Of the samples tested, 84% resulted positive for at least one pesticide residue, comprising a total of 63 different active ingredients out of 373 screened. The predominant types were fungicides and insecticides, accounting for 46% and 44% of the total, respectively. Additionally, 15 compounds were not approved in the EU as plant protection products (PPPs), prompting concerns regarding potential illegal use or contamination via beeswax recycling. TWC exceeded the risk threshold in seven samples, primarily attributed to one or two active ingredients. Furthermore, the TWC calculated for individual compounds surpassed the threshold for four insecticides: Carbaryl, Fipronil, Imidacloprid, and Thiamethoxam. Moreover, both TWC and the number of active ingredients demonstrated moderate to high positive correlations with the percentage of area covered by orchards. Considering that TWC does not account for potential synergistic effects among different molecules, and that we found an average of five different active ingredients per sample, our results indicate a concrete toxicological risk for bees associated with agricultural practices, highlighting the urgent need to implement eco-sustainable and pollinator-friendly strategies.

# ASSESSING THE EFFECTIVENESS OF MITIGATION MEASURES ON POLLINATOR DECLINE: AN INTEGRATED MULTI-BIOMARKER APPROACH (ÆM-POLLY PROJECT)

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## Abstract

The aim of the ÆM-POLLY project is to develop and validate an integrated protocol as a tool to verify if the mitigation measures foreseen by the EU Green Deal for agricultural practices, are effective in halting and reversing the decline of wild pollinator biodiversity. This monitoring protocol will integrate endpoints in terms of presence, abundance and diversity of wild pollinator species with endpoints able to assess the state of health at the sub-individual, individual and population level. These endpoints are selected and designed to diagnose biological alterations in wild pollinators due to chemical stress from pesticides, stress linked to climate changes, food and water deficiencies, habitat loss and diseases. Such an integrated approach using a set of biomarkers has never applied before to wild pollinators. The monitoring protocol is applied in 4 orchards and 4 vineyards characterised by the presence or absence of mitigation measures, and in 4 adjacent natural areas. Surveys for assessing pollinator diversity are performed in spring and late summer. During this activity individuals of the most abundant/representative species are sampled for pesticide levels, morphological (body size, fluctuating asymmetry variations) and biomarker analysis (immune, reproductive, nervous systems, oxidative stress, metabolism, detoxification processes and genotoxicity, energy mobilisation and feeding performance). From our project we expect to: obtain a dataset of baseline biomarker values related to the health status of representative pollinator species; assess the effectiveness of the mitigation measures by comparing the pollinator diversity and the biomarkers related to their health in two different crops; define a final integrated monitoring protocol to assess the health status of wild pollinators and their biodiversity; develop user-friendly guidelines to assess the effectiveness of the different mitigation measures in different agroecosystems, to guide decisions and inform policies.

*ÆM-POLLY is a PRIN PNRR 2022 project, funded by European Union – Next Generation EU.*

# ASSESSING THE IMPACT OF 2-HEPTANONE ON HONEYBEE HEALTH: IMPLICATIONS FOR VARROA MITE CONTROL

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## Abstract

Synthetic acaricides have been used for decades to control V. destructor, but their negative effects on bees are well documented. Naturally derived acaricides are an alternative with lesser adverse impacts. Among these, 2-heptanone (2-H), a bee pheromone, has shown great potential with its proposed efficacy as a Varroa mite repellent due to its local anesthetic effects on smaller parasitic arthropods. Despite this potential, the impact of 2-H on honeybees remains largely unexplored. In this study, we investigated the effects of a 5-day exposure to various concentrations of 2-H on honeybee survival, as well as the activities of the neuronal enzyme acetylcholine esterase (AChE) and the detoxifying enzyme glutathione S-transferase (GST). The TUNEL apoptosis analysis provided additional insights into the neurotoxic potential of 2-H. A commonly used natural acaricide, thymol, was used for comparison. Our results reveal a significant impact on honeybee survival at concentrations of 2-H → 0.8 ppm and thymol → 25 ppm, suggesting that 2-H exhibits greater toxicity towards bees. Furthermore, effects on the neuronal system were only observed following exposure to 2-H, with a reduction in AChE activity observed even at the lowest concentration (0.8 ppm) and induction of brain apoptosis at 1.2 ppm. Notably, the activity of GST, an indicator of detoxification processes, remained unaffected by both 2-H and thymol. Based on our findings, we conclude that 2-H poses a risk to bee health and caution against its use for acaricidal purposes.

# ASSESSING THE IMPACT OF RF-EMF EXPOSURE ON WILD BEES

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## Abstract

The current study aims at investigating the effects of radio-frequency electromagnetic fields (RF-EMFs) on honey-bee health and wild bee communities. For wild bees, work is carried out in Greece and Cyprus. In Nea Moudania- Chalkidiki- Greece, a field study investigates the effects of 3.4 and 24-26 GHz RF-EMFs on insect biodiversity, with emphasis on wild bee communities. Pan traps are being set up at different distances from an emitting RF-EMF antenna and the abundance and diversity of wild bees captured in the traps is assessed in control and exposed areas.

In Cyprus, a field study is taking place using artificial wild bee nests shielded or unshielded from RF-EMF radiation. The nests consist of an outer PVC pipe (19 cm diameter and 38 cm length) and an inner pipe (12 cm diameter and 18 cm length) filled with dry reed stems, 17 cm long, 5-10 mm in diameter. RF-EMF shielding is achieved via covering the inner pipe with a fine metal instead of plastic mesh as for the unshielded nest. RF-EMF-shielded and RF-EMF-unshielded trap nests were placed at 25 sites near 3.4 GHz 5G-NR antennas at different locations on the island. The nests are monitored systematically for bee occupation. In addition, sampling of bees along a transect is carried out at each location, while simultaneously the RF-EMF exposure pattern of each location is characterized using a specialized instrument for measuring environmental RF-EMF (ExpoM-RF4). The abundance and diversity of bees at each location both in nests and along transects will be correlated to the measured RF-EMF characterization of each site to assess potential RF-EMF effects on wild bees.

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# ASSESSMENT OF THE EXPOSURE OF HONEYBEE COLONIES IN POLAND TO PESTICIDE RESIDUES - LIVE BEES MONITORING

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## Abstract

As a part of the monitoring programme of the health status and losses of bee colonies in domestic apiaries (which has been in operation in Poland for 10 years) in addition to the diagnosis and recording of bee poisoning, monitoring of pesticide residues in the hive environment and in bees is also carried out. The results of the tests on live bees provide a reference for tests carried out in situations of suspected poisoning of bee colonies with plant protection products.

To date monitoring studies indicate that pesticide residues are found in live bees, but both the variety of substances found and the levels vary widely from poisoned bees.

In 2023, 63 samples of live bees taken at supervised apiaries during summer inspection visits were subjected to toxicological analysis. The analysis showed that 56% of the live bee samples contained pesticide residues. Residues of 22 pesticides were determined in live bee samples, the most common being acetamiprid, difenoconazole, DMPF, DMF, tau-fluvalinate, tebuconazole, azoxystrobin, chlorpyrifos and fludioxonil. Residues of up to 4 pesticides were determined simultaneously in live bee samples.

The results of these studies are very valuable in interpreting the analytical results of poisoned bees. The best example is clothianidin, residues of which have so far never been found in live bees, making any residue of this highly toxic insecticide found in dead bees clearly indicative of clothianidin exposure as the cause of the poisoning.

# BEE-COMING DISTURBED: THE IMPACT OF ELECTROMAGNETIC FIELDS ON BUMBLEBEE SLEEP-WAKE RHYTHMS

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## Abstract

Potential anthropogenic factors threatening insect pollinators include, but are not limited to, magnetic (MF) and electromagnetic fields (EMF) generated by all electronic devices. In insects, there is evidence of the effect of EMFs on circadian rhythms. In this study, we focused on the effect of static MFs on sleep-wake rhythms in the bumblebee *Bombus terrestris*.

We monitored the free-running locomotor activity of individually kept bumblebees for 10 days under constant conditions in the absence of visible light. Three types of static MF (0, 50 and 120 µT) were used. The movements of each individual were monitored by a camera and the images were processed by image analyzing software. Both the period length and the power of periodicity were compared between groups. Our preliminary data show that there was no difference in period length in the different MFs, but we found significant difference in the power of periodicity, which was stronger in the hypomagnetic field (0 µT) compared to the rhythm in 120 µT MF.

Overall, the work shows that the insect circadian rhythm was indeed sensitive to unnatural MFs. Since *B. terrestris* shares the same type of potential magnetic sensor, the circadian clock protein Cryptochrome, with vertebrates, our results primarily highlight the potential risk of EMFs to pollinators, but may also be important for research on the sensitivity of vertebrates to human-induced EMFs.

# CONCENTRATIONS OF TOXIC ELEMENTS IN HONEYBEE (*APIS MELLIFERA*) WAX DURING ITS PROCESSING TO COMB FOUNDATIONS

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## Abstract

Beeswax is a product of honeybees (*Apis mellifera* L.) that they synthesize in their bodies. It is crucial for the colony's physiology, as well as the health and safety of honey and bee pollen stored in honeycomb cells. Beeswax is continuously recycled in intensive beekeeping by processing old (dark) honeycomb into comb foundations, which are essential for establishing productive honeybee colonies and preparing them for the main honey pastures. It's important to ensure that honeycombs and food supplies in the hive are not contaminated with harmful environmental substances. Long-term accumulation of toxic metals in the hive and the inability to decompose them in honeycombs can lead to physiological disorders. The study aimed to determine variations in concentrations of As, Cd, Cr, Hg, Ni, and Pb in wax samples during processing using the ICP-MS technique for multi-element analysis. Liquid wax samples were taken after 24 hours from the beginning of the four-level deposition phase directly from the tank. After the first sampling, a dark precipitate of contaminated beeswax was removed from the processing, and the remaining bright beeswax was poured into a clean steel tank for re-deposition. The next sampling was carried out after seven days from the beginning of the repeated deposition phase at 75 °C. The statistical analysis showed significant differences in element concentrations between the wax samples taken after day 1 and day 7 of processing, except for Cr in the second phase. The results indicate that wax's long-term cooling and deposition during processing had a positive effect, leading to a significant decrease in high concentrations of toxic elements when removing the fourth sedimented layer of wax before the second stage of processing.

# DIVERSITY PATTERNS OF P450 GENES IN 17 HONEY BEE SUBSPECIES

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## Abstract

Honey bees (*Apis mellifera*) inhabit a vast geographical range, spanning diverse natural and agricultural ecosystems. They are exposed to different levels and types of natural (such as plant allelochemicals) and synthetic (such as pesticides) xenobiotics within this range. Several genes have been implicated in the resistance of insects to pesticides, including the P450 monooxygenases superfamily that contains 46 genes. Here, the sequences of P450 monooxygenases from >1500 individuals representing 17 subspecies of the four honey bee main lineages will be analyzed. The functional annotation and effects of each variant will then be predicted using SnpEff and the allele frequency and FST (fixation index) of each SNP per population and evolutionary lineages will be calculated. It is expected to have highly differentiated SNPs among the different subspecies/lineages.

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# DNA DAMAGE AND MORPHOLOGICAL ABNORMALITIES IN SPERMATOZOA AMONG APIARIES WITH REGISTERED HONEY BEE COLONY LOSSES

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## Abstract

The honey bee, *Apis mellifera* (Linnaeus, 1758), is a species sensitive to environmental pollution. The use of pesticides in beekeeping and agricultural activities is a significant contributing factor to the observed increase in mortality rates amongst honey bee colonies. The assessment of DNA damage in reproductive cells is crucial for the survival of species. The aim of this study was to assess *in situ* the genotoxic potential of pesticides on the spermatozoa of *A. mellifera* populations experiencing colony losses by applying the sperm comet assay and morphological analyses. The presence of pesticides as genotoxic agents in bees and food stocks within the hives of the test and control localities under investigation was revealed by chromatography analysis. The values of the comet assay parameters, namely Tail Intensity (TI%) and Olive Tail Moment (OTM, µm), were found to be significantly higher in two of the five studied test apiaries, which exhibited the highest colony loss (53.3 and 63.5%). In parallel with this, a variety of abnormalities were observed in the acrosome, head, and flagellum of spermatozoa in all tested apiaries, which could be attributed to the consequences of the observed sperm DNA damage. The percentage of spermatozoa exhibiting morphological abnormalities was found to be positively correlated with the level of DNA breaks and mortality rates. The current study demonstrated that the sperm comet assay and sperm morphological analyses could serve as reliable biomarkers for general toxicity and genotoxicity in *A. mellifera*, indicating an impaired reproductive potential of honey bee populations.

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# EFFECTS OF MOSQUITO LARVICIDES AND ADULTICIDES APPLIED VIA TRUCK MOUNTED AND AERIAL SPRAYING ON HONEY BEES (*APIS MELLIFERA*) IN FLORIDA

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## Abstract

The use of Ultra Low Volume (ULV) mosquito adulticides is common in Florida, particularly in urban areas, as they are highly effective at controlling mosquitos. Additionally, there are many Florida mosquito control districts in which water bodies are treated with mosquito larvicides. The impact of some of these chemicals on honey bees has been studied, but not as much is known about how simultaneous exposure to these chemicals in realistic field scenarios will impact overall honey bee colony strength. We hypothesize that residues of mosquito control products used in the truck-based and aerial applications will be found inside honey bee colonies, though at levels low enough not to cause acute toxic responses. In this study, we determined the impact of field realistic mosquito control practices on colony strength parameters by placing 15 new honey bee colonies in three "hotspot" treatment sites (five colonies per site) in which mosquito control operators treat three to five times each site. We also placed 15 new colonies in three sites (five colonies her site) receiving little to no mosquito treatment applications (negative control sites). The "hotspot" treatment sites were treated with Mosquito Mist II, Naled, and Vectobac12AS. Colony strength parameters were measured before and after the three-month treatment period and samples of hives matrices (pollen, nectar, bees, brood) were collected from all colonies and analyzed to determine the residue levels of mosquito control product present in the hives. No significant differences were observed in colony mortality and health parameters between colonies located in treated and control sites. The resulting data will be used to inform best management practices for mosquito control programs and apiculture in the future.

Keywords: mosquito control products; honey bees; ULV application; non-target impacts; pesticide residues

# ENHANCING HONEY BEE HEALTH: *AGARICUS BISPORUS* PROTECTIVE EFFECT IN CHEMICALLY AND BIOLOGICALLY INDUCED STRESS

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## Abstract

Global climate changes, environmental pollution, and the frequent use of insecticides in agriculture are some of the potential causes leading to disruptions in the ecosystem, affecting production of food intended for bees, and therefore bee colony losses. Additionally, *Nosema ceranae* infection as well as exposure to agricultural or urban insecticides have proven negative impact on bee health, leading to significant losses. All of this promotes disruption of honey bee oxidative status, which is usually monitored through the concentration of malondialdehyde (MDA) and the activity of the antioxidative enzymes catalase (CAT), glutathione S-transferase (GST), and superoxide dismutase (SOD). Previous studies show that the extract of *A. bisporus* exerted protective potential in bees exposed to *N. ceranae*. Current research employed *A. bisporus* mushroom extract to assess its efficacy in mitigating oxidative stress and enhancing bee survival and health in *Nosema*-infected bees treated with deltamethrin (widely used insecticide in mosquito control). Parameters such as bee survival, *Nosema* spore counts, and antioxidative enzyme activities were recorded and analyzed. The results indicated that deltamethrin adversely affected bees, while co-administration with *A. bisporus* extract provided a noticeable protective effect, reducing oxidative stress markers and improving survival rates. Our findings suggest that *A. bisporus* extract has the potential as a natural supplement to enhance bee health and resilience against chemical and biological stressors. This could have significant implications for beekeeping and agricultural practices and management of various insecticides, promoting a more sustainable approach to managing bee populations that are crucial for pollination and biodiversity.

# EVALUATION, ISOLATION, AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM FIVE PLANTS FOR THE CONTROL OF VARROA MITE

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## Abstract

Varroa mites are a major pest of honeybees, causing significant damage to hives and contributing to colony collapse disorder. The use of synthetic acaricides to control *Varroa* has led to the development of resistance and concern about residual effects on the honeybees themselves, the honey, and other hive products. The study aimed at finding the acaricidal activity of natural products (plants) as an alternative to the conventional acaricides that are in use. Information about the plants was acquired from herbalists from Bauchi, Nigeria. Five plants (*Calotropis procera, Securidaca longipedunculata, Jatropha curcus, Boswelia dalzielii,* and *Lannea acida*) frequently mentioned by the respondents were selected, extracted (methanolic and aqueous extract ) and subjected to bioassay test on the Varroa mite. *S. longipedunculata* showed to be the most effective ($P \leftarrow 0.05$). Further investigation to isolate and characterize the bioactive compounds of *S. longipedunculata*, firstly, the phytochemical of the plant extract reveals the presence of alkaloids, tannins, flavonoids, cardiac glycosides, xanthoproteins, terpenoids, steroids, and phenol. Secondly, TLC was conducted to determine the best solvent combinations to run the column. Different solvents were used but the best solvent combinations that gave the best spots were pet ether, diethyl, and ethyl acetate (6:3:1). 14 pure isolates were collected from the column, the isolate will be tried on the Varroa and honey bees again to determine which isolate is responsible for killing the mites and the bees before structural elucidation as the work is still ongoing.

# EVALUATION OF THE ECOTOXICOLOGICAL STATUS OF HONEY BEES (*APIS MELLIFERA*) FROM ENVIRONMENTS WITH DIFFERENT ANTHROPOGENIC IMPACT, USING A MULTIBIOMARKER APPROACH

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## Abstract

*Apis mellifera* is a keystone pollinator of wild plant species and crops. The massive use of pesticides in agriculture, associated with pollution from other human activities and the presence of parasites, can cause toxicological effects in bees including a decrease of the immune defences, leading to the collapse of the colonies. Effective assessment of the ecotoxicological impacts of anthropogenic contaminants requires an approach that combines different biomarkers, enabling a more precise diagnosis of sublethal effects of environmental stressors through a combination of different biological responses. This study aimed to apply a set of biomarkers to study the ecotoxicological status of honey bees. Adult honey bees were collected, in 2019 from apiaries located in four areas, and in 2020 and 2021 in nine areas. The locations were selected depending on the different anthropogenic inputs: a wood, a clover field, an orchard, a sunflower field, a rural area, a vineyard, a field with wheat crops, a wildflower field, a suburban area, and two areas with berries. Variation in glutathione S-transferase (GST), carboxylesterase (CaE), acetylcholinesterase (AChE), alkaline phosphatase (ALP), and lysozyme (LYS) activities, nuclear abnormalities (NA) frequency and differential haemocytes count (DHC) were evaluated. Throughout the 3 years, honeybees from the cultivated areas revealed alterations in neurotoxicity, metabolic, and genotoxicity biomarkers. In the urban area, in 2019, the biomarker results indicated immunosuppression in the honey bees, while in 2020 the specimens from the suburban area were undergoing some neurotoxic effects and alteration of metabolic and immune responses. After this 3-year monitoring, the bees sampled in the cultivated areas seem to be more exposed to chemical stressors than those sampled in wood or in orchard environment. This approach resulted to be a useful tool for a better understanding of the sublethal effects that exposure to contaminants can have on the health status of these insects.

# EXPLORING THE POPULATION DYNAMICS AND ECOTOXICOLOGICAL IMPACTS ON TWO APIARIES FACING VARYING LEVELS OF ANTHROPOGENIC PRESSURES

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## Abstract

The health of bees and, more generally, of pollinating insects is increasingly challenged by anthropogenic impact, represented by multiple factors such as climate change, habitat destruction, parasitosis and diseases, but especially due to environmental pollution and the use of pesticides in agriculture. Population dynamics, the presence of *Varroa destructor* mites and ecotoxicological impact, by means of biomarkers, were investigated in two apiaries facing varying levels of anthropogenic pressures. Neurotoxicity (acetylcholinesterase (AChE) and carboxylesterase (CaE)), metabolic (alkaline phosphatase (ALP)), biotransformation (glutathione S-transferase (GST)), and immune system (lysozyme (LYS), phenoloxidase (POx) and prophenoloxidase (proPOx)) biomarkers were performed on the various worker sub-castes of *Apis mellifera*. Specifically, we evaluated the biomarker responses based on the sub-castes (newly formed, adult builders, and foragers), seasonality (spring, summer, and autumn), and we investigated whether enzyme activities could be influenced by potential sources of contamination. The results showed a physiological oscillatory trend in population dynamics and varroa, thanks to the treatments carried out to control it. The enzymatic activity values in the different worker sub-castes observed in the three periods of the year, particularly the responses of AChE, showed lower activity in newly formed bees and builders compared to foragers, while GST showed higher activity in newly formed bees. The use of synthetic pesticides has likely produced toxicological effects in treated bees. This work contributed to improving the understanding of the physiological activities of the investigated enzymes in the different castes, allowing a deeper knowledge of the sub-lethal effects caused by pesticides and environmental contaminants, and how climate change and other stress factors can affect the population dynamics of these insects.

# EXPOSURE OF BUMBLEBEES TO MICROPLASTICS DEPENDING ON HABITAT TYPE

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## Abstract

Microplastics have been found in almost every location around the world and are known to be harmful when ingested. Most of the research about microplastic pollution in Estonia are related to the Baltic Sea. Bumblebees could be a good indicator for assessing the presence of microplastic pollution on land, as they meet many surfaces during their life, fly several kilometers in one day, and their fur produces static electricity that can attract other particles in addition to pollen. In this study, bumblebees were chosen as research objects to assess microplastic pollution in Estonian cities, fields, and forests. Microparticles were washed off the bumblebees, organics were removed from the washing water according to the Fenton protocol, and then the microparticles on the filters were examined under a microscope. The results revealed that bumblebees living in cities had more microplastics on their fur than bumblebees living in fields and forests. For cities, significantly more microplastics were observed near a busy highway, but bumblebees collected during the rainy season were cleaner than bumblebees collected during the hot, dry season. Since there are no previous research works of this kind in Estonia, it would be possible to take the results of this work into account in future evaluations of terrestrial microplastic contamination.

# GENOTOXIC EFFECT OF ACARICIDES APPLIED IN CONVENTIONAL AND ORGANIC BEEKEEPING

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## Abstract

In recent years, alarming data have been published on the honey bee colony losses worldwide. The reasons for this are varied and complex, most often related to disease, environmental impacts and unknown nature.

Methods used in beekeeping to control *Varroa* include hive treating with acaricides, some of which are also applied as pesticides in agriculture to control plant pests. These are amitraz, coumaphos and tau-fluvalinate. Chemical analysis detected 0.0015 to 0.0054 mg/kg amitraz in bee body samples, 0.049 to 0.93 mg/kg coumaphos and 0.015 mg/kg tau-fluvalinate in samples of honey, wax and pollen in a hive. In organic beekeeping, an alternative effective method for *Varroa* control is treatment with oxalic acid.

The aim of the present study was to investigate the mutagenic effect of amitraz, coumaphos, tau-fluvalinate and oxalic acid applied as acaricides in beekeeping. In this aspect, the frequency and specificity of chromosomal aberrations occurring after exposure to the tested acaricides was analyzed. As an object in laboratory conditions the *Allium cepa* root meristem has been used and an anaphase method and a micronucleus test have been applied.

The pesticide treatment of the samples produced significant changes in the chromosome structure and organization, demonstrating clear genotoxic potential. The acaricides tested induced abnormalities of clastogenic and aneugenic type, including chromosome bridges and fragments, 'wandering' chromosomes, C-mitoses and micronuclei. Oxalic acid, used in the recommended for organic beekeeping concentration, has not expressed mutagenic effect. Our subsequent *in situ* studies confirm these findings to a significant extent.

## Acknowledgments

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# IMPACTS OF A SINGLE EXPOSURE TO PICOXYSTROBIN DURING THE LARVAL STAGE ON HONEY BEES

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## Abstract

Bees are known for their vital role in natural ecosystems and agricultural settings due to their pollination prowess. Nevertheless, pesticide use remains a big problem in agriculture, contaminating natural ecosystems and affecting bees. Fungicides have been widely used worldwide, and honey bees can bring pollen and nectar contaminated to the colony, exposing larvae. Studies focusing on larvae exposed to fungicides are relatively limited compared to adult studies. Therefore, the work aimed at the effects of larval exposure to the fungicide picoxystrobin (strobilurin group) on the biological parameters and the cellular stress in the fat body. The larvae were single exposure on the fourth day (D4) to picoxystrobin 5 ng a.i./µL (PCX5), 45 ng a.i./µL (PCX45), 135 ng a.i./µL (PCX135), and 400 ng a.i./µL (PCX400). The effects on larval and pupal mortality, pupation rate, and emergence were evaluated. Additionally, the cellular stress in the fat body was evaluated in the newly emerged bees exposed during the larval stage. The exposure to PCX400 increased by 26% larval mortality when compared to CTL (p=0.013), as well as a negative influence of picoxystrobin exposure was noticed on the emergence rate (Binomial GLM, $\chi^2$=21.311, df=5, p=0.0007), reducing the number of newly emerged adults when exposed to PCX400 (p=0.0001). The other concentrations did not alter the larval and pupal mortality and pupation and emergence rates. Cytotoxicity effect was observed in newly emerged bees from PCX400 by positive immunolabeling of HSP70. Thus, a single exposure to picoxystrobin can impair larvae's development, induce a cellular stress response, and may interfere with colony dynamics. Studies like this reinforce the relevance of intensifying efforts to develop protective actions against larval exposure to fungicides.

# NOVEL METHODS FOR IN VITRO REARING AND ECOTOXICOLOGIC RISK ASSESSMENT OF HONEY BEE DRONES

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## Abstract

Honey bee drones are often neglected in ecotoxicologic risk assessment of pesticide safety, although this caste is essential for colony reproductive fitness. Accordingly, to enhance regulatory assessment of gonadotoxicity in honey bee drones, we developed a successful protocol to rear honey bee drones in vitro from larva to adult, with 74% survival to adult eclosion. Moreover, we established novel methods for evaluation of the reproductive effects of chronic, in vitro, drone pupal exposure to the synthetic miticide amitraz. Using this approach, drone pupae exposed to incremental, environmental doses of amitraz in vitro demonstrated significant decreases in survival to adulthood, developmental delay, as well as significant, dose-responsive, decreases in sperm viability and total sperm count. As proof of concept, these methods to rear drones in vitro and expose them chronically to miticides during pupal development will support future inclusion of the drone caste in ecotoxicologic risk assessment by pesticide regulators.

# ORAL EXPOSURE-DRIVEN EFFECTS OF A NOVEL SPIDER VENOM-BASED BIOPESTICIDE ON SURVIVAL, GUT MICROBIOME AND HEAD TRANSCRIPTOME OF ADULT WORKER HONEYBEES

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## Abstract

The escalating use of plant protection products contributed to the degradation of numerous ecosystem services by exerting heightened pressure on non-target organisms(NTOs). To overcome this, the European Union through the Farm2Fork Strategy and its aim of reducing the use of chemical and more hazardous pesticides by 50% by 2030, targeted the development of more environmentally friendly products. Biopesticides derived from natural molecules of animals are considered a promising alternative to conventional chemical pesticides. One source of molecules currently receiving attention are proteins from insect antagonists, such as the neurotoxin from the venom of the spider *Segestria florentina*(SFI6), which proved to be toxic to different insect pests of important crops. Although biopesticides are often considered to have reduced environmental impact, their effects on NTOs can be challenging to predict, mainly because of the lack of appropriate tools incorporated into the regulatory framework. The study aimed to determine the effects of the fusion protein SFI6-BSA upon oral exposure on *Apis mellifera*, the key model organism for pesticide ERA to pollinators. The effects were evaluated, after acute and chronic exposure, on the survival, gut microbiome and head transcriptome of adult worker honeybees. Oral toxicity tests were performed according to the OECD Guidelines for the Testing of Chemicals - No. 213 and 245, respectively. DNA(gut) and RNA(head) extractions were made using NZY Isolation kits(NZYTech). Sequencing was made using an Illumina NovaSeq platform. We demonstrated that acute oral exposure to SFI6-BSA had no adverse effects on honeybees. On the other hand, when bees were subjected to chronic exposure, it caused no significant effects on survival but induced alterations in the gut microbiome and gene expression profiles. The responses revealed by the "omic" approaches, coupled with regular testing procedures could be considered valuable tools for further research and inclusion in regulatory frameworks.

# POLYPOLL: AN INTERDISCIPLINARY APPROACH TO UNRAVEL THE EFFECTS OF COMBINED EXPOSURE TO CHEMICAL POLLUTANTS ON INSECT POLLINATORS

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## Abstract

Insect pollinators play a vital role in maintaining wild plant diversity and crop yields, essential for global food security. However, these invaluable species are facing alarming declines due to several causes, with chemical pollution emerging as a key driver. While many ecotoxicological studies focused on the effects of insecticides, pollinators are routinely exposed in agricultural settings to cocktails of other chemicals such as fungicides, herbicides, heavy metals, and veterinary drugs. The combined ecotoxicological effects of these pollutants on pollinators remain poorly understood.

Moreover, chemical pollutants can elicit sublethal effects in pollinators, including physiological and behavioural alterations. While previous studies have primarily focused on honeybees, there is a pressing need to extend this research to other insect pollinators. Utilizing multi-biomarker approaches may offer insights into the impacts of pollutants on diverse physiological pathways and enable the development of early warning signals to assess pollinator health.

Additionally, chemical pollutants can disrupt the gut microbiome, a crucial component of the insect holobiont. Given the importance of microbial symbionts in insects, understanding the effects of pollutants on the microbiome is essential for all pollinator species.

To address these knowledge gaps, the POLYPOLL project adopts a holistic approach, investigating the effects of various chemical pollutants on three representative species: social honey bee (*Apis mellifera*), solitary red mason bee (*Osmia bicornis*), and common drone fly (*Eristalis tenax*). By studying different species, representing different lifestyles and taxa, the project aims to thoroughly assess the impact of chemical pollutants.

By integrating multiple stressors, innovative methodologies and diverse pollinator species, the project aims to improve our understanding of the complex interactions between pollutants and pollinator health. Ultimately, these findings will refine environmental risk assessment protocols and help conserve pollinator populations and their vital ecosystem services.

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# POTENTIAL USE OF HONEY BEES FOR BIOMONITORING OF POWDERY PHOSPHOGYPSUM STACKS IN CROATIA

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## Abstract

European honey bee can serve as a continuous biomonitoring species at industry polluted locations. Forager bees, exposed to contaminants, are carrying them to their hives where they can be detected and quantified in bees and hive matrices. In this pilot study we evaluated the feasibility of using honey bees for biomonitoring of fine powdery phosphogypsum (PG) stacks at the deposition site in Kutina (Croatia). In July 2023 two honey bee colonies were placed in the PG deposition site close proximity and compared to four control colonies located in larger distance from the PG stack. The set up considered similar plant species cover at all locations. By measuring 20 macro and trace elements levels with ICP-MS in bees, honey, propolis and pollen, as well as natural radionuclides measurements in honey and pollen using gamma-ray spectrometry, the experiments aimed to better understand which sample matrix could best be used to measure potential airborne releases of fine PG powdery particles. First campaign also investigated the effects of PG on honeybees' health. Our results indicate that bees and honey can indeed be used to indicate PG stack airborne emission. Specifically, nickel, molybdenum and uranium concentrations were 2-10 times higher in bee and honey samples taken from the colonies in the vicinity of the PG stack, if compared to measured concentrations in the controls. Measured radionuclide activities were below the detection limit in most of the samples. However, in pollen samples, $^{214}$Bi and $^{214}$Pb were measurable and differences between two sites were observed, which should be further investigated. The bee PG exposure showed no effects on the bee foragers' health determined by the stress enzymes glutathione S-transferase and acetylcholine esterase activity analysis compared to bees from the controls. It can be concluded that the bee-based biomonitoring approach is promising and deserves further systematic development.

# SENSITIVITY OF DIFFERENT LEARNING FORMS TO PESTICIDE TREATMENT IN THE HONEY BEE *APIS MELLIFERA*

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## Abstract

Honey bees play a crucial role in terrestrial ecosystems thanks to their contribution to pollination. A significant decline of honey bee populations has been observed worldwide, owing to several factors such as habitat loss, negative effects from invasive species and pesticide use. While pesticides have been regularly associated with cognitive deficits in honey bees, they belong to numerous chemical classes with a range of different molecular targets. Therefore, different phytosanitary treatments may have dissimilar effects on bees' perception, learning and memory performances. In addition, more complex learning tasks may be more sensitive to perturbations than more simple ones. We used the conditioning of the proboscis extension response (PER), which mimics bees' foraging behaviour in the lab, to assess the effect of sublethal doses of 2 pesticides, cypermethrin and chlorantraniliprole on bees' ability to associate floral cues (such as odours) with a nectar reward. Bees were subjected to a series of learning tasks revealing different aspects of bee cognition. In detail, non-associative learning was assessed through habituation of the PER, while associative learning and olfactory generalisation were assessed through absolute conditioning. In addition, bees' olfactory discrimination abilities were measured through differential PER conditioning. Our results show different patterns of effects for the two pesticides on the different tasks, but bees' ability to discriminate between similar odours was generally more sensitive to pesticide treatment than simpler forms of learning. Generally, these results show that low doses of pesticides may impact bees' ability to find fruitful floral sources while foraging.

# SPERM MORPHOLOGICAL ABNORMALITIES IN DRONES FROM APIARIES WITH DIFFERENT MORTALITY RATES

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## Abstract

The production of morphologically and functionally normal drone sperm is a determining factor in the reproductive success of honey bees (*Apis mellifera*). The present study aims to evaluate the reproductive potential of drones from test apiaries with variable colony mortality. The morphological analysis of spermatozoa was conducted on sperm smears obtained from six apiaries: five with different mortality rates and one control. For each sample tested, more than two hundred gametes were analyzed. Various abnormalities were observed, and the ratio between spermatozoa with normal morphology and those with morphological abnormalities was determined. Dysmorphogenesis was identified in the spermatozoon's three main parts: the acrosome, the head, and the flagellum. The acrosome exhibited a bent, curved, or missing configuration. Abnormalities observed in the head portion of the spermatozoa included alterations in nuclear shape or the absence of the head. The most prevalent morphological alterations were scored in the spermatozoa flagellum, including those with bent, coiled, fragmented, bifurcated, multiple, and broken structure tails. A positive correlation was observed between the percentage of abnormal spermatozoa and the reported rate of colony losses in honey bees. The results thus far are consistent with our previous findings regarding the correlation between pesticide contamination in the test apiaries and the observed DNA damage in *A. mellifera* spermatozoa.

## Acknowledgments

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# STINGLESS BEES: A SYSTEMATIC REVIEW ON THE TOXICOLOGY OF THESE IMPORTANT BUT NEGLECTED POLLINATORS

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## Abstract

Stingless bees, comprising over 600 known species within the tribe Meliponini, are essential for maintaining biodiversity, as they pollinate a wide range of native flora. Furthermore, although many species possess a close and interdependent relationship with pristine environments, often relying on native trees with large cavities as nesting sites, some species possess huge plasticity, nesting even in deeply anthropized environments. These bees play critical roles in ecosystem functioning through their pollination services, contributing to the reproduction of numerous plant species, including many economically important crops such as cacao, coffee, and fruits. Beyond their ecological significance, stingless bees hold cultural and economic importance for many indigenous communities, where they are bred and managed for their honey, pollen, and propolis. The overwhelming majority of studies on pesticide toxicity and risk assessment on bees are conducted on the model species *Apis mellifera* in the United States and Europe, where stingless bees are absent. In May 2023, the European Food Safety Authority (EFSA) published its revised guidance document on the risk assessment of plant protection products (PPPs) and bees, where finally other social and solitary bees were included (i.e. *Bombus* and *Osmia*). However, the impact that pesticides have on stingless bees remains largely unknown. Here, we present the results of a

systematic review of toxicological studies concerning stingless bees, which highlights a substantial knowledge gap. Up to 2024, only 129 studies have been identified on the effect of pesticides on stingless bees, with over 80% conducted in a single country (Brazil). Additionally, the number of species and PPPs tested is extremely low. Therefore, it is important that stingless bees are included in risk assessment guidance to fill this significant knowledge gap and to protect these vital pollinators.

# STUDY OF PYRETHROID RESISTANCE IN UAE POPULATIONS OF VARROA DESTRUCTOR

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## Abstract

The ectoparasitic mite, *Varroa destructor*, is considered the main pest of *Apis mellifera* worldwide, causing the collapse of honey bee colonies in few-year interval, if left untreated. In order to control the mite population below the economic injury level, commercial beekeepers often rely on very limited pool of synthetic acaricides that are registered and available on the market. Since the first report of decreased Varroa susceptibility to pyrethroids in 1990s, the massive use of tau-fluvalinate and flumethrin favored the spread of resistant mites to different countries. Most recent studies reported association between point mutations in the voltage-gated sodium channel (VGSC) gene and observed resistance in varroa mites in North and South America, and Europe. However, the data about acaricide resistance in varroa mites from the Middle East countries are lacking. Therefore, the aim of our study was to estimate the current status of pyrethroid resistance in *V. destructor* mites from the United Arab Emirates.

Adult Varroa females were collected from different apiaries located across the country. After DNA extraction, the partial sequence of the VGSC gene was PCR amplified and specific amplicons were subsequently subjected to the RFLP analysis by using *Sac*I restriction enzyme, and bidirectional sequencing.

Products of the *Sac*I test showed the RFLP pattern with one, two, or three bands on agarose gel, corresponding to *V. destructor* resistant homozygotes, sensitive homozygotes, and sensitive heterozygotes, respectively. Sequence analysis of the PCR products from resistant mites detected a point mutation at the nucleotide position 1710 of the *V. destructor* VGSC, indicating amino acid substitution L925V.

Our data show the existence of VGSC mutations associated with acaricide resistance in local *V. destructor* populations, which requires comprehensive revision of the current mite control programs.

# URBAN BIOMONITORING OF HEAVY METALS AND PESTICIDES WITH HONEYBEES ACROSS ITALY: 7 YEARS IN 7 CITIES

Lucia Lenzi, Fabio Sgolastra, Claudio Porrini

Department of Agricultural and Food Sciences-University of Bologna, Italy

## Abstract

The use of honeybees (*Apis mellifera* L.) as bioindicators of environmental pollution has been widely adopted to study different contaminants in both agricultural and urban areas in the last decades. In the present study, financed by Conapi, a large beekeepers' cooperative, we performed a widespread monitoring of 10 heavy metals and 400 pesticides (including glyphosate) in 7 Italian cities, from north to south, for 7 years. Each station consisted of 2 beehives provided with "*underbasket"* cages to monitor the mortality of honeybees, and two sampling (one in summer and the other in autumn) of fresh honey and bee foragers were performed each year. If the mortality overcame the threshold of 250 bees/station/week extra analysis was carried out. Glyphosate was found in periodic sampling of bees and honey in 5 out of 7 cities and in 5 out of 7 years. Only in 2020 and 2021 it was not found, probably due to restrictions on human activities due to the current pandemic in those years. In the seven years of the project, the mortality threshold was exceeded 4 times. The chemical analysis have always highlighted the presence of glyphosate, except in 2023 where permethrin, acetamiprid and DEET were found. The results of heavy metals were compared with reference values calculated *ad hoc* and classified into 3 concentration levels (low, medium, high); the metals that recorded the highest values over the 7 years were Cu, Pb, Cr, Ni and Fe. As with pesticides, in 2020 and 2021 most values were at low concentration levels. Our study indicates the constant presence of pesticides even in urban areas and reflects the impact of human activities on the environment. Furthermore, it confirms the role of bees as bioindicators to monitor contamination in our cities and guide the decision-making process of stakeholders.

# 7 Communication and behavior

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## Oral presentations

**Moderator:**
Fabio Manfredini

# ANTENNAL TRANSCRIPTOME ANALYSIS AND BEHAVIORAL STUDIES TO DECIPHER THE GENETIC BASIS OF OLFACTION IN *BOMBUS TERRESTRIS L.*

Fatih Dikmen[1], Tunç Dabak[2], Çiğdem Özenirler[3], Burcu Daşer-Özgişi[3], Vahap Eldem[1]

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## Abstract

The insect antenna is a highly complex and multisensory organ that mediates chemosensory, mechanosensory, gustatory, and olfactory information. Pollination success is largely dependent on this sensory mechanism. However, the genetic mechanism of perception and odor discrimination in the antenna is still not fully understood, especially in bumblebees. In this study, we aimed to explore the differentiation of antennal chemosensory gene repertoires among different developmental stages (from larvae to adult) of *B. terrestris* and gene expression profiles among eugenol treated forager bumblebees. In the first step we tried to explore the correlation between gene expression levels at developmental stages with increasing and decreasing trends, respectively. Combined with the mRNA RNA-Seq and miRNA-seq data, the results of both positively and negatively correlated genes revealed that antennal transcriptomes are highly dynamic at different developmental stages. We also examined the expression profile of chemosensory and olfactory receptor genes and found that their expression was mainly positively correlated. 114 chemosensory-related genes were identifed including 68 ORs, 15 GRs, 19 IRs, nine OBPs, two CSPs, and two SNMPs in antennal tissue. Using adult antennae as a comparison, the number of differentially expressed genes were 87, 18, and 31 in larvae, early-stage pupae, and late-stage pupae, respectively. Secondly a set of bumble bee foragers were trained to give reflexive responses for eugenol and the other set was used as a control group which was not exposed to any sort of specific odor. The experiment group showed significant changes in the expressions of the *OR2*, *OR4*, *OR47a*, *OR65a, OR49b* and *OR22c* genes. We believe that our data will expand the understanding of antennal-specific functions and gene signatures by measuring global transcriptome dynamics.

# SOCIAL IMMUNITY THROUGH SOCIAL LEARNING

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## Abstract

Social learning has been documented in a variety of animal taxa, including insects. In the past decades, a growing number of studies have shown that social bees can acquire behavioural traits through social learning. However, whether social immunity traits relevant for colony health can be learned from nestmates remains unexplored. Here, we show data suggesting that social immunity in Western honey bees (*Apis mellifera*) can be significantly enhanced via social learning. In a laboratory cage experiment, grooming behaviour of adult workers against ectoparasitic mites, *Varroa destructor*, was tested. Newly emerged individuals ("learners") were placed in cages with other newly emerged ("controls") or with older workers ("demonstrators"). Grooming behaviour of learners paired with mite-infested controls or demonstrator workers was categorized and recorded before, during and after a series of 10 demonstrations. Demonstrators expressed grooming significantly more often and faster than controls, suggesting that this behaviour can be enhanced by experience. Further, learners kept with demonstrators reacted significantly faster to a mite than the controls. Given that the laboratory data can be confirmed in field colonies, these findings suggest that enhancement of immunity via social learning could constitute a key mechanism to promote colony health in wild and managed honey bees and in other social insects.

# IMPACT OF PARASITE AND PESTICIDE EXPOSURE ON THE PRODUCTION AND PERCEPTION OF PHEROMONES IN THE HONEY BEE

Fanny Mondet[1], Amelie Noel[1], Louise Jeandroz[1], Jean-Luc Brunet[1], Benjamin Basso[1], Charlène Dumas[1], Emilien Rottier[1], Benoit Lapeyre[2], Yves Le Conte[1], Cedric Alaux[1]

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## Abstract

Honey bees are exposed to a large variety of abiotic and biotic stressors, the effects of which are not easy to assess. Stressor interactions have been identified for several years, however non-lethal effects of such associations remain rarely described. In particular, interactions between the main biotic stressor of honey bee colonies, the parasite *Varroa destructor*, and other abiotic factors such as pesticides have seldomly been addressed. In the present study, individual honey bees have been exposed to varroa and environmental doses of two pesticides (chlorpyrifos and boscalid), to assess the impact of these stressors on the social regulation within honey bee colonies. Using chemical analyses, electroantennography recordings and behavioural assays, we compared several chemical communication aspects in honey bee workers. We show that varroa, chlorpyrifos and boscalid, alone or in combination, can affect both pheromone production and perception in stressed workers. These effects seen at the individual level, are likely to have consequences at the colony level, considering the importance of chemical communication for the performance of the honey bee colony superorganism. This study brings new knowledge to understand the complexity of stressor interaction and their impact on honey bee health.

# INVESTIGATING THE EFFECT OF DEFORMED WING VIRUS INFECTION IN THE BRAIN ON HONEYBEE LEARNING

Tesni Houlston, Giulia Pipolo, Alan Bowman, Fabio Manfredini

University of Aberdeen, United Kingdom

## Abstract

Deformed Wing Virus (DWV) is one of the most prevalent honeybee pathogens worldwide, and in addition to causing physical deformities, is reported to alter behaviour. DWV drives accelerated behavioural maturation and alters olfactory learning, both of which can negatively impact foraging efficiency. Previous studies investigating the effects of DWV on associative learning in honeybees, used abdominal DWV injections and found that it reduced learning ability compared to control bees. However, recent work performed in our research group, studying how natural DWV infection altered reversal learning ability, found that bees with higher DWV levels in the mushroom body, were better learners. To address these contrasting results and further investigate cognitive symptoms, reversal learning ability was assessed in control and DWV-injected honeybee foragers. This study found no difference in associative learning ability between treatment groups, but in the reversal learning phase DWV-injected bees performed better, with more injected bees learning the second odour and responding earlier than control bees, supporting the findings of our recent work. Interestingly, despite learning the second odour more efficiently than control bees, injected bees did not perform better in the short-term memory test. Quantification of viral loads in the mushroom bodies is ongoing, these levels will be compared to DWV levels in the abdomens to validate injection as a suitable method for inducing high level DWV infections in the brain. Mushroom body DWV loads will also be correlated with learning performance to better understand the link between DWV infection and olfactory learning. To broaden our understanding of these cognitive impacts, a visual learning protocol will be implemented this summer, as DWV has been found in the optic lobes of the honeybee brain, and this assay will help assessing the impact of DWV on a different type of learning that is also invaluable for efficient foraging.

# SPECIFICITY OF VARROA SENSITIVE HYGIENE BEHAVIOUR

Amélie Noël, Joachim De Miranda, Barbara Locke

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## Abstract

The parasite *Varroa destructor* is a threat to the honey bee (*Apis mellifera*) and to beekeeping worldwide. Varroa infestation of a colony can quickly compromise its survival if left untreated. A key factor in this are the severe virus epidemics transmitted by varroa, particularly when feeding on honey bee pupae during its reproduction. For example, emerging adult bees that have been parasitized by varroa during the pupal stage have a higher rate of Deformed Wing Virus (DWV) infection than non-parasitized individuals. However, some colonies have developed resistance to the parasite and are able to regulate the growth of the varroa population within the colony. To do so, they display specific hygienic behaviours specifically directed towards varroa-infested brood cells, such as VSH (Varroa Sensitive Hygiene). Bees performing this behaviour detect parasitized brood cells, uncap and clean their contents, thus disrupting mite reproduction. The mechanisms underlying these behaviours are not fully understood, but require the emission of semio-chemical signals, for both the initial detection and for triggering the appropriate behavioural response. This identification of parasitized brood cells is linked in part to the type and level of virus infection of the pupa. Several compounds are already been described as being more emitted by DWV infected brood than uninfected brood, and triggering VSH behaviour. The aim of this project is to understand whether VSH responds differentially to different virus species transmitted by varroa, and whether this can help explain the changing virus distributions in bee colonies over time, geography and with respect to the adaptation of certain honey bee populations to natural co-existence with varroa.

# CHARACTERIZING THE PHENOTYPIC MECHANISMS OF NATURALLY ADAPTED VARROA MITE RESISTANCE IN HONEY BEES

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## Abstract

The honey bee population on the island of Gotland, Sweden, have survived for 25 years with little to no varroa treatment. This population has lower mite reproductive success compared to non-resistant populations. However, the mechanisms of resistance in this honey bee population are still a mystery. By excluding the access of adult bees to brood cells using excluder cages, we were able to determine that the presence or absence of adult bees did not affect the rate of mite reproductive success in this and other mite resistant populations. This result clearly suggested that brood traits, independent of adult intervention, should be investigated further for mechanistic explanations of mite resistance. The mite's reproduction window is tightly linked with the brood's development, and intercepts chemical pheromones produced by the brood to synchronize the timing of egg laying. By using gas chromatography on extracted brood pheromones produced after capping, we have found that our resistant population produces significantly lower quantities of brood ester pheromones (BEP) immediately after capping compared to a non-resistant population. This is in combination with an overall trend of lower BEP produced for up to 36 hours after capping. These results suggest that the brood are using chemo-camouflage, more specifically, what we have termed chemo-whispering to disrupt or interfere with the initiation of mite reproduction. These results deepen our understanding of how the Gotland honey bee population is able to survive without varroa intervention, and expands our fundamental knowledge on the phenotypic mechanisms underlying varroa resistance.

# 7 Communication and behavior

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## Poster presentations

# A COMPARISON OF RESEARCH METHODS USED IN RECORDING AND ANALYSING THE VIBRO-ACOUSTIC COMMUNICATION OF HONEY BEES

Sylwia Łopuch, Adam Tofilski

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## Abstract

Research methods for the vibro-acoustic communication of honey bees (*Apis mellifera*) have changed over the years. In the past, signals were recorded using microphones, then accelerometers or laser vibrometers, and recently with high-speed cameras. Therefore, the aim of this study was to assess the efficiency of the detection of vibro-acoustic signals produced by honey bees using a microphone, an accelerometer, and a high-speed camera to register sounds, vibrations, and wing movements.

Vibro-acoustic communication of honey bees was recorded in an observation hive. In the middle of a comb, located behind a glass wall, an accelerometer was placed next to the end of a microphone probe. The behaviour of honey bees inside that part of the comb was recorded using a high-speed camera. Communication among bees was registered simultaneously with the use of the microphone, accelerometer, and high-speed camera.

The number of vibro-acoustic pulses significantly differed ($p<0.001$) among the compared methods. The high-speed camera was able to record two times more pulses of wing movements compared with the microphone or accelerometer, which registered pulses of sound or vibration generated by bees present in their vicinity. Short signals consisting of a few wing movements were only detected by the high-speed camera. A marked advantage of recording with the use of the microphone or accelerometer was the possibility of precise analysis of the characteristics of sound or vibration pulses, including their frequency or amplitude.

In conclusion, a decision about which method to use to study vibro-acoustic communication should be made based on the type of data needed.

# BUZZING UNDER PRESSURE: INVESTIGATING THE EFFECTS OF ANTHROPOGENIC DISTURBANCE ON THE INTRASPECIFIC BEHAVIOUR OF *BOMBUS TERRESTRIS* WITH COMPUTER VISION-BASED APPLICATION

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## Abstract

Human disturbance and anthropogenic noise can adversely affect a multitude of pollinators' behaviour by hindering physiology, nervous function, and development. Noise as an understudied type of pollution can also disrupt the perception of environmental signals and intraspecific acoustic communication, thereby it can weaken pollination services. Thus, here we investigate the impact of two types of disturbances on wild bumblebees' behaviour under field conditions. Alongside a control group, we used a noisy (simulating traffic noise through a speaker) treatment and one with continuous human presence. We video-recorded foraging bumblebees (*Bombus terrestris*) on *Lotus creticus* flowering patches in five-minute-long slots in urban green areas of Terceira Island (Azores, Portugal). For the automated bumblebee detection, we created computer vision models based on a deep learning algorithm (YOLOv5), with custom datasets. We used linear mixed models to compare the differences between the treatments in the distance between individuals (Multiple Comparisons of Means, Tukey Contrasts) and the average number of bumblebees visiting the patches. The average distance between individuals showed significant differences ($p < 0.01$) between treatments, with human presence showing the greatest and the noisy treatment the least interindividual distances. However, there were no significant differences in the average number of bumblebees on the patches, indicating that although these disturbances do not impact bumblebees' pollinating activity but likely disrupt intraspecific communication. Our findings confirm both the presence of humans and anthropogenic noise impacts bumblebees' behaviour. Yet, these effects may be more intricate than first assumed, emphasizing the necessity for additional research to thoroughly understand direct and indirect anthropogenic disturbance on pollinator behaviour.

# EFFECT OF URBANIZATION GRADIENT ON THE BEE COMMUNITY OF THE APPIA ANTICA REGIONAL PARK IN ROME

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## Abstract

The ongoing urbanization process is growing all over the world, increasing the impact of the urban ecosystem on the environments and living communities. Quantifying the effect of urbanization is not easy, due to the great complexity given by the presence of natural elements and anthropic structures. Studies show that urban areas can represent favorable environments for bees' conservation, maintaining the community diversity and functionality, even compared to apparently more natural peri-urban areas which are often more human impacted. In this work we studied the bees' community of the Appia Antica Regional Park of Rome. The Appia Antica Regional Park extends, without interruptions, from the city center to the rural areas, offering a unique urban-rural gradient with a true ecological continuity. We collected bees in three areas within the Regional Park, representing different parts of the urbanization gradient (urban, suburban, rural). We carried out samplings in spring and summer, from April to September 2023, using pan traps. We focused on *Apis mellifera*, wild bees in general, and bees belonging to Halictidae family in particular. For all the 3 groups considered, a significative difference between abundances in seasons and sites ($p<0.05$) and the interaction of these two variables ($p<0.05$) emerges. *A. mellifera* is more abundant in spring season, irrespective of the considered area. In spring there is no significant difference in the abundance of wild bees along the gradient, while in summer the abundance increases in all sites, especially in the suburban area. For Halictidae, the trend is similar to the wild bees in general, but they are more abundant in urban area in spring, while in summer the abundance peak shifts towards suburban areas. These studies can help us better understand how to conserve pollinators in urban environments.

# EXPLORING THE SUMMER FORAGING PATTERNS OF HONEYBEES IN AN AGRICULTURE LANDSCAPES

Mari Gigauri[1], Soraia Sousa Santos[1], Rubén Mina[1], Artur Sarmento[1], Nuno Capela[1], Henrique M.V.S. Azevedo-Pereira[1,2], Joana Alves[1], António Silva[1], Lucie Mota[1], Silvia Castro[1], José-Paulo Sousa[1]

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## Abstract

Fragmentation of the natural habitats in intensive agricultural landscapes has led to a significant decline in abundance and quality on the food sources for the honeybees and other pollinators. This study analyses the effects of temporal and spatial variation of flower resources on the foraging patterns of honeybees (*Apis mellifera iberiensis*) in an intensive agricultural landscape. Waggle dance decoding was used to identify the main foraging locations of honeybees. Monthly flowering species quantification was performed to assess possible floral resources changes through landscape monitoring in a 10×10km. The importance of plant species for honeybees was classified according to their nectar and pollen production and quality. The study revealed spatial and temporal variations in floral resources' availability. With a variety of plants species, instead of dominance of a given plant (mono-varieties), this landscape provided diverse food resources for honeybees. Consequently, results show variability in the foraging locations of honeybees; they foraged closer to the hive the beginning and farther later in summer. Considering floral resources in beehive's surrounding area, wildflowers resource availability decreased during the season, while sunflower presented a later flowering period, which affected honeybees foraging behaviour. We conclude that honeybees have shown a clear preference for wildflower resources, when available. Crops were visited only when higher quality floral resources were lacking. Our results support the need of bee-friendly practices in the agricultural landscape, while promoting sustainable beekeeping.

# THE EFFECT OF THE REARING TEMPERATURE ON THE THERMAL PREFERENCES OF HONEY BEE DRONES (*APIS MELLIFERA*) AT DIFFERENT AGES

Krystyna Czekońska, Sylwia Łopuch

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## Abstract

Honey bee males, called drones, are responsible for the production and transfer of semen to the queens during mating flights. Their quality depends on many factors, including the temperature prevailing in the nest during their individual development. Drones reared in unfavourable thermal conditions achieved lower body mass. Such conditions may also affect their thermal preferences during adult life. For this reason, the aim of our study was to assess the effect of changing temperatures (33-35°C or 35-33°C) during the post-capped development of drones on their body mass at eclosion and thermal preferences.

Combs with capped brood of drones from three colonies were kept in incubators in changing temperatures from higher to lower (35-33°C) or from lower to higher (33-35°C). After the emergence, 882 drones were individually weighted. The thermal preferences of drones were assessed at the ages of 1, 5, 10, 15, 20, and 25 days on a temperature platform in the range of 20°C - 46°C.

Drones reared in temperatures changing from higher to lower (35-33°C) were significantly heavier after emergence ($p<0.001$) compared with those reared in temperatures changing from lower to higher (33-35°C). Moreover, drones from group 35-33°C preferred a temperature 1°C lower than those from group 33-35°C ($p=0.001$). The thermal preferences of drones significantly changed with their age ($p < 0.001$). These findings indicate that the rearing conditions affect the body mass of drones at eclosion and their thermal preferences in adult life.

# THE SELECTION OF VARROA MITE RESISTANT BEES IN ALGERIA: IMPORTANCE OF HYGIENIC BEHAVIOR IN APIS MELLIFERA INTERMISSA

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## Abstract

Hygienic behavior is considered an important factor in resistance of the honey bee mite Varroa destructor, Hygienic bees have the ability to detect, uncap, and remove diseased brood from the nest before the causative organisms reach the sporulating stage. Hygienic behavior in the honey bee, Apis mellifera L., is highly variable among and within populations and subspecies. the objective of this study was to determine the frequency of this criterion in the local race Apis mellifera intermissa. A study on the selection of local bee colonies Apis mellifera intermissa is carried out on 50 colonies in the arid steppe zone of Djelfa, the apiary has not been treated against varroa for 5 years. During these years, the resistance criterion was evaluated 4 times a year by the method of Marla Spivak. The study points out the importance of this criterion in the intermissa breed to resist varroa. Out of 50 colonies, 20 hives remain characterized by a hygienic behavior which exceeds 90%. A queen breeding was carried out on these colonies, swarms were installed in three apiaries. Another evaluation of this criterion shows that this behavior is still present and constitutes a good basis for the selection of colonies resistant to varroasis. The study of the dynamics of varroa populations in the colonies studied shows a very weak development. The results show that hygienic behavior and temperature are two factors that limit the growth of the mite in this region. The only problem is the availability of honey and nectar resources all year round in order to have a good production of honey.

# 8 Evolution and population genetics of bees

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## Oral presentations

Moderator:
Silvio Erler

# ON THE LIMITS IN USING A SINGLE REFERENCE GENOME FOR POPULATION GENOMICS: TOWARDS PAN-GENOMES

Alain Vignal[1], Eynard Sonia[1], Chibani Bahi Amar Amira[2], Emmanuelle Labarthe[1], Pierre Faux[1], Thibault Leroy[1], Kamila Canale-Tabet[1]

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## Abstract

Since the advent of the so-called next generation sequencing (NGS) methods, whole genome sequencing has been used for population genomics, including in honey bees. In this approach, relatively cheap parallel sequencing is used to generate large quantities of short reads (usually 150 bp long) for individual samples that need to be aligned onto a reference genome. Genetic markers, usually SNPs, are then detected by observing sequence divergence between the aligned short reads and the reference genome. The limits to this approach, is that it depends highly (i) on the quality of the reference genome and (ii) on the fact that it is built from only a single individual. Quality issues have now been addressed with the great progress made in long-read sequencing technologies, but the fact of relying on a single individual sample for the reference remains a major issue. We have produced a reference genome for the black bee *Apis mellifera mellifera* by PacBio long-read sequencing and used it in a small population genomics study. Results show that the origin of the reference genome used will have some influence on the quality of results obtained. This is especially the case when studying mitochondrial DNA, as mis-alignment of reads can be caused by the presence of nuclear mitochondrial (NUMT) segments. In the future, such problems may be solved by pan-genome approaches.

# OBSERVATION OF GENETIC GAIN WITH INSTRUMENTAL INSEMINATION OF HONEYBEE QUEENS

Ségolène Maucourt[1], Andrée Rousseau[2], Frédéric Fortin[3], Claude Robert[1], Pierre Giovenazzo[1]

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## Abstract

Controlling mating in the honeybee (Apis mellifera) is part of one of the greatest challenges for the beekeeping industry's genetic selection programs due to specific characteristics of their reproduction. Several techniques for supervising honeybee mating with relative effective control have been developed over the years to allow honeybee selection. As part of this project, we compared the genetic gains for several colony performance traits, obtained using the BLUP-animal method, according to the selection pressure applied in controlled reproduction (directed fertilization versus instrumental insemination). Our results show similar genetic gains for hygienic behavior and honey production between colonies whether queens were fertilized naturally or via instrumental insemination, as well as similar or lower genetic gains for colonies with queens inseminated for spring development. In addition, we noticed greater fragility in queens following insemination. These findings show that instrumental insemination is an effective tool for reproductive control in genetic selection and for estimating breeding values more precisely. However, this technique does not result in queens of superior genetic quality for commercial purposes.

# EXPLORING POPULATION STRUCTURE AND ADAPTATION IN HONEY BEE SUBSPECIES FROM SOUTHERN GLACIAL REFUGIA: *A. M. IBERIENSIS* AND *A. M. LIGUSTICA*

Carlos A. Yadró[1,2,3,4,5], Dora Henriques[1,2], Giovanni Cilia[6], José Rufino[1,2], Raquel Martín-Hernández[7], Antonio Nanetti[6], M. Alice Pinto[1,2]

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## Abstract

Glacial refugia harbor populations with complex diversity patterns. In honey bees, the Iberian and Italian Peninsulas served as two of the most important glacial refugia in Europe. Here, we analyzed whole genomes generated from drones to infer population structure, genetic diversity, and the molecular basis of the local adaptation for the two native subspecies of these Peninsulas: *A. m. iberiensis* (N=86; M-lineage) and *A. m. ligustica* (N=225; C-lineage). For *A. m. iberiensis*, Admixture analysis revealed a strong cline between two genetic backgrounds from Southwest to Northeast and no C-lineage introgression was detected. For *A. m. ligustica,* introgression with *A. m. carnica* occurred in Central and Southern Italy (median q-value$_{carnica}$=0.069; IQR=0.187), away from the natural hybridization zone in Northeastern Italy where higher admixture proportions were detected (median q-value$_{carnica}$=0.229; IQR=0.262). *A. m. mellifera* introgression was detected especially in the Northwest (median q-value$_{mellifera}$ 0.053; IQR=0.030), and with lower values in Central and Southern Italy (median q-value$_{mellifera}$ 0.014; IQR=0.041). *A. m. iberiensis* showed higher diversity when compared to *A. m. ligustica*: ℗ (℗$_{iberiensis=}$0.325, ℗$_{ligustica=}$ 0.245, p-value<0.001); He (He$_{iberiensis=}$0.319, He$_{ligustica=}$0.319; p-value<0.001) but lower relatedness (IBD kinship$_{iberiensis=}$0.002, IBD kinship$_{ligustica=}$0.014; p-value<0.001). Selection signatures were detected and cross-validated using PCAdapt, SAMBADA, and RDA. SNPs with q-adjusted p-values < 0.01 detected by at least two methods were considered strong candidates. For *A. m. ligustica*, 133 candidate SNPs annotated to 125 genes were detected by all three methods, including dnaJ homolog subfamily C member 9, nephrin, and the diuretic hormone receptor, and these were correlated with precipitation. For *A. m. iberiensis*, 528 SNPs annotated to 527 genes were detected, and these included proteins related to heat-shock response, such as Cyp40 and rrp45. While no common candidate SNPs were detected

between both subspecies, 20 common genes containing candidate SNPs were detected, such as 4-coumarate-CoA ligase 1, CPR9, and alpha-mannosidase 2.

## GENOMIC INSIGHTS INTO MIDDLE EASTERN HONEY BEE SUBSPECIES: POPULATION STRUCTURE AND GENETIC INTEGRITY

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### Abstract

The genetic patterns of Middle Eastern *A. mellifera* subspecies have been understudied, hindering a comprehensive understanding of honey bee evolutionary history. Here, we studied the genetic integrity of five Middle Eastern subspecies across a broad geographical range: Turkey (*A. m. anatoliaca*, N=97; *A. m. caucasia*, N=75; *A. m. syriaca*, N=18), Jordan and Lebanon (*A. m. syriaca*, N=238 and N=29), Iran (*A. m. meda*, N=75), Oman, and the UAE (*A. m. jemenitica*, N=13 and N=10). ADMIXTURE and PCA analyses were conducted on SNPs detected from whole-genomes. Our findings reveal concerning conservation statuses for many populations/subspecies. In *A. m. caucasia* and *A. m. anatoliaca*, only 10 and 28 samples, respectively, were pure (introgression < 90%). In the *A. m. caucasia* range, 60 samples were hybrids of *A. m. caucasia, A. m. syriaca*, and *A.*

*m. ligustica*. In the *A. m. anatoliaca* range, 69 samples showed high hybridization degrees with *A. m. syriaca*, and *A. m. caucasia*. Only six samples in the Turkish range of the *A. m. syriaca* range were identified as pure, while the rest were also hybrids. All samples from Jordan and Lebanon showed variable *A. m. ligustica* introgression. In Iran, 23 samples were classified as pure *A. m. meda*. The rest showed introgression primarily due to *A. m. ligustica* and *A. m. caucasia*. In the UAE, two main groups were identified: the first comprised hybrids of *A. m. jemenitica*, *A. m. lamarckii* and *A. m. ligustica*, and the second group mainly consisted of hybrids of *A. m. lamarckii* and *A. m. ligustica*. Oman stands out as the sole location where all samples were identified as pure *A. m. jemenitica*. This study indicates widespread hybridization across various regions and underscores the urgent need for targeted conservation efforts for Middle Eastern subspecies.

*This work was funded by PRIMA project MEDIBEES.*

# GENETIC DIVERSITY AND DELETERIOUS MUTATION LOAD IN APIS

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## Abstract

Among the *Apis* species, the Western honey bee (A. mellifera) is the most widely used species for both honey production and crop pollination. The ecological and economical importance of these uses explains the human-mediated importation of colonies to regions outside of the Western honey bees' original range (Africa, Europe and the Middle East), where thirty subspecies are described. In this work, we gathered genetic data from hundreds of *Apis* genomes (mostly from A. mellifera, but also from *A. cerana*, A. laboriosa and *A. dorsata*), to investigate the demographic history, genetic diversity and impact of the beekeeping practices. Inter-crossing of different honey bee subspecies seems to have contributed to increase the diversity and reduce the genetic load of some genetic groups particularly appreciated by beekeepers. Conversely, black bee insular conservatories effectively prevented hybridization with the other subspecies, maintaining pure genetic backgrounds, but at the cost of lower diversity and increased genetic load, thereby questioning the long-term success of these conservation efforts. Looking at the variation of recombination and diversity along the genome, we confirm that the recombination landscape is highly heterogeneous in honey bees and shapes the genetic landscape of diversity. Remarkably, this landscape is well-conserved both among the different A. mellifera subspecies, but also among the different *Apis* species, even after more than 15 million years of divergence. We eventually discuss some perspectives on future options of better monitoring the evolution of the genetic diversity in honey bees.

# A HIGH-DENSITY AND TIME DYNAMIC DISTRIBUTION MAP OF HONEY BEE MITOCHONDRIAL DNA LINEAGES COVERING THE ENTIRE ITALY

Valeria Taurisano[1], Anisa Ribani[1], Dalal Sami[1], Kate Elise Johnson[1,2], Valerio Joe Utzeri[1], Samuele Bovo[1], Giuseppina Schiavo[1], Luca Fontanesi[1]

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## Abstract

Growing interest has emerged in the need to monitor the genetic integrity of the European *Apis mellifera* subspecies, which could be threatened by the human-mediated dispersion of non-native populations and lines. Honey contains environmental DNA (eDNA) traces from all organisms that directly or indirectly were involved in its production, including the DNA of the honey bees that produced it. Specific mitochondrial DNA (mtDNA) lineages (known as mitotypes) characterize several *A. mellifera* subspecies. Different mitotypes can be detected using honey as a source of honey bee DNA, providing approximate population genetic information useful for estimating the diffusion and frequency of honey bee mitotypes. In this study we present a snapshot of the diffusion of the main *A. mellifera* mtDNA lineages in Italy over five years (2018-2022). DNA was extracted from more than 3200 honey samples produced in all regions of the Italian peninsula, Sardinia and Sicily, during these five years. PCR products were analysed using a fragment size-based assay. Results confirmed that the C lineage was the most frequent mitotype throughout Italy except in Sicily where the A lineage was always highly represented. Other mtDNA haplotypes (A and M lineages) were present in almost all Italian regions. The updated distribution map of honey bee mtDNA lineages obtained in this study can be useful for designing and evaluating the potential effectiveness of conservation policies aimed at maintaining the integrity of honey bee genetic resources in Italy. Furthermore, we demonstrated that this approach based on the entomological footprint left in honey can provide a simplified yet extensive population genetic analysis, considering that each honey sample may contain the DNA of many different honey bees, from different colonies or even different apiaries.

# EXPLORING THE DIPLOID MALE VORTEX IN *APIS MELLIFERA*: A SIMULATION STUDY OF CSD GENE DYNAMICS AND POPULATION EXTINCTION RISK

Robert Mroczek[1], Pawel Blazej[2], Agnieszka Łaszkiewicz[1], Pawel Mackiewicz[2], Joanna Niedbalska-Tarnowska[1], Aleksandra Ertman[1], Kinga Adamczyk-Weglarzy[1], Malgorzata Cebrat[1]

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## Abstract

Bees heterozygous for *csd* gene develop into females, hemizygous into males, while homozygous into nonviable diploid males. Complete lack of fitness of homozygotes leads to selection of *csd*, resulting in a significant level of its intraspecific polymorphism. Diploid drones are unfavorable for the bee colony because they are produced instead of worker bees. The decrease in the number of worker bees weakens the colony and may lead to colony collapse. The decreased number of colonies in the population is followed by a further decrease in the diversity of csd, forming a positive feedback loop leading to population extinction. This loop has been predicted earlier in computer simulations which characterized the most significant factors influencing its dynamics but did not take into account the specifics of *Apis mellifera* reproduction, such as the actual number of *csd* alleles present in the population and intracolonial genetic diversity. Therefore we have created a simulation model that takes into account the specifics of the colony life cycle and reproduction of this species. In this model, we have simulated the population dynamics using a set of variables: swarming and mating distances, the number of *csd* alleles in the population, colony death probability influenced by the degree of heterozygosity between the *csd* alleles in the queen's genome and her spermatheca and the probability of colony death independent from *csd* status. Our main observations are: the population survival is not influenced by the number of *csd* alleles exceeding 40; the collapse of the population cannot be predicted by the overall number of *csd* alleles present in the population; the populations prone to extinction start to exhibit an imbalance in *csd* allele frequency several hundred generations prior to their death; the population can be rescued by increasing the mating distance and, to a lesser extent, the swarming distance.

# CHARACTERISATION OF *APIS MELLIFERA MELLIFERA* IN IRELAND: A MULTI-DISCIPLINARY APPROACH

Alexandra Valentine[1], Arrigo Moro[1], Chiara Binetti[1], Brandon Collier[1], Kenneth Sandoval[1], Keith A Browne[1], David Wragg[2], Matthew Richardson[2], Mark W Barnett[2], Grace P McCormack[1]

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## Abstract

*Apis mellifera mellifera* (Amm) has a native range spanning across most of Northern & Western Europe, and much of the early honeybee systematics was conducted on this subspecies. Ireland boasts one of the largest remaining populations of Amm in Europe but this subspecies is now also under significant threat of introgression with non-native subspecies In Ireland. The extensive native range of Amm encompasses a variety of habitats and climatic conditions, driving phenotypic adaptation and the effects of the relatively unique mild damp temperate oceanic conditions of Ireland is thought to have resulted in distinctiveness of the Irish Amm population. However little systematic work has been carried out on this population. Using multiple morphological and molecular approaches we determined the current hybrid status of the managed population whilst assessing the intraspecific variation within the remaining Amm in Ireland. This study revealed that only 69% of the 466 colonies assessed were now classified as Amm via a SNP assay at $Q > 0.95$ purity threshold indicating a rapid increase in levels of introgression. Several unique mitochondrial haplotypes were identified. A suite of 12 morphological traits suggest evidence for distinctiveness of Irish Amm in total leg length, proboscis length and cubital index from European honey bees. This research points to a honey bee population that is uniquely adapted to life in Ireland but under threat given a fourfold increase in introgression rates in recent years.

# 8 Evolution and population genetics of bees

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## Poster presentations

# CO-PHYLOGENY OF SMALL HIVE BEETLES AND HONEYBEES IN THEIR NATIVE RANGE

Michael N.K. Muturi[1], Peter Neumann[2], Alexis Beaurepaire[2,3]

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## Abstract

A fundamental question in evolutionary biology is how micro-evolutionary processes translate into species diversification. Co-phylogeny provides an appropriate framework to address this for host-parasite interfaces, but data remain scarce for parasites of bee colonies. Indeed, no study has investigated the factors underpinning genetic differentiation of small hive beetles, *Aethina tumida*. Here, we investigated the co-evolutionary interactions between these parasitic beetles and honeybee hosts in their native range in Africa. Previously published sequences from several countries in Western, Eastern and Southern Africa were used to assess the co-phylogeny of these hosts and parasites. Patterns of divergence, convergence and co-speciation between the two species were determined. The data will be presented and discussed.

# HIGH-RESOLUTION mtDNA-BASED PHYLOGENY OF HONEY BEES (*APIS MELLIFERA CARNICA*) FROM SERBIA

Pavle Erić[1], Marija Tanasković[1], Katarina Erić[1], Aleksandra Patenković[1], Ljubiša Stanisavljević[2], Slobodan Davidović[1]

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## Abstract

The western honey bee (*Apis mellifera*) is recognized as an important pollinator in nature and agricultural ecosystems. It is the most commonly managed insect, and although it was believed to be extinct in Europe in nature, the sightings of free-living colonies are becoming more frequent, raising the interest of the scientific community. Free-living honey bee colonies, which can survive in the wild without human intervention, represent an important genetic resource that needs to be properly valued and conserved. One of the ways to identify specific maternal lineage and follow it through the generations is by analysing its mitochondrial DNA (mtDNA). A standard method for detecting different mtDNA lineages relies on sequencing the intergenic $tRNA^{leu}$*-cox2* region, but sequencing complete mitogenomes provides higher resolution and allows better identification of specific lineages.

We sequenced 14.5 kb long mitogenomes from 237 autochthonous *Apis mellifera carnica* worker bees originating from different free-living or managed colonies from a wide range of Serbian regions. Sequencing was performed using Illumina's NovaSeqXPlus platform. The first complete mitogenome of *Apis mellifera* (NC_001566) was used as a reference genome for haplotype determination. Among 237 worker bees, 187 unique haplotypes belonging to the C lineage were detected and classified into two main groups according to phylogenetic relationships. Free-living colonies showed high diversity even though the majority of samples originated from Belgrade. In most cases, we could not identify region-specific clustering of lineages, since the majority of branches contained individuals from different regions, however in a few branches region-specific clustering of lineages could be observed.

The results indicate a strong admixture between populations from different regions of Serbia and the presence of unique mtDNA lineages in free-living colonies. This finding suggests the importance of free-living colonies as a reservoir of genetic diversity, which requires further investigation.

# INSIGHTS INTO POPULATION STRUCTURE AND CONSERVATION STATUS OF NORTH AFRICAN HONEY BEES

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## Abstract

The population structure of North African *A. mellifera* subspecies has been overlooked. Here, WG generated from drones were analyzed to assess population structure and the conservation status of three A-lineage subspecies: *A. m. lamarckii* (Egypt, 68 samples), *A. m. intermissa* (Algeria, 69 samples), and *A. m. sahariensis* (Algeria, 68 samples; Morocco, 26 samples). Population structure was assessed by SNMF and PCA. For *A. m. lamarckii*, variable C-lineage introgression was detected (median q-value$_{\text{C-lineage}}$=0.068; IQR=0.074) and PCA showed a well-grouped cluster slightly shifted towards C-lineage. Moroccan samples showed a highly compact group close to, but distinct from, the Algerian samples, and without signals of C-lineage introgression. In Algeria, only 28 *A. m. sahariensis* samples showed high purity values for this subspecies, and the remaining 48 samples showed variable introgression from *A. m. intermissa* (q-value$_{intermissa}$=0.127; 0.021). A more concerning scenario was found for *A. m. intermissa*, in which only 17 samples were classified as pure *A. m. intermissa*, 21 samples showed variable *A. m. sahariensis* introgression (q-

value$_{\textit{sahariensis}}$=0.125; 0.016), and the remaining 32 samples showed to be pure *A. m. sahariensis.* In the PCA analysis, a large portion of Algerian samples formed a cluster containing individuals of both subspecies. The remaining Algerian samples formed five well-defined isolated clusters: three containing *A. m. sahariensis* samples and two containing *A. m. intermissa* samples. SNMF runs at K=7 for the Algerian samples also recovered the existence of these high-purity isolated clusters. While most *A. m. intermissa* samples were located in the northern part of Algeria, some were located farther south. For *A. m. sahariensis,* a large portion of the samples were located close to the Mediterranean coast. These results suggest that, in addition to the C-lineage introgression (especially in *A. m. lamarckii),* the geographical delimitation originally described by Ruttner does not exist anymore in North Africa.

# PHYLOGEOGRAPHY OF THE GIANT HONEYBEES BASED ON MITOCHONDRIAL GENE SEQUENCES

Chet Bhatta[1], Sarah Cluff[2], Deborah Smith[3]

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## Abstract

We carried out a phylogenetic analysis of the giant honeybees using mitochondrial COI and COII gene sequences analyzed with maximum likelihood methods. Our goal was to resolve phylogenetic relationships among *Apis laboriosa*, and the *Apis dorsata* subspecies: *A. d. dorsata, A. d. binghami*, and *A. d. breviligula*, the last two of which have been proposed as full species by several authors. We obtained strong support for four clades within *A. dorsata*: the three subspecies mentioned above, and a fourth from south India, but our analysis did not resolve the phylogenetic relationships among the four clades within *A. dorsata* in the broad sense. Recognition of these distinct lineages is important for conservation planning, so that their individual ecologies and migration patterns can be taken into account.

# SCREENING FINNISH HONEYBEE (*APIS MELLIFERA*) POPULATIONS FOR RECAPPING BEHAVIOUR (REC) AND SUPPRESSED MITE (*VARROA DESTRUCTOR*) REPRODUCTION (SMR)

Kati Tunkkari

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## Abstract

*Varroa destructor* mite is evolutionarily new parasite to European honeybee (*Apis mellifera*), it started to migrate to *A. mellifera* only about half a century ago. Honeybee evolution can be fast, but host-parasite evolution hasn't proceeded naturally due to chemical treatment of *Varroa*, and other measures and actions done by beekeepers. These include the habit of bringing new queens from abroad and mixing the bee breeds. More natural and ecologically sustainable means to deal with severe varroa problem is to create local bee populations that will adapt not only to their environment but to *Varroa* due natural selection. Also, *Varroa* evolution will be guided more by the parasite-host interaction and less by the beekeeper. In Finland this work was started by one single beekeeper about twenty years ago. Recently this way of breeding has gained more interest. In my master's thesis I study Finnish bee populations and breeds for two traits that have been found around the world in honeybee populations coexisting with *Varroa destructor*. These resistant bee populations have higher levels of REC behaviour: uncapping and recapping of sealed brood cells, and SMR: suppressed mite reproduction, which means over 20% failure in mother mites' reproduction. REC is performed by worker bees: they open the wax cap and the silky cocoon covering the brood and cover the hole with wax later. REC behaviour is one of the factors causing SMR. In this work I use the COLOSS RNSBB (Research Network for Sustainable Bee Breeding) SMR & REC -brood investigation protocol. I scan hives from three old locally adapted stock, one of them surviving *Varroa* without chemical treatment. Plain REC scanning has been done to a wider set of populations and breeds, including Dark European honeybee (*Apis mellifera mellifera*). Preliminary results show variation from zero to low levels of REC.

# 9 Beekeeping issues

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## Oral presentations

Moderators:
Sigmar Naudi
Erki Naumanis

# PERSPECTIVES ON WELLBEING AMONG COMMERCIAL BEEKEEPERS IN NEW ZEALAND

Pike Stahlmann-Brown[1], Patrick Dawkins[2,3], Jane Pierce[4], John Berry[2], Barry Foster[2,5], Ricki Leahy[2,5], Jane Lorimer[2,6,5], Russell Marsh[2], Will Trollope[2]

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## Abstract

The destructive nature of pests and diseases on *Apis mellifera* have prompted many countries to undertake annual surveys of colony losses. The New Zealand Colony Loss Survey is modelled on the COLOSS survey, but has been adapted to the NZ apicultural context.

In particular, the 2023 New Zealand Colony Loss Survey included two assessments of subjective wellbeing that are commonly used in the psychology literature, namely, The World Health Organisation's "WHO-5" inventory and the "Cantril Ladder". The WHO-5 inventory assesses mental wellbeing over the past two weeks and has been used to screen individuals at risk for mental health issues such as depression. The Cantril Ladder measure evaluates overall life satisfaction and has been validated in more than 160 countries. To facilitate comparisons, these questions were also included in a nationally representative survey of New Zealanders and a large-scale survey of New Zealand farmers, foresters, and growers.

While commercial beekeepers report high life satisfaction in an international context, beekeepers' life satisfaction lags behind that of all other types of primary producers in New Zealand as well as the general public. Moreover, beekeepers' mental wellbeing is low, on average, with nearly half of all commercial beekeepers falling below the recognised for potential risk of mental health issues.

The survey further asked beekeepers to reflect on specific aspects of beekeeping that may impact on wellbeing. Beekeepers identified dramatic reductions in honey pricing in recent years, costly compliance issues, severe weather events, growing pressure from pests and disease, competition for floral resources, and the aging population of beekeepers as negatively influencing beekeeper well-being. Other aspects of the job, such as working outdoors and the physical demands of the job, are viewed positively by beekeepers.

# METHODOLOGICAL OVERVIEW OF THE VALUATION OF HONEY PROVISIONING CAPACITY AS AN ECOSYSTEM SERVICE – RESULTS OF AN INTERPRETIVE SYNTHESIS REVIEW

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## Abstract

Ecosystem services are the contributions of ecosystems to the well-being of society. Pollinators are key ecosystem service providers, and the European honey bee (*Apis mellifera* L.) has a prominent role among them. While pollination is relatively well covered in recent ecosystem service research, there is much less focus on honey provisioning capacity - despite the large body of traditional and practical knowledge in beekeeping. In this presentation we would like to address this gap and discuss honey provisioning in the light of the ecosystem service approach, by providing an overview of models applied in scientific literature. To this end, a special kind of systematic literature review, the so-called Critical Interpretive Synthesis was applied to analyse the published valuations of honey provisioning capacity.

Three main types of models were identified: rule-based matrix models, extended rule-based matrix models and predictive statistical models. Key decision points in the mapping and assessment process were identified as well as the uncertainties inherent in them, along with their possible solutions. Such decision points are: the floral resources considered, the incorporation of multiannual weather-related fluctuations as well as seasonal phenological variation, and the incorporation of landscape patterns and certain environmental variables. The results contribute to the methodological clarification of the valuation of honey provisioning capacity as an ecosystem service and to the theoretical and methodological grounding of future assessments.

General conclusion of our review is that besides the main honey flow resources, habitats that provide a continuous and diverse source of nectar and pollen are critical for honey provisioning capacity. The needs of honey bees in this respect are broadly similar to those of wild pollinators, and our results emphasise the need for widespread adoption of nature-friendly landscape management practices.

# TOWARDS DOMESTIC POLLINATION WITH BUMBLEBEES

Anna-Maria Borshagovski, Claude Flener

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## Abstract

Laboratory-bred bumblebees are sold around the world to pollinate in commercial gardens. Finland has no domestic commercial bumblebee production, so we are completely dependent on imported bumblebees, while the demand for them is increasing. This total dependence is a risk to our national security of food supply. The use of imported bumblebees also causes risks to natural pollinators through the spread of foreign diseases and resource competition. To mitigate these risks, and to respond to the increasing need of pollination, the *Domestic Pollinators for Commercial Horticulture* project aims to investigate the possibilities of breeding domestic bumblebees.

Our breeding experiments in 2023 and 2024, building on the experience of several professionals, revealed that the breeding of local bumblebee species is possible when certain factors are carefully taken care of, including right breeding conditions, good quality bee food, good hygiene and thorough scheduling of the year-round breeding. Regarding commercial production, the greatest challenge is to turn the breeding operation into a financially viable business. Entrepreneurs should carefully consider whether they can compete with the large European produces, or whether they could approach other customer segments with new products and services. If successful, domestic bumblebee production would help to decrease the risks to natural pollinators, increase our self-sufficiency and our adaptability to potential crises affecting bumblebee production and importation to Finland, as well as to create new possibilities for pollination services.

## EUROPEAN BEEKEEPING: THREATS, CHALLENGES AND PERSPECTIVES

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### Abstract

Number of managed bee colonies in Europe is continuously growing (in EU 20 million + approx. 10 million in other European regions). Most of colonies in Europe are kept by hobby beekeepers, total number of people involved in beekeeping is around 1 million (of this number, more than 700 thousand in the EU). European beekeepers must face challenges that negatively affect the development of beekeeping, among the most serious are pests and diseases, including invasive ones, changes in land use, pesticides, contaminants and the unstable market for honey and other products of hives. Activities like young beekeepers' involvement, research and extension programmes, cooperation between beekeepers and promotion of sustainable agriculture and increased biodiversity have the ambition to reverse these negative trends. Varroosis remains the most widespread threat to beekeeping, while the bee survival threshold is dramatically reducing. *Varroa* control strategies across Europe are not uniform. New predators and pests have entered European continent, including Small Hive Beetle, *Tropilaelaps* mite and Asiatic species of hornets. Actual situation of legislative changes related to beekeeping and honey import/export development in the EU will be discussed, including adulteration issues. EU remains only 63% self-sufficient in honey. Average export price for European honey is close to be 6 euro, while average import price of honey is just slightly above 2 eur/kg.

# VESPA VELUTINA - A NEW THREAT TO HONEY BEE COLONIES IN SOUTHWEST GERMANY

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State Institute of Bee Research, Germany

## Abstract

The Asian hornet (*Vespa velutina nigrithorax*), a wasp species originally native to Asia, was accidentally introduced to Europe in 2004 and is an invasive threat that voraciously feeds on various insect species including honey bees. Currently classified as an invasive alien species of concern, it falls under EU regulation 143/2014. Assigned to Article 16 of the regulation through the Federal Agency for Nature Conservation in Germany, states must take immediate eradication measures.

While worker hornets feed primarily on carbohydrate-rich sources such as nectar and fruit, larval development requires protein-rich sources, often obtained from flying insects, including honey bees. This predation weakens honey bee colonies and jeopardizes their winter survival. Beekeepers, already concerned about the parasitic mite *Varroa destructor*, are further alarmed by the spread of this new predator, which in other countries has led to colony losses, reduced pollination efficiency and negative impacts on agricultural production.

Initially rare, its sightings have increased dramatically since 2023, signaling a rapid expansion in Germany, especially along waterways. To curb further spread a central coordination station was established at the State Institute of Bee Research (LAB) at the University of Hohenheim, Stuttgart. We will validate sightings and coordinate nest removal with local specialists for southwest Germany. In 2023, a total of 550 secondary nests were reported. The majority of these have been successfully removed. At the conference, we will present data on the number of founder nests and secondary nests reported in 2024 and provide insights into the management measures employed.

# THE UKS RESPONSE TO YELLOW LEGGED ASIAN HORNET (*VESPA VELUTINA*) INCURSION

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## Abstract

The Yellow Legged Asian Hornet (*Vespa velutina)* is a non-native species originating in Asia which predates on insects. It has caused significant honeybee colony losses in mainland Europe, where it became established after accidental introduction 20 years ago. *V. velutina* was first detected in the UK in 2016 and the UK government's National Bee Unit (NBU) is responsible for contingency planning and responding to reports. Between 2016 and 2022 the UK detected on average 2 nests per year and all reported sightings and nests have been successfully tracked and destroyed. 2023 saw a significant increase in reports of *V. velutina* with several thousand triaged reports leading to 72 confirmed sightings of single hornets, which ultimately led to the detection and eradication of all 72 nests.

The NBU has developed a sophisticated triaging system for sightings and a "track and trace" process to locate nests from confirmed sightings. These systems utilise techniques such as mobile reporting applications, drones, thermal technology, and radio telemetry. Flight lining and triangulation data gathered during incursions has led to the development of an ArcGIS based "track and trace" mobile application for in field use. This app generates preprogramed flight times and directional indicators from real time data input, as well as recoding samples through barcoding for traceability through the laboratory process.

Genotyping of individual trapped hornets and hornets found within destroyed nests has established no relatedness, all have become established from separate incursions from the European *V. velutina* population. The NBUs contingency response has been instrumental in preventing this invasive pest from becoming established in the UK and this work offers valuable insights to other countries who have yet to encounter this invasive pest or are in the early stages of incursion.

# IDENTIFYING THE MAIN REASONS FOR THE HONEY BEE COLONY LOSSES IN BULGARIA

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## Abstract

Honey bees have a high sensitivity to environmental pollution and are successfully included in different biomonitoring studies. Their genetic richness is under danger by the global climate changes and the new pathogens, the stress of the changing environment and its pollution.

The results reveal that for the period 2017 – 2023, the losses of bee colonies in Bulgaria increased from 2.04% in 2017 to over 14%. During the critical 2022, 21.28% of the included in the study honey bee colonies have been lost due to different reasons, as follows: problems with the queen bee – 3.16%; natural disasters – 0.17%; death or reducing in number to a few hundred bees in the hive – 18.19%. Survey data show that sunflowers, rape, maize, orchards and autumn forage crops are predominantly grown round the apiaries with the greatest losses, on vast agricultural areas.

By usage of complex approaches the general toxic, cytotoxic, genotoxic and histopathological effects of agrochemicals applied in beekeeping and agriculture and the possible synergistic interactions between them have been analyzed. Beekeepers report that different pesticides have been applied for plant protection in the areas of their apiaries, as well as that oxalic acid, coumaphos, amitraz and tau-fluvalinate are the most frequently applied against *Varroa*.

A toxic effect of the chemicals included in the study has been found both under laboratory and *in situ* conditions. Reduced cell proliferation, various chromosomal aberrations, DNA damages in spermatozoa and histopathological changes in the drone's testes have been established.

The results reveal the need to develop measures for conservation of the national genetic resource of *A. mellifera* and to conduct activities for further detailed studies of the risk factors for the honey bees health and viability in Bulgaria.

## Acknowledgments

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# FLUCTUATION OF WINTER LOSSES IN FINLAND AND VARROA FREE ÅLAND

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## Abstract

Finnish Beekeepers' Association has been collecting information about winter losses by Lime survey questionnaire and also from special group by phone as a part of COLOSS monitoring group since 2008.

Åland, the autonomous archipelago province of Finland, got official varroa free status in EU in 2013. Since winter 2017-18 Åland winter losses have been reported separate from mainland Finland in order to see differences due to varroa free beekeeping.

Winter losses fluctuate in Åland like in mainland Finland but always lower level. In a year of high winter losses there is a lot of weakened colonies also in Åland. Winter with very low losses showed higher percentage of weakened colonies than the losses itself in Åland. This is never the case in mainland Finland.

The main cause of winter losses seems to be varroa and the consequences of varroatosis as expected in mainland Finland while in Åland the queen problems and weakened colonies appear to have a greater impact. Further research is needed to determine whether bacterial or virus infections could contribute to the weakening of the colonies, especially in Åland.

# ADVANCING BEE HEALTH: FIELD SCREENING FOR EUROPEAN FOULBROOD USING HIVE DEBRIS

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## Abstract

One significant threat to bee colonies is the European Foulbrood (EFB), a bacterial disease caused by the pathogen *Melissococcus plutonius*. Early detection and swift management of EFB are critical to mitigating its spread and preserving bee colonies.

Here, we present a novel approach for the early detection of EFB by analysing hive debris using Polymerase Chain Reaction (PCR). This method offers a simple, cost-effective, and rapid means of screening bee colonies for the presence of *M. plutonius* DNA in the apiaries. The early detection of EFB is based on field screening of apiaries from suspected areas. Pooled hive debris samples are sent to a laboratory for PCR detection of *M. plutonius.* Positive apiaries are then inspected by experienced beekeepers or bee advisors, who can suggest the next step to minimise the adverse effects of EFB.

We used hive debris screening of the presence of *M. plutonius* in Czechia to detect suspected apiaries in areas with EFB outbreaks. In total, 44 apiaries were sampled, and 12 (23 %) were evaluated positive by PCR and then by clinical inspection, 10 (23 %) were PCR positive but without clinical symptoms. False positives by PCR indicated the pre-clinical phase of EFB. No false negatives by PCR were recorded. The sensitivity and specificity of PCR testing of hive debris were 1.000 and 0.667, respectively. Our study demonstrates the feasibility and efficacy of this approach. It highlights its potential as a valuable tool for beekeepers, researchers, and regulatory authorities in monitoring and managing EFB outbreaks. Thus, EFB screening is being performed in further regions of Czechia.

In conclusion, hive debris PCR screening represents a cheap and significant advancement in bee health management strategies, offering a proactive and scalable solution for early detection and containment of EFB.

# SAMPLING STRATEGIES OF EXTRACTED HONEY USED FOR AFB RISK ASSESSMENT AND SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN COMMERCIAL BEEKEEPING OPERATIONS

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University of Saskatchewan, Canada

## Abstract

American foulbrood (AFB) risk assessment has been focused on *Paenibacillus larvae* spore (AFB-spore) concentrations in samples collected from individual hives (e.g. adult bees, brood-chamber honey, or bottom-board debris). These approaches are quite effective in small beekeeping operations but they are logistically impossible for large commercial operations which, consequently, rely on antibiotic metaphylaxis for control of AFB despite of development of antimicrobial resistance in beekeeping industry in the USA and Canada.

We have demonstrated that AFB-spore concentrations in samples of pooled, extracted honey (conveniently collected during end-of-season extraction) have prognostic value for assessment of the risk of AFB at commercial operation level and for surveillance of antimicrobial resistance in *P. larvae* (AMR-AFB isolates).

The first objective was to comparatively evaluate two different sampling strategies of pooled extracted honey from large commercial beekeeping operations in Western Canada. The first strategy sampled 6 random geographically distant apiaries/yards in each commercial beekeeping operation (n=52) during 2019 and 2020, and the second strategy sampled every fifth barrel of extracted honey (~275 kg) during the end-of-season extraction cycle in 2023. The first sampling strategy revealed the higher overall AFB-spore concentrations in extracted honey, whereas the second sampling strategy identified higher number of commercial operations with AMR-AFB isolates in Saskatchewan, Canada.

The second objective was to present results of our on-going world-wide surveillance of AFB-spore concentrations and AMR-AFB in honey samples (n=174) purchased via www.amazon.com from 39 countries on 5 continents. Preliminary evaluation revealed that honey samples from the USA and Canada, where antibiotic metaphylaxis is commonly used, have similar range of AFB-spore concentrations as international honey samples, but AMR-AFB isolates were identified only in honey samples from the USA and Canada but not from the rest of the world.

Extracted honey seems to be appropriate and convenient sample for AFB risk assessment and AMR-AFB surveillance.

# EFFECT OF HARVESTING PRACTICES ON THE MICROBIAL LOAD OF FRESH COLLECTED POLLEN

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## Abstract

Over the past few years, bee pollen has become a popular dietary supplement due to its high nutritional content. However, the quality of the final product is greatly influenced by the methods used by beekeepers during the collection process. Given that in Greece fresh bee pollen is predominantly sold and consumed, in the present study we examined the impact of harvesting periods in different environmental conditions, the use of various types of pollen traps (outdoor front, indoor bottom, and indoor roof), and the duration of time spent in these traps (0, 1, 2 days) as potential sources of contamination in the final product. The microbial load of the collected pollen samples were analyzed through spread and pour plating for the following parameters: Mesophilic total viable count (MTVC) (PCA, incubation time 24 h, temperature 37 °C), Yeasts and Moulds (Y&M) (RBC, incubation time 48h, temperature 25°C), Enterobacteriaceae (ENT) (VRBGA, incubation time 24h, temperature 37°C), Lactic acid bacteria (LAB) (MRS agar, incubation time 72h, temperature 37°C) and Escherichia coli (TBX, incubation time 24h, temperature 37°C). The comparative study of different types of pollen traps highlights the advantages of using indoor traps and suggests avoiding early spring collection. Delayed harvesting had an impact on the microbial load of pollen, especially on the populations of MTVC and ENT exceeding the market limits as defined in food hygiene standards, with values higher than 5 log cfu/g and 2 log cfu/g respectively. Indeed, the highest counts of MTVC and ENT were detected after 2 days of pollen staying in outdoor front traps (5.98 and 4.81 log cfu/g, respectively). The results of the study will be a helpful guide in giving the appropriate beekeeping practices for the production of fresh pollen with microbial load harmonized with hygienic standards.

# THE GERMAN BEE MONITORING PROJECT. WHAT PESTICIDES DO WE FIND IN STORED POLLEN

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## Abstract

The German Bee Monitoring, launched in 2004 to better understand drivers of colony losses, is the longest continuously running survey monitoring honey bee health. We have been collecting health measures, such as *Varroa destructor* infestation, pathogen presence, viral infections, and overwintering success for twenty years. Over 120 beekeepers are currently involved nationwide with more than 1,200 colonies monitored annually. Bee, honey and pollen samples are taken up to three times per year in spring, summer and fall for disease and residue analyses. Pesticide residue analysis has been a regular part of the annual analysis since 2009.

We report the preliminary pesticide residue results for N = 2,410 apiary samples. Our multi-residue analysis has investigated residues in stored pollen for up to 478 different pesticides and their metabolites. Honey bees function like environmental monitors, foraging in a wide radius from their point-source colony. The residues detected thus essentially reflect current agricultural practices within a 2-6 km radius of the apiary, the typical foraging distance of honey bees.

A total of 85 of the 478 active substances and metabolites were detected in the bee bread samples from 2023. The residues were mostly in the trace range, though 61 active substances were detected at least once above the limit of quantification. 93.7% of the samples contained at least one pesticide residue, though only 81.1% of the samples had at least one active substance above the limit of quantification. We will also report on the overall trends of pesticide residues from 2009-2023, where insecticide residues are decreasing over time, while fungicide residues are increasing, a trend that matches current agricultural plant protection policies. As a pollen analysis was conducted for each sample, we can determine what plant sources are correlated with higher pesticide exposure risk.

# APPLICATION OF BEEKEEPING PRACTICES TO PRODUCE STRAWBERRY TREE HONEY AND INVESTIGATION OF THE FINAL PRODUCT'S QUALITY

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## Abstract

Strawberry tree (*Arbutus* sp.) plays a vital role in beekeeping during the autumn (October-December) by providing essential resources for bees to gather and store food for the winter, ensuring their successful overwintering. Recently, consumers have shown a preference for strawberry tree honey, leading beekeepers to relocate their colonies to areas where the plant thrives. However, due to the extended flowering period, strawberry tree poses risks for honey production as adverse weather conditions can disrupt the collection and ripening processes, resulting in immature and potentially sour honey. In the present study, 15 bee colonies were moved to an area abundant with strawberry tree plants in Western Greece, and specific beekeeping practices were applied in order to collect monofloral strawberry tree honey. The unnecessary frames were removed from the hives and two empty frames were added for honey collection, always maintaining crowded conditions. Nectar influx was observed from the first days of locating the bee colonies in the area, but a significant increase in reserves was detected 30 days after the beginning of flowering, leading to the honey collection. The honey was collected after the combs were ¾ sealed, and the average amount collected was 4.1 kg/hive. The flowering of strawberry tree remained stable and unaffected by prevailing weather conditions. During the experiment relatively high average temperatures (>9º C) prevailed, keeping the bees active and allowing the collection of honey. The collected monofloral honey samples had average moisture 20.3%, electrical conductivity 0.72 mS/cm, HMF 7.03 mg/kg, diastase activity 11.37 DN, pH 3.95, and acidity 42.06 meq/kg. The volatile profile included .alpha.isophorone (percentage >60%), .beta.isophorone (10%), and keto-isophorone (3%). The data suggest that the utilization of strawberry tree flowering with appropriate beekeeping practices can lead to the production of a highly valued honey.

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# THE SOCIAL AND ENVIRONMENTAL CONDITIONS CONTROLLING HONEY BEE QUEEN REARING

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## Abstract

The honey bee queen is the most important individual in the colony, laying thousands of eggs daily and controlling the genetic qualities of the colony. Beekeepers grow and trade queens to increase their colonies' health and honey harvest. Artificial queen rearing is done in queenless colonies in the field and has remained largely unchanged for over a century. The control of the breeder on the environmental conditions of the growing larvae is very limited. since, the hives are placed in the open field and depend on the season and the environment in which they are placed, in terms of nutrition and the ability to raise brood in general and queens in particular. In addition, the hives are exposed to various hazards such as pathogens, which can harm the queen rearing process and the health of the entire colony. To address this issue, our research focuses on developing a laboratory-based method for queen bee rearing. We established a protocol for rearing queens in cages supplied with pollen and honey, placed in a chamber with control environmental conditions similar to the hive (T=34°C, RH=60%). We tested how many workers are needed to grow a single queen. We used cages with 50, 100, 200 and 300 bees. Our research revealed that optimal queen rearing occurred with 200 workers similar in weight and size to traditional reared queens. Using this protocol, we tested the effect of pollen nutrition on the rearing success. We found that high pollen concentration is crucial for queen rearing. We tested the effect of the larval age on the rearing success rate, we found that it's better to use larva younger than 48 hours. These findings lay the groundwork for rearing queen bees under controlled, pathogen-free environment. Future research will focus on evaluating the field performance of laboratory-reared queen bees.

# APIOMIC, HONEY BEE BREEDING USING GENOMICS

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## Abstract

Each year our Canadian beekeeping industry produces nearly 75 million pounds of honey and offers pollination services that make an annual contribution to agricultural worth an estimated $4.5–6.1 billion. Unfortunately, since the last 15 years, beekeepers across Canada suffer an average winter colony loss of 26%. The highest ever recorded loss occurred during winter 2022 and imported 360,000 queens and 60,000 packages of bees from various countries to rebuild their honey bee livestock. Thus, supporting the Canadian honey bee queen rearing and breeding is a sustainable solution to ensure Canadian honey bee stock self-sufficiency.

The ApiOmic project uses genomic tools within Canadian breeding programs to produce honeybee stock that is winter hardy, productive and disease resistant thus ensuring efficient pollination services, superior honey production and increasing sustainability of our beekeeping industry. ApiOmic uses the Illumina Honey bee 100K SNP array that provides a cost-effective genomic tool that can be applied to breeding and selection. This array was created from a list of genomic regions that are linked to a range of honey bee traits. We have tested the Illumina Honey bee 100K chip with our honey bee selected lineages and results show the presence of 71K differentially expressed SNPs with a call rate of 99% and the existence of significant genomic differences between our bee lineages and unselected lineages.

ApiOmic is divided in 3 related activities. Activity 1 implements genomic selection in our honey bee breeding program. Each year we select breeder colonies using genomic breeding values and produce 12 new lines (180 colonies). Activity 2 optimizes honey bee colony DNA sampling. Activity 3 studies genomic parameters, such as genetic diversity and inbreeding coefficients that guide the selection process. Activity 3 also studies paternal lineage and the efficacy of our mating yard.

# OPTIMIZING HONEYBEE BREEDING SCHEMES: REDUCING THE GENERATION INTERVAL OF DAMS TO ONE YEAR WITH PARTIAL PHENOTYPING

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## Abstract

INTRODUCTION
In honeybee breeding schemes, the generation interval is often two years on the dam path. This allows phenotyping of certain traits, such as honey yield, during a full beekeeping season to measure early to late-season harvests. Other traits, however, such as hygienic behavior (HYG), can be phenotyped early, within the first year of birth of queens. Using simulation, we investigated the impact of reducing the dam's generation interval from two to one year on the genetic gain and inbreeding build-up, after 20 years of selection.

METHODS
We simulated populations under selection, with individual queens, worker groups, and drones, using an polygenic model adapted to bee specificities. We simulated two breeding schemes. In the 'base' scheme, the dam and sire generation interval were both two years, allowing the phenotyping of honey yield in addition to HYG on both potential dams (breeding queens, BQs) and potential sires (drone-producing queens, DPQs). In contrast, in the 'alternative' scheme, the dam generation interval was reduced to one year, allowing for the phenotyping of only HYG on BQs, while the sire generation interval remained two years with complete phenotyping of DPQs. A yearly winter mortality randomly eliminated 25% of all BQs and all DPQs. The genetic correlation between honey yield and HYG was either 0, -0.6, or +0.6. The breeding goal was the sum of breeding values of honey yield and HYG with the same weights.

RESULTS
In all scenarios, the alternative scheme outperformed the base scheme, yielding 40 to 45% greater genetic gain. However, this additional genetic gain was accompanied by an increase in inbreeding of 20% to 35%.

CONCLUSION
Reducing the generation interval proved to be a powerful way of increasing genetic gain even though it meant that honey yield could not be phenotyped on potential BQs. However, it also brought more inbreeding.

# BREEDING, SELECTION AND QUEEN PRODUCTION OF APIS MELLIFERA CARNICA ON A KOVAČIĆ FAMILY BEE FARM IN CROATIA

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## Abstract

Breeding and selection of honey bees is a complex, long-term process that require several crucial components for success. Briefly, these include the accurate evaluation of desirable traits, proper selection of maternal and paternal colonies based on performance tests, and controlled mating practices. After successful breeding and selection work, it is essential to produce high-quality queens in quantities sufficient to meet market demands for both quality and price. This process involves two major steps: the production of high-quality queen cells, which is fundamental for obtaining phenotypically superior queens, and the production of a significant number of drones from selected colonies to ensure effective mating. During the presentation, I will demonstrate how we implement these critical breeding steps at our family farm, where we produce approximately 20,000 mated queens annually.

# 9 Beekeeping issues

## Poster presentations

# ASSESSING BEEKEEPER BELIEFS AND BEHAVIOURS IN RELATION TO EUROPEAN FOULBROOD

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## Abstract

European foulbrood (EFB) is a serious bacterial disease of honey bee larvae that is notifiable in many countries across the globe. Despite operating a honey bee health programme for the surveillance and control of EFB since the 1940s, the disease remains a problem in certain regions of England and Wales. The views and practices of beekeepers are rarely studied when considering EFB epidemiology and spread, despite clear risks from certain beekeeping practices. Here we surveyed over 300 beekeepers from across two English regions where EFB is present, to ascertain whether they considered the disease a local risk. We also gathered data on their beekeeping experience, practices, and history of contact with the national bee health programme. We build a conceptual model to explore the direct and indirect relationships between experience, education, beliefs and behaviours, and tested these hypotheses using structural equation models.

We found that those beekeepers who read more literature and had more beekeeping experience were more likely to practice improved apiary hygiene. Previous experience of seeing EFB was consistently associated with an increased likelihood of perceiving EFB as a local risk. Interestingly, there was no consistent increase in the perception of EFB as a local risk in those beekeepers who had been visited by national bee health inspectors. It was also interesting to note how beekeepers often blamed those with disease for having poor beekeeping practices, which is a behaviour consistent with other human diseases.

Our results demonstrate the importance of considering social elements of honey bee disease control, and highlight important gaps in both educational materials and evidence that link disease with certain beekeeping practices. Similar surveys could help improve the impact of national disease control efforts by focusing targeting training and educational efforts.

# B-THENET, THE THEMATIC NETWORK FOR MODERNISING THE EU BEEKEEPING SECTOR THROUGH THE CO-CREATION AND VALIDATION OF BEST BEEKEEPING PRACTICES

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## Abstract

B-THENET is the first Horizon Europe Thematic Network for modernizing the EU beekeeping sector by describing Best Beekeeping Practices, based on a multi-actor network that connects beekeepers, researchers, advisors, and other stakeholders. The Best Beekeeping Practices are distilled through the cascade of the 4 interlinked B-THENET platforms, using the B-THENET bottom-up methodology. The validated and tailored Best Beekeeping Practices are available in local languages on the Repository platform, forming the base of the B-THENET national and international training activities.

The description of the Best Beekeeping Practices occurs at the 13 National B-THENET centres involving the national beekeepers in each step of the co-development process. Three International

B-THENET Centres (for beekeepers, for advisors, and for stakeholders) were also embedded into the training scheme.

The Best Beekeeping Practices are going to be co-developed in 4 rounds during implementing B-THENET. In the first round, altogether 71 practices and research findings were collected in two themes: "Apiary set-up and management/maintenance" and "Varroosis". Having those practices prioritised by the EU beekeepers, the priority practices were subjected to a complex socioeconomical analysis for further selection. For determining the communication channels preferred by the EU beekeepers, two sociological surveys were carried out. The 13 National B-THENET Centres will finally organise 130 online and on-field events to ensure the tailored, interactive co-development and rating of the practices. By April 2024, the first set of validated Best Beekeeping Practices (altogether 284 practices in 13 languages) were transferred to the Repository platform. In the second round, 28 beekeeping practices will be collected in the following two themes: "Colony set-up and daily management/maintenance" and "American Foulbrood". Following the same methodology, currently altogether 364 beekeeping practices are being developed on the "Exchange" platform and the validated best practices will be available on the Repository platform by January 2025.

# BEE COLONY LOSSES IN BULGARIA - REGIONAL ASPECTS OF THE PROBLEM

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## Abstract

During past years the Bulgarian beekeeping is under great danger. For a period of the last 5 years honey bee colony losses have increased from 2.04% to over 14%. Two main reasons could be mentioned for that: 1) the uncontrolled imports of honey bee queens with foreign origin, although the Bulgarian law forbids it and 2) the usage of different pesticides in the agricultural activities which seem to be in relation to the reported high mortality rate of bee colonies in some regions in Bulgaria.

The rate and the reasons for honey bee colony losses were investigated among all regions of Bulgaria by the standardized international COLOSS questionnaire.

By the survey, data were summarized for about 50 apiaries in locations throughout the country. The most significant losses were reported in apiaries located in northern and central Bulgaria.

The following losses were detected among the total lost colonies due to objective reason: problems with the queen bee –14%, natural disaster – 1% and mortality in the hive or a sharp decrease in the number of bees with the family to several hundred – 85%.

The most significant losses were found for apiaries located near agricultural areas with sunflower, rapeseed, corn, orchards and autumn fodder crops. Oxalic acid, coumaphos and amitraz and less frequently – flumethrin, thymol and lactic acid were the most frequently applied against varroatosis among the beekeepers surveyed. Residual amounts of 27 pesticides were found in the areas with the highest mortality. Among the group of the pesticides detected were insecticides (including acaricides), fungicides, herbicides and growth regulators.

The presented and analyzed data should be considered when developing activities to protect the honey bee health status in Bulgaria.

## Acknowledgments

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# COLOUR-BASED TRACKING OF HONEY BEE POLLEN LOADS DIVERSITY

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## Abstract

Pollen plays a crucial role in the nutrition of honey bees, and the nutritional quality of the pollen pellets depends on the botanical origin of the pollen. Therefore, determining the diversity of pollen during the vegetation period provides insight into the botanical origin of the pollen and, indirectly, the nutritional health of the bee colony. Pollen colour is one way beekeepers can determine the botanical origin of pollen pellets. However, the colour of a pollen type may vary by region, depending on the species involved in the pollen type. Therefore, the colour charts of various regions may not be comparable and universally applicable.

We studied pollen colour and its variation in Estonia across three regions (Southern, Western and Central Estonia) where different bedrock (Devonian sand and limestone) and landscape (agricultural and semi-natural) provide habitats for different plant communities and, hence, different nutritional opportunities for honey bees. During the vegetation period of 2024, three independent pollen pellet samples from each of the three regions were collected using pollen traps. From May to August, pollen pellets were collected for one day every two weeks. The pellets were sorted by colour to determine colour diversity; each colour sample was analysed palynologically to determine the botanical origin of the pollen. The preliminary results will be presented and briefly discussed.

# EXPLORING BIVOUAC TRAPS: UNDER-UTILIZED SWARM-CATCHING DEVICES

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## Abstract

Despite beekeepers' best efforts, swarms happen, reducing desirable traits in one's queens, the honey crop, and harassing the neighbors. Most swarms could be caught in bivouac traps (aka Russian scions), but this practice is not widespread, and their effectiveness is unknown. The optimal design of bivouac traps has never been researched and their potential benefits remain unrealized.

A few examples of successful bivouac traps have been posted on the internet. These diverse bivouac traps have a variety of features whose details could be investigated to determine what would work best to attract swarms. Features mentioned include:
- Location: 10 meters in front of the hives, at the edges of the apiary, upwind of the hives, near or in the trees that swarms prefer, in the middle of the apiary
- Number of scions per apiary: one, two, 4 of more
- Height: 2 meters, 5 meters, higher.
- Odor: Old black comb, beeswax, propolis, Nasonov scent, QMP, lemongrass, catnip, pine, a combination.
- Scion: A long pine tree branch, a sturdy coniferous branch, a short pine tree, a frame of old black comb, a round rod, a square rod, a flat board, a charred board, a bundle of rags, commercial bag. (Boards and rods about 60 cm in length.)
- Scion protective cover: none, any scrap lumber, a 5-gallon bucket lid, a flat shape, an inverted V shape.
- Scion support: a rope hanging from a branch, a long pole, a tripod of long poles, a PVC pipe in the ground.
- Scion attachment: solid/rigid, loose
- Effective time period: until mid-June, the swarm season

The limited sample on the internet, without success rates, precludes hypotheses of optimal design, but the similarity of the structures provides a feature set amenable to systematic investigation, and the results would be broadly applicable.

# HOW TO DEFINE BEST BEEKEEPING PRACTICES (BBPs) USING A BOTTOM-UP AND MULTI-ACTOR APPROACH (MAA) IN THE EU

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## Abstract

The EU beekeeping sector needs practical solutions to achieve sustainable and resilient apiculture, including the reduction of colony losses. One of the keys to improving colony health is the proper application of the beekeeping practices, adapted to local conditions: to the geographical (e.g., climatic and environmental conditions) and beekeeping context (e.g., bee genetics, beekeeping equipment, and honey bee diseases). The Horizon Europe project “B-THENET”, is a Thematic Network based on a Multi-Actor Approach. It envisions to provide tailored Best Beekeeping Practices co-created and evaluated by the EU beekeepers to reach a more sustainable EU apiculture with a bottom-up approach. To identify and prioritize Best Beekeeping practices, we follow the B-THENET methodology: a survey for EU beekeepers, associated with an inventory of practices developed from previous EU projects and networks as well as from scientific publications.

During the first 18 months of implementation of the project, two EU-wide surveys were spread in 13 languages among beekeepers, in cooperation with the different project’s partners. The

practices prioritized by the beekeepers were then validated against the B-THENET socioeconomical criteria.

In the first round, practices were collected and rated in two themes: "Apiary set-up and management/maintenance" (Good Beekeeping Practice category) and "Varroosis" (Biosecurity Measures category), resulting in 2,094 answers from 22 Member States. The second round of collection of practices in the themes "Colony set-up and daily management/maintenance" and American Foulbrood", applying the same methodology, reached 22 countries and yielded 1,054 of replies to the survey.

# ITS2 DNA-METABARCODING AS A TOOL FOR THE BOTANICAL AUTHENTICATION OF HONEY: COMPARISON WITH PALYNOLOGY ANALYSIS USING MOCK MIXTURES

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## Abstract

Honey is appreciated globally for its nutritional and sensory attributes. However, it faces significant challenges due to widespread adulteration, with mislabeling of botanical origin being one of the most frequent frauds. Determining the botanical origin of honey traditionally relies on melissopalynology, a laborious method requiring expertise, and often providing only family-level identification. DNA-based pollen identification would allow a simpler and more accurate determination, and DNA-metabarcoding is emerging as one of the most promising approaches. However, the accuracy of the qualitative results as compared to melissopalynology and the reliability of using the number of sequences to estimate pollen percentages in honey still needs further evaluation.

Herein, 13 individual pollen samples representing different species were used to prepare mock mixtures for methods comparison. First, each pollen was analysed by microscopy to determine the number of pollen grains per milligram. Then, four pollen mock mixtures were created, two containing only 5 species and two 13 species. In each case, one of the mock mixtures was prepared with an equal mass of each pollen (corresponding to varying amounts of grains), and the other was prepared to contain a similar percentage of each pollen. Additionally, each of these mock mixtures was individually mixed with agave syrup (naturally pollen-free) to mimic the honey matrix. The pollen and agave mock mixtures were subjected to ITS2-metabarcoding and palynology analysis in a specialized laboratory.

The metabarcoding results align well between the pollen and agave mock mixtures, although certain species showed overrepresentation while others were underrepresented. In contrast,

palynology results closely matched the expected composition of the pollen mixtures, whereas significant discrepancies were observed in the agave samples.

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# MIGRATORY HABITS OF SLOVENIAN BEEKEEPERS AND THEIR ECONOMIC EFFICIENCY

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## Abstract

Beekeepers often migrate colonies to access forage on sources normally unobtainable at their locations. In the past, the most common way of hive migration was an either apiary in a fixed structure on a lorry or in the truck container, using traditional fixed-volume AŽ hives which are very suitable for building in. Increase of engine power in family cars allowed spread of small 10 – 12 hive trailer-apiaries which in turn started a migration boom. All the migrations must be recorded with the Veterinary authority; the records contain location of origin and destination, date of migration and number of colonies moved. We have analyzed available data for the past ten year.

There important forages in Slovenia, are acacia in early May, followed by linden and chestnut in June. Of the dew forages the most important is fir (*Abies alba*) and spruce (*Picea abies*. Following these forages, beekeepers register yearly between 2000 and 3000 hive migrations in which between 60.000 and 80.000 hives are moved. Using data about origin and destination of migration we were able evaluate the distance travelled using the Graphhopper OpenStreetMap container: 120.000 to 180.000 km. However, due to implementation of record keeping these data are underestimated. Likely number is at least 30 % higher.

We used survey among migratory beekeepers to collect additional information on migrations: how many forages they pursue per season and of which type. Additionally, we have collected information on the age and gas consumption of their traction vehicles and the quantity of hives per migration. On average, migratory beekeepers have 2 mobile units, allowing them to pursue more forages (2 on average, 4 as maximum). Most desired forage is acacia (23 %), followed by chestnut (22 %) and fir (20 %). Dynamics of migration differs between years due to the weather.

# QUEEN RINGING AS AN ALTERNATIVE METHOD FOR WINTER QUEEN BANKING

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## Abstract

Having available backup queens is of great importance for beekeeping operations, especially over winter or early spring when queen rearing is not yet taking place. Usually, queens are placed in the hive in individual small cages to protect them from attacks by other queens or workers but also to allow the bees to feed them. Due to the cages' fixed position that may lead to problems especially during winter, when caged queens might not be able to follow the in-hive movement of the winter cluster.

This study aimed to find a solution of keeping multiple fertile queens in one colony over the winter. To prevent queens from fighting and stinging each other, we used stiff plastic tubes to cover their abdomen, a technique known as queen ringing. This also allowed all ringed queens to move freely in the colony. The trial was part of larger international study on winter brood interruption by queen ringing.

In late September, we established colonies as queen banks (n=3). First, the original queens were caged using standard-transportation cages. After the brood was completely capped, we removed the original queens and introduced ringed queens (n=6) using same type of cages. In one colony, only one of six queens was accepted. In two of the colonies, all the ringed queens were accepted and cared for. On some of the queens, we observed wing damages. However, all ringed queens (n=12) in of both queen banks overwintered successfully and were used in February for requeening other queenless colonies, which lost their queens during the winter. All these queens were accepted and started immediately to lay eggs.

The experience and results gained during this experiment open up new possibilities for successful queen banking. Further studies with a higher number of colonies are planned in the framework of COLOSS Varroa-TF.

# SOCIOLOGICAL ANALYSIS OF BEEKEEPERS' PREFERENCES FOR COMMUNICATION AND DISSEMINATION OF BEST BEEKEEPING PRACTICES IN THE EU

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## Abstract

The European Union's beekeeping sector requires practical solutions to attain sustainable and resilient apiculture, encompassing the mitigation of colony decline. One of the keys to improving colony health is sharing the knowledge ready to be put into practice and fostering best practices exchanges. The Horizon Europe project "B-THENET", is a Thematic Network based on a bottom-up and multi-actor approach that supports both of those objectives. The project's ambition is to create a European knowledge-based network where beekeepers are directly and actively involved. The co-creation and co-description as well as the long-term storage and dissemination of Best Beekeeping Practices and ready-to-use research and innovation solutions are built on a cascade of four interlinked BTHENET platforms which engages all relevant actors from beekeepers, advisors in beekeeping to various stakeholders.

To understand and scope beekeepers' preferences for the channels, means and type of communication and dissemination of the Best Beekeeping Practices to maximize their penetration into the everyday, local EU beekeeping practice, B-THENET developed a targeted sociological study. Within the study, two surveys were distributed through the EU Survey System in 15 languages in 2023. We received a total of 1,366 answers originating from 22 EU countries. The analysis of survey responses revealed that visual materials are the most favored means of communication by the EU beekeepers: live-action videos of 5-10 minutes in length, in the English language with subtitles in the local language, presented by fellow beekeepers or advisors, and hosted on YouTube. However, the study also underlines the importance of translating the Best Beekeeping Practices and related materials into local languages.

Our results highlight the significance of adopting a multifaceted approach, integrating both visual and linguistic considerations, to effectively disseminate best beekeeping practices across the diverse landscape of the EU beekeeping sector.

# SUSTAINABLE BEEKEEPING: OVERVIEW OF CONCEPTS REGARDING HONEY BEE MANAGEMENT

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## Abstract

The poster presents a review of concepts about the terminology concerning honeybee management, developed with several activities of EU research projects (BPRACTICES, B-THENET), explaining their meaning and showing their interrelationships.

Modern beekeeping is strongly linked with sustainable. According to FAO, sustainable beekeeping is the farming sector that produces or uses natural resources in a manner that results in no net decline or negative impact on those resources over time. It should, therefore, meet the needs of present and future generations, while ensuring profitability, environmental health, and social and economic equity.

Sustainable apiculture requires good knowledge on the proper management of bees to optimize the natural systems and resources beekeepers rely on (FAO - Good beekeeping practices for sustainable apiculture) reducing the use of veterinary medicines and impact on environment.

“Good beekeeping practices” (GBPs) are those integrative activities that beekeepers apply to obtain optimal health for humans, honeybees and the environment.

“Biosecurity measures in beekeeping” (BMBs) are all the operational activities to reduce the risk of introduction and spread of specific honeybee disease agents.

“Honey bee welfare practices” are recommendations adapted to supporting bees amid stressor challenges, prioritizing bee health and natural behaviours as the best ability to achieve coping. These practices ensure colony resilience while promoting ethical and sustainable beekeeping.

“Best beekeeping practices” (BBPs) are “those practices (as GBPs and BMBs) that have been adapted to a specific geographical context (e.g., different climatic and environmental conditions) and a specific beekeeping context (e.g., different bee genetics, beekeeping equipment, and diseases)”. They represent the state of the art of beekeeping for a given area.

# TRUSTABLE AND SUSTAINABLE OPEN PLATFORM FOR SMART HONEY VALUE CHAINS

Aleksejs Zacepins[1], Mehmet Aydin[2], Ewa Ziemba[3], Maria Alejandra Palacio[4], Sahin Aydin[5], Vitalijs Komasilovs[1], Ewa Maruszewska[3], Enrique Bedascarrasbure[4], Olvija Komasilova[1], Samet Okuyan[6], Anna Karmańska[3], Graciela Rodriguez[4]

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## Abstract

The honey business as an agri-food sector faces sustainability challenges to cope with complex relations from farm to fork, climate change, increasing competitive pressures, varying consumer diets, and food safety. The TOP4HoneyChains is a collaborative project funded within the ICT-Agri 2022 call. Within the project challenge to generate a smart value chain, driven by market preferences and consumer demands, based on the quality that has been preserved from the apiary, with records and transparency throughout the entire honey-food business including the apiary and honey processing, building trust between buyers and sellers, will be tackled.

TOP4HoneyChains project presents an innovative approach to address the challenges faced by the honey industry regarding transparency, trust, and sustainability. The primary concern of TOP4HoneyChains is to increase effectiveness and efficiency of traceability and transparency for achieving smart honey value chains as a whole honey-food system supported by a trustable open data platform along data-driven innovative digital services. Additional services will provide the users with feed-forward (from the apiary) as well as feed-back mechanisms along well-documented and verifiable information regarding the apiary, the origin and production practices, quality metrics, and “the story of honey” as higher value-end products. Additional data-driven intelligent services will provide multi-actors of the chains with the insights and trends regarding quality requirements, critical points-of-interests during the production process, supply dynamics, consumer perception and willingness to pay in relation to the degree of transparency along accessibility of the data.

Consumers benefit from access to detailed information about the honey they purchase, including its origin, production methods, and sustainability credentials, empowering them to make informed choices and support ethical beekeeping practices.

More information about the project can be found on the website: https://top4honeychains.eu/

# 10 Bee diversity, conservation and interactions among species

## Oral presentations

Moderator:
Helena Wirta

# *BOMBUS TERRESTRIS TERRESTRIS* AS A THREAT FOR THE PROTECTION OF *BOMBUS XANTHOPUS* IN THE TUSCAN ARCHIPELAGO (ITALY)

Chiara Benedetta Boni[1], Francesca Coppola[1], Marino Quaranta[2], Francesca Giannini[3], Antonio Felicioli[1]

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## Abstract

*Bombus terrestris* is a ubiquitous Palearctic species occurring in Italy with four morphological subspecies: *B. t. terrestris* and *B. t. dalmatinus* in North-Central Italy, *B. t. calabricus* in South Italy and *B. t. sassaricus* in Sardinia. *Bombus xanthopus*, present in Corsica (France), Capraia and Elba Islands (Tuscan Archipelago, Italy), and considered as a subspecies of *B. terrestris* until 2015, has been elevated to the status of endemic Corsican species due to its molecular and eco-chemical features.

Since the end of the 1970s, the presence of *B. t. terrestris* has been ascertained on all Tuscan Archipelago islands except Capraia. On Capraia Island, the last sighting of *B. t. terrestris* dates back to 1917. Since then, only *B. xanthopus* and *B. pascuorum melleofacies* were detected.

In 2021 *B. t. terrestris* was again detected on the island raising two main hypotheses: (i) *B. t. terrestris* has always been present with a low population density, such as not to be detected in previous investigations, or (ii) its presence is the result of a more recent recolonization. The recolonization event may be promoted by either intentional or unintentional introduction or it may be the result of a natural migration from the mainland or surrounding islands. Hybridization between *B. t. terrestris* × *B. xanthopus* on Capraia Island has been also ascertained. These new finding provides insight on the distribution range of B. t. terrestris in the Tuscan Archipelago and raise concern on the conservation of the endemic *B. xanthopus* population.

# TROPHIC REPARTITION OF THE POLLEN SOURCE BETWEEN MANAGED HONEY BEES AND UNMANAGED BEES

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## Abstract

Wild bees population is in severe decline worldwide although its key roles in maintenance of ecosystems. Managed honey bees density and beekeeping are usually considered to have a negative impact on wild bees populations. Trophic competition between managed honeybees and wild bees is believed to be one of the main factors affecting different aspects of the wild bees biology. In this study trophic competition was investigated trough the still little exploited approach of the palynological analysis combined to metabarcoding of pollen gathered by both managed honey bees and wild bees in three Italian National Parks. Sampling was performed twice a year in summer (June-July) and autumn (September-October) 2021. Taxonomic recognition of captured wild bees carrying pollen was also performed. Pollen gathered by honey bees was collected directly from pollen traps previously set up in the apiaries located near wild bees sampling sites. The overall trophic network resulted highly specialised ($H_2$'=0.933) with a high trophic partitioning between each wild bee species and honey bees suggesting that wild bee species are usually sustained by different pollen pastures from those of honey bees. A high degree of pollen source sharing between honey bees and wild bees was only recorded in summer in Maiella National Park on *Rubus* f. which resulted the most widely representative flowering in the sampling site. In the other Park low or absence of pollen source sharing were recorded. The low sharing of pollen resources could be due to the natural trend occurring in natural populations, where species tend to minimize competitive overlap as a result of a co-evolution between wild bees and managed honey bees. These results despite preliminary and not exhaustive, provide a useful knowledge base for further investigation to better understand phenomena that led to the presence of this strongly pollen partition between wild and managed bees.

# MORPHOLOGICAL ADAPTATION IN A SOLITARY BEE (*ANDRENA VAGA*) - ENDOPARASITE (*STYLOPS ATER*) SYSTEM

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## Abstract

The grey-backed mining bee *Andrena vaga* is one of the hosts for the Strepsipteran endoparasite *Stylops ater*, which is known for extreme sexual dimorphism and hypermetamorphosis. For most regions in Europe, were both are occurring, the population structure, genetic diversity and morphological adaptations are unknown. Here, we sampled hosts and parasites of both sexes at several nest aggregations in and around Braunschweig (Germany) to study their biology and distribution. Genetic analysis revealed the absence of local variation within *Stylops*. Host bees emerged earlier than non-parasitized bees. Individual male and female bees hosted up to four parasites in their bodies. A trend was detected in the *Stylops*' preference for hosts of their own sex and the position of extrusion from the host abdomen. Morphological adaptations upon parasitation include: ovary reduction and reduced head width for bees infested by male *Stylops*, host masculinization (indicated by the shape of the metabasitarsus), and intensified tergal hairiness (most strongly near the point of parasite extrusion). Parasites responded to the hosts' sex and multiple infestation with reduced size of cephalothoraxes. Future studies are needed to understand the mechanisms behind parasite-induced host manipulation.

# WILD BEE MONITORING IN AGRICULTURAL LANDSCAPES – A CONCEPTUAL APPROACH INCORPORATING DNA ANALYSES IN BIODIVERSITY MONITORING

Wiebke Sickel[1], Swantje Grabener[1], Niels Hellwig[2,1], Toni Kasiske[1], Lasse Krüger[1], Leonie Lakemann[1], Lara Lindermann[1], Sophie Ogan[3,1], Frank Sommerlandt[1], Johanna Stahl[1], Petra Dieker[4,1]

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## Abstract

Wild bees are vital pollinators essential for functioning agro-ecosystems. Yet, they have faced strong population declines caused mainly by land-use change and climate change. The increased awareness for the importance of wild bees among policy-makers and the general public, together with a lack of robust data on wild bee populations has led to the development and partial implementation of a nation-wide wild bee monitoring in agricultural landscapes in Germany. This monitoring scheme is, amongst others, based on non-lethal species detection via citizen science.

This approach is combined with DNA analyses in order to obtain more comprehensive data on wild bee communities in agricultural landscapes. For example, *COI* (meta-)barcoding is used to achieve species-level identification for cavity-nesting wild bees and bumblebees. Additionally, pollen metabarcoding allows reconstructing plant-pollinator interactions in the surrounding agricultural landscapes. Last but not least, (phylo-)genetic diversity is assessed using different molecular biological methods and indicators.

Our approach incorporates DNA-based analyses into the overall monitoring scheme and thereby achieves an ecosystem-wide understanding of wild bee communities that reaches far beyond species inventories. Therefore, our aim is to inspire researchers and policy-makers to integrate genetic methods into other long-term monitoring programmes.

# WHAT MAKES THE ELTONIAN NICHE OF HONEYBEES CHANGE?

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## Abstract

The Eltonian niche of a species consists of its set of interactions with other taxa. How these interactions vary due to biotic, abiotic and human-induced factors is a core question of modern ecology. In seasonal environments, the realized Eltonian niche would vary according to changes in time in the occurrence and abundance of interaction partners and the species own choices. Further we can expect management to have a large impact on the interactions of species. As we wanted to compare the effect of the time of the season with that of management, honeybees provide an excellent species to study.

As traces of DNA from the taxa honeybees have interacted with store in honey, we can assess the interactions of honeybees with flowers and microbes in the surrounding habitats, their hives, and themselves by using honey as a sample. We sampled beehives from different regions and managed by different beekeepers repeatedly during a summer in Finland. We used shotgun sequencing to identify the taxonomic origin of the DNA in the honey samples. We used joint species distribution modeling to assess the impacts of the time of the season in comparison to the effect of location, management, and the colony itself in determining the interactions of honeybees.

The honeybee interactions with other taxa varied largely among taxonomic and functional groups. In comparison to the effects of the colony, management and location, the impact of the time was relatively small on the interactions. Yet using DNA in honey offers unique insights to the variation in the identity and abundance of interaction partners among honeybees. During the summer, the interactions with different taxa changed tremendously, but overall the manager and colony identity appear as important as the time of the season in determining honeybee's realized Eltonian niche.

# URBAN ORNAMENTALS, PARKS, AND GARDENS: EXPLORING THE INTRICATE INTERPLAY BETWEEN BEE POLLINATORS AND THE URBAN ENVIRONMENT

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## Abstract

Urbanization has reshaped landscapes, isolating peculiar green spaces arising from private gardens or parks, which serve as crucial habitats for Hymenoptera in the cities. These new environments may allow the colonization of different pollinators, influenced by the resource availability (plant composition and period of blooming) nesting ecology and movement capabilities. Moreover, urbanization has altered the dynamics of floral-bee interactions that have evolved over millennia. The introduction of exotic plants, not co-evolved with local pollinators and that of cultivated varieties, often selected for aesthetic appeal but bearing altered colours and forms, forced the establishment of new interactions. The forced occupation of relatively small niches by different pollinators can potentially influence disease transmission among them. In this context, our research aim is to shed light on the intricate network of interactions in a large city in Northern Italy, a potential model for the study of inter and intraspecific interactions that involve bees, plants and disease in urbanized environments, with an emphasis on the role of urban green spaces in sustaining bee biodiversity. Monitoring was carried out in 90 study sites selected over 3 years (2019–2021), which represent various "green categories". On-site monitoring included records and collection of bee individuals. A sample of specimens of 6 out of the most common pollinator species, collected in 6 parks were tested for the presence of honey bee viruses in 2020 and 2021. The presence of various bee viruses was detected in both social and solitary bees at different monitoring times and locations. By analysing bee-plant interactions, adaptability of bee species to different plant species, the impact of exotic species on pollinator presence, and how different species can harbour disease we aim to shed light on the importance of urbanized environments as biodiversity hotspots and their potential contributions to pollinator conservation efforts.

# SPATIO-TEMPORAL POPULATION GENETICS OF SOLITARY BEES TO ASSESS THE IMPACT OF MANAGEMENT

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## Abstract

Over the past decades, a growing number of bee species have been managed to compensate for pollinator declines. The introduction of such managed bees may result in hybridization with wild conspecifics thereby disrupting local adaptations. In Europe, solitary bees *Osmia cornuta* and *Osmia bicornis* are reared *en masse* and transported over large distances. Despite the ubiquity of these species, the consequences of their management on local wild populations have not been investigated yet. Here, we assess for *O. cornuta* and *O. bicornis* whether management can affect the genetic structure of local wild populations through spatio-temporal analyses. Samples were collected using trap nests in urban, rural, and semi-natural areas of Switzerland thereby reflecting a gradient of *Osmia* spp. management. Samples from two commercial breeders active in the study areas were also analyzed. In addition, museum specimens before the beginning of *Osmia* spp. management (i.e., early 90s) were studied. 11 microsatellite markers were used to compare genetic diversity and population structure of both species across space and time. The analysis of contemporary samples revealed strong gene flow between areas and managed populations for both species, suggesting a considerable admixture possibility due to management. The ongoing analysis of museum specimens will allow assessing the situation before management began and will tell whether management is indeed a main driver of genetic population structure of these bee species. The data will be presented and discussed.

# THE ISLES OF SCILLY – A MICROCOSM FOR EUROPEAN HONEY BEE CONSERVATION

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## Abstract

The Scilly Isles are a small group of five inhabited and many smaller islands off the south west tip of Cornwall, UK, beyond the reach of flying bees or swarms except by human transportation. They have a mild climate with few if any frosts, but winds are harsh and there is little natural tree cover. Honey bees may have been kept there since medieval times. Today a small number of managed colonies are kept on the five main islands, and it is believed that the islands remain free from varroa and foulbrood. It is known that there are also a significant number of wild colonies. In recent years a small number of exotic queens have been brought to the islands, but for biosecurity reasons the beekeepers now wish to maintain a sustainable local bee population without further imports or an increase in total hive numbers. Genetic analysis shows that two islands have near native bees, one shows signs of recent imports, and the others have mixed populations. Experiments involving mark – recapture of drones at drone congregation areas have so far not demonstrated that drones can travel between the islands, although swarms are known to do so. A strategy for long-term sustainable beekeeping on the islands is now being developed with the involvement of all stakeholders. Many of the considerations that are important to the Scilly bee population mirror those of larger island honey bee populations elsewhere, and are therefore applicable to honey bee conservation in general.

# THE USE OF CROPS AS HABITAT PLANTS SUPPORT WILD POLLINATORS IN MOROCCAN FARMLANDS

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## Abstract

Global food security is heavily reliant on crop pollination. However, evidence on pollinator decline has been reported in all continents. Globally affordable conservation strategies need to be developed, as high-cost measures like European agri-environment schemes are not scalable in all countries. Here, we test, if a low-cost conservation approach named “Farming with Alternative Pollinators” (FAP) can benefit wild pollinator abundance and richness in agro-ecosystems and in crops, through establishment of marketable habitat enhancement plants (MHEP). The study was carried out in four Moroccan agro-climatic regions, during two years, using 6 main crops (pumpkin, zucchini, faba bean, tomato, eggplant and apple) and 201 sites.. Based on 7097 recorded specimens, our results show that the wild pollinators of the entire FAP fields (i.e. 75 % main crop and 25 % MHEP) were significantly more abundant and species-rich than those of control fields (i.e. 100 % main crop). Considering the main crop wild pollinators, FAP did not display any significant effect either on wild pollinator abundance or on pollinator richness. Our study provides strong evidence that FAP constitutes a relevant method for wild pollinator conservation in agro-ecosystems.

# APPLYING GENETICS TO SUPPORT CONSERVATION ASSESSMENTS OF MOROCCAN BIODIVERSITY: THE SAHARAN HONEYBEE (*APIS MELLIFERA SAHARIENSIS*) AS A CASE STUDY

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## Abstract

In Morocco, there are two well-recognised honey bee (*Apis mellifera* L) subspecies: *A. m. intermissa* (the Tellian honey bee) in the north and *A. m. sahariensis* (the Saharan honey bee) in the south-east. Honey bee diversity of the latter subspecies is under threat due to anthropogenic factors such as transhumance and the introduction of allochthonous honey bee subspecies to recover from colony losses. To assess the genetic diversity and population structure of the Saharan honey bee, we used a set of 12 microsatellite loci and analyzed 148 colonies which were clustered into seven populations representing the expected distribution of *A. m. intermissa* and *A. m. sahariensis*, and reference samples from two European subspecies, *A. m. carnica* and *A. m. mellifera*. The average number of alleles per locus in the sampled populations ranged from 2.417 (*A. m.* carnica) to 10 (East Sahara). Per-locus expected heterozygosity (He) fluctuated between 0.820 ± 0.028 in the High Atlas population and 0.404 ± 0.072 in *A. m. carnica*. Bayesian clustering analyses as revealed by structure analysis suggests two distinct clusters in Morocco separated by the High Atlas Mountains (FST = 0.05). Even though high levels of admixture in honey bees with *A. m. intermissa* jeopardize the genetic integrity of the Saharan honey bee from the Saharan region, no evidence of introgression was detected from the European reference subspecies. Results of this study reveals that beekeeping practices have strongly influenced the genetic structure and diversity of honey bees from southeastern Morocco, and that introductions of non-native subspecies represent a serious threat to the genetic integrity of native honey bee populations.

# SPREAD OF *VESPA VELUTINA* IN SPAIN, SUCCESSFUL CONTROL ON THE BALEARIC ISLANDS

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## Abstract

The yellow-legged hornet, *Vespa velutina nigrithorax*, originally from Asia, has become a problematic invasive species in Europe due to accidental introduction. In Spain, it has established populations in northern regions such as Navarra, the Basque Country, Galicia, Cantabria, and Catalonia. Recently, it has also been detected in Extremadura, La Rioja, Andalucía, and Valencia. Our study focuses on the first field investigation of eradication strategies conducted in the Balearic Islands. This case serves as a model for meticulously planning and executing eradication programs for the yellow-legged hornet in newly invaded regions.

We outline the step-by-step process from the initial detection of the species to the formation of a multidisciplinary team and the implementation of field and laboratory methodologies. Specifically, we examined various methods including spring trapping of queens, using traps to detect adult presence, utilizing citizen science data, employing triangulation methods to locate nests by tracking adult movements from artificial feeding points, and employing mechanical nest destruction techniques.

The Spanish Ministry of Environment and Energy for Ecological Transition, as the governing body responsible for managing invasive alien species, declared the eradication of *V. velutina* on the island in 2020. However, our team continued awareness efforts, recognizing the potential for reinfestation if not vigilantly monitored. Indeed, in 2021, a new nest was discovered in Mallorca. Genetic analysis indicates two distinct introductions of this invasive hornet to Mallorca at different times and from different European regions: Italy in 2015 and mainland Spain in 2021. These findings underscore the importance of ongoing surveillance and proactive management strategies to mitigate the impacts of invasive species.

# A METABARCODING TOOL TO ESTIMATE PREY SPECTRUM OF *VESPA VELUTINA* (HYMENOPTERA: VESPIDAE) ON THE LOCAL ENTOMOFAUNA

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## Abstract

The invasive yellow-legged hornet, *Vespa velutina*, is a social Hymenoptera native from Asia and an invasive species in Europe, where it was first detected in France in 2004. This species poses a significant threat to local ecosystems and economies in Europe due to preys on a wide variety of insects, mainly honeybees. Although this species was detected on the European continent approximately 20 years ago, information available on its trophic spectrum is quite limited. This study aimed to decipher the prey spectrum of *V. velutina* of the local entomofauna using metabarcoding tools. We collected meconium samples from yellow-legged hornet nests removed in 2017 in Mallorca (Balearic Islands, Spain) and amplified mini barcodes using an arthropod-specific primer set. Metabarcoding analysis revealed a fraction of the diet its nest exclusive, highlighting preference for Apidae, Calliphoridae, Vespidae, Muscidae, and Sarcophagidae insect preys. Moreover, the study identified potential impacts of *V. velutina* on local insect populations with diverse functional roles, shedding light on the ecological implications of this invasive species. By utilizing advanced molecular techniques, we were able to assess the dietary diversity of *V. velutina* and its potential role in shaping local entomofaunal communities. This research contributes to our understanding of predator-prey interactions in invaded ecosystems and underscores the value of metabarcoding tools in studying the foraging behaviour of invasive species.

# PATHOGEN PRESENCE AND PREVALENCE IN OCEANIC ISLANDS: IMPLICATIONS FOR BEE CONSERVATION IN THE CANARY ISLANDS

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## Abstract

Oceanic islands such as the Canary archipelago, stand out as crucial global hotspots of endemism, hosting numerous unique species of both animals and plants, especially vulnerable to anthropogenic impacts, due their small territory and isolation. They serve as simplified ecosystems, offering natural replicas that make them exceptional natural laboratories. They, therefore, represent a great opportunity to study the complex interaction between the main drivers of bee decline, such as pathogen spillover and land-use change.

The aim of this study was to analyse the pathogen abundance and prevalence of three microsporidia species (*Variomorpha bombi, Variomorpha ceranae* and *Variomorpha apis*) and three species of trypanosomatids (*Crithidia mellificae, Crithidia bombi,* and *Lotmaria passim*). More than a thousand specimens of 10 species of wild bee species and the western honey bee (*Apis mellifera)* from six different localities in Tenerife and Gran Canaria, including coastal and high mountain habitats were analysed. Our data reveal a low prevalence of microsporidia in honey bees, except in high-mountain habitats, while they are almost absent in wild bees in both habitats. However, a higher trypanosomatid prevalence was observed in both managed and wild bees. Preliminary analysis suggests that there is a higher pathogen abundance in both wild and honey bees in areas with higher density of honey bees. Furthermore, pathogen abundance is negatively correlated with floral resources abundance in both bees guilds.

These results underline the importance of appropriate management and conservation practices of island ecosystems to prevent the spread of pathogens in both wild and honey bees populations.

Keywords: wild bees; honey bees; pathogens; islands

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# INTERACTIONS BETWEEN GLYPHOSATE USE AND ENVIRONMENTAL FACTORS ON THE PREVALENCE OF PATHOGENS IN MANAGED AND WILD BEES

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## Abstract

An alarming pollinator decline is currently occurring worldwide, threatening the reproduction of entomophilous plant species in both wild and agricultural environments. The causes of this decline are multifactorial, with the effects of pesticides and the transmission of pathogens playing an important role. This is especially relevant for pollinators active in agrifood crops, as they are exposed to a greater amount of pesticides than other pollinators and may be more susceptible to contact with pathogens. Therefore, it is crucial to understand how exposure to these substances affects pathogen dynamics to ensure the conservation of both managed and wild bees.

Our hypothesis is that the prevalence of pathogens in wild bees increases with the presence of managed bees and that environmental factors act as aggravators (glyphosate) or mitigators (floral resources) of this impact on both managed and wild bees.

To verify this, samples of different managed and wild bee species were collected by trapping at different times and taxonomically identified by barcoding. The presence of common pathogens in managed species (microsporidia, trypanosomatids and ascomycetes) was detected by PCR amplification of specific regions. Only trypanosomatids showed differences in prevalence between bee species and between study periods, showing higher prevalence in honey bees at the end of the flowering period. Analysis of the interaction between infection and glyphosate application in the crop showed that the risk of pathogen infection does not seem to be influenced by the use of glyphosate. However, other factors related to the environment of these plots may influence trypanosomatid dynamics. These results show that there are different variables involved in the transmission of pathogens in agricultural areas that should be taken into account to establish actions to mitigate these factors.

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# DRIVERS OF WILD BEE AND HOVERFLY COMMUNITIES IN URBAN CONTEXTS

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## Abstract

Insects play a vital role in pollinating a wide range of wild flora and food crops worldwide, thereby supporting many ecosystem services. Despite their importance, the ongoing decline in pollinator populations jeopardises the continuity of these services in both managed and natural contexts. Urbanisation is a catalyst for land cover transformations, leading to adverse effects on biodiversity and ecosystem integrity through the conversion of natural habitats into landscapes dominated by built structures and impervious ground cover. As such, it is imperative to determine the variables that drive pollinator communities in urban contexts to adopt adequate and sustainable conservation strategies that safeguard ecosystems throughout future city development. This study surveyed wild bees (Hymenoptera: Apoidea) and hoverflies (Diptera: Syrphidae) in 15 locations, encompassing 8 urban parks and 7 urban agricultural sites, in Bologna, Italy. Insects were sampled by transect plots combined with hand netting, to analyse plant-pollinator interactions, as well as pan traps to obtain a broader scope of the pollinator diversity in each site. This research aimed at performing a city-wide monitoring scheme to determine the richness of pollinators and catalogue their functional role in urban green spaces. It also aims to assess the relative importance of local-scale (such as plant richness, bloom cover and the type of urban green space) and landscape factors (surrounding land cover) in influencing the composition of pollinator communities within this urban environment. The findings of this study will provide valuable knowledge for green area management strategies focused on establishing conducive habitats and refuges for local pollinator populations.

# HONEYBEES' EFFECTS ON SPECIES DIVERSITY AND PLANT POLLINATOR INTERACTIONS: INSIGHT FOR IMPLEMENTING SUSTAINABLE BEEKEEPING PRACTICES

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## Abstract

Beekeeping is frequently criticized for the potential that managed honeybee' high abundance and foraging behavior can prevent wild pollinator species from foraging on the plant hosts they need to acquire adequate resources. While changes in wild pollinator community composition (i.e., species turnover and loss) can constitute clear evidence of honeybees' negative impacts on wild bee diversity, disruptions of plant-pollinator interaction networks (i.e., "rewiring") are also a possible outcome. The potential for honeybees to cause either wild pollinator species turnover or interaction rewiring will generally depend on levels of local resource availability and degree of wild pollinator generalization/ specialization. We present results from a study in Southern Norway where honeybee hives are normally absent. Beekeeping is restricted within 20 km of the Norwegian Beekeeping Association's facility in Nesbyen as a measure to protect the genetic lineage of the queens reared there. This area thus provides a unique opportunity for a manipulative experiment to test hypotheses for how variation in the honeybee density can affect native bee species assemblies along gradients of both floral resources and nesting site availability. We used both netting and pan traps at 40 sites to measure the area's pollinator abundance and diversity, and document plant-pollinator species interactions. Data from 2022 describe baseline conditions, and data from 2023 describe effects of introducing beehives at varying density within the study area. We present results of analyses exploring how spatial and temporal species turnover and plant-pollinator interaction rewiring reflect the conditions under which competition from honeybees has the greatest potential to disrupt pollination wild pollinator communities. We will also describe how findings are used within a model for prescribing appropriate honeybee colony stocking densities, accounting for local floral resource availability and nesting sites, to avoid negative effects from competition with local wild pollinators.

# 10 Bee diversity, conservation and interactions among species

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## Poster presentations

## AUSTRIAN WILD BEE INVENTORY: EXPLORING NATIONAL DIVERSITY

Sabine Schoder[1], Johannes Pfrommer[1], Manuel Ankel[2], Katharina Thierolf[3], Johann Neumayer[3], Gernot Neuwirth[3], Stefan Dötterl[2], Bärbel Pachinger[1]

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### Abstract

Various studies have shown a significant decline of wild bees in recent decades. Austria is the home of about 700 species of wild bees. However, little is known about the trends in populations and the endangerment status of the species, making it difficult to develop effective protection measures for wild bees. Therefore, the aim of our project is to establish a monitoring system with systematically collected data on the distribution of wild bee species throughout Austria.

The main focus is the documentation of the status quo of wild bees in 205 selected sites in the Austrian cultural and natural landscapes (farmland, grassland, protected areas) in the period from October 2022 to April 2025. Obtained data will be linked to biodiversity monitoring data of other organisms (e.g., plants, butterflies, grasshoppers), which are collected by the Environment Agency Austria. The method used will be explained and discussed in the light of European pollinator monitorings. First project results will be presented, such as the distribution of selected rare species in the context of land use.

As a further pillar of monitoring, bumblebee monitoring is carried out by Citizen Scientists. In the first year of the surveys (2023), 35 participants collected data at 38 transects across Austria once per month. More than 550 individuals and 18 species were found. In 2024 46 participants generate data at 59 transects.

# COMPOSITION OF BEE SPECIES IN JULY-FLOWERING FIELD CROPS IN LATVIA

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## Abstract

In Latvia, since 2019, bees (Hymenoptera: Anthophila) have been studied in various agrocenoses to clarify the composition and diversity of species. This paper summarizes the research results obtained in 2020–2022 in the various July-flowering field crops: spring rapeseed (*Brassica napus*), buckwheat (*Fagopyrum esculentum*), white melilot (*Melilotus albus*), white clover (*Trifolium repens*) and red clover (*Trifolium pratense*). The studies used two methods - tricolored (yellow, white, blue) pan traps and Malaise traps - which were set and held in field crops for six sunny days around mid-July each year. The captured bees were removed from the traps in the evening of each day. A total of 111 species of bees were found in July-flowering crops: 20 Andrenidae, 32 Apidae, nine Colletidae, 29 Halictidae, 16 Megachilidae and five Melittidae species. The European honey bee (*Apis mellifera*) had the highest proportion (53.43%) in the species composition. Also, 13 wild species were relatively common: *Andrena bicolor*, *A. dorsata*, *Bombus hortorum*, *B. lapidarius*, *B. lucorum*, *B. soroeensis*, *B. terrestris*, *B. veteranus*, *Halictus tumulorum*, *Lasioglossum calceatum*, *L. morio*, *L. pauxillum* and *Dasypoda hirtipes*. The proportion of their individuals in the species composition was within 1–8%. Species diversity rates varied daily within each studied field and among different fields each year. For example, in one buckwheat field in 2022, species richness (R) fluctuated between 5 and 20 species (median 12.5), but the effective number of species ($^1D$) – between 2.76 and 14.87 (median 6.83) in six days. Among the different fields, in 2020, the median values of R were between one and eight, and the $^1D$ values – between 1.00 and 3.78. In 2021, median values of these parameters varied between 9 and 18 (R) and between 3.47 and 8.74 ($^1D$), respectively, while in 2022, they were 4.0…16.5 (R) and 2.48…6.83 ($^1D$).

## eDNA DETECTION TOOLS FOR MONITORING *VESPA VELUTINA*

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### Abstract

The yellow-legged hornet (*Vespa velutina*) has recently emerged as a novel threat to pollinators in North America. The invasive hornet has been shown to disrupt plant-pollinator networks in regions outside its native range and exhibits a distinct predation preference for the western honey bee (*Apis mellifera*), thus threatening honey bee colony health. Here, we investigated the efficacy of environmental DNA (eDNA) approaches to monitor the recent invasion of the yellow-legged hornet in Savannah, Georgia, USA. We swabbed various apiary substrates (including hive entrances, hive tools, hornet bait traps) and collected soil samples beneath honey bee colonies in apiaries where the invasive hornets were confirmed present. We were able to detect yellow-legged hornet DNA from soil samples, validating our eDNA methodology as an effective way to monitor the continued invasion of the hornet. Swabs of hive substrates provided less conclusive results and require additional work to determine which substrates reliably yield hornet DNA. Our results demonstrate the potential utility of an eDNA monitoring tool for identifying areas where the hornet has established, allowing one to allocate resources towards nest discovery and destruction efficiently.

# EXPLORING SPECIES DIVERSITY OF WILD BEES ON THE CULUCCIA PENINSULA (NW SARDINIA, ITALY) THROUGH AN INTEGRATED TAXONOMIC APPROACH

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## Abstract

The Mediterranean basin supports some of the most diverse pollinator communities in the world and is one of the most species-rich areas for bees. The limited knowledge of wild bees in Mediterranean areas represents a significant constraint to their conservation. Particularly in Sardinia, the second largest island in the Mediterranean, information of the bee fauna is still limited. For this reason, we compiled a first checklist of Apoidea Anthophila of the Culuccia Peninsula, approximately 3 km$^2$ situated in north-western Sardinia (Italy) near the Maddalena Archipelago.

The specimens were identified through an integrative taxonomy approach, combining morphological recognition and the use of DNA barcoding. Bees were sampled from April to October 2022-2023 along seven fixed transects of 400 x 2.5 m. In addition, two Malaise traps were installed from May to October 2022. Total genomic DNA was extracted from one leg of 286 specimens to amplify a fragment of the mitochondrial COI gene. COI sequences were compared with those deposited in BOLD (BLAST) and Neighbor-Joining (NJ) phylogenetic trees were built only for those species whose identification is in doubt.

In total, 1071 individuals of wild bees belonging to 73 different species were collected. Of these, 208 COI sequences from 58 different species were obtained. Eight species are reported for the first time in Sardinia.

The phylogenetic tree analysis revealed that *Bombus terrestris sassaricus* and *B. xanthopus* collected in Culuccia do not distinguish themselves at the molecular level. Similarly, for as do the sequences deposited in BOLD of *Andrena afzeliella* and *A. ovatula* , although previous phylogenetic analyses recognise these as distinct species. This work represents the first study on the diversity of Apoidea Anthophila on the Culuccia Peninsula and contributes to enriching the current limited knowledge on wild bees in Sardinia.

# GEOGRAPHIC VARIATION WITHIN LINEAGE M OF HONEY BEES

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## Abstract

Lineage M of honey bees (*Apis mellifera*) naturally occurred in western and northern Europe. Within its range, there are two recognised subspecies: *A. m. mellifera* and *A. m. iberiensis*. Both of these subspecies are endangered by the mass importation of non-native queens by beekeepers. To protect the native bees, they need to be reliably identified. The identification can be based on both molecular and morphological markers. Morphometric identification of honey bees is faster, cheaper, and does not require sophisticated equipment; therefore, it is available to both scientists and beekeepers. The main limitation of this method is the lack of reference samples, which can be used for comparisons between subspecies or lineages.

We have collected more than 13 thousands wing images of honey bee workers. They represented 750 colonies or locations in the natural range of *A. m. mellifera,* including Colonsay Island, Norway, Poland, and Belarus. Additionally, we have used publicly available data related to the Iberian Peninsula, Cuba, and south-eastern Europe for comparison. On the wing images, 19 landmarks were indicated, which were analysed using geometric morphometrics.

We found significant differences between the countries. As expected, honey bees from the Iberian Peninsula differed markedly from bees from north-eastern Europe. There was also a clear difference between lineages M and C. The obtained data can be used as reference samples for reliable morphometric discrimination between lineages and countries. Moreover, it can assist in prioritising conservation efforts by identifying populations distinguished by low levels of introgression with non-native genes.

# INVESTIGATING SURVIVAL AND ADAPTATION OF WILD HONEYBEES IN FOREST HABITATS IN THE UK

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## Abstract

Until recently it was considered that honeybees did not live in the wild in the UK and the rest of Europe. However, recent observations have documented free-living *Apis mellifera* colonies thriving in some protected forest areas of Britain. These colonies have been gaining significant importance given their apparent ability to survive without chemicals and the unique nature of the habitats they reside in. In recent years, over 100 wild colonies have been monitored by collaborating beekeepers. The data were added to the Galway Honey Bee Research Centre citizen science webtool to allow long-term monitoring.

Locations, winter survival and nest site characteristics have been documented. Samples of bees were collected from many of the colonies, largely within three different areas in the UK. DNA was extracted from freeze-dried thoracic tissue of 30 individuals per colony and DNA was sequenced using illumina technology and a pool-seq approach with 50X genome coverage. Following an established bioinformatics pipeline, introgression was measured using F3 statistics and population structure assessed using Fst approaches and network analyses. Phenotypic traits were assessed and correlations between colour, morphological traits and genotypic data were carried out using a suite of R packages.

In the first year of monitoring, colonies showed a good (>80%) winter survival on average. Genomic data suggest a high rate of introgression within the colonies but some colonies show a significant background of *A. m. mellifera* (native) alleles. Correlation between these results, wing morphometry, proportion of banded bees and survival will also be discussed. Data comparisons between forest, natural cavities and man-made structures will be made.

This study shows that free-living honey bee colonies nesting naturally in tree cavities and surviving without management are common in the UK. Also highlighted is the critical importance of preserving and studying those populations, offering significant implications for bee conservation efforts.

# LOCATION OF EMPTY CELLS IN THE NESTS OF *CHELOSTOMA FLORISOMNE* AND *HERIADES TRUNCORUM*

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## Abstract

Above-ground nesting solitary bees often use naturally available linear structures to build their cells for their offspring. Empty cells are often found in such nests. Empty cells built behind the nest entrance are called *vestibulum* in mason bees and were shown to serve as protection against parasitic species. Empty cells, however, may also appear in other locations in such linear bee nests. The aim of our study was to describe the nest structure and the location of empty cells of the large scissor-bee (*Chelostoma florisomne*) and the large-headed resin bee (*Heriades truncorum*) both being part of the Osmiini tribe. Bees were bred on the premises of the III. Campus of the Jagiellonian University, Kraków, Poland.

We have dissected 17 nests of large-headed resin bee containing 1 – 18 cells each, a total of 154 cells. Only 38 empty cells were found in these nests. Except one cell, all these were located behind the wall, closing the nest's entrance. The number of these cells ranged between 1 – 4, suggesting a protective function similar to *vestibulum* present in other mason bee species. Only one nest lacked empty cells. The few available nests did not allow us to draw further conclusions.

Also 293 nests of the large scissor bee were collected. The nests contained 1-15 cells. In total, 1454 cells contained bee or bee remains, and 664 were empty cells. We found that empty cells were regularly located in every other position in the nest, not only before the entrance, as in other species. Their presence negatively correlated with the level of nest parasites ($r = -0.4023$; $p = 0.0001$). This result suggests that they are also built to prevent attacks by cleptoparasites such as the wasp *Sapyga clavicornis*, which invades the nest after the cells are closed, *via* the side wall of the nest.

# MATERNAL ORIGIN OF HONEYBEE (APIS MELLIFERA) COLONIES FROM ACROSS EUROPE

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## Abstract

Worldwide commercial beekeeping poses a threat to the native origin of the honeybee (*Apis mellifera*), with beekeepers favouring subspecies of Eastern European C-lineage ancestry, due to their docile behaviour and high honey production traits. In many parts of western and northern Europe, queens of Western European M-lineage ancestry have been massively replaced by queens of C-lineage ancestry, and this has led to the development of conservation programs aiming at recovering native lines. The maternally-inherited mitochondrial DNA (mtDNA), particularly the intergenic region tRNA$^{leu}$-cox2, has been the marker of choice for assessing honey bee variation at large geographical scales. Herein, we will show the results of the mtDNA analysis of over 850 colonies collected across 28 European countries. These samples were subjected to DNA extraction, followed by PCR, and Sanger sequencing. The analysis of the sequences was conducted in Mega 11. The results indicated that, apart from Portugal, Spain, and the conservation centres in France and Denmark, where the colonies exhibited African or M haplotypes, the remaining countries are dominated by colonies of C-lineage maternal ancestry. In conclusion, this unprecedented mtDNA analysis conducted across Europe underscores the worrying dominance of C-lineage genetic variation, highlighting the urgent need for strategic conservation efforts to preserve the native genetic diversity of *Apis mellifera*.

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# MONITORING OF FREE-LIVING HONEY BEE COLONIES IN SLOVAKIA

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## Abstract

The occurrence and survival of free-living honey bee (*Apis mellifera* L.) colonies has received more attention worldwide in recent years. Ten years ago (2014), the western honey bee was listed in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, with a "data deficient" status. The goal of the scientific community is to gradually fill this gap in knowledge based on mapping the occurrence of free-living colonies, investigating their population and viability. Since 2017, we have started searching for colonies of free-living bees in Slovakia and evaluating their overwintering. So far we have documented 93 cavities in which we have made 199 observations of the survival of bee colonies. In seven observation seasons, 41 bee colonies (x=20.6%) successfully survived the winter until the first larger pollen collection and nectar flow. The vast majority of observed locations are located in urban areas (parks, cemeteries, street alleys). For each location of occurrence, we record the GPS coordinates, the height of the opening above the ground, the orientation of the opening, the size of the opening. In most cases, these are cavities in trees, where we record the type of tree, its circumference at a height of 1.3 meters above the ground. We took samples of bees for genetic tests from many observed bee colonies. The ongoing results of monitoring and related research should help define the degree of protection of free-living honey bee colonies.

# MOTIVATION AND ACTIVITIES FOR THE CONSERVATION OF THE LOCAL BULGARIAN HONEY BEE *A. M. RODOPICA*

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## Abstract

Different scientific tendencies occur in the apidology field. Two of them are much important: selection activities in order to create "the best honey bee" and preservation of the genetic diversity by conservation of the local honey bees. The valuable qualities that should be achieved for the selected "best bees" are also the main characteristics for the adapted local honey bees – high queen fertility, good honey productivity and overwintering, low defensive and swarming behavior and clearly expressed hygienic behavior. But low amount of brood during the winter and occurrence of unwanted qualities in the second generation are two serious weaknesses reported for the hybrids. Local honey bee populations are better adapted and enough productive in the specific conditions of the environment.

Local Bulgarian honey bee *A. m. rodopica* has high levels of queen fertility, productivity, overwintering and hygienic behavior, and low level of defensive and swarming behavior. Investigations during the years on the local honey bee populations from all over the country gave evidences that they could be discriminated from the other European subspecies and origins. A scientific system has been developed including different biological, morphometric, allozyme and DNA markers. In addition, a DNA bank has been established which could be used for future genetic studies.

The protection of the local honey bee in Bulgaria is based on the conservation activity of the National Bee Breeding Association and the existence of a functioning Law on Beekeeping in Bulgaria. It contains provisions ensuring the protection of the Bulgarian honey bee gene pool, as well as those prohibiting the importation of bee queens and colonies with foreign genetic origin.

## Acknowledgments

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# NO EVIDENCE ON COMPETITION IN FOOD PLANTS OR CO-OCCURRENCE BETWEEN MANAGED AND UNMANAGED POLLINATOR GROUPS ON CALCAREOUS GRASSLANDS IN ESTONIA

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## Abstract

In the recent decades, we have witnessed a decline in biodiversity across almost all ecosystems and taxa, including pollinating insects. Insect pollination is an important ecosystem service contributing to both agricultural success and yield increase as well as the sustainability of ecological systems both directly and indirectly. One emerging concern might be competition between managed and non-managed pollinators and this study focuses on the co-occurrence of pollinators in semi-natural habitats.

Calcareous grasslands have been proven to be one of the most biodiverse ecosystems in Europe and preserving these grasslands could maintain biodiversity and create sustainable nesting and foraging sites for pollinators.

30 sites were chosen across the islands and coastline of West Estonia, most of which were restored to open grasslands in 2016. Sites were chosen to create relevant environmental gradients, including area of crop fields, forests, calcareous grasslands, and size of monitored sites. We conducted transect walks to count pollinators (honey bees, bumble bees, hoverflies, butterflies, solitary bees) and their interaction with their food plants. Transect walks were conducted three times during the summer of 2022. Floral abundance was also recorded and used in the study.

We created statistical models to show species co-occurrence between all studied groups, in relation to the surrounding environment and the models showed no negative correlation between managed and unmanaged pollinator groups. Variables such as restoration status, grazing, beehives or apiaries also didn’t have any negative correlation between the groups. Bipartite network analyses showed that preferred food plants differed between groups which could be interpreted as lack of competition on food resources.

Calcareous grasslands offer abundance in food plants thanks to diverse flora which, in turn, supports having very diverse pollinator communities. The surrounding landscape provides with enough resources that managed and unmanaged pollinator groups appear to show no competition for food resources.

# UNRAVELING THE EFFECTS OF GRAZING ON POLLINATOR COMMUNITIES IN GRAN CANARIA'S PINE FORESTS

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## Abstract

Pollinators play a crucial role in both natural and agricultural ecosystems. However, numerous anthropogenic factors, such as land use change and intensification, have led to the decline of many pollinator groups. Grazing, a widespread land use practice, significantly alters landscapes worldwide and directly impacts pollinators by reducing their primary food sources. Despite being a longstanding activity since the arrival of the first settlers, the effects of grazing on the vulnerable ecosystems of the Canary Islands remain largely unexplored.

This study aimed to assess the abundance and diversity of wild bees and dipteran pollinators in the pine forests of Gran Canaria, considering both grazed and ungrazed areas. Various vegetation units, as well as climatic and landscape variables, were considered. A total of 777 specimens, representing 51 pollinator species, were collected using pan traps. Our findings revealed a negative correlation between grazing and the abundance and richness of endemic wild bee species, while no significant effect was observed for Diptera. These results highlight the importance of understanding the response of pollinators to grazing practices in pine forest ecosystems.

Keywords: pollinators; grazing; land use change; biodiversity; wild bees; dipterans; Canary Islands; pine forests

# WHERE TO PLACE ASIAN HORNET TRAPS TO PROTECT APIARIES AND WHICH BAIT TO MAXIMIZE EFFICACY AND SELECTIVITY?

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## Abstract

*Vespa velutina nigrithorax* is widely recognised as a threat to honeybees and requires management through preventive and curative beekeeping measures. To reduce the presence of the Asian Hornet (AH) in apiaries, beekeepers turn to mass trapping in summer. A common question arises regarding the positioning of traps and the bait selection. To provide some insights, we deployed 2 sets of 6 trap combinations in six apiaries across Wallonia, Belgium, from July to November: one within the apiaries and another 50 meters away. The traps utilised included five ‘lid traps' with different baits: beer/wine/grenadine (BWG), Trappit Wasp Bait from Agrisense, VespaCatch from Vétopharma, homemade mixture with bananas (MixCRAW), as well as water and one Vetopharma trap with VespaCatch as control items. In total, over the six sites 4549 insects were captured with 1.7 times more in the apiary than at 50m. Regardless of the bait used, 2 to 3 times more AH were captured in the apiary than at 50m. In the apiary, the best bait was Trappit Wasp Bait (n=745) followed by MixCRAW (342), Vespacath (229), and BWG (150). No AH were caught with water and catches with Vetopharma trap with VespaCatch (321) were similar in lid traps with MixCRAW, Vespacath, BWG. Regarding selectivity, for all baits, more non-target insects were caught far the apiary in comparison to AH catches. The best selectivity (avg. 87%) was achieved in the apiary with the lid trap - Trappit Wasp Bait combination. The Vetopharma trap was less selective towards non-target insects both near (avg. 48%) and far (avg. 23%) capturing European hornets, wasps, and other insects rather than AH. From these results, we recommend for summer trapping installing traps close to beehives and using Trappit Wasp Bait to enhance the AH capture efficiency and limit the impact on insect biodiversity.

# WILD BEE SPECIES RICHNESS AND PATHOGEN PREVALENCE IN AREAS WITH AND WITHOUT HONEY BEES ON AN ISLAND ECOSYSTEM

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## Abstract

Pollinators provide essential and beneficial services that sustain natural ecosystems. The current widespread decline of pollinators and particularly of bees (Hymenoptera: Anthophila) is of global concern. Multiple anthropogenic pressures, which interact with each other, threaten the decline of both managed (honey bee) and wild bee populations. Factors related to this decline include climate change, habitat loss and fragmentation, malnutrition due to shortage of floral resources, the spread of exotic pathogens and the introduction of species and/or subspecies. The aim of this research is to advance in the knowledge of these last two factors to reconcile the development of a productive activity such as beekeeping with the protection of natural resources such as wild bees. The oceanic island of La Palma (Canary archipelago) represents an ideal natural laboratory as honey bee hives transhumance has only recently started and the introduction of foreign subspecies of domestic bees is at low levels.

We present here preliminary results of the biodiversity of wild bee species found as well as the prevalence of bee pathogens (Trypanosomatids and Microsporidia) in two areas, with and without honey bee presence. These data will be used to estimate temporal changes in the prevalence of pathogens in wild and honey bees, the correlation of their prevalence with the transhumance and with the introgression of foreign subspecies of honey bees on La Palma. Finally, we aim to propose management measures and protocols for annual monitoring of the beekeeping activity to minimise the impact of transhumance and the introduction of foreign subspecies on the populations of the local honey bee ecotype and the different species of wild bees in the Canary archipelago.

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## WING GEOMETRIC MORPHOMETRICS OF EUROPEAN HONEY BEE POPULATIONS

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## Abstract

Wing venation patterns have long been used to identify honey bee subspecies, singly or in combination with other morphological traits, using different approaches. Beekeepers have traditionally used identification methods that only require estimations of the Cubital Index, Hantel Index, and/or Discoidal Shift Angle. However, these measures do not consider all the information carried by wing patterns, and a more accurate approach is geometric morphometrics, which requires the annotation of 19 landmarks in the forewing vein junctions. While manual wing annotation is a time-consuming and error-prone endeavour, the recently developed software DeepWings© performs this task automatically, allowing a fast and relatively accurate identification of European honey bee subspecies, and is appropriate for large-scale projects. In this study, we analyse the patterns of forewings extracted from over 850 colonies sampled across 29 European countries. To that end, the right forewings of five workers per colony are photographed with a digital camera attached to a stereomicroscope. The images are then analysed using DeepWings©. Our main objective is to assess (i) how closely the colonies identified by DeepWings© match the endemic evolutionary lineages and subspecies; and (ii) the association between the identification produced by DeepWings© and that inferred from mitochondrial DNA. While geometric morphometrics does not replace molecular tools for the identification of colonies, it can provide a preliminary estimation of their genetic integrity.

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# 11 Bee nutrition

## Oral presentations

Moderator:
Bjørn Dahle

# SUNFLOWER'S REWARD: CURRENT STATE OF KNOWLEDGE OF SUNFLOWER NUTRITION ON BEE HEALTH AND DEVELOPMENT

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## Abstract

Sunflower, *Helianthus annuus*, is a prominent global oilseed crop with rising cultivation in Europe as a bee-friendly crop by providing abundant floral resources for pollinators. Breeding efforts have renewed interest in sunflower floral resources due to their potential to enrich ecosystem services. With pollinator's reliance on pollen, as the primary source of nutrition (protein, lipids, and other micronutrients) and nectar as the primary source of energy (carbohydrates), it is important to review the current state of knowledge regarding sunflower nutrition and subsequent impacts on bees. This would contribute a baseline for future breeding efforts with emphasis on pollinator attractive hybrids. The aim of this review was to summarize the reported nutritional components in pollen and nectar of *Helianthus annuus* and reported impacts of sunflower pollen diets on bees' survival, development, and health. Although the nutritional quality of sunflower pollen was often questioned due to reported lower protein levels, various studies showed strong variances between other nutritional components. Moreover, results concerning the developmental and survival parameters of *Bombus* and *Apis* species have been inconsistent. Conversely, studies quantified a positive impact on pollinator health due to a frequently reported reduction of the gut parasite, *Crithidia bombi*, in *Bombus* species. These findings probe the question on how to precisely define the quality of floral resources, emphasizing the need for caution when categorizing sunflower as a low quality nutritional resource, especially through a single species perspective. This review also brings into light a prominent gap in scientific knowledge regarding nectar quality on bee nutrition and health, thereby warranting further research to gain a more comprehensive view of sunflower nutritional resources for pollinators.

# SPRING FLORAL DIVERSITY ENHANCES WINTER SURVIVAL PROBABILITY OF HONEY BEE COLONIES IN DIFFERENT CLIMATES

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## Abstract

Winter is a vulnerable time for honeybee colonies and high rates of winter mortality are a pressing concern for beekeepers, giving the importance of fostering colonies capable of restarting brood and nectar collection come spring. Key factors for survival are the implementation of measures against varroa and the feeding of the hives during the pre-winter phase. However, weather conditions, during the growing season and winter season, and landscape context may also influence winter survival. For our study, we monitored colonies from summer 2021 until spring 2023 in three different countries in Europe that represent different climatic areas. Health and development of the colonies were assessed before and after the overwintering period for both winters. We also recorded external temperature and precipitation. Moreover, we collected and analyzed pollen samples for diversity and nutritional values (protein content and fatty acid diversity), and we checked the pathogen loads in our colonies. Survival varied strongly across year and country, implying that climatic and geography should be taken into consideration when studying honey bee overwintering. We also found that winter survival was positively related to the diversity of pollen collected during spring of the previous foraging year. These results underline the importance of the floral diversity in the landscape around the apiaries, and especially how spring has a carry-over effect, determining the health of the colonies later during the season.

# HOW DOES THE DIVERSITY AND AVAILABILITY OF FLOWERS AFFECT BEE NUTRITION?

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## Abstract

Bees, like most animals, need diets that provide all required micro- and macro-nutrients in appropriate amounts and ratios in order to thrive and reproduce. They obtain most nutrients from floral pollen and nectar. Nectar provides primarily carbohydrates, while most other nutrients are obtained from pollen. Surprisingly, the nutritional requirements of most bee species apart from some ‘model’ bee species, i.e. honey bees (*Apis mellifera*) and several bumble bees (*Bombus terrestris* and *B. impatiens*), are largely unknown, although this knowledge is essential to support bee health and design conservation actions in our landscapes.

We aim to better understand the nutritional requirements of wild bees and how changes in the diversity and composition of flowering communities affects the nutrition available to different bee species and hence their well-being. To answer these questions, we applied a novel approach based on a combination of pollen metabarcoding and chemical analyses to infer bee species-specific pollen nutritional niches.

Our results indicate variation in nutritional requirements of different wild species, potentially related to their specific life-history traits. They also highlight the importance of bees having access to a diverse spectrum of flowering species and to an appropriate composition of floral communities, in particular at the foraging landscape scale.

# INSIGNIA-EU: ITS2 METABARCODING MONITORING OF THE FLORA VISITED BY HONEY BEES IN EUROPE

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## Abstract

Numerous studies have explored honeybee ecology and health by analysing the botanical diversity of pollen brought into the colony by foragers. Pollen-metabarcoding, which allows rapid simultaneous analysis of large numbers of samples, can be used to identify the botanical origin of the bee-visited plants. Within the INSIGNIA-EU project ITS2-metabarcoding analysis of over 2,500 pollen samples was performed. These samples were collected biweekly between May and August 2023 from pollen traps placed in apiaries across the 27 EU-countries. Apiaries were selected based on three main land-use types: agriculture, natural, and urban (artificial). A total of 111 families, 501 genera, and 1,042 species were detected across the EU. Spatial analysis of alpha diversity showed that the mean Richness varied between 6.47 (Finland) and 10.52 (Hungary), while the Shannon Index (H') varied between 1.43 (Finland) and 1.90 (Slovenia). Furthermore, mean Richness was 8.52,

8.02, and 9.06, and H’ was 1.70, 1.65, and 1.75 in agriculture, natural, and urban landscapes, respectively. However, these metrics did not differ among countries (p=0.46 for Richness and H’) or landscapes (p=0.37 for Richness and H’). Temporal analysis showed higher Richness and H’ in the last samplings (mid-July to late-August: 9.83, 10.14, and 9.76; 1.85, 1.89, and 1.84, for Richness and H’, respectively) on a European scale. However, there were no statistical differences between sampling rounds, with mean Richness ranging from 7.20 (early May) to 10.13 (mid-August) and mean H’ from 1.56 (early May) to 1.88 (mid-August) (p=0.43, for Richness and H’). Cluster analysis of beta diversity showed a strong geographical structure, with the bee-collected flora of the Mediterranean countries being the most differentiated in Europe. This study shows that honeybees collect pollen with similar levels of diversity across different landscapes and countries. Moreover, the Mediterranean countries have a more distinct bee-collected flora compared to the rest of Europe.

# EFFECTS OF PROVIDING SUPPLEMENTARY VITAMINS, AMINO ACIDS AND FATTY ACIDS IN HONEYBEE NUTRITION

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## Abstract

Honeybees collect nectar, honeydews, and pollen to ensure their nutritional requirements for carbohydrates, amino acids, fats, vitamins, and minerals. In the frame of increasing productivity and dealing with the limitation of natural supplies from the plants that due to climate change, beekeepers use synthetic feeds, with often unclear direct and indirect effects on bees and colonies. The aim of the present study was the application of different nutritional schemes (vitamins, amino acids, fatty acids) to honeybees both in cages and in field conditions and their evaluation through the determination of parameters such as lifespan, consumption rate, morphometric characteristics (dimensions of wings, abdomen, head, thorax), hypopharyngeal gland area, lipid and protein content of tissues and bee colonies growth. For this purpose, two experimental feeding designs were carried out. In the first, honeybees in cages were fed with syrups to which vitamins, proteins and oils were added, while in the second, implemented in the field, to bee colonies were provided supplements enriched with proteins and oils. It was found that supplementing honeybee nutrition with vitamins had a positive effect on their lifespan, the provision of amino acids and oils increased the protein and lipid content of the larvae and led to the production of bees with a larger size of hypopharyngeal glands. The results of the research showed that the nutrition provided by plants to bees cannot be replaced only by synthetic carbohydrate and protein food, but care is required for bees to consume all the necessary biomolecules in order the colony to be viable and productive.

# HEADSPACE SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR DETERMINING THE VOLATILE PROFILE OF HONEYDEW HONEY FROM NORWEST OF IBERIAN PENINSULA

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## Abstract

Honeydew honey becoming one of the most sought-after honeys in the beekeeping industry and by consumers. This honey is distinguished by its botanical origin, derived from an extrafloral secretion excreted by the acorns of *Quercus pyrenaica*. The increasing prevalence of long, dry summers has allowed a taxon such as *Quercus* to become an important contributor to bee production. The characterization of this type of honey is of great commercial interest and remains a pending task. In recent years, innovative and rapid techniques such as Headspace Solid Phase Microextraction (HS-SPME), are increasingly employed for the determination of honey's aroma, thus providing a useful tool for typification studies.

The main objective of this research was to describe the volatile organic compound profile of 16 samples of honeydew honey from the northwest of the Iberian Peninsula. Honey samples were identified by melissopalynological analysis. A 65-µm-thick polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was employed for the extraction and subsequent analysis of volatile compounds via gas chromatography-mass spectrometry (GC-MS). Identification of the compounds were detected by comparing their mass spectra and retention index.

The volatile organic compounds (VOCs) present in more than 70% of the samples were as follows: 1-Nonanol; phenyl-Acetaldehyde; Benzaldehyde; Benzyl alcohol; Oak lactone; Thymol; Alpha-terpineol; Linalool; Isoborneol; Linalool oxide; Phenylethyl alcohol; and Hotrienol. The relative concentration of these compounds varied according to the accompanying pollen types present in honeydew samples. However, the highest relative concentrations were observed for 1-Nonanol and Linalool oxide. The VOCs identified in this study belong to the following categories: aromatic alcohols, aldehydes, ketones, monoterpenes, monoterpenoids, and lactones. This profile may

represent the volatile profile of these honeydew honeys and could be used to typify this type of honey.

## Acknowledgements

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# IMPACT OF DIFFERENT DIETARY PROTEIN SOURCES ON THE COMPOSITION OF HONEY BEE HAEMOLYMPH

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## Abstract

The honey bee (*Apis mellifera*) faces significant challenges due to poor-quality nutrition and biotic stressors that compromise colony health and disease resistance. Insufficient dietary protein can detrimentally affect honey bee immunity. Supplementing bee diets with alternatives such as pollen substitutes or microalgae like Chlorella, known to enhance colony productivity, may address these nutritional deficiencies. However, the specific effects of diet on honey bee metabolism remain underexplored. This study examines how three protein sources—pollen, Ultra Bee pollen substitute, and Chlorella—affect the levels of amino acids and related compounds in honey bee haemolymph.

Using the MetAmino® kit (Chromservis) optimized for LC-MS analysis of amino acids, we assessed haemolymph samples from bees fed different protein diets. Or results revealed distinct variations in the amino acid profiles depending on the protein source provided. Most notably, the levels of the essential amino acid valine and methionine were significantly higher in bees fed with Chlorella compared to other protein sources. In bees fed with UltraBee, we observed increased levels of leucine and the non-essential amino acid cysteine. In contrast, bees consuming natural pollen had elevated levels of carnosine, a dipeptide known for its protective effects against free radical accumulation in *Drosophila melanogaster*.

These findings provide a preliminary understanding of how different dietary sources impact the honey bee metabolome. The observed variations in amino acid profiles suggest that specific protein sources can differentially influence honey bee physiology and potentially their health and disease resistance.

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# UNRAVELLING THE IMPACT OF NUTRITIONAL STRESS ON HONEY BEE COLONIES

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## Abstract

Agricultural intensification and changes in land use have led to a significant increase in the areas dedicated to monoculture plantations. Consequently, the diversity of polliniferous and nectariferous resources available for honey bees has decreased. In a previous study, we showed evidence that nutritional stress, associated with the consumption of Eucalyptus grandis pollen, a nutritionally poor food resource, negatively affects adult bee and brood population while promoting Nosema infection. In this study, we delve into the question of how nutritional stress affects colony strength and health. Two groups of colonies were placed in an Eucalyptus grandis plantation in autumn, at the beginning of the flowering period. Both groups had access to E. grandis pollen, while one group was also supplemented with a polyfloral pollen patty throughout the flowering period. At different times, we monitored the honey bee nutritional status, colony strength, and health. The field assays were conducted twice, in 2017 and 2022. Honey bees under nutritional stress (which mainly consumed E. grandis pollen) showed lower vitellogenin expression, suggesting precocious forager activity and a shortened lifespan; and lower glucose oxidase expression, suggesting a compromised social immunity. Those bees also exhibited a smaller area and perimeter of hypopharyngeal glands, as well as lower head and thorax weight. At the colony level, colonies under nutritional stress showed lower brood and adult bee population compared to supplemented colonies. Interestingly, RNA viral levels were higher in supplemented colonies, but no negative effects were detected. In conclusion, nutritional stress had a severe impact on honey bee colony strength. Those results may be useful to understand the impact of agricultural intensification on honey bee well-being and colony losses.

# A NOVEL MALDI-MS PROTOCOL FOR IDENTIFYING POLLEN IN HONEYBEE CORBICULAE AND ANALYSING THEIR TROPHIC POTENTIAL

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## Abstract

The nutritional richness of pollen is crucial for bees' metabolism, conferring resistance or tolerance to stressors such as agrochemicals and pathogens. No single plant species is capable of providing all the essential amino acids required by honeybees, therefore, the importance of a diversified and balanced diet can't be minimized. Pollen sources depletion, caused by a high level of anthropization of the bees' habitat, climate change and loss of biodiversity, is forcing honeybees to forage on one or few pollen varieties, making a nutrient and complete diet dreamlike. Studies focalized on, a fast evaluation of botanical origin and trophic value of corbicular pollen, has never been developed. In this work we present a fast method for pollen botanical identification and its potential nutritional profile assessment. To achieve this goal, corbicular pollen from 5 Italian regions was collected during spring 2023. Pollens were identified with microscopy-based techniques and DNA sequencing, and finally analysed with Matrix-Assisted Laser Desorption Ionization-Mass Spectrometry (MALDI-MS). Four different chemical extraction solutions and two physical disruption methods were tested to achieve an effective protocol for the MALDI-MS. Moreover, a correlation between the nutrients available in corbicular pollen, their botanical origin and the monitored region was performed. The best performances for corbicular pollen were obtained using a sonication disruption method after extraction with acetic acid or trifluoroacetic acid. The obtained pollen molecular mass fingerprints (MFPs) allowed us to establish a new rapid, reliable, cost-effective methodology for the identification of the corbicular pollen botanical origins. The analysed pollen revealed wide differences in the vitamins amount, lipids and protein content from the same botanical species collected in different regions, confirming a major influence of environmental conditions upon the plant potential for pollen quality. This new approach opens to a wide range of environmental studies spanning from plant biodiversity to ecosystem trophic interactions.

# THE TROPHIC POTENTIAL OF POLLENS WITH DIFFERENT BOTANICAL ORIGIN: A COMPREHENSIVE PROTEOMIC AND MICROBIOLOGICAL APPROACH

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## Abstract

Agricultural practices, pollutants and climate change can influence plant metabolic functions as well as the spatial distribution and abundance of trophic resources for pollinators. Nectar and pollen availability or changes in their dietary profile are may also occur and lead to nutritional deficit in pollinators, and trigger additional threats to ecosystem stability. The absence of a balanced diet, concerning the pollen source diversity and quantity and quality of protein content, has been proven to be detrimental for honeybee health, gut microbiome fitness and haemolymph protein content. Our research aims at investigating the potential of different trophic sources, i.e.  of different botanical origin, on the stimulation of honeybee's immune and proteomic response and modulation of the gut microbiome development. For these objectives, a holistic approach was proposed using MALDI-MS (MALDI BeeTyping) for antimicrobial peptides (AMPs) and SDS-PAGE for vitellogenin detection in haemolymph. Moreover, q-PCR was used for gut microbiome analysis. Newly-emerged caged honeybees were daily fed with a fresh suspension of pollen and sugar syrup (1:1 w/v) for a total of 40 monofloral and 13 polyfloral pollens belonging to 8 botanical families (Cistaceae, Asteraceae, Cornaceae, Boraginaceae, Fagaceae, Leguminosae, Rosaceae and Salicaceae) collected in 5 Italian regions. After 5 days, haemolymph and guts were extracted for the MALDI-BeeTyping, SDS-PAGE, and qPCR analysis after DNA extraction. Our results showed that different pollen sources can affect both AMPs and vitellogenin content in haemolymph but without significant variations. However, using a genetic algorithm on MALDI BeeTyping spectra, we developed a model able to discriminate various small size proteins correlating with vitellogenin. Finally, qPCR results highlighted that all botanical families could influence significantly the gut core microorganisms within the genera *Bifidobacterium*, *Bombilactobacillus*, *Lactobacillus*, *Frischella*, *Bartonella*, *Gilliamella* and *Snodgrassella*. These results represent a first step in understanding the complex interaction between pollen sources, microbiome and honeybees' health.

# 11 Bee nutrition

## Poster presentations

## A NEW FAST AND EFFICIENT METHOD TO ESTIMATE POLLEN FLORAL RESOURCES

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### Abstract

Interactions between entomophilous plants and insect visitors are considered mutualistic. Entomophilous plants require insects for pollination and reproduction while insect visitors depend on floral resources for nutrition. Bees need pollen throughout their life cycle, from larvae to adults. Growing studies suggest that both nutritional quality and quantity of pollen resources are essential to sustain declining bee populations.

Quantifying pollen resources at landscape scales requires precise measurements of pollen production per flower for each entomophilous species, as well as the density of these species per biotope. The most commonly used method involves estimating the volume of pollen produced by multiplying the mean volume of individual pollen grains by the number of pollen grains per flower. However, the nutrients available in floral pollen grains are assessed in terms of mass (mg of proteins, lipids or amino acids). We therefore developed a rapid and efficient method for estimating the mass of pollen grains. A known mass (2 mg) of floral pollen is injected in a flow cytometer that counts the number of grains. Based on multiple sampling, we demonstrated the robustness and replicability of this new method. Despite similar average volumes, pollen grains from different floral species do not have the same density and mass. In consequence, different floral species offer different nutrient quantities to their visitors.

# ANALYZING THE ELEMENTAL MAKEUP OF HONEY: A COMPARATIVE STUDY OF BEE DIETS

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## Abstract

Honey bees produce natural food called honey. It is a complex mixture of proteins, sugars, pigments, organic acid, minerals and other elements. Honey is usually produced from nectar. However, in extreme circumstances bees can use other food sources to produce honey. In this study we examined how different food sources effect elemental composition of honey in an extreme nectar dearth. Each bee hive was fed a different diet: commercial sugar patty consisting of ground table sugar mixed with invert syrup was fed to the sugar group (SG); commercial sugar patty was mixed with yeast to make yeast group (YG) feed, pollen to make pollen group (PG) feed and *Tenebrio molitor* flour to prepare Tenebrio group (TG) feed. Honey was collected from each hive and analyzed for its elemental composition using with inductively coupled plasma mass spectrometry. Elements Ag, Hg, Tl and U were not detected in any of the samples. The sum of all analyzed elements had the highest value in honey from PG, followed by similar amounts in YG, TG and lowest in SG. We have found V and Cd only in pollen hive honey. Cr was found in honey from pollen and Tenebrio hives. While Co and Zn were in pollen and yeast hives. Highest concentrations of S, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se and Sn were observed in PG; Li, Na, Mg, Al, P, Mo and Pb in YG and of B, K, Ba and Sb in TG. Honey bees in SG did not accumulate higher concentrations of any element compared to other three groups. This research suggest that sugar syrup will not provide honey bees with necessary micro- and macro-element needs. They should always be supplemented also with either pollen or pollen substitutes such as yeast or Tenebrio flour.

# DRONE BROOD FLOUR: A SUSTAINABLE APPROACH TO ENHANCING HONEY BEE NUTRITION AND COMBATING VARROA MITES

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## Abstract

The global honey bee population is facing numerous threats, including parasites such as the Varroa mite and insufficient nutrition due to dwindling forage opportunities. To overcome these challenges, we propose a novel approach that utilizes drone brood as a valuable source of proteins, fats and minerals during pollen is scarce. Through a comparative analysis, we found that drone brood flour has a comparable mineral composition comparable to Tenebrio flour, a known pollen substitute. In addition, the removal of capped drone brood helps to combat the Varroa mite, as these parasites prefer to reproduce on drone brood. This method not only ensures bee nutrition, but also contributes to the health and sustainability of hives by reducing Varroa mite infestation and minimizing the use of pesticides. In addition, the use of drone brood offers economic benefits for beekeepers, as they are less reliant on expensive commercial supplements and can potentially increase their profits through surplus pollen. The simplicity of the process allows for easy implementation in apiaries, making it a practical solution for beekeepers worldwide. Overall, our study represents a promising strategy to tackle two major threats to honey bee colonies — poor nutrition and Varroa mite infestation — simultaneously, thereby promoting the sustainability and resilience of beekeeping in the face of global challenges.

# EFFECTS OF ADMINISTRATION OF COMMERCIAL PROTEIN DIET IN HONEY BEES NUTRITION

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## Abstract

Protein diets is well known to significantly affect honey bees physiology resulting in a larger dimension of mandibular glands, acini of hypopharyngeal glands and higher proteolytic enzyme activity. Use of protein-supplemented artificial diets is a common practice in beekeeping in period of low food availability and no data are yet available on its effects on bees health. Effects of administration of two commercial protein diets respectively enriched with 1.7% and 7.7% of protein on bees survival rate, social (i.e., glucose oxidase activity, GOX) and individual (i.e., phenoloxidase activity, PO) innate immune systems were preliminary investigated. Protein diets effects were tested on two bees imago ages (i.e., newly emerged, NE and forager bees, FO) to assess whether protein diet could compensate for lack of pollen sources in NE bees and improve the health of FO. Beet sucrose candy diet without protein was used as control diet.

Administration of high-protein diet determined a significantly higher mortality of NE bees compared to bees fed control diet and low-protein diet. Administration of a high-protein diet in NE bees stimulated GOX production which resulted significantly higher at 10th day of feeding than those fed low-protein diet and control and a significantly reduction of PO activity at 20th day. After 20 days, GOX activity significantly decreased in all diet group to level like T0 except in the control group. Overall, FO bees survival resulted lower than NE with a higher survival rate recorded in control group than in experimental ones and no differences were recorded on immune systems.

In conclusion, administration of high-protein diet determined a significant reduction of lifespan in both NE and FO bees and indicate that administration of commercial high-protein diets is not able to compensate pollen diet in NE bees and negatively affect the health of FO, determining an increase of mortality.

# EFFECTS OF PROTEIN DIETS ON BROOD DEVELOPMENT AND NOSEMA SPORES IN CARNIOLAN HONEY BEES

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## Abstract

The honey bee is the most well-recognized managed species for pollination services. Nevertheless, multiple stressors or pathogens, such as the microsporidia Nosema, can impact colonies health and productivity. One way to boost colonies health and their fitness is by offering a high-quality food. We aimed to determine if different amounts of protein added to the honey bee diet, during late summer, after honey harvest,  affect brood and the appearance of *Nosema* sp. spores in *Apis mellifera carnica* colonies. Experimental honey bee colonies in five locations in Slovenia were fed with different diets: sugar patty—control, 5% raw protein, or 24% raw protein added to sugar patty. Each treatment group composed of seven honey bee colonies. The area of capped and uncapped broods were measured before and after consuming the diets (2kg/colony). Additionally, forager bees from each colony were sampled to quantify *Nosema* sp. spores.
The  abdomens of the bees ($n = 20$) from each experimental colony were ground in distilled water (1 mL per bee), and the suspension was microscopically (400 X) examined and spores counted, using a Neubauer chamber. The results showed that colonies in three of the five locations had a reduction in capped and uncapped broods after treatment with the protein diets ($p<0.05$). On the other hand, the number of *Nosema* spores in forager bees showed an indication of a decrease after treatment with diets for all five locations. However, statistical differences between diets before and after feeding were only observed in two out of five locations ($p<0.05$). Moreover, no differences were observed between the 24% raw protein and 5% raw protein diets ($p>0.05$). The present study highlights that protein diets had effect on reduction the amount of *Nosema* spores. Further studies are needed to demonstrate the protein diet effect the brood development in different beekeeping seasons.

# INCREASED METABOLIC AND ANTIOXIDANT ACTIVITY OF ENZYMES IN HONEY BEES AFTER FUNGAL EXTRACT SUPPLEMENTATION

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## Abstract

Several stressors are linked to the detriment of honey bee health and colony decline. Studies about fungal extracts' antimicrobial and antioxidant properties have shown that they can prevent viruses' infections and may be used as a dietary supplement for bees. However, there is limited research on the effects of fungal extracts on immune system enzymes. Our study aimed to investigate the effect of fungal extracts on the activities of antioxidants and biological markers in treated honey bees' hemolymph. Ten experimental honey bee colonies (*Apis mellifera carnica*) were divided into five groups, each consisting of two colonies. The colonies were fed with the following fungal extracts: *Ganoderma lucidum* 4%, *Inonotus obliquus* 4%, *Trametes versicolor* 4%, and *Hericium erinaceus* 4%, diluted in sugar syrup (sugar: water; 1:1). The control, untreated colonies received only sugar syrup without fungal extracts. Colonies were fed three times a week over three weeks, totaling three liters of food provided to each colony by the end of the experiment. Hemolymph (3 μL) was individually extracted from 10 bees from each treatment group at 7, 14, and 21 days respectively. It was then added to a sterile microtube containing 127 μL of 0.6% NaCl and promptly chilled at -25°C for biochemical analyses. Three metabolic markers (Aspartate transaminase, Alkaline phosphatase, Alanine aminotransferase) and three antioxidant activity enzymes (Total antioxidant capacity, Glutathione peroxidase Superoxidase dismutase) were analyzed. Our results demonstrate that using any fungal extracts as a supplement increased the activities of metabolic markers and antioxidant activities ($p<0.0001$) after 14 days for all tested enzymes compared to bees fed with sugar syrup. The outcomes of our study showed that fungal extracts improve the natural immunity of bees. We conclude, however, that adding fungal extracts can ensure an effective supplementary feeding during spring honey bee colonies development.

# INVESTIGATING THE EFFECTS OF BEEELIXIR AND SPIRULINA SUPPLEMENTATION ON HONEY BEE NUTRITION: A LABORATORY EXPERIMENT

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## Abstract

Honey bee nutrition plays a crucial role in colony health and survival, particularly during periods of dearth and in preparation for wintering. In this study, we examined the impact of two nutritional supplements, BeeElixir and spirulina, on worker bee health and physiology under laboratory conditions.

Worker bees were divided into several groups: a control group fed with a standard sugar syrup (SS, 1:1), BeeElixir (1:2, 1:3, 3:1; BeeElixir:water), and spirulina (1 g of dry spirulina in 100 ml SS). Survival rates and consumption patterns were monitored over the course of the experiment. Body and head weight were measured and hemolymph was sampled on day 18, to assess the effects of the supplements on bee vitality and development.

Our findings revealed notable differences among the groups. Bees fed with SS or spirulina exhibited enhanced survival rates and increased consumption compared to BeeElixir groups. Moreover, BeeElixir (1:3) supplementation led to increased consumption levels and BeeElixir (3:1) significantly affected mortality rates. Interestingly, body and head weight measurements indicated variations in nutrient assimilation and resource allocation among the groups, with spirulina and BeeElixir (3:1)-fed bees demonstrating a more efficient utilization of resources.

Furthermore, we conducted hemolymph analysis using MALDI (Matrix-Assisted Laser Desorption/Ionization) mass spectrometry to delve into the molecular underpinnings of the observed effects. Results from the MALDI analysis provided insights into the metabolic pathways and biomolecular profiles associated with BeeElixir and spirulina supplementation, shedding light on the physiological mechanisms underlying their impact on honey bee nutrition.

Overall, our study highlights the potential of spirulina and BeeElixir as a viable alternative to traditional sugar syrup supplementation in bee colonies, offering improved survival outcomes (spirulina) and efficient nutrient utilization (spirulina and BeeElixir). Additionally, our findings underscore the importance of considering diverse nutritional strategies in apiculture practices to enhance bee health.

# ITS2 METABARCODING OF HONEY BEE-COLLECTED POLLEN: A COMPARISON OF TWO DNA EXTRACTION PROTOCOLS

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## Abstract

Honey bee-collected pollen provides valuable environmental information on available plant sources and diversity over space and time. Since high-throughput sequencing is becoming increasingly affordable, pollen metabarcoding is thriving as an alternative to the time-consuming palynology, especially for projects involving a large number of samples. This is the case of the INSIGNIA-EU project, which encompassed over 2,500 pollen samples collected across the EU. A bottleneck in the metabarcoding laboratorial pipeline for large projects is DNA extraction. Commercial kits for DNA extraction involving manual processing have been widely used in metabarcoding. However, for large numbers of samples, manual processing is time-consuming and prone to cross-contamination, requiring an automated alternative. Herein, we compared the performance of the Maxwell® (Promega) automatic extractor using the Maxwell® RSC PureFood GMO and Authentication Kit DNA extraction protocol with that of the manual Macherey–Nagel NucleoSpin Food Kit on 41 mixed-pollen samples collected by honey bees in Austria, Denmark, France, Greece, and the UK. We compared the relative abundances obtained from ITS2 metabarcoding between both DNA extraction protocols. Similar results were achieved, with the manual extraction detecting 112 families and 50 genera and the automatic detecting 113 families and 49 genera. The automatic and manual extraction protocols detected the same top-5 families

with similar relative abundances: Brassicaceae 13.8% vs 14.0%; Fabaceae 13.1% vs 14.2%; Asteraceae 12.4% vs 11.5%; Rosaceae 10.8% vs 12.8%; Plantaginaceae 10.8% vs 9.0%, respectively. The Shannon diversity index did not differ (p-value = 0.479, Kruskal-Wallis test) between the two extraction protocols (median: family = 1.5 for automatic and 1.6 for manual; genus = 1.7 for both protocols). Our results indicate that automatic extraction can confidently replace the manual extraction method, allowing easy scaling up of pollen analysis (1 hour for extracting 48 samples as compared to ~5 hours to extract 24 samples).

# POLLEN COLLECTION OF CARNIOLAN HONEY BEE COLONIES DURING A BEEKEEPING SEASON

Helga Déri[1], Judit Horel[1], Tünde Kiss[1], Lídia Lennert[1], Tamás Sipos[2], Márton Flórián[1], Tiborné Szonda[1], Gábor Bárczi[1], Edit Zajácz[1]

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## Abstract

Main food sources of Carniolan honey bee colonies are demonstrated by analysing the pollen loads collected from April to September during 2021 and 2022, in Gödöllő, Hungary. In 2021, the average weight of pollen collected by bee colonies ranged from 77 to 319 grams during each collection period, while in 2022, the range was from 24 to 275 grams. The apparently high variability in pollen quantity was influenced not only by the characteristics of foraging periods, but also by the developmental stage of the colonies. Considering botanical origin, in 2021, pollen from a total of 157 plant taxa was found in the samples, while in 2022 from 210 taxa. In both years one-quarter part of these plant species were solely pollen sources, while their three-quarters part were also nectar donors. The most frequently visited plant families were Asteraceae, Fabaceae and Rosaceae. The pollen samples largely consisted of pollen of one so-called "predominant" plant species. In 2021, 72 predominant species were identified, whereas, in 2022, 81 of such taxa. More than half of the species were predominant in both years. The most typical plant species of the spring growing season, whose pollen was present in at least half of the samples examined, were: *Acer* spp., *Pinus* spp., *Salix* spp. and *Taraxacum officinale*. In summer, pollen from *Tilia* spp., *Parthenocissus* spp., *Helianthus annuus*, as well as *Zea mays* was most often collected by honey bees. In the autumn period, *Ambrosia artemisiifolia*, *Carduus* spp., *Hedera helix* and *Helianthus annuus* were the most characteristic plants. The number of identified plant taxa suggests that bee colonies find diverse food sources in the studied foraging area during each beekeeping season. However, the variability in the amount of pollen collected, proposes that continuous monitoring of their food supply is essential for their health.

# RELIABILITY OF POLLEN METABARCODING IDENTIFICATION: ASSESSING THROUGH CROSS-VALIDATION WITH VARIOUS TAXONOMIC ASSIGNMENT APPROACHES

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## Abstract

Metabarcoding is a powerful tool to study the taxa composition of biological samples. However, to assign a taxon to a DNA sequence, bioinformaticians need to choose between different strategies or parameter values and these choices sometimes seem rather arbitrary. We present a case study on ITS2 and rbcL databases used to identify pollen collected by bees in Belgium. We blasted a random sample of sequences from the reference database against the remainder of the database using different strategies and compared the known taxonomy with the predicted one. This in silico cross-validation (CV) approach proved to (1) assess the relative accuracy of taxonomic predictions, (2) define rules to discard dubious taxonomic assignments and (3) provide a more objective basis to choose the best strategy. We obtained the best results with the best blast hit rather than by selecting the majority taxon from the top 10 hits. The predictions were further improved by favouring the most frequent taxon among those with tied best bit scores. We obtained better results with databases containing the full sequences available on NCBI rather than restricting the sequences to the region amplified by the primers chosen in our study. Leaked CV showed that when the true sequence is present in the database. Classical 10-fold CV offers a different yet more realistic view of the true error rates. Taxonomic predictions with this approach worked well up to the genus level, particularly for ITS2. Using a database containing only the local flora of Belgium did not improve the predictions up to the genus level for local species and made them worse for foreign species. At the species level, using a database containing exclusively local species improved the predictions for local species by ~12% but the error rate remained rather high: 25% for ITS2 and 42% for rbcL.

# SEASONAL VARIATION OF THE PROTEIN CONTENT OF POLLEN COLLECTED BY HONEY BEES IN HUNGARY

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## Abstract

Pollen is the primary protein source for adult honey bees, an essential component in their nutrition and crucial for the development of brood.

The aim of the present study was to investigate the changes in the protein supply of honey bees during a beekeeping season in Hungary. Pollen was collected from bee colonies in Gödöllő (central Hungarian region) from April to October in both 2021 and 2022. Samples were collected at three-week intervals, for a total of eight periods per year. A total of 248 pollen samples were collected (in 2021: 120, in 2022: 128). At each period, the quantity and botanical composition of the pollen samples were determined for each colony. The moisture content was determined by lyophilisation, with values ranging from 8.7% to 26.8%. The total protein content of the pollen samples was determined for each period and for the predominant species of the periods using the Kjeldahl method. The protein content of the pollen samples ranged from 13.3% to 29.4% in 2021 and from 12.5% to 30.2% in 2022. During the beekeeping season, the average protein content of the collected pollen exhibited an upward trend from April, reaching its highest levels in early June in 2021 and mid-May in 2022, with mean values of 25.5% (2021) and 26.5% (2022), respectively. Subsequently, there was a decrease in protein content until September, with values of 14.2% in 2021 and 13.7% in 2022. The average protein content of pollen collected in the final period (late September to early October) was once again at high levels, at 27.7% (in 2021) and 25.8% (in 2022), respectively.

In consideration of the quantity of pollen collected, the highest amount of protein was available to the bee colonies in late spring, which facilitated their growth and development before the swarming season.

# SUITABILITY OF STRAWBERRY TREE HONEY FOR BEE WINTERING IN WESTERN GREECE

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## Abstract

Strawberry tree (*Arbutus unedo* L.) is a perennial shrub widely distributed in Greece. The honey it produces is highly valued by beekeepers as it is the final honey collection before winter (November – December). Usually, beekeepers opt not to harvest it and instead leave it in the hive to support the bees' nutrition and aid in their successful overwintering. However, there are concerns among beekeepers due to the honey's high moisture content, which can lead to souring and dysentery problems in bees. In the present study, two experimental apiaries with 15 hives each were established in Western Greece, where strawberry tree plants are abundant. Honey frames were removed from the hives to ensure the collection of strawberry tree honey. Three harvests-samplings were conducted during winter and changes in moisture, HMF, pH, and acidity of the honey were analyzed, with a focus on its suitability for bee nutrition during overwintering. The samples were firstly subjected to pollen and organoleptic analysis to verify their botanical origin. The results revealed relatively high levels of moisture (21.2 %) and acidity (47.2 meq/Kg), while HMF and pH values fell within normal ranges (2.3-18.4 mg/kg and 3.8-4.1, respectively). Furthermore, the presence of isophorone compounds (alpha.isophorone, .beta.isophorone and keto-isophorone), which are known for their antimicrobial, antibacterial, and antioxidant properties and may contribute to enhancing the bees' immune system and supporting their successful wintering, was identified. All the experimental bee colonies managed to overwinter successfully, with only their reserves of strawberry tree honey, with the weather conditions being favorable during the study period. However, further research under different weather conditions is required to draw more conclusive findings about the bees' overwintering success solely on their strawberry tree honey reserves.

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# 12 Information flow from research to public and practice

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## Oral presentations

Moderator:
Robert Brodschneider

# DIAL "B" FOR BEEKEEPING: A GLOBAL SURVEY ON APICULTURAL EDUCATION AND INFORMATION SERVICES TO IMPROVE HONEY BEE COLONY VITALITY

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## Abstract

The COLOSS (prevention of honey bee COlony LOSSes) B-RAP (Bridging Research and Practice) group studies the means for the effective transfer of the latest beekeeping knowledge from scientists and extension workers to practising beekeepers. A purpose-designed questionnaire was translated and published online to collect data on the information sources preferred and most used by beekeepers, to understand the best means for communication and beekeeping education.

The study coordinated by 29 countries and one region (Latin America) obtained 11,351 responses, mainly from Europe, Asia, North America and Latin America. Information transfer to beekeepers differed significantly between all eight beekeeper characteristics, showing the biggest effects for the factors: continent, beekeeper age and experience as well as beekeeping education.

Avoiding honey bee colony losses requires healthy colonies. An important contributor to maintaining good colony health and vitality is effective colony management, but individual beekeepers vary greatly in their knowledge and application of optimal management practices. Beekeepers become knowledgeable through their acquisition of reliable information, therefore beekeeping advisors must reach out to them most efficiently. Best practices for knowledge transfer to a very diverse group of beekeepers in the apicultural sector, which is largely unregulated in most countries, must be studied further.

This study presents the largest comparison of an international survey of beekeepers to collect information concerning which information sources different types of beekeepers use and prefer, and how this varies among countries and continents. Such knowledge is essential for communicating good beekeeping practices to beekeepers most effectively.

# EMERGING THREAT OF ANTIMICROBIAL RESISTANCE IN *PAENIBACILLUS LARVAE*: CONSEQUENCES FOR HUMAN HEALTH AND FOOD SAFETY

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## Abstract

American foulbrood, caused by *Paenibacillus larvae*, poses a significant threat to global honey bee populations. While managed through various methods, the emergence of antimicrobial resistance (AMR) in *P. larvae* strains presents a challenge. Despite its widespread prevalence, American foulbrood does not currently pose a direct zoonotic threat. However, ineffective use of antibiotics in managing infections has led to the exponential contamination of hives with spores, which serve as reservoirs for new infections.

Antibiotics such as penicillins, oxytetracyclines, and tylosin have been used in beekeeping to manage American foulbrood. Efficacy of these antibiotics has reduced over time as bacteria develop mechanisms to evade their effects. Inadequate governmental control policies have perpetuated the misuse of antibiotics in beekeeping practices, contributing to development of antimicrobial resistance.

Honey harvesting activities accidentally contribute to dissemination of *P. larvae* spores, which can carry antimicrobial resistance genes. Consumption of honey contaminated with spores presents a potential risk, as interaction between P. larvae spores and human digestive tract remains poorly understood. Despite quality requirements regarding antibiotic residues in honey, inclusion of P. larvae in microbiological criteria is overlooked in food safety regulations.

This paper serves as a call to action for research aimed at understanding and mitigating threat of antimicrobial resistance in *P. larvae* for the benefit of honey bee health, human consumers, and environment. Our research in Republic of Srpska suggests that current control strategies have been effective in preventing the acquisition of antimicrobial resistance in *P. larvae* isolates. However, further investigation is needed to explain complex interactions between *Paenibacillus* spores, human microbiome, antimicrobial resistance and human health. Ultimately, addressing this "invisible quality" of raw food aligns with principles of One Health, emphasizing interconnection of human, animal, and environmental health and imperative for AMR stewardship identification.

# HONEY BEE WATCH DEVELOPS PARTICIPATORY MONITORING PROTOCOL TO STUDY FREE-LIVING *APIS MELLIFERA* COLONIES

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## Abstract

Honey bees are predominantly perceived as a domesticated animal for agricultural purposes, while their role and importance as a wild species is often overlooked. In contrast to managed populations, wild cohorts are shaped by natural selection, which is why they can potentially cope better with threats like *Varroa destructor* and a changing environment. However, the IUCN's Red List classification of Apis mellifera in Europe remains Data Deficient, underscoring the knowledge gap about the life of these essential pollinators in their natural habitats and highlighting the urgent need for more comprehensive data about their density, distribution, and survival.

A group of researchers and experts have united to address this data deficiency, aiming to identify self-sustaining wild populations through the monitoring of their nests, genomic sequencing, and comparative testing of pests and pathogens against specimens from local managed colonies. Motivated by the lack of reference methods for studying *A. mellifera* outside of apiaries, a standardized protocol was developed for their monitoring. Designed as an open-access, participatory questionnaire intended for use throughout the year by researchers and citizen scientists, it captures key data about the colonies and their habitats, including bees' observable activity, survival rates, weather conditions, and other relevant environmental characteristics. Collecting these valuable data throughout Europe (and beyond) is indispensable in addressing their unresolved Red List status and is expected to inspire future studies.

Ultimately, this project represents a successful model of pan-European cooperation that enhances the research toolkit available for studying wild pollinators, which will also answer the crucial

question of whether self-sustaining populations of *A. mellifera* still exist in the wild. The development of this monitoring protocol and overall coalition-building has been the mission of Honey Bee Watch, a global citizen-science study focused on better understanding how and why free-living honey bees survive with little to no human intervention.

# TOOLS AND INITIATIVES TO INCREASE PUBLIC AWARENESS AND ENCOURAGE BEST PRACTICES FOR POLLINATOR CONSERVATION IN THE LIFE 4 POLLINATORS PROJECT

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## Abstract

LIFE 4 Pollinators (LIFE18/GIE/IT000755) is a Governance and Information project aiming at raising awareness of the importance of pollinators and promoting good practices for their conservation in the Mediterranean area. The project developed multiple actions to involve different stakeholders in Italy, Spain, Greece and Slovenia.

Tools in 6 languages, downloadable at the project website, were distributed at training and dissemination events: 2 animation videos, 6 field guides to plants and pollinators and 4 handbooks for farmers, managers of protected areas, managers of urban green areas, citizens and educators.

27 *Pollinator bioblitz* with a total of 900 participants were organised in natural parks of the four countries, and the project *Student4pollinators* reached 300 teachers and 1600 secondary school students in Italy, Spain and Greece. Ex-post surveys showed a significative increase in knowledge and a change in behavior and awareness concerning wild pollinators.

A *web platform* to collect photos of insects on flowers sent by citizens and identified by experts: almost 2000 photos were submitted, mainly taken in Natura 2000 sites during bioblitzes and in urbanized areas during educational activities.

A *Pollinator-friendly declaration* was proposed to local authorities to help them creating local pollinator strategies and until now it was signed by 6 municipalities.

To promote more sustainable farmland ecosystems, a pilot project for farmers was developed. A training cycle on pollinator-friendly management involved 350 farmers and 24 agricultural organization consultants. A *Code of conduct* for farmers was proposed to obtain a *Certificate of pollinator-friendly farm*; 22 farms signed it and 4 of these successfully completed the verification

process and received the certificate. In 12 farms that voluntarily applied our pollinator-friendly actions, their effectiveness was monitored.

Finally, a *BEE indicator* based on bee biodiversity was developed to evaluate the effectiveness of CAP green measures and eco-schemes and was tested in 13 farms.

# LEVERAGING COMMUNITY SCIENCE FOR LARGE-SCALE MONITORING OF INSECT POLLINATORS: INSIGHTS FROM ABEILLES CITOYENNES

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## Abstract

While concerns about the decline of insect pollinators persist, pinpointing at-risk pollinator communities remains challenging due to the significant time, cost, and expertise needed for the collection and identification of wild insect pollinators, notably bees. These challenges are especially significant over multiple years and across extensive geographic areas. The *Abeilles citoyennes* (abeillescitoyennes.ca) project, a pollinator monitoring initiative, was launched in Quebec in 2019 to monitor the diversity of wild bees and hover flies within the province of Quebec over time. Specifically, volunteers are provided with a kit containing materials such as pan-traps and net for sampling pollinators, as well as instructions and materials for preserving the samples and sending them to the lab. The sorting, processing, and identification of pollinators are carried out by the university team. From 2019 to 2021, 131 volunteers collected insects at 161 sites across the province. A total of 13,558 bees and 2,486 hover flies were collected and identified to species. In 2023, we received samples from 61 volunteers and 112 sites, while we have 105 volunteers and 139 sites registered for 2024. In this presentation, we use it as a case study leveraging the complementarity of community science and taxonomist expertise for large-scale monitoring of insect pollinators. In addition to presenting potential data uses, we will discuss the benefits and challenges of the *Abeilles citoyennes* approach and opportunities for improvement.

# THE CITIZEN SCIENCE MONITORING OF IRISH FREE-LIVING COLONIES: A MODEL FOR FUTURE LARGE-SCALE STUDIES FOR ASSESSING WILD HONEY BEE SURVIVAL

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## Abstract

Ireland harbors the largest recorded number of free-living *Apis mellifera mellifera* colonies known in Europe, yet their survival is considered at risk due to the pressure imposed by the invasive parasites *Varroa destructor*. However, recent research suggests long-term survival in some Irish free-living colonies, possibly indicating the evolution of host resistance against the parasite. These locally adapted colonies may represent a potential asset for replenishing and improving local managed stocks that perish due to the parasite. With the aim of better understanding survival of free-living colonies, a multi-year citizen science study focused on the reporting and long-term monitoring of their nests was initiated in 2021. So far, 454 verified nests have been reported by 320 contributors. Notably, we also received 99 reports from the UK. Citizen scientists have been recruited to repeatedly document colony activity for complementing genomic testing aimed at verifying continual nest occupancy by the same colony, or its direct descendants. Using a digital platform to upload field observations based on a standardized monitoring protocol, volunteers have been monitoring 102 nests (18% of the total) since the start of the monitoring aspect of the study in 2023. The majority of these nests (66%) have been monitored more than once, suggesting a sustained interest by volunteers to contribute to our project. We ascribe these positive results to our recruitment and communication strategies focused on community building, which will be discussed during the presentation. Data on winter survival are being collected and will also be presented. Given the extensive scope of our study as well as the rate of volunteer' recruitment and retention, this project has the capacity to serve as a dependable model for future research aimed at the monitoring of free-living colonies across large scales.

# ADDRESSING CHALLENGES IN BEEKEEPING DATA COLLECTION: LESSONS FROM ITALIAN COLOSS MONITORING

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## Abstract

Honeybee colonies are essential for agriculture and the environment. Beekeeping is practised in all EU countries and is characterised by diverse production conditions, yields and beekeeping practices.

Since 2008, the COLOSS (Prevention of honey bee COlony LOSSes) Association Questionnaire is administered annually to beekeepers across Europe, enabling international data comparison and a deeper understanding of colony loss risk factors and work towards more effective strategies for honeybee conservation.

Colony loss rates vary in any one year and between years, for many reasons. Therefore, the representativeness of survey respondents for the beekeeper population in their country is of pivotal importance.

Over time, the number of participating countries represented in these surveys has gradually increased involving 27 countries.

The number of participants, about 5% of the European beekeepers to date, varies considerably between countries (from <1% to 17%) with marked differences among regions (NUTS2 level) within the country, representing a limit to the reliability of the results.

Using Italian national beekeeping registry (size of the beekeeper population, distribution and average number of hives per beekeeper) and the COLOSS 2022-2023 data, we tried to define the representative sample per each region that should participate in the questionnaire in order to make statistical inference with different degree of confidence levels (CL) and confidence intervals (CI).

The participation of 8.9% of the Italian beekeepers in the monitoring survey (6,914 participants), ca. 346 beekeepers/region, would be sufficient for obtaining a reliable result giving info on approx. 141,000 apiaries (95% CL; 5% CI).

Next COLOSS challenges in countries already participating in the survey questionnaire are:
i) encourage targeted data collection, based on national beekeeping registry, to obtain

representative samples in each country;
ii) overcome the limited and scattered participation of beekeepers within the country ensuring at least the national coverage of respondent beekeepers.

# IMPROVING BEEKEEPING RESILIENCE: AN INTRODUCTION TO THE BEEGUARDS EU PROJECT

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## Abstract

After a competitive two-stage evaluation process, the BeeGuards project was selected as one of two research projects for the "Resilient beekeeping" call funded under the Horizon Europe framework (HORIZON-CL6-2022-BIODIV-02). The BeeGuards consortium, coordinated by Dr Cecilia Costa from the Italian Agricultural research Council (CREA), consists of 27 partners including scientists, beekeepers and beekeeping associations, consulting and technology companies, from 16 countries. The 4-year project starting in October 2023, aims to provide sustainable management practices, novel breeding strategies, and digital and forecasting tools that will allow the beekeeping sector to adapt to a changing environment. In the field, BeeGuards will perform studies in 11 countries in Europe and beyond, applying innovative threshold-based management practices, using hives equipped with digital sensors. This will support the work of beekeepers by providing guidelines for the adaptation of beekeeping practices to present and future challenges. BeeGuards will perform complementary immunological, behavioural, pathological, genomic and ecological studies that will serve to elucidate how management, climate and environment act on honey bees and other pollinators. Beekeepers, farmers and citizens will be involved via a WikiBeedia and by citizen-science and carbon footprint studies to ensure that research findings are directly translated into useful/good practices for end users.

# HOW TO IMPLEMENT MODERN METHODS IN BEE BREEDING

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## Abstract

Beekeepers are keen to improve their bees. Classical tools of measuring colony growth, gentleness and honey production have been supplemented by assessment of disease prevalence and hygienic behavior. Varroa mites is a particular challenge, causing a range of major disease issues in colonies, which are mainly controlled by chemical treatment.

Here we investigate how to motivate beekeepers to partake in developing and testing of bees with better resistance traits. The understanding of the complicated genetics of honeybees is poor amongst beekeepers in general, even amongst queen breeders, but there is a desire for further education.

We will describe how we have developed tools to demonstrate colony evaluation, complemented by molecular genetics and a modern assay to for hygienic behavior towards varroa infested brood, and are trying to involve beekeepers in these undertakings

# SUPPORTING DIGITAL TRANSFORMATION EFFORTS IN AUSTRIA THROUGH PARTICIPATION OF BEEKEEPERS AND BIDIRECTIONAL INFORMATION FLOW

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## Abstract

Digital technologies can transform the way we work, especially when it comes to processes where greater efficiency and speed make a difference. This also applies to the Austrian beekeeping sector, which has made comparably limited use of these. Austria is a country characterised by small-scale structures and locally shaped infrastructure. Hence, Austrian beekeepers could benefit particularly from digital technologies to react more precise, efficient and faster to beekeeping challenges. Among these are e.g. disease-recognition and treatment, but also identification of best feeding and harvesting times to avoid colony loss. The Austrian Agency for Health and Food Safety (AGES) is therefore currently running a project called "Imkerei-Digital", which aims at collecting and sharing available information and opinions on digital tools *by* and *for* all Austrian beekeepers.

When it comes to digital transformation opportunities for Austrian apiculture, our project's motto is "beekeepers know best for themselves what they really need or want". We therefore utilized bidirectional to multidirectional information and communication flow (AGES to beekeepers, beekeepers among beekeepers, beekeepers to AGES), encouraging beekeepers to define for themselves which information is most useful, from their practical point of view and in the light of their own reflections regarding general cost-effectiveness. To share and discuss information, opinions, and thoughts regarding available or potential digital technologies, beekeepers were encouraged to participate in i) workshops, ii) online-questionnaires, iii) personal discussions (on site or via the phone or video-communication), as well as iv) information/opinion-sharing via conventional mail or e-mail.

Since the project is still ongoing, we aim at presenting the results which will be by far more elaborate this autumn. However, we also aim in presenting some typical challenges which we encountered during realization of the current project and share potential solutions.

## Acknowledgements

*The current project is funded by the Austrian Ministry for Finances.*

# MANDATORY INFORMATION OF HONEY BEE COLONIES LOCATION GIVES ADDED VALUE TO BEEKEEPER-PROVIDED EPIDEMIOLOGIC COLONY MORTALITY DATA

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## Abstract

Regular monitoring of honeybee colony losses has become crucial over the past few decades, with the COLOSS research association's approach being widely adopted. This method relies on voluntary reports from beekeepers regarding their management practices and overwintering outcomes, specifically the number of colonies wintered and lost. Winter honey colony losses have been annually monitored in Czechia since the winter 2013-2014 by COLOSS survey and moreoverall honeybee colonies and beekeepers are mandatorily registered in Czechia. The response rate increased from 1.0 % to 4.8 % of beekeepers since we started the monitoring survey. Variations in response rates can impact loss rate calculations and multi-year comparisons, potentially introducing bias. To address this, we have introduced the parameter of representativity for the first time in our calculations of colony losses across different regions, aiming to mitigate biased results. Although the survey responses cover mostly the entire Czech Republic, response clusters in certain areas and missing responses in others pose significant challenges for data evaluation and interpretation. By including representativity, we aim to enhance the validity of our findings, providing more accurate and reliable estimates of honeybee colony losses. This adjustment ensures a more comprehensive understanding of colony loss dynamics across Czechia. Additionally, it allows for better observations across the years in regions with comparable data quality information, ultimately contributing to better-informed strategies for honeybee conservation and management.

# 12 Information flow from research to public and practice

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## Poster presentations

# CENTRE FOR INFORMATION AND IMPROVEMENT OF KNOWLEDGE ABOUT POLLINATING INSECTS - POLISH PROJECT TO RAISE AWARENESS AMONG SOCIETY

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## Abstract

The National Institute of Horticultural Research, Apiculture Division in Puławy, is the largest Polish centre for comprehensive research and deepening knowledge of the biology of pollinating insects, mainly honey bees, mason bees and buff-tailed bumblebees, as well as wild bee species occurring in Poland. The highly qualified scientific staff of the Apiculture Division constantly undertakes popularization activities, but in order to increase their effectiveness, it is necessary to complete them with practical aspects, supply didactic materials and develop a base for educational activities. The project includes, among other things, the preparation of new nesting structures, beehives, flower meadows, pollinator-themed playground, etc. The recipients of the activities will represent various social groups, including: children, schoolchildren, curious adults, students of natural sciences and educational studies, students of the University of the Third Age, nature enthusiasts, allotment holders, fruit growers, planters, amateur beekeepers and professionals, as well as members of the local government responsible for managing urban greenery. The goal of the project is to provide expert knowledge on the biology and conservation of the most important groups of pollinating insects and their importance in maintaining ecosystems and food production. The workshops proposed in the project are therefore perfectly in line with the current environmental protection issues. The researchers will impart relevant know-how and encourage real pro-pollinator actions by protecting and creating new nesting sites, supplementing the forage base with appropriately selected nectar- and pollen- producing plants. Effective initiatives to protect bees in Poland depend on society’s sensitivity and ecological awareness, which will be shaped through media promotion of the entire project.

*Project number NdS/547976/2022/2023 funded by the Ministry of Education and Science Republic of Poland, Science for Society Programme.*

# COLMENA: A NETWORK FOR BEE RESEARCH, TRANSFER OF KNOWLEDGE, AND DISSEMINATION IN LATIN AMERICA

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## Abstract

Latin America (LA) is a major producer and exporter of animal and plant-based food products, of which plant product yields rely heavily on entomophilous pollination; with bees being the major pollinators. In LA, the management of honeybees (apiculture) and native stingless bees (meliponiculture) serve as tools for development, generating employment and supporting regional economies. Both production systems have enormous potential but face significant challenges. The COLMENA Network, born within the Latin American Society for Bee Research (SOLATINA), gathers bee researchers from eight LA countries and is financed by the CYTED Programme. Its aims are: i) to generate scientific and technical knowledge linked to honeybees and native stingless bees; ii) to promote the training of human resources in both scientific and technical areas; iii) to strengthen beekeeping and meliponiculture in the countries where those are emerging activities; iv) to transfer knowledge and capabilities to producers; v) to disseminate research results to the scientific community and the public. Here, we will share some of the network's achievements. We aim to promote the integration of apiculture and meliponiculture into diversified agricultural systems, aided by knowledge-based actions, to produce quality foods for our populations; taking into account environmental conservation, the local cultural heritage, and the rational use of our natural resources.

# ECONOMIC LOSSES AND EXPENDITURES ASSOCIATED WITH HONEY BEE HEALTH

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## Abstract

Uruguay is a territorially small country but with a beekeeping sector economically and socially relevant. With more than 200 colonies per beekeeper, Uruguay exports 95% of the honey produced, placing the country among the top 20 global honey exporters. However, pest and pathogens of honey bees are widely distributed, affecting colony productivity and survivorship. The objective of this study was to analyze the expenditures and economic losses in Uruguay due to honey bee health problems. Three of the most prevalent diseases of honey bees were considered: varroosis, nosemosis and chalkbrood disease. For expenditures calculations, public and private expenses were considered (health campaigns, official veterinary services, and investments in materials and equipment, and veterinary products and services according to the cost structures of the two mostly used technological alternatives applied by beekeepers and other logistic and human resources, respectively). For the economic losses' calculations, a model was designed, which considers direct deaths of the colonies, losses of production because of dead colonies and because of disease prevalence, and losses in rapeseed crop yield because of the deficit of honey bees. Total expenditures on animal health for the period under analysis (2012-2021) were on average USD 2.530.000 per year and 80% comes from the private sector. Beekeeping losses due to animal health were on average USD 20.000.000 per year, being significantly higher than the expenditures. These losses are equivalent to the 71.5% of the national incomes of bee products exports. The main source of losses was the reduction in production, particularly honey. Finally, the varroosis was the disease with greater economic damage for the chain. These results are a first snapshot of the quantification of the economic cost of bee health and should be considered in the design of politics for improve the sustainability of the beekeeping sector.

# ENGAGING CITIZENS IN INSECT CONSERVATION: THE INSECT FESTIVAL INITIATIVE IN THE BALEARIC ISLANDS (SPAIN)

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## Abstract

Insects play pivotal roles in ecosystem conservation and biodiversity, yet public awareness of their importance often falls short. The Insect Festival aims to bridge this gap by fostering engagement between researchers, policymakers and the public. Throughout the event, attendees immersed themselves in a series of interactive workshops and engaging activities designed to foster a deeper understanding of insects and their ecosystems. Participants embarked on a journey of discovery led by researchers and naturalists from exploring insect diversity to understanding their crucial role in pollination and beyond. The festival's activities were diverse and captivating. Children and families delved into the intricacies of insect morphology through hands-on workshops, where they had the opportunity to learn about the fascinating world of insect anatomy. Meanwhile, participants were invited to contribute to a collective art installation inspired by pollinators, highlighting the symbiotic relationship between art and science. For those with a penchant for cinema, "Polli-Cinema" offered a captivating cinematic experience, showcasing documentaries that underscore the importance of conserving pollinator species and their habitats. Through storytelling and visual narratives, attendees were inspired to take concrete actions to protect these vital creatures. The festival also served as a platform for innovation, with the presentation of the "Invasapp" mobile application and website, developed as part of the STOPInvasores project. This app empowers citizens to contribute to invasive species management efforts, underscoring the importance of citizen science in addressing environmental challenges. From workshops and art installations to film screenings and mobile apps, the Insect Festival was an immersive and enlightening experience for attendees of all ages. By fostering a deeper appreciation for insects and their ecosystems, this festival seeked to empower individuals to become stewards of biodiversity and environmental conservation.

# UTILIZING SCALE HIVE DATA IN BEEKEEPING IN FINLAND

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## Abstract

The data collected from the scale hive monitoring was analysed and explanatory factors were sought for the timing of the harvest season and the annually varying amount of honey harvest. The Finnish Beekeepers' Association (SML) maintains a public scale hive monitoring website, where observers have reported measurement results of their own hive scale weight development, as well as free-form observations on the weather, crops and measures taken at the hive.

The harvest seasons studied were from 2014 to 2023. By combining numerical and verbal data, it was possible to observe the cumulative weight development of the hive, the plants of the harvest season, the effect of weather conditions, and the timing of the harvest season and harvest. The results showed large differences in the measurement results of the scale hives and the timing of the harvest season, which are primarily explained by geographical differences between Northern and Southern Finland. Observations of plants during the harvest season were similar in different localities and varied according to weather conditions. Some suggestions for improving the hive scale monitoring system of SML were made.

# 13 Open Topic

## Oral presentations

Moderator:
Janez Prešern

# CAN THE MASON BEE *OSMIA BICORNIS* ACT AS A BIOINDICATOR FOR THE ENVIRONMENTAL SPREAD OF ANTIMICROBIAL RESISTANCE?

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## Abstract

Antimicrobial resistance (AMR) is a critical global public health threat. The antimicrobials overuse in human and animal health has led to the spread of resistant strains in urban and natural environments. Additionally, antimicrobial resistance genes (ARGs) are often transmitted horizontally between different strains, enhancing environmental resistance. Monitoring the environmental occurrence of AMR and its effects is therefore essential. Bees are effective environmental bioindicators, commonly used to monitor pollutants and pesticides. Also, bees can intercept atmospheric particulates and microorganisms on their body hairs during foraging activities and acquire ARGs from contaminated water. This study aimed to evaluate the potential of *O. bicornis* as a bioindicator for the spread of AMR in the environment. This study was conducted in the Emilia-Romagna region (Italy) across eight sites, varying from highly urbanised to completely natural. *O. bicornis* cocoons were placed in each site until natural emergence occurred. Two weeks post-emergence, 15 nesting females per site were sampled. Microbiological cultures were obtained from the surface of 12 bee bodies and their gut microbiota. Each sample was streaked onto a chromogenic non-selective medium and incubated at 37°C, isolating 98 bacterial strains identified using MALDI-TOF, covering a wide diversity of plant, animal, and human bacteria. AMR was phenotypically tested using the Kirby-Bauer method with 19 common antibiotics in Italy. The highest resistance levels were found for amoxicillin and penicillin, while some of them exhibited multiple AMRs. Additionally, DNA extracted from other 5 bees per site and all isolates were tested for 31 ARGs, to describe the relationship between phenotypic and genotypic resistance s in *O. bicorins*. Results show significant resistance variability, particularly for genes coding resistance to colistin and beta-lactam antibiotics. These findings highlight the utility of *O. bicornis* as an important bioindicator for monitoring environmental AMR, emphasizing the need for ongoing surveillance and mitigation efforts.

# CONSUMERS' PREFERENCES FOR HONEY PRODUCTS IN THE EU

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## Abstract

Honey has been an important component of human culture for thousands of years, both as a nutrient and a natural remedy. In the EU, honey production satisfies approximately 63% of EU market demand, with the remainder imported from non-EU countries. Despite not fulfilling its own market needs, the EU is the second largest honey producer after China in the world. The B-THENET Horizon Europe project aims at modernising the EU beekeeping sector by improving its sustainability at all stages. To gauge consumer preferences, perceptions, and attitudes regarding honey consumption and the sustainability of beekeeping in the EU, we launched a consumer survey in 2023 among EU citizens. We collected a total of 2,193 responses across 15 EU Member States. The majority of EU consumers include honey products in their weekly diet primarily for its organoleptic features. The possibility of honey fraud might hinder some consumers from buying honey products. Consumers have a well-established knowledge base of the most common honey types and a basic awareness of the elements of honey production. Consumers value the importance of a short supply chain and prefer to buy honey produced in apiaries committed to

sustainable beekeeping practices, even if that results in a higher price for such products. Consumer preferences could influence EU beekeepers and help them find unique market niches, thus improving their economic sustainability. Consumer preferences should also be incorporated into EU research, innovation and policy-making to support of the development of good beekeeping practices.

# BIOLOGICAL ACTIVITIES OF HONEY. INFLUENCE OF CHEMICAL EXTRACTION AND GASTROINTESTINAL DIGESTION

Miguel Ángel Fernández Muiño, Marta Alevia Antón, Maria Teresa Sancho Ortiz, Sandra María Osés Gómez

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## Abstract

**Introduction:** Evaluation of antioxidant, anti-inflammatory and antimicrobial properties of honey, after digestion is important because during the digestive process, degradation of compounds, formation of new ones and changes in their bioaccessibility may occur and therefore the biological activities evaluated in fresh honey may differ from those that occur after digestion.

Aims: Total phenols and flavonoids and the biological properties (antioxidant, antimicrobial and anti-Inflammatory activities) of 15 honeys collected in Castilla y León (Spain) as well as the effect of different extractions, chemical and physiological (in vitro digestion) on these properties.

**Materials and Methods**: Total phenolics content was determined using the Folin–Ciocalteau reagent. The total flavonids content was assessed by measuring the colour of aluminium-flavonoid.complexes in alkaline medium. The antioxidant activity was evaluated against three radicals (ABTS•+, ROO•, •OH), anti-inflammatory activity was evaluated using a modified hyaluronidase inhibition.Assay and the antimicrobial activity were studied against five bacteria (Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Streptococcus mutans and Pseudomona aeruginosa) and one yeast (Candida albicans). (DOI.: 10.3390/foods.2021.10061412).

**Results**: The results show that in comparison with raw honeys, the methanolic extracts exhibited lower values for antioxidant activity and higher anti-inflammatory and antimicrobial activities against L. monocytogenes. The digested honeys showed higher antioxidant activity than the pre-digested honeys, as well as higher antimicrobial activity against S. aureus and L. monocytogenes, which underlines the possible antioxidant and antimicrobial effects of honey after digestion.

Conclusion: Heather honeys showed the highest values of antioxidant, anti-inflammatory and antimicrobial activity. Methanolic extracts showed a lower antioxidant and antimicrobial activity (except L. monocytogenes) but higher anti-inflammatory activity than crude honeys. The soluble fraction after in vitro digestion showed a higher antioxidant and antimicrobial activities against S. aureus and L. monocytogenes but lower anti-inflammatory activity.

**Keywords:** honey; in vitro digestion; phenolic extract; antioxidant activity; anti-inflammatory activity; antimicrobial activity

# ENVIRONMENTAL DNA IN HONEY: GENOMIC TOOLS FOR ENTOMOLOGICAL AUTHENTICATION OF THIS PRODUCT

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## Abstract

Honey is one of the most commonly frauded food products, with the most frequent frauds involving adulteration and mislabeling of its origin. The entomological origin of honey can be considered from two perspectives: i) the subspecies of *Apis mellifera* that produced the honey; and ii) the footprints derived from plant-suckling insects that produce honeydew, which are always present in authentic honey. Identifying the *A. mellifera* subspecies provides an important link to boost the conservation and integrity of honeybee populations and also offer indirect information on the geographic origin of the honey, based on the natural distribution of the different subspecies. Plant-suckling insects belonging to the Rhynchota order provide multidimensional information related to the plant origin of honeydew, based on the botanical specialization of these plant parasites, indirectly indicating the geographic origin of the honey. To identify the honey bee subspecies, we set up assays to analyse DNA extracted from honey that targeted regions of the *A. mellifera* mitochondrial DNA (mtDNA). These regions are informative to distinguishing different subspecies carrying the mtDNA lineages A (e.g. *A. m. siciliana*) and M (e.g. *A. m. mellifera*), as well haplotypes C1 (*A. m. ligustica*) and C2 (*A. m. carnica*). Another assay was designed to genotype approximately100 subspecies-informative honey bee single nucleotide polymorphisms (SNPs) using DNA extracted from honey and a genotyping-by-sequencing approach. For identifying plant-suckling insects, we designed a targeted metabarcoding assay based on two conserved regions of Rhynchota mtDNA using a next generation sequencing approach, along with bioinformatic pipelines to interpret sequenced reads and assign them to Rhynchota families. These approaches were applied to more than 150 honey samples, revealing that these methods are highly informative for authenticating the honey based on their entomological origin.

# PHYSICOCHEMICAL CHARACTERISTICS OF BEESWAX

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## Abstract

The purpose of the study was to develop and validate methods for the determination of physicochemical parameters of beeswax: melting point and acid, saponification and iodine numbers. Methodologies for the determination of the above parameters were developed using instrumental techniques with modern apparatus that allow to obtain better resolution, accuracy and repeatability than the classical physicochemical methods recommended by Polish Standards for Beeswax.

For the developed methodologies, uncertainties of the measured parameters for natural wax and beeswax comb foundation samples were determined. The samples were obtained in 2022-2023 from the ecological apiary of the Department of Apiculture of National Institute of Horticultural Research located in the Polesie National Park. To compare the physicochemical properties, samples of beeswax comb foundation and wax from other known sources were also tested, including wax from conventional apiaries, virgin wax, wax and beeswax comb foundation stored for about 20 years, and beeswax comb foundation from outside the national territory.

The average results of individual parameters for all samples were within the following ranges: melting point - 63.5°C ± 0.8°C, acid number - 17.2 mg KOH/1 g ± 0.9 mg KOH/100 g, saponification number - 91.8 mg KOH/1 g ± 7.9 mg KOH/100 g, iodine number - 9.9 g I2/100 g ± 1.2 g I2/100 g. The obtained values for the individual parameters were in accordance with the requirements of the Polish Standards for Beeswax and with the literature data.

*Research conducted within the framework of a targeted grant from the Ministry of Agriculture and Rural Development Task Republic of Poland: Optimization of ecological apiary management in order to increase the efficiency of beekeeping production and improve the health of bee colonies.

# CONSIDERING HONEY BEE WELFARE: KNOWLEDGE GAPS, INDICATORS AND THE WAY FORWARD

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## Abstract

Animal welfare science is occupied with assessing the welfare of animals, and has been mostly focusing on mammals and birds in farm contexts. The field is mainly following the paradigm of sentientism, implying that suffering of sentient beings should be avoided, and that positive emotional states are favorable. With insect farming for food production becoming more abundant, more interest for insect welfare has emerged. Recent evidence suggests that insects are sentient, thereby capable of experiencing pain and positive welfare states.

Bees occupy a complex space in this landscape of insect farming: they are not equally physically restricted or killed for harvesting as are insects kept as feed. Yet, they are exposed to human impacts, including disease exposure, management and housing, frequent handling, and breeding. Bees' functions and presence in ecosystems have been considered through conservation concerns, and their health is a central interest of research and beekeeping. Yet, their welfare, as individuals and as livestock, has been explored seldomly so far by animal welfare research.

We conducted a literature review synthesizing existing knowledge, knowledge gaps and opportunities to advance honey bee welfare. Results include suggestions for indicators that could now be validated through empirical work, and which data are required for such undertaking. We thereby stimulate specific actions, which is required for the newly emerging field of insect welfare and give specific recommendations both for future research and practical beekeeping.

We conclude that recent advances in animal welfare science including concepts and applied methodologies can now be expanded to all taxa, including insects and that welfare concepts can advance through such expansion. Considering welfare of honey bees will strengthen conservation efforts by providing earlier indicators for need for action, and will help to inform bee keeping practices and advance the field of insect welfare as a whole.

# MICROBIAL BIO-FERTILIZERS CAN ALTER THE SUNFLOWER' VOCS PROFILE AND SUGAR PASSIVE TRANSPORTERS GENE EXPRESSION, INFLUENCING POLLINATOR ATTRACTION

Daniele Alberoni[1], Chiara Braglia[1], Loredana Baffoni[1], Riccardo Favaro[2], Sergio Angeli[3], Diana Di Gioia[1]

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## Abstract

Sunflowers (*Helianthus annuus*) are plants rich in nectar and pollen and are generally considered beneficial for pollinators. However, a decrease in sunflower nectar secretions has been described in many regions of the globe lately. This decline is attributed to drought, but another factor that might affect plant performance is the decreased microbial diversity of soils in the agroecosystems, which does not allow the establishment of a beneficial "dialogue" between sunflowers and soil microorganisms. Soil microorganisms may be responsible for the solubilization of microelements useful for nectar and volatile organic compounds (VOCs) emission, altering pollinator attraction. Plant Growth Promoting Bacteria (PGPB) are widely used bio-fertilizers in agriculture to increase plant growth and resistance to stress factors. However, it is currently unknown how PGPB can modify the plant physiological processes leading to nectar secretion or VOCs emission in sunflowers, thus influencing the plant-pollinators network. To shed light on these aspects, a trial involving hybrid and non-hybrid sunflower varieties was established in Italy. Sunflowers were sown in soil plots, which were inoculated with different PGPB such as *Bacillus*, *Lactobacillaceae*, and *Pseudomonas*. About 200 flowers were collected from the different experimental plots and analyzed for VOC emission (SPME-GS) and for the gene expression of SWEET passive sugar transporters (RT-qPCR). Simultaneously, sunflower root samples were collected, and epiphytic microbial community sequenced on Illumina Hi-Seq. Results showed that the colonization of the rhizosphere was mainly driven by the sunflower variety and not by the PGPB treatments. Conversely, some PGPB significantly altered the VOCs profile and the pollinator attraction, with *Pseudomonas* that was found to increase pollinators attraction. Finally, the alteration of gene expression of SWEET passive sugar transporters were increased and driven by some PGPB. These results represent a first step in understanding the role of soil microorganisms and PGPB on sunflower attractiveness to pollinators.

# A SUPERORGANISM APPROACH TO HONEYBEE COLONY HEALTH

Peter Neumann

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## Abstract

Honeybee colonies can be considered as superorganisms consisting of individual bees analogous to cells in metazoans. Despite superorganism resilience, high colony losses have been reported in many areas globally. Here, I suggest applying more rigorously the superorganism concept to better understand colony health and eventually mitigate these losses.

Individual workers conduct all core tasks necessary to maintain colonies, e.g. collaborative brood care, foraging and thermoregulation. Cohorts of workers conducting such core tasks constitute organs of the superorganism. Therefore, organ failure assessment scores, which are routine in medicine, may constitute reliable predictive markers of colony death. To achieve such scores, the number of workers and their efficacy to perform the respective task must be known, because both parameters together underly functionality of such organs, i.e. a minimum number of functioning workers is required to avoid organ failure. However, due to numerous and often ubiquitous stressors (parasitic mites, virus infections, sublethal poisoning, malnutrition, etc.), workers will often display compromised functionality (e.g. reduced feeding glands) or even non-functionality (e.g. clinical virus symptoms). While worker mortality due to such stressors is in general reasonably well understood, their sublethal impacts on task performances of workers are often poorly understood. Further, interactions between stressors can range from antagonism to synergism, are virtually inevitable, but are even less well understood especially in their roles of compromising worker functionality. Taken together, this calls for more dedicated and concerted research efforts on colony health with a much stronger focus on worker cohort functionality. As colony losses in the temperate regions occur mostly over winter, more detailed investigations on the functionality of thermoregulation by long-lived workers appear very promising.

In conclusion, effects of interacting stressors on core tasks of workers, which constitute essential organs of a functional honeybee superorganism, deserve more attention to better understand the health of colonies.

# PROTEOMICS OF HONEYBEE WORKERS IN THE SUBTROPICS

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## Abstract

In honeybees, workers must outlive broodless periods to ensure the survival of the colony. From, the temperate regions, it is known that short- and long-loved workers are linked with the presence/absence of brood respectively and display different proteomes. For example, long-lived winter bees display higher vitellogenin titres. Despite broodless periods occurring outside of the temperate regions, the respective proteomics are currently unknown.

Here, we show in field colonies approaching the broodless period in the subtropics that workers display a significantly higher abundance of vitellogenin indicating that the underlying mechanisms for longevity are universal.

We investigated proteomic differences between workers as they approach broodless periods from different locations in a sub-tropical region, Sicily (Italy). Workers were sampled from brood frames from August to October (2023) and their heads were processed for proteomic extraction. Initial data analysis highlighted significant differences in protein expression between the three locations. We found that protein groups such as vitellogenin and ribosomal proteins were significantly differentially abundant between locations indicating unique protein profiles for each location, i.e. a higher vitellogenin abundance in one location.

For the first time, our results indicate similarities between temperate and subtropical honeybee populations with respect to the mechanisms underlying worker longevity. Since worker longevity is fundamental for colony survival, a conserved mechanistic approach across the entire species seems plausible irrespective of environmental factors. In light of climate change triggering brood less periods, it seems essential to further expand research on worker longevity outside of the temperate regions.

# HOW TO OPTIMIZE HONEYBEE BREEDING SCHEMES VIA INSTRUMENTAL INSEMINATION

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## Abstract

Breeding honeybees ideally consists of selecting genetically superior queens for reproduction and pairing them with equally superior drones. The most secure way to ensure the latter is to use instrumental insemination which provides a maximum of control for the breeder. We examined how this control can be exploited to optimize breeding schemes. Particularly, we investigated how to ideally choose insemination drones and how to distribute them to the virgin queens.
Three different insemination strategies were considered. In Mating Station Emulation (MSE), drones for a queen are taken from a sister group of drone producers; in Single Colony Insemination (SCI), each queen was fertilized with drones from a single colony, and in Pooled Semen Insemination (PSI), sperm of drones from many genetic origins was pooled and all queens were inseminated from this semen pool. Using the software BeeSim, we performed stochastic simulations of honeybee breeding with the different insemination strategies.

After 70 years of breeding, strategy SCI led to up to 44% higher genetic gain than both MSE and PSI schemes – sometimes at the cost of high inbreeding rates. For PSI, we observed that mixing the semen lost too much pedigree information, so that selection for estimated breeding values no longer outperformed phenotypic selection. The lower inbreeding rates could not sufficiently compensate for this disadvantage.

In comparison with SCI, strategy MSE suffered from a lower accuracy of paternal pedigrees and a longer generation interval. We thus concluded SCI to be the preferred mode of instrumental insemination. However, particularly for small populations, strategies of inbreeding avoidance need to be implemented. These could be achieved via stretching paternal generation intervals, performing Within Family Selection, or Optimum Contribution Selection.

# 13 Open Topic

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## Poster presentations

# A NEW APPROACH TO HONEY BEE WELFARE DEFINITION

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## Abstract

Honey bee populations face increasing threats from stressors, biotic (e.g. pathogens, pest, and invasive species such as Vespa velutina and Aethina tumida) and abiotic (e.g. habitat loss, climate change, pollution, nutritional imbalances, and pesticide exposure), collectively impacting honey bee health, often leading to conditions such as colony depopulation and colony loss (CL). These conditions may not have always singular direct causes but frequently result from a combination of factors (potentially subclinical or less severe) accumulated over time, demanding prevention measures, such as Good Beekeeping Practices (GBPs) and Biosecurity measures (BSMs).

For this reason, a collective state of general balance in Welfare may offer a better approach to modern beekeeping, averting imbalances and CL, with welfare models emerging as promising preventive measures. Welfare Models are specie-specific protocols currently aiming to enhance conditions and protect individuals based on needs, and risk assessment. Honeybees lack tailored models, and welfare definitions are often generalistic.

Given the limited understanding of honey bee mental states, encompassing cognition, behavior, intelligence, and experiences beyond nociception, it is mandatory to adopt the precautionary principle as outlined in the Terrestrial Animal Code. This principle supports preventing unnecessary suffering while meeting fundamental needs and it reflect in colony personalities and behavior, respecting the colony preferences evident in Dawkins's valenced states.

Since honey bees are managed by humans, their Welfare must be contextualized within managed agroecosystems and semi-natural environments, aligning with the One Health and One Welfare approach. Honey bee welfare can be defined as "a balanced and dynamic state in the most natural condition possible considering the managed context and the environmental condition, where the superorganism have the freedom to express their roles and preferences, meet their fundamental needs and adapt positively to variable external stress without enduring unnecessary suffering in order to return to their original state of equilibrium (homoeostasis)".

# BIOGEOGRAPHIC ORIGIN INFLUENCES THE PHYSICO-CHEMICAL PROPERTIES OF MEDITERRANEAN BASIN HONEYS

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## Abstract

The environmental qualities of the Mediterranean basin draw distinctive plant peculiarities. Thus, MEDIBEES has undertaken the study of the honeys of this region in order to recognize the patterns of differentiation between them. The aim is the possible definition of quality marks associated with geographical origin.

Honey samples are available from each of the partner countries (Portugal, Spain, Italy, Malta, Turkey, Jordan, Lebanon, Algeria) and collaborators (Egypt, UAE). The proposed sampling collects honey from all the biogeographical regions existing in each country. About 276 honeys have arrived at the CIAPA laboratory for analysis. The analytical work is carried out in Italy, Algeria and Spain. The consistency and comparability of the data obtained will be verified through interlaboratory tests. The physicochemical parameters will be assessed according to the methods proposed by the International Honey Commission (Bogdanov et al., 2002). The botanical origin and its pollen spectrum will follow the method proposed by Louveaux et al. (1978) with modifications adapted to each laboratory.

Orange blossom and eucalyptus honey are produced in Italy, Jordan, Portugal and Spain, while Sidr, Tinder thistle, cornflower and mesquite honey were found in Jordan. Humidity averages were higher in Portugal and Turkey (±16.50%) and lower in Jordan (←15.50%). Diastatic activity was higher in those from Italy, Jordan and Spain. Distinctly, honey from UAE has high electrical conductivity (1.40 mS/cm), pH (5) and free acidity (60 meq/kg), together with pfund of 120 mm and sugar content of 60%.

The results obtained so far are yielding interesting results, which will lead to the success of the stated objective. The study under development will bring added value to the Mediterranean beekeeping market.

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# HONEY BEE HEALTH STATUS SCREENING IN THE MAIN BEEKEEPING REGIONS OF THE KINGDOM OF SAUDI ARABIA

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## Abstract

Apiculture has a long-term tradition in the Kingdom of Saudi Arabia as well as in the Middle East. Beekeeping plays a key role in rural viability and the domestic demand for honey products is high. The majority of the bee hives are traditional, migratory beekeeping is still a widespread practice, and the indigenous honey bee subspecies, Apis mellifera jemenitica prevails in the colonies. To assess the honey bee health status in the five main beekeeping regions of the Kingdom of Saudi Arabia, we collected 134 adult honey bee samples from a total of 24 apiaries. From the samples collected, we screened the prevalence of various honey bee pathogens: parasites (Varroa destructor, Acarapis woodi, Lotmaria passim, Crithidia mellificae), fungi (spore counts of Nosema apis and Nosema ceranae), bacteria (Melissococcus plutonius, Paenibacillus larvae), and viruses (Acute Bee Paralysis Virus – ABPV, Deformed Wing Virus – DWV, Chronic Bee paralysis Virus – CBPV, Lake Sinai Virus 1 – LSV-1, Lake Sinai Virus-2 – LSV-2, and Black Queen Cell Virus – BQCV). Most of the apiaries tested positive for at least one investigated honey bee pathogen. In the samples collected, Varroa destructor, Nosema ceranae, and the honey bee viruses were the most prevalent. The high Varroa mite infection level could lead to the presence of ABPV and DWV, while nosemosis could be associated with CBPV infection.

# HONEY QUALITY, SAFETY AND GEOGRAPHIC TRACEABILITY: A PRELIMINARY INVESTIGATION ON ITALIAN CITRUS HONEYS

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## Abstract

Citrus honey (CH) is regarded as an exceptional monofloral apicultural product which, as well as other animal food product, must meet the quality, safety, and traceability rules set at EU level. This study, carried out within the PRIMA PLANT-B Project (grant number 1812/PLANT-B), aimed to assess the quality, safety and any honey characteristics linked to origin of CH samples from three Italian regions, playing a role in the national CH production. In total, 34 CH samples from the 2020 and 25 samples from the 2021 beekeeping seasons coming from project-based study cases in Sicily (38 samples) and in Campania (11), and from Sardinian (10) retail stores were analysed for free acidity, pH, colour, and electrical conductivity (EC). For all the samples, the trace elemental analysis targeted Pb, Cd, Hg, Cr, and As. Overall, the obtained results were in line with those reported for the same traits in other studies on Mediterranean CHs. Furthermore, all samples complied with the EU limits for moisture and EC (Dir. 2001/110/CE), and for Pb (EU Reg. 2015/1005). On the whole 2020-2021 sample set, there were significant differences due to the geographical origins, particularly between Sicilian and Campanian samples, regarding pH, colour, and Pb content ($p<0.05$), and between Sicilian and Sardinian samples as far as EC and Cd content ($p<0.05$). However, testing the geographical origin×year interactions, significant effects ($p<0.05$) were found on the Cd and Pb contents and on the EC values, thus limiting the suitability of these parameters for the univocal geo-traceability of the CH samples studied. In conclusion, the samples analysed met the EU honey quality standards and, with an eye towards future researches, other elements are under study for their discriminatory potential among CH samples coming from the three Italian regions.

Keywords: Italian honey; monofloral honey; food quality and safety; traceability

# HONEYBEE COLONY LOSS MONITORING IN LITHUANIA: THREE-YEAR RESULTS

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## Abstract

Every year, the beekeeping sector suffers from the loss of honeybee colonies during the winter season. The international network COLOSS (Colony Collapse Occurrence Prevention and Safety), which brings together scientists, veterinarians, and agricultural consultants seeks to understand the causes and trends of this phenomenon through one of its core projects, the Monitoring of honeybee colony losses.

The participating countries conduct a large-scale survey of beekeepers to assess the losses of the previous year overwintering of bee colonies and the factors that may have influenced it. Each spring, beekeepers, as citizen scientists, use the LimeSurvey questionnaire to provide data on the number of their honeybee colonies, the condition of the colonies, the treatment they have used for Varroa mite control, and any other information that may be relevant to determining the causes of colony losses.

Lithuanian beekeepers first participated in the Monitoring survey in 2013 and again in 2016, however the data was not analysed in detail due to insufficient number of respondents. The Lithuanian beekeepers' survey was relaunched in 2021 with ~2% of beekeepers now participating in the survey each year. In this presentation, we will look in more detail at the survey results in Lithuania over the last three years, showing that colony losses during overwintering have varied between 8.1% and 13.9%.

# POLYANDRY AND INFESTATION LEVELS OF SMALL HIVE BEETLE (*AETHINA TUMIDA*) IN NATIVE AND INVASIVE POPULATIONS

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## Abstract

Increased population density may lead to higher levels of polyandry by reducing costs of mate finding. However, the importance of polyandry is poorly understood for most invasive species, including the small hive beetle (*Aethina tumida*, hereafter referred to as SHB), is a parasite of honey bee nests. The recent discovery of polyandry in SHB in the USA raises the question whether polyandry levels differ across native and invasive populations, and whether polyandry may correlate with population density. Here, we investigated SHB polyandry levels and population density across native (South Africa, Nigeria, and Kenya) and invasive (Brazil, Australia, Italy, and USA) populations. From each location, 8-12 field-caught SHB females and up to 25 laboratory-reared offspring from each female were genotyped at 13 DNA microsatellite loci to estimate the number of mates for each female. Population densities at their apiaries of origin were recorded during colony inspections. Initial results confirm polyandry in Italy, Australia and South Africa in addition to the USA. This study will enhance our knowledge of mating behaviour in the invasive SHB and lay the foundation for understanding the role of polyandry in biological invasions.

# PROFITABILITY OF APICULTURE IN FINLAND

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## Abstract

Finnish Beekeepers' association and the Statistical services of Natural Resources Institute Finland have in co-operation collected economical information from beekeeping companies since 2013. In 2024 data from 15 companies will be collected. All companies have more than 15 beehives. Data collected includes data of income, expenditure, number of hives, honey yield, working hours in apiculture, etc. All participants get a report of their data. Results are also presented in the Economy doctor portal: www.luke.fi/economydoctor/beekeeping.

# QUALITY ASSESSMENT OF HONEY FROM CENTRAL ITALY: A MICROBIOLOGICAL AND CHEMICAL INVESTIGATION

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## Abstract

In honey production, several sources can introduce microbial and chemical agents adverseing to quality and safety of the product. This study examined 21 honey samples from Central Italy (Gubbio, Umbria region) collected in 2018, in terms of microbial profile and trace elements (Pb, Cd, and As) occurrence. Regarding the microbiological contamination, only one sample had a microbial load exceeding 10 CFU/g, but *Pseudomonas aeruginosa*, *Escherichia coli*, and coliforms were not detected. For yeast determination, only one sample presented a value of 160 CFU/g, a value significantly higher than the average (32.0 ± 41.9 CFU/g) of the sample set. The moulds analysis did not produce detectable results on all the samples. The results confirm that honey does not provide suitable conditions for the development of microorganisms, especially due to high sugar content, low moisture, acidity, and glucose oxidase activity. Regarding the undesired trace elements, the results for Pb (0.06 ± 0.06 mg/kg) were compared with the Maximum Residue Limits (MRL) established by the EU Regulation 2015/1005 (0.10 mg/kg). Only three samples (0.11 - 0.13 mg/kg) exceeded the MRL for Pb. As far as the arsenic content (0.058±0.202 mg/kg), only four samples showed levels higher than the average found in the literature (0.007±0.002 mg/kg). On average, the Cd content (0.006±0.005mg/kg) was higher than the levels reported in the literature (0.004±0.004 mg/kg). Based on a GIS analysis, no clear relationships were found between the results and the degree of anthropization of the apiary areas within a radius of 1-2 km. It suggests that numerous, and sometimes unpredictable, factors complicate the occurrence of contaminants in honey. However, geolocation could still play a crucial role in minimizing the risk of marketing products that do not meet the highest quality standards expected for honey.

# SUSTAINABLE BEEKEEPING NETWORK: STRENGTHENING FAO-OMAN AND IZSLT PARTNERSHIP

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## Abstract

Omani honey-production has recently increased due to Government efforts, but still faces several obstacles such as lack of innovation, adoption of good beekeeping practices and laboratory training for honey quality control. The Omani Ministry of Agriculture, Fisheries and Water Resources (MAFWR), together with the Food and Agriculture Organization (FAO) in Oman, decided to develop a cooperation program in collaboration with the Istituto Zooprofilattico Sperimentale Lazio e Toscana “Mariano Aleandri” (IZSLT), that is FAO Reference Centre for Animal Health and Food Security – Discipline Apiculture, Health, and Biosecurity. The outcomes of this project included creating a network between researchers and technicians from Italy and Oman, improving the capabilities of laboratories in bee health and honeybee products analysis. Moreover, a “*Manual on general good practices and specific biosecurity measures in the Omani context”* was written to improve the technical capabilities of beekeepers. The *“Manual”* was organized into multiple sections concerning: the peculiarities of Omani apiculture, constraints and issues facing beekeeping, pests and diseases, human disturbance, laws, general Good Beekeeping Practices, specific Biosecurity Measures, and outlooks. Moreover, to improve the Omani laboratory capabilities both theoretical lessons and practical activities were organized including two training visits between the two countries, one in Oman (in March 2023) and one in Italy (in February 2024). Objects of the training: flower structure and pollination, nectariferous and non-nectariferous plants, morphology of pollen grains, melissopalynological and palynological preparation of slides and analysis, structure and microscopic identification of flower and honey pollen, nomenclature, percentage count, pollen spectrum, types of honeys and their properties and pollen composition, sensory analysis, identification of the botanical and geographical origin, palinoteca setup. Upon completion of the program, an examination was administered to assess the acquired knowledge and skills of participants.

# THE USE OF BUMBLEBEES AS POTENTIAL POLLINATING AGENTS IN VERTICAL FARMING

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## Abstract

Vertical farming (VF), the cultivation of plants in multiple layers in climate-controlled environments supplemented with artificial lights, is reforming modern agriculture. Although VF technologies offer promising contributions within sustainable plant production frameworks, they are constrained by high labour and development costs. They are also limited to cultivating crops or varieties that do not require insect pollination, or that would necessitate manual or wind pollination, further increasing costs. This ongoing research explores the feasibility of insect pollination in VF to moderate labour costs and produce high-quality products. Buff-tailed bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), were introduced to a climate-controlled room with artificial lighting, simulating a VF system. Basil plants were placed under different light treatments in two tests. The first test exposed the bumblebees to the indoor environment using monochromatic lights of full white, red, blue and green to assess their response and behaviour and whether they effectively visit the flowers under each treatment. The second instead, presents a choice test using combinations of lights commonly used in VFs: red and blue wavelengths (RB) in a ratio of 3, RB in a ratio of 3 with an addition of green, RB in a ratio of 3 with an addition of white and full white. This test will determine which spectra are most conducive to maintaining flower visitations and pollination services. The current findings suggest that bumblebees could be a helpful resource in VF but, to eventually be integrated within an active VF, the protocols need much refinement. Adding UV into the light treatments could be a solution to stimulate flower visitations. Given the paucity of research related to this topic, the limitations, practical considerations and suggestions on optimising the methods and experimental design for prospective research are also discussed.

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