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# COLLOQUIUM ASIA OKINAWA & Mini-Symposia

## ABSTRACT BOOK

Hosted by the Okinawa Institute of Science and Technology  
Ecology and Evolution Unit



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# ABSTRACT OIST Mini-Symposium 22-24 March 2021

Amy Paten, PhD  
Postdoctoral Research Fellow, CSIRO, Australia

**Brief Bio:** I am a postdoctoral Research Fellow in Environmental Systems Biology at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in the Land and Water business unit – in Australia. Previous experience working in nutrigenomics in livestock before making the switch into honey bee health and environmental systems biology.

**Research Interest:** I am particularly interested in how organisms and microbial communities respond to stressors in their environment and using multi-omics and machine learning approaches to better understand molecular mechanisms, predict responses and develop interventions.

**Keywords:** systems biology, ecotoxicology, honey bee, multi-omics, neonicotinoids

## Honey bee ecotoxicogenomics

Ecotoxicogenomics is an emerging discipline that aims to link phenotypes to environmental perturbations through characterizing multi-omic changes in biomolecule profiles. This presentation explores the value of using a multi-omics approach to ecotoxicology and honey bee health. We exposed larvae to sublethal concentrations of a commonly used neonicotinoid pesticide (imidacloprid) and characterized the resulting molecular changes through integration of transcriptomic, proteomic and metabolomic data. This approach enabled us to connect molecular events to developmental and metabolic impacts of sublethal pesticide stress in larvae that may have longer term consequences for honey bee colony health.

Nurit Eliaash, PhD  
Postdoctoral Researcher, Okinawa Institute of Science and Technology, Japan

**Brief Bio:** I am a Postdoctoral researcher at the Ecology and Evolution unit, OIST, Japan, and a fellow of the Program for Outstanding Postdoctoral Students of the Council for Higher Education, Israel. My main research interest is host - vector - pathogen coevolution, specifically in the tripartite system of bee - Varroa - virus interaction. I completed my PhD. at the Hebrew University and the Agricultural Research Organization (ARO), at the Soroker Chemoecology Lab. Combining chemical ecology, molecular biology, omics and bioinformatic approaches, I am investigating the interaction between the honeybee, Varroa and its vectored viruses, aiming to reveal the mechanisms underlying host-parasite coevolution.

**Keywords:** Varroa, viruses, vector-pathogen interaction, RNAi silencing, network analysis

### Varroa mite genes interact with its viruses' load

Co-authors on this work: Nurit Eliaash\*, Miyuki Suenaga and Alexander Mikhayev  
\*Lead Author

Pathogenic honey bee viruses are in large part responsible for worldwide honey bee population collapses, threatening global food security. Varroa plays a key role in the spread of viruses and without it viruses are typically present at low non-lethal levels. However, little is known about the interaction between the viruses and the mites. Here we studied the interaction between varroa gene expression and the load of several viruses it carries.

We used a network analysis approach to identify groups of genes that share a similar expression pattern across a large set of available varroa transcriptomic data (RNAseq). Correlating these co-expressed genes groups to Varroa viral load, we detected biological processes in Varroa that interact with specific viruses, mainly of the Iflavivirus family. Interestingly, the nature of the interaction depends on the virus type: bee-pathogenic viruses (Deformed wing viruses type A and B) correlate in the opposite direction to that of Varroa specific viruses (such as Varroa destructor virus-2). To experimentally validate these predicted interactions, we silenced specific genes that are central players in the Varroa-virus correlations (using RNAi). We found that reduction in Varroa gene expression was accompanied by a change in viral load. These findings suggest that Varroa does interact with specific viruses and shed light on its role as a vector of bee-diseases.

in knowledge of social immunity \ detoxification.

andApis populations \ species with and without health issues and the apparent major gaps landscape level. Focus should probably be given to a more systematic comparison of areas BEEBOOK) into joint concerted large-scale experiments ranging from cell cultures to the issues to be tackled, I herewith suggest doing globally beyond agreed protocols (aka

and time may provide false negative evidence. In light of the apparent complexity of the Accordingly, individual laboratories just cannot cover all aspects and snap-shots in space honeybee colony ranging from individual bee, over caste to colony and population level. instead multiple possibly interacting mechanisms are governing the overall health of any in both space and time for any given mechanism. It is further apparent that not a single, but complex interactions between (too) many known and unknown factors as well as variation products) are debated, but a consensus has not been reached. This is probably due to nutrition and beekeeping management (incl. breeding, import\export of bees and hive solutions are still not at hand. Moreover, the roles of other pathogens, agrochemicals, agreement that ectoparasitic mites and their associated viruses are key players, sustainable losses are variable in both space and time for so far unknown reasons. Even though there is losses in many areas globally. Coordinated surveys ( [www.coloss.org](http://www.coloss.org)) revealed that these global attention. For over a decade, beekeepers are noticing unsustainably high colony It is evident that the uncertain health of managed honey bees, Apis mellifera, requires

## A holistic view of global honey bee health

**Keywords:** apiculture, evolution, host-pathogen, social insects, xenopioitics

please refer to his google scholar.

of COLOSS (prevention of honeybee Colony Losses, [www.coloss.org](http://www.coloss.org)). For further details, Department of Zoology and Entomology, University of Pretoria, South Africa and President Institute of Bee Health, Vetsuisse Faculty, University of Bern; Extraordinary Professor, ecotoxicology. Peter currently is Foundation-Vincent Prof. of Bee Health; Director of the species on all continents except Antarctica focusing on honey bee pathology and (love) to honey bees. Over the past 30+ years, he has worked with various social insect fascinated by social insects since his childhood and eventually moved from ants (his first Brief Bio: Peter Neumann has a background in chemistry, ecology and evolution. He is

Institute of Bee Health, University of Bern, Switzerland  
Peter Neumann, Prof. Dr.

**Assistant Professor, Purdue University**  
**Brock A. Harpur, PhD**

**Brief Bio:** Brock Harpur is an Assistant Professor in the Entomology Department of Purdue. Brock arrived to Purdue after completing a National Science and Engineering Research Council Postdoctoral Fellow at the Donnelly Centre, University of Toronto. His work explores the evolution and genetics of honey bees. Brock completed his PhD on population genomics of honey bees at York University. Brock has been awarded the prestigious Julie Payette Research Scholarship from the National Science and Engineering Research Council, an Ontario Graduate Scholarship, the Entomological Society of Canada's President's Prize, and was an Elia Research Scholar during his time at York University.

**Keywords:** Genomics, evolution, immunity, sociality, polyandry

## **Would a gene drive work in Varroa destructor?**

Co-authors on this work: Nicky Faber\*, Yoni Meibord, Gus MacFarlane, and Gredor Gorjanc  
Lead Author

The greatest threat to honey bee populations and the beekeeping industry globally is Varroa destructor (Varroa mites). Since their introduction into the United States in 1987 and their subsequent spread, Varroa mites have been responsible for the loss of millions of colonies and the near extinction of feral honey bee populations. Beekeepers have the option to treat their colonies with acaricides; however, mites are evolving resistance to some of these treatments and others harm honey bees. New targeted methods are needed to cope with Varroa mites. Genetic pest management tools such as CRISPR/Cas9-based gene drives may provide one option. Gene drives increase the probability of a specific allele being inherited in the offspring of gene drive carriers. By 'driving' alleles that reduce the fitness of homozygous females or males, it is possible to dramatically reduce the size of target populations. Such systems have been successfully modelled and tested in other arthropods. Here, we test a model of CRISPR/Cas9-based gene drives for Varroa mites to understand how realistic such a system would be given the unique biology of Varroa mites and predict which gene drive system and management practices would maximize the effectiveness of spreading within a colony and an apiary.

Dennis vanEngelsdorp, PhD  
Associate Professor, University of Maryland

**Brief Bio:** Dennis, an Associate Professor at the University of Maryland, has a broad interest in pollinator health. The focus of his current work involves the application of epidemiological approaches to understanding and (importantly) improving honey bee health. Dennis is the past and founding president of the Bee Informed Partnership (BeeInformed.org) which attempts to provide a platform to collect "big data" on the state of managed honey bee colony health. Analysis of these data is providing important insights into the role management practices and environmental factors (such as landscape, pesticides, and climate) have on colony health.

Dennis was born in the Netherlands but emigrated to Canada when he was two. He grew up on the outskirts of Toronto before completing his undergraduate and master's degrees at the University of Guelph. His undergraduate degree was in Horticulture and International Agriculture. While at Guelph, he took an undergraduate beekeeping course. Beekeepers have a saying "once stung – you know you are a beekeeper or not – it gets in your blood". That was certainly the case for Dennis, who ended up doing a master's degree on Honey Bee Health as a result of that introductory class.

Dennis has traveled extensively studying and speaking on bee health. His lab helps run several honey bee health monitoring programs including US national honey bee disease survey. His work has been featured in numerous documentaries, print and electronic media (including a story in the New Yorker and Time magazine), and he has given a TED talk ([https://www.ted.com/talks/dennis\\_vanengelsdorp\\_a\\_plan\\_for\\_bees](https://www.ted.com/talks/dennis_vanengelsdorp_a_plan_for_bees)).

## Varroa – challenges in management

Varroa are considered the most important threat to honey bee health globally. In the US the impact of this mite is considered by beekeepers and researchers alike as the leading cause of high rates of mortality documented over the last 13 years. While several Varroa treatment strategies are available, their utility is often less than required and often – less than advertised. Some of these inefficiencies are the result of varroacide resistance development. Treatment failure, however, is not explained by resistance development alone. Rather the movement of mites across the landscape, between colonies and apiaries also drives mite population growth in colonies. Fortunately, mite migration may be managed with inexpensive modifications such as robbing screens.



declines.

varos and viruses are complex with multiple proximal factors possibly leading to observed significant, but relatively minor. However, the arrival of Varos has increased the number of found that the effects of bee genetics on viral levels and community composition to be investigate their genetic background as well as to uncover viral population dynamics. We while investigating the effects of genetic background. RNA-seq of the bees allowed us to examine changes taking place in populations over the time course of early varos infestation varos mites arrived near-simultaneously in each. As a result, these samples allow us to Africanized bees indeed arrived in each population, though at different times. By contrast Africanized honey bees, with samples from feral colonies cryogenically frozen. The Department of Agriculture between 1988 to 2001 in order to monitor the arrival of populations, in Mexico and the southern US. These populations were sampled by the US investigated this process using a unique historical time series data set where from three bee vector deadly viruses, making them an excellent model to study these processes. We have faced numerous anthropogenic stressors, including the arrival of varos mites that host genetics affects the progression of disease remains poorly understood. Honey bees populations must resist the arrival of new parasites and diseases or face extinction. How

### Bees facing Varos and viruses over space and time: a historical study

**Keywords:** Social insects, genomic, evolution, honey bees, host-parasite-virus interactions

**Brief Bio:** Sasha started his research as a bee behavioral biologist at Cornell, publishing his first papers with his friend and mentor, Tom Seeley. After a decade in the wilderness, studying mostly ants, Sasha re-engaged with bees, their parasites and diseases thanks to an Australian Research Council Future Fellowship, which brought him to the Australian National University.

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 Adjunct Professor, Okinawa Institute of Science and Technology, Japan  
 Alexander (Sasha) Mikhayev, PhD

thought to be the most important issue confronting Japanese beekeeping. going to introduce Japanese beekeeping, focusing on the problem of bee diseases, which is mites, malnutrition, lack of flowers, as it does in other countries. In this symposium, I am still faces many problems in Japan, e.g. damage due to insecticides, bee diseases, parasitic imported honey, and the demand for honey bees as pollinator remains steady, beekeeping strawberry. Even though domestic honeys are traded in the market at higher prices than than 200,000 bee hives have been introduced into greenhouse production of crops such as Honey bees are used as pollinators for production of fruit such as apple and plum, and more to be very important in agriculture due to the role of honeybee as pollinator in horticulture. consumed in Japan is imported from abroad. Nonetheless, in Japan beekeeping is thought local governments, and most of them keep less than 100 hives. In addition, most honey Beekeeping is not a large industry in Japan. Only about 2,000 beekeepers register with

## **The current status of beekeeping in Japan, focusing on the influence of bee diseases on Japanese beekeeping**

**Keywords:** Beekeeping, honey bee diseases, insecticides, genome analysis and editing

research. am convinced that these are very important technologies for the advancement of honey bee currently focusing my efforts on research related to genome editing and genome analysis. I mainly been studying the honey bee diseases and damage caused by insecticides. I am have conducted research in various fields related to honey bees. In recent years, I have continue to work as a contract researcher at the same research institute. As a researcher, I engaged in research on honey bees and beekeeping. I retired in March last year, but I the research institute of the Ministry of Agriculture, Forestry and Fisheries, I have been **Brief Bio:** I started my career as a researcher in *Drosophila* genetics, but ever since I joined

Fisheries, Japan  
Researcher at the research institute of the Ministry of Agriculture, Forestry and  
Kiyoshi Kimura, PhD

Mari Ogihara, PhD  
Researcher at the Institute of Livestock and Grassland Science, NARO, Japan

**Brief Bio:** Mari H. Ogihara, PhD, is a researcher of the Institute of Livestock and Grassland Science, NARO in Japan. Her areas of research interest are physiology of Varroa mites and honeybees, interaction between Varroa mites and honeybees. In addition, she is working on nationwide surveillance of Varroa damage in the Japanese aparies to understand actual situation of Japanese aparies. Dr. Ogihara majored in physiology of Acari and received bachelor's degree, master's degree, and PhD in agriculture from Tsukuba University, in 2004, 2006, and 2009, respectively. She worked at Tsukuba University as a JSPS research fellow (tick physiology) and worked at Tokyo University as a post-doc and a JSPS research fellow (insect physiology).

**Keywords:** Varroa destructor, Apis mellifera, Apiculture, Acari physiology

## Surveillance and genetic characterization of Varroa mites in Japan

Varroa destructor is an important pest of Western honeybee Apis mellifera in Japan. Damage of honeybee by V. destructor infestation is well-recognized in Japanese beekeepers, but intensive surveillance of the mite has not been conducted in Japan. Our group conducted surveillance of V. destructor from 2018 in Japanese aparies. We focused on three perspectives: Varroa infestation rates in A. mellifera colonies, genetic backgrounds and possibilities of acaricide resistance of V. destructor in Japan. V. destructor was detected from over 90% of investigated aparies in Japan. The analysis of genetic background showed that spreading a single haplotype of V. destructor, called Korea haplotype, among A. mellifera colonies in Japan. Though earlier studies detected another haplotype, called Japanese haplotype, from A. mellifera, no mites of Japanese haplotypes were detected in recent surveillance of A. mellifera colonies. In addition, Japanese haplotypes of V. destructor were rarely detected from Japanese honeybee, A. cerana, indicating Korea haplotypes of V. destructor become a dominant haplotype in Japan. The surveillance also showed that the possibility to develop acaricide resistance in V. destructor among Japanese aparies. Fluvastatin is one of the permitted acaricides for V. destructor control in Japan. We found some of Varroa mites in the Japanese aparies has a specific amino acid substitution in a fluvastatin-targeted sodium channel, which substitution has been known to confer fluvastatin resistance. These results reveal actual situation of Varroa infestation in Japanese aparies and indicate the need of more effective Varroa control for Japanese aparies.

Réunion. Such as its interaction with other pathogens on the indigenous honeybee are on going in La Réunion. More precise observations on the dynamics of colonisation of this ectoparasite, we found high mortalities were in Madagascar, Mauritius and La Réunion after N. destructor observed in the Seychelles, not colony mortality were registered, the only territory where no P. larvae were detected in the 2WIO area. Despite the high prevalence of DWV different islands. *Vosema ceranae*, BQCV, CBPV and DWV were detected in each country, CBPV, DWV, KBV and SBV. Different pathogen patterns were observed within the larvae), 2 *Nosema* species (*Nosema apis* and *N. ceranae*) and 6 viruses (ABPV, BQCV, Tropilaelaps sp. and Braula sp.), 2 bacteria (*Melissococcus plutonius* and *Panurginus* (wherever possible). The pathogens surveyed included 3 ectoparasites (N. destructor, destructor invasion such as the diversity of other pathogens before and after its invasion conducted several epidemiological and mortality surveys to evaluate the evolution of N. Mauritius (2012), and most recently in La Réunion (2017). Since its arrival we had pollination. In 2010, the invasion of Varroa destructor was reported in Madagascar, then in that A. m. unicolor significantly preferred native flora and could play a major role in its further ecological studies (interaction with the native flora) made in La Réunion suggested proving its indigenous status in the Mascarenes, Seychelles and Comoros archipelagos. differentiation of this subspecies in the surrounding islands before the Human colonisation, Our recent genetic (mitochondrial and nuclear) and morphometric analyses supported the unicolor (African lineage) has ancestrally been described as the honeybee of Madagascar. biodiversity with high rate of endemism where the endemic subspecies *Apis mellifera* The South West Indian Ocean (2WIO) archipelagos and Madagascar constitute a hotspot of

Johanna Clémence

Henriette Rasolofiariso, Tsiory Rasolofiariso, Julien Galateau, Benoit Jobart, Nicolas Blot, Co-authors on this work: Delatte Hélène\*, Lepretre Gérard, Esnault Olivier, Maéva Techer,

## An overview of the honeybee genetic diversity and health in the Indian Ocean Islands

**Keywords:** Ecology, biology, population genetics, evolution, honeybee health.

research on honeybee-Varroa interactions in Réunion island. the islands of the Indian ocean, she is currently based in Madagascar, but also develop and their interaction in their environment. She has worked on several arthropod models of honeybee indigenous of the Indian Ocean; *Apis mellifera unicolor* and the carpenter bee) their settlements in new areas; ii) on bioecology and genetic diversity of pollinators (the knowledge i) of the ecological and genetical factors triggering invasive arthropod pests and insect ecologist and population geneticist. She dedicated her research questions to the in the French research center of CIRAD since 2008 in the UMR PVBMT unit. Helene is an the University of Wageningen in 2005, and her HDR in 2013. She has a permanent position at the University of Wageningen from her PhD in virology and population genetics at

Researcher at CIRAD – Réunion Island (based in the moment at Madagascar)  
Helene Delatte, PhD, HDR

**Robert Paxton, Prof. Dr.**  
**Chair of General Zoology, Institute for Biology, Martin Luther University Halle-**  
**Wittenberg, Germany**

**Brief Bio:** Prof. Dr. Paxton is a graduate of Sussex University, where he undertook a PhD on sex ratios in solitary wasps in 1985, Robert subsequently held postdoc positions at Cardiff University, researching bees and pollination, Uppsala University, working on bee population genetics, then the University of Tübingen (Germany), researching the genetics of social evolution. In 2003 took up an academic post at Queen's University Belfast from where, in 2010, he moved to the University of Halle (Germany), to take up a chair in zoology and where he focuses on insect evolutionary ecology, with a taxonomic focus on bees.

**Keywords:** social evolution, host-parasite relations, pollination, conservation genetics, bee

## **The dynamics of Deformed wing virus genotypes in Varroa-infested honey bee colonies**

The ectoparasitic mite *Varroa destructor* is widely recognised as the scourge of beekeeping with *Apis mellifera* across the world. Colony mortality of *Varroa*-infested colonies is often attributable to infection by viruses that the mite transmits, particularly Deformed wing virus (DWV). The originally described DWV genotype A (DWV-A) has been shown to have spread across the world, likely accompanying the dispersal of *Varroa* mites. A second genotype B (DWV-B) has more recently been described, a genotype that we have shown to be apparently more virulent in adult honey bees than DWV-A. Evidence points to its rapid worldwide spread as well as its marked increase in prevalence, particularly in the UK. Here I present evidence from my own group and others that tests the hypothesis that DWV-B may be spreading, potentially at the cost to DWV-A, with grave consequences for honey bee health in the coming years.

Emily Remnant, PhD  
Lecturer and Academic Fellow, University of Sydney

**Brief Bio:** Dr Emily Remnant is an Academic Fellow and Lecturer in the School of Life and Environmental Sciences at the University of Sydney. Her research investigates evolution in action, using genomics to explore how insects respond to strong selective pressures like parasites and pesticides. She is particularly interested in investigating novel ways to improve the health of beneficial insects like honey bees by examining host-parasite interactions, virus discovery and evolution and honey bee immune responses to viral disease.

**Keywords:** Virus evolution, antiviral immunity, honey bee health, host-parasite interactions, RNA interference

### **Virus silencing in honey bees and their mites**

Viruses are some of the most common pathogens contributing to declining honeybee health worldwide. Since the emergence and global spread of the parasitic mite *Nurse* destructor, one of the most striking observations has been the concurrent increase in virus levels in honeybee colonies. *Nurse* provides an alternate route of transmission as a mechanical vector, enabling virus particles to directly breach the cuticle of developing bees and replicate to higher levels. Some evidence also exists to suggest that viruses can actively infect mites, facilitating increased transmission via biological vectoring. To better understand the role of *Nurse* as a vector, we investigated if honeybee viruses actively replicate in *Nurse* by determining whether they trigger the mite's antiviral immune response. Using deep sequencing of small RNA in honeybees and *Nurse*, we examined the RNA interference (RNAi) pathway, a major antiviral immune pathway in insects. We find that multiple honeybee-infecting viruses are actively silenced in *Nurse*, however mites show distinct degradation profiles that differ from the profiles observed in bees. Our results indicate that viruses are silenced differently between bees and their mite parasites and point to alternate viral RNAi pathways within these two *Arthropod* lineages, potentially identifying a novel target that may be exploited in *Nurse* biocontrol. Characterising the antiviral response of the honeybee's most significant parasite will contribute to a better understanding of the role of this vector in the evolution and spread of honey bee viruses.

Tetsuro Inamoto, D.V.M. PhD  
Kyoto Prefecture Livestock Division, Department of Agriculture, Japan

**Brief Bio:** My name is Tetsuro Inamoto and I'm a veterinarian. I work for Kyoto Prefectural office, Japan. While at work, I have been a guest researcher at Kyoto Gakko University since 2017. I'm interested in the infectious diseases of honeybees and pathological analyses. I'm good at observing the cells microscopically. In my free time I enjoy and learn Japanese tea ceremony.

### **New method of injecting solution into the abdomen to overcome fixation delay of the midgut on making the histological specimens of honeybee worker**

Few histological studies have been performed to evaluate infectious diseases in honeybees as their water-resistant exoskeletons make the preparation of whole-body specimens difficult by blocking the permeation of fixative and substitution solutions. Additionally, it remains difficult to fix samples when specimens are collected far from the laboratory, as delays in fixation lead to autolysis of abdominal organs. In this study, we propose an intraperitoneal fixative injection method forApis mellifera to overcome these problems. Particularly our method is to inject 30μL fixative solution (3:2 solution of 80% ethanol and Bouin's fixative) into the abdomen on the day of sampling. This method prevents fixation delay and provides honeybees' tissue specimens in good condition for histopathological examinations. The results of the injection were favorable by the microscopic observations of the midgut because microvilli were stained well and cellular components corresponding to the terminal web and tight junctions around cell boundaries were clearly distinguished. Besides, this method doesn't need a temperature control since the storage temperature after fixative injection didn't affect the results. We concluded this is a simple and effective method. This is the content which I have recently studied in Kyoto Gakko University. This study is published in Applied Entomology and Zoology (2020) 55: 321-324, introducing as a new method for preparing tissue specimens for histopathological examinations.

regulates patterns of social interactions that are at the root of honey bee colony performance. Our results suggest that the gut microbiota – brain axis of pairwise social interactions. The colony social network structure by increasing the frequency and altering the patterning of gene expression changes in the brain and no marked change in odorant profiles, it affects under laboratory conditions. We show that while the gut microbiota induces relatively small foraging, which we found to play a major role in honey bee physiology and behavior even honey bee. Our experimental design allowed us to control for the transition from nursing to automated behavioral tasking, we investigated the gut microbiota – brain axis in the through a combination of gene expression analyses, cuticular hydrocarbon profiling, and host, and the experimental tractability of its gut symbionts.

microbiota – host interactions due to the eusocial lifestyle and ecological importance of the honey bee presents an attractive and relevant model to study such aspects of gut changes in social responsiveness affect the emergent properties of animal societies. The whether these effects are widespread in the animal kingdom and how individual-level shown to increase host responsiveness to social stimuli. However, it remains unclear neurodevelopment and behaviour. In vertebrate model organisms, gut symbionts have been The gut microbiota influences many aspects of animal physiology including

## The gut microbiota affects the social network of honey bees

**Keywords:** Microbiome, honey bee host

approaches to further our understanding of microbiota-host interactions. This allows us to combine bioinformatics and genomics with experimental their gut microbiota is evolutionarily conserved, functionally relevant, and experimentally tractable. Social bees are our favorite study system as the ecology and evolution of host-associated microbiomes and to unravel the molecular the University of Lausanne, Switzerland. My major research interests lie in understanding the Department of Fundamental Microbiology at the University of Lausanne, Switzerland

**Brief Bio:** I am an Associate Professor at the Department of Fundamental Microbiology at the University of Lausanne, Switzerland. My major research interests lie in understanding the ecology and evolution of host-associated microbiomes and to unravel the molecular

Philippe Engel, Prof.



native honey bees in both individual and social levels. pathogens and parasites in different species of Apis and resistance mechanisms of the more resistant than A. mellifera. In this context, our team has studied the epidemiology of honey bees are also affected by microbial diseases and parasitic mites but appear to be Southeast Asia. However, A. mellifera is prone to many diseases and parasites. The native replaced the eastern honey bee (Apis cerana) in agriculture in Thailand and in much of western honey bee. The western honey bee (Apis mellifera) has been introduced and Southeast Asia is a homeland to at least nine recognized honey bee species including the

### **When East meets West: The prevalence of pathogens and parasites in honey bees**

**Keywords:** Honey bee pathology, disease resistance, immune responses, host-parasite interactions

**Brief Bio:** Dr. P. Chantawannakul has worked on bee pathology for almost 20 years and collaborated with bee researchers at USDA (Baton rouge and Beltsville, US), University of Bern (Switzerland), National Institute of Livestock and Grassland Science (Japan), Chinese Academy of Agricultural Science (China), CSIRO (Australia), Free University of Bolzano (Italy), and Bulgarian Academy of Science (Bulgaria). Dr. Chantawannakul is co-Vice president and one of executive committee members of COLOSS network ([www.coloss.org](http://www.coloss.org)).

Department of Biology, Faculty of Science, Chiang Mai University, Thailand  
Panwan Chantawannakul, Prof. Dr.

such a data portal for the bee research community will be presented. During this presentation challenges to finance, develop and maintain 140 Apis hosts. Currently the data portal contains 11019 Sequences Read Archive (SRA) experiments for to all currently available metagenomic datasets involving bee-associated microbes. The BeeBiom Consortium established a publicly available online resource, namely the BeeBiom data portal ([www.beebiom.org](http://www.beebiom.org)), which provides an overview of and easy access

hinders integrative analysis. datasets are stored in different repositories and are often not readily accessible, which associated with bees and their link to bee health. The challenge of such approach is that datasets provides an unprecedented opportunity to obtain a global picture of the microbes each of these datasets by itself has provided important insights, the integration of such about the composition, genomic content, and dynamics of bee-associated microbes. While researchers around the world have collected a huge amount of sequencing ('omics') data and detrimental pathogens, with implications for bee health. Over the last few years, Bees can be colonized by a large diversity of microbes, including beneficial gut symbionts

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Rehavia2,3, Philipp Endels  
 Benjamin Dainat1, Valentine Rech de Lavall2,3, Vincent Doublet4, Marc Robinson-

## Challenges to get it done and long term maintenance The BeeBiom Data Portal: a web-based resource to facilitate bee microbiome research.

(<http://wp.unil.ch>) that developed the beeBiom data portal [www.beebiom.org](http://www.beebiom.org). bee microbiome and together with a core team is coordinating the BeeBiom consortium for few years at the Swiss Honey Bee Health Extension service. He is also interested on the Post doc at the USDA in Beltsville, Maryland and USDA before working as an extension specialist of Bern, Switzerland, performing his thesis at the Swiss Bee Research Centre. He did his Landesanstalt für Bienenkunde Baden Württemberg. He earned his PhD from the University Hohenheim in Germany; where he worked as scientific auxiliary at the Honey Bee Institute achieving his Masters in Agronomy specialized in animal science at the University of biology, population genetics and ecology at the University of Montpellier in France, before the breeding and selection section of the institute ([www.apis.admin.ch](http://www.apis.admin.ch)). He studied honey bee health, head of the national reference laboratory for bee disease and in charge of Brief Bio: Benjamin is a research scientist at the Swiss Bee Research Centre specialized on

Swiss Bee Research Centre,roscope, Switzerland  
 Benjamin Dainat, PhD

COLOSSA AIA OKINAWA 25-26 March 2021

# COLOSSA AIA

## JAPAN OKINAWA 2021



25 MARCH 2021

ONLINE SCIENTIFIC TALKS

26 MARCH 2021

BEEKEEPERS SEMINAR



Drawing by M. Techer

# Perspective of genome editing aiming at building the better strains of the honey bee *Apis mellifera*

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The Western honey bee, *Apis mellifera*, is an excellent pollinator utilized worldwide, and critically link to agricultural production. However, it is serious issues that threat of the honey bee health caused by viral infection, parasites, and exposure to chemical substances. One possible solution would be establishing healthy strains by breeding, although it is time consuming and laborious. The other option is introduction of beneficial traits by genetic manipulation. Recent development of a convenient and versatile genome editing technology, CRISPR/Cas9, in the honey bee enables to introduce a pin-point mutation in any target genes resulting in their functional alteration. The CRISPR/Cas9-mediated gene manipulation was, however, limited so far to functional analysis of genes that have biological interests. This technology could be used to bring beneficial traits into the honeybee. Since whole genome information of the honey bee is available now, genes to be targeted will be selected relatively easily.

Here, we demonstrate how to introduce a mutation (gene knockout) by using the CRISPR/Cas9 in the honey bee. As the initial step, we targeted three genes: the ommochrome biosynthesis enzyme kynurenine 3-monooxygenase (kmo), the major venom protein melittin (melt), and the ATP-binding cassette (ABC) transporter family scylet (st). A single guide RNA (sgRNA) targeted 20-base sequences of each gene and the Cas9 endonuclease protein were mixed to form ribonucleoproteins (RNPs). The RNP was microinjected into eggs or pre-embryos. More than 50% of G<sub>0</sub> larvae were somatic mosaics for kmo revealed by PCR and RFLP analysis, and several types of mutations (indels) were detected at the targeted site. Similarly, we found somatic mosaics of the G<sub>0</sub> queens developed in the colony when melt and st were targeted. After allowing oviposition to these mosaic virgin queens, the st gene-knockout G<sub>1</sub> drones were obtained. Trials to produce further generations are underway.

**Keywords :** genome editing, CRISPR/Cas9, breeding, beneficial trait

## What have we learnt studying Varroa destructor in Asia?

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The ectoparasitic mite *Varroa destructor* is the greatest threat to the survival of its novel host, the Western honey bee, *Apis mellifera*. In contrast, it causes little harm to its original host, the Eastern honey bee, *Apis cerana*, since these species share a long co-evolutionary history. However, this coevolution is poorly studied. To better understand the mechanisms underlying the balanced host-parasite interaction in this system, we combined behavioural, genetics and omics approaches to investigate the resistance mechanisms of *A. cerana* as well as host specificity and population dynamics of *N. destructor*.

The *N. destructor* K1-1/2 haplotype that shifted host to *A. mellifera* was found infesting *A. cerana* in eastern China. Microsatellite analyses and experimental infestation identified differences between mites infesting original and new hosts. The invasive lineage infesting *A. mellifera* shows a low host specificity since it reproduced on all host sexes and species tested. In contrast, all variants infesting *A. cerana* were only able to reproduce on drone brood of their original host. Natural infestations of *A. cerana* worker brood were rare and significantly less frequent than infestation of drone brood, except for the *N. destructor* K haplotype. The low occurrence of mite reproduction on worker brood, which constitute the main resistance trait of *A. cerana* is neither due to a lack of host attractiveness nor of reproduction initiation by the invasive parasite. Successful mite reproduction was instead prevented by abnormal development of the host. Adult *A. cerana* workers recognized this state and removed both hosts and parasites, which reduces the fitness of the parasite. Infestations of *A. cerana* colonies by multiple mite lineages did occur and led to hybridization. Gene flow was however unidirectional, from the host-shifted native mites, thereby setting the stage for speciation of the invasive lineage of *N. destructor*. Genomic and transcriptomic analyses identified a set of genes that might underlie host specificity and promote host shifts.

These results provide new insights into the coevolution between hosts and parasites in this system, and may contribute to mitigating the large-scale colony losses of *A. mellifera* due to *N. destructor*. Hybridization between shifted and non-shifted mites also indicates a potential threat to the original host of *N. destructor*.

**Keywords :** *Varroa destructor*; *Apis mellifera*; *Apis cerana*; coevolution; host specificity

# The untold story of Varroa mite successful invasion unraveled by worldwide population genomics

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**Keywords :** Varroa; global invasion; host switch; whole genome sequencing; population genetics

success could help us in predicting future outbreaks.

miters are continuously spreading over from Asian Apis to A. mellifera, our knowledge on N. destructor will help us to further elucidate the origin and evolution of N. destructor on its new host. As a parasitic least 10 genetic populations co-exist worldwide (nuclear genomes). I will explain how these results (metagenomics), (2) reveal previously undetected host switches (mitogenomes) and (3) show that talk, I will briefly present how from a single mitre library, we were able to (1) infer host information (Jacobson, N. Linderoth, N. Underwood and Varroa sp. type "Luzon" and type "Mindanao"). In my populations was confirmed and compared to genomes of sister species and non-switching miters (N. ~3.3 million variable sites throughout the genome. The identity of Varroa present on A. mellifera whole-genome sequencing of 1,350 V. destructor (including 32 miters from A. cerana), we found Varroa species on different hosts - and with a sampling period spanning from 1989 to 2021. Using worldwide. To date, the collection gathers 1,660 miters from 61 countries - including all known and native range and involved honey bee researchers, beekeepers as well as veterinary services build in the past five years. The sampling participation network covers most of N. destructor invasive advantages of our Vd3.0 reference genome and the unique Varroa mitre collection that we steadily To identify the key demographic and genetic mechanisms behind Varroa invasive success, we took

evolve new traits despite an initial loss of genetic diversity?

and independently develop novel traits such as acaricide resistance. How can this specialist parasite originated from two clonal strains. Yet, Varroa miters are persisting after 80 years of invasion genetic diversity following host switch led to the assumption that N. destructor invasive populations the most damaging pest in beekeeping. The high infestation in combination with an important loss of honeybees to the Western honeybee, it has successfully spread almost worldwide and is considered as introduced parasitic Varroa miters. Since Varroa destructor switched from its original host the Eastern In the last decades, Western honeybee populations all over the world are in continuous battle against

# gene expression analysis Establishment of a simple method for ex vivo honey bee cell culture capable of

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The honey bee, *Apis mellifera*, is one of the most important insects in agriculture as a pollinator of cultivated and natural plants, as well as a model insect for biological research. A colony decline is ongoing concerns for threatening the stable supply of pollinator worldwide, and one of the causes is infection of viral pathogens. The *in vitro* systems are the valuable tools to investigate the mechanisms of interactions between viruses and host cells. Therefore, development of honey bee cell culture has been attempted and several primary cell cultures derived from variety of tissues were developed. However, there are few reports on establishment of stable cell lines and development of system to introduce genes in cultured cells.

In the present study, we demonstrate a simple method of ex vivo cell culture system in the honey bee. Hemocyte cells obtained from late instar larvae could be cultured in a commercial insect medium more than two weeks in healthy conditions. An expression vector plasmid DNA which has the enhanced green fluorescent protein (eGFP) gene driven by the immediate early 2 (IE2) viral promoter was transfected to the cells by using commercial transfection reagent. The eGFP fluorescence was detected in one-third of cells in average. For gene functional analysis, gene silencing by double-stranded RNA-mediated RNAi in cell cultures has been performed exclusively using established continuous cell lines. It is possible to introduce dsRNA corresponding to a part of the eGFP gene into ex vivo cultured cells by both transfection and soaking. The eGFP gene expression was interfered with and the eGFP fluorescence was detected only in a small portion of cells. A convenient and high reproducible method of ex vivo cell culture fully practicable of gene expression assays was established in the honey bee.



# Application of thiamethoxam in mustard adversely affects honey bee colonies

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Bees, the important pollinators, have been reported to get affected by thiamethoxam used on mustard for managing sucking pests. To evaluate this, higher tier studies i.e. field trials were conducted by applying thiamethoxam 25WP (Actara®) at recommended (250 g a.i./ha) and double of the recommended dose (500 g a.i./ha) on Brassica napus (G2C7) following the standard guidelines mentioned in COL022 Beepack. At the recommended dose of thiamethoxam, adult bee mortality was very high (134.81-331.88 bees/colony) for the first three days which decreased thereafter and became statistically at par with untreated control on 8<sup>th</sup> day of application. Over a period of 10 days after application, there was an average loss of 1194 bees/colony. This affected the mean bee strength and nuptial honey hoarding in a colony. These parameters started increasing on 7<sup>th</sup> day of application (692 bee-days & 2787.41 g, respectively) and became significantly higher than pre-treatment on 14<sup>th</sup> day of application (819 bee-days & 3780.96 g, respectively). The mean nuptial honey hoarding did not decrease below pre-treatment. The mean nuptial worker brood also showed similar trend i.e. reduction upto 7<sup>th</sup> day of application (612.09 cm<sup>3</sup>/colony) which became statistically at par with pre-treatment on 14<sup>th</sup> day of application (688.20 cm<sup>3</sup>/colony) but the pollen hoarding decreased upto 14 days (290.30 cm<sup>3</sup>/colony) and on 21<sup>st</sup> day of application (372.79 cm<sup>3</sup>/colony), it increased significantly more than pre-treatment. The effect of double the recommended dose was more pronounced. Overall, it is concluded that thiamethoxam even at recommended dose had negative effect on A. mellifera colonies. Hence, farmers must not apply thiamethoxam on mustard blooms during peak activity of bees.

**Key words:** Apis mellifera, colony development, mustard, thiamethoxam

Ecological treatment of Varroosis: Brood interruption techniques and application of oxalic acid

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This study aims to compare the efficacy of oxalic acid combined with non-chemical treatments (biotechnical methods), as green caging and trapping comp, to control Varroa destructor infestations in honeybee colonies. A randomized clinical trial to determine the efficacy against Varroa of management or biotechnical practices as brood interruption (green caging and trapping comp method) associated with the application of an organic acid (oxalic acid 3%), over 40 days, in autumn, in 9 hives strongly affected in the island of Gran Canaria. Three colonies were randomly assigned to each one of the following three groups. **Group 1 (G1): Green Caging + treatment with oxalic acid (OA).** **Group 2 (G2): Trapping comp + treatment with OA.** **Group 3 (G3): Treatment with OA.** The Varroa treatment with oxalic acid, combined with brood interruption techniques, has a higher varroacide efficacy. In the clinical trial, an analysis of variance between the three experimental groups is carried out. The results show that the most efficient method is the applied in group 2 (trapping comp technique + OA). In this group, the level of infestation decreased from very severe to slight, and with an efficiency rate that varies between 81-91%. **G1:** Efficacy: 64%-67%. **G2:** Efficacy: 81%-91%. **G3 (Control):** Efficacy: 28-54%. The results obtained from this research allow, on the one hand, the development of more practices to be applied as varroacides, as organic acids, and, on the other hand, effective biotechnical controls with fewer adverse effects on the colony. The experiment revealed significant reductions in the level of infestation of Varroa in colonies with "trapping comp" method during 25 days. It is an option to alternate medicines authorized for organic beekeeping and supplement with methods that allow the application of treatment in the absence of breeding in order to achieve maximum effectiveness of treatment.

**Keywords:** Varroa, oxalic acid

# Study of the main causes of mortality of honey bee colonies Apis mellifera intermissa in Algeria

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The bee is an essential part of the environmental balance, it also has other interests such as the  
production of honey, propolis, royal jelly and wax. The aim of this study is to take stock of the  
main causes of mortality recorded among beekeepers. The study was carried out in the central  
region of Algeria known for its beekeeping vocation. 108 beekeepers in this region are surveyed  
using a questionnaire and field trips. For the detection of beekeeping pathologies in the  
laboratory, 89 samples are taken from all beekeepers in the regions studied. The diseases sought  
are: varroa, American foulbrood, nosemosis and some viruses.

The survey shows that most beekeepers report mortalities of more than 15%. The study indicates  
the presence in the colonies of a very high infestation rate by Varroa, the presence of foulbrood  
nosemosis and the virus of deformed wings. It is found that the maintenance of beehives by  
beekeepers is very low. Indeed, very few of them are those who protect their colonies from  
inclement weather and humidity, who respect the orientation of the colonies. All of these  
unfulfilled factors contribute to a weakening of the colonies. One of the most common causes of  
colony loss is the action of pesticides such as herbicides, fungicides and insecticides. Thus, 13%  
of beekeepers declare that they are victims of treatments carried out by farmers.

**Key words:** Honey bee, mortality, virus, Varroa, conservation

**Keywords :** Decontamination, residues, veterinary treatments, bees

treatment history in surrounding crops.  
Fenampiros, detected at lower concentration levels depending on their origin and pesticide compounds such as Propiconazole, Bromopropylate, Chlorpyrifos, Cypermethrin, Diazinon and products of Amitraz, frequently detected in beeswax. Similarly, it is efficient in removing further  $\mu\text{g/kg}$ , respectively. This process is also able to eliminate other residues, such as the transformation concentration ranges of 1800 – 2600  $\mu\text{g/kg}$ , 100 – 300  $\mu\text{g/kg}$ , 140 – 300  $\mu\text{g/kg}$  and 160 – 470 and other acaricides such as Coumaphos, tau-Fluvalinate, Chlorfenvinphos and Acrinathrin at reduce the beeswax contamination load by values of around 80 % – 90 % for veterinary treatments with methanol at 65 °C followed by an Adequation Process with water at 70 °C. This procedure can A decontamination process has been developed. The beeswax is treated by an Extraction Process

of the hive and affect the honey bee colony.  
mainly due to the use of recycled compds. Such residues may potentially migrate to the food reserves acaricides applied are lipophilic, non-volatile and lead to persisting of residues in the beeswax  
One of the biggest problems facing the beekeeping sector is the appearance of varroosis. Most of

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## Removal of veterinary treatments and other residues from beeswax

# **Tachyspermum ammi against Varroa destructor in Laboratory Tests** **Cymbopogon citratus, Eucalyptus globulus, Rosmarinus officinalis, Thymus** **Toxicity Effect of Five Essential Oils and their Components Derived from**

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The ectoparasitic mite *Varroa destructor* has become a major worldwide pest of the honey bee *Apis mellifera* L. Intensive use of various kinds of chemical acaricides against these mites has led to the development of the mites' resistance, thereby reducing the acaricides' efficacy and also causing contamination of hive products. Thus, natural products such as essential oils can become an important alternative to synthetic products in controlling *Varroa* mites. In our study, five essential oils and their components derived from *Cymbopogon citratus*, *Eucalyptus globulus*, *Rosmarinus officinalis*, *Thymus schimperi*, *Tachyspermum ammi* were investigated for its chemical composition and toxicity activity against *Varroa* mites through the complete exposure tests (laboratory test). Hydrodistillation of the leaves of these five plant materials yielded essential oil (T. ammi: 3.2% v/w; T. schimperi: 0.80% v/w; C. citratus: 0.62% v/w; E. globulus: 1.10% v/w; R. officinalis: 0.83% v/w). Analyzed by GC-MS, carvacrol, thymol, p-cymene, 1,8-cineole, citral and  $\alpha$ -terpinene were found to be the main constituents in the oils. In the toxicity assay, three oils (T. ammi: 4h-LC50=48.75  $\mu$ g/ml (2.44 mg oil/L Air volume); T. schimperi: 4h-LC50=109.16  $\mu$ g/ml (5.46 mg oil/L Air volume); C. citratus: 4h-LC50=586.49  $\mu$ g/ml (29.32 mg/L Air volume)) showed high acaricidal activity against *Varroa* mites. However, two oils from E. globulus and R. officinalis had a weak acaricidal activity. Among the main constituents, thymol (4h-LC50=70.71  $\mu$ g/ml (3.54 mg thymol/L Air volume)) and carvacrol (4h-LC50=106.10  $\mu$ g/ml (5.31 mg carvacrol/L Air volume)) induced high toxicity to *Varroa* mites. And citral (4h-LC50=322.51  $\mu$ g/ml (16.13 mg citral/L Air volume)) also possessed a mild acaricidal activity. However, p-cymene and 1,8-cineole were less effective in toxicity testing (4h-LC50 > 5000  $\mu$ g/ml). The results showed that thymol and carvacrol-bearing essential oils were highly acaricidal and can be a useful tool in controlling *Varroa* mite infestations.

## Beekkeeping industry in Iran: Current status and future directions

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Modern beekkeeping in Iran started in the late 1940s. Since then, it has played an important role as a source of food, employment, environmental conservation and supplementary income to a large number of rural, horticulturists, agriculturists etc. Today, Iran is one of the top ten honey producers in the world with over 100,000 tons of honey a year and 100,000 beekkeepers organized in more than 200 co-operatives throughout the country. Most of beekkeepers produced honey as their primary product until ten years ago. Then over the last decade, advancements in training programs, modern technical facilities and other fields made beekkeeping more efficient. As a result, other products such as pollen, royal jelly, beeswax and propolis took bigger parts in the beekkeeping economy. Despite the presence of many young beekkeepers, vast areas of rich floral and favorable climate which has made Beekkeeping as an ideal activity, there are many challenges facing Iranian beekkeeping industry, e.g., natural disaster, bee diseases, low honey production, predators, pesticides, malnutrition and high colony losses. This mini review discusses the current status of beekkeeping industry in Iran, focusing on the challenges and future directions.

**Keywords:** Iran, beekkeeping industry, Honey bee

**Keywords:** Evolutionary Biology; Host-Parasite interactions; Population genetics; Host jumps

bees globally. underlying host jumps ultimately helping to mitigate their impact on both managed and wild honey need for a better understanding of the biology of native Asian mites to decipher the mechanisms to their original host may threaten endemic Asian honey bees. Altogether, these findings stress the previously believed to be distinct species may be a single one. Finally, the spillover from *A. mellifera* introduction of alleles caused by multiple infestations further suggest that lineages of mites *N. jacobsoni*. The host jumps observed here indicate that such events have been underreported. of single colonies by two *N. jacobsoni* species and introduction of alleles between *N. jacobsoni* and Thailand. In Thailand, we also detected spillovers of mites from *A. mellifera* to *A. cerana* infestations contrast, native mites appeared to have jumped to *A. mellifera* colonies in the Philippines and were only infested by native mites and *A. mellifera* only by the invasive *N. jacobsoni* haplotype. In Vietnam and Thailand were compared. In Vietnam, strict host specificity was found, where *A. cerana* of mite populations infesting sympatric colonies of *A. cerana* and *A. mellifera* in the Philippines, sympatric populations of Eastern and Western honey bees. To do so, the origin and genetic structure nuclear and mitochondrial DNA analyses to investigate the host specificity of *N. jacobsoni* across species and haplotypes, such jumps may have occurred but remained undetected. We here combined provides opportunities for further ones. However, given morphological similarities between *N. jacobsoni* and *N. jacobsoni* have been reported, but the large *A. mellifera* stock exploited unintentionally in Asia *A. cerana* to imported Western honey bees, *A. mellifera*. Very few cases of successful the case in Asia after ectoparasitic mites, *N. jacobsoni* managed to jump from Eastern honey Lack of host-parasite coevolution can cause devastating effects after parasite host jumps. This was

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## with population genetics THERE AND BACK AGAIN – Deciphering the host specificity of *N. jacobsoni*

**Keywords:** beekeeping; honeybee health; Asia; Europe; Turkey

applications will be interpreted.

the scientist and beekeeper's approach. Different results of different approaches and current Nosema prevalence, bee nutrition, queen production, local races and ecotypes will be described with points between the two continents, transition of pathogens, migratory beekeeping, Varroa treatment, disease are even more susceptible to the second one. It is assumed that Turkey is one of the crossing life affect the biology of bees and make them susceptible to diseases. Honeybees infected with one but a result in the disappearance of colonies or honeybee decline. The different adversities in colony affected by beekeeper activity or an environmental factor. Therefore, bee diseases are not a cause bee health occurs as a result of degradation of colony development, bee physiology or biology being beekeeping will directly affect colony development, colony biology and colony outcomes. Loss of in terms of the application of scientific approaches. Any kind of application to the colony in constitutes an advantage in terms of diversity, the discontinuation of certain habits is a disadvantage of traditional beekeeping in Asia and modern beekeeping in Europe. Although this integrity of Beekeeping can be defined by a sentence at the meeting point of the continents; It is a combination

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Aslı Özkirim

## Beekeeping in the meeting point of continents: From Asia to Europe



# Polyorphism of COL gene in honey bees from different regions of Ukraine

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The rapidly evolving mitochondrial COL gene is widely used in the molecular taxonomy of insects to identify closely related forms. Accordingly, to assess the distribution of subspecies \ breeds of Apis mellifera in Ukraine, PCR amplification, sequencing and comparison of this gene was performed for bees from different geographical regions. Breed-specific mutations in the COL gene have been identified for the Dark European, Carpathian and Ukrainian Steppe honey bees, which are widely distributed in Ukraine. It was found that the current distribution of these breeds does not correspond to the traditional zoning. The widespread practice of importing the genetic material of Apis mellifera from different regions of Ukraine leads to uncontrolled hybridization and represents a threat to the conservation of aporiginal breeds of honey bees.

**Keywords:** biodiversity, molecular markers, cytochrome oxidase

# Observation of monthly metabolic changes in honey bee workers using <sup>1</sup>H NMR

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In temperate climates, honey bee colonies show distinctive seasonal states. The series of events in honey bee colony such as brood rearing, population growth, food storing, and overwintering affect their physiology, metabolism, and behavior. A snapshot of metabolites at specific time may have a potential in prediction of colony health.

This study was conducted to observe the monthly and seasonal metabolic features of honey bee workers (Apis mellifera) using proton nuclear magnetic resonance (<sup>1</sup>H NMR). A total of 330 honey bee workers were collected over the course of a year, five bees were pooled into one sample. Samples with head, gut, and poison sack removed were extracted with methanol, analyzed by 2D <sup>1</sup>H NMR. A total of 36 metabolites were identified and quantified by Chenomx NMR Suite 8.6.

The results showed that worker bees could be distinguished based on their metabolic profiles by season. The identified metabolites were projected as monthly variation graph. The majority of the metabolites peaked in concentration during May and January and showed the lowest levels in March. Essential amino acids exhibited similar fluctuation over a year. Four out of seven essential amino acids: leucine, lysine, phenylalanine, valine showed significant increase from "August–October" to "November–February" ( $p < 0.05$ ). The metabolites involved in lipid metabolism: choline and glycerophosphocholine, exhibited similar fluctuations. Both of them showed a gradual upward trend from summer, reaching its highest levels in winter ( $p < 0.05$ ). In contrast, phosphocholine remained significantly lower in winter compared to summer ( $p < 0.05$ ). The most remarkable biomarker was a CH<sub>3</sub> singlet signal at  $\delta$  3.83 ppm. The peak showed significantly high concentrations only in summer "June–July".

The results give us a broad perspective of views in honey bee metabolism, and may be useful in guiding future research and providing practical helps to the apiculture industry.

**Keywords:** Apis mellifera, nuclear magnetic resonance, metabolome, honey bee metabolites

# Combined effects of temperature and nutritional stress on TBARS level in Apis mellifera

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The aim of our study was to evaluate the thiobarbituric acid reactive substances (TBARS) level (as a marker of oxidative stress in general) in summer generation worker bees of Apis mellifera in laboratory conditions under the prolonged combined effects of stress low temperature and nutritional. One-two-days-old bees were transferred from the frames to the cages (about 300 bees each) and kept during 3 days at +28 °C on a diet with mixture of 25% glucose + 25% fructose. After that, the insects were transferred for 5 days to diets with various carbohydrate components: (1) 50% sucrose, (2) 50% glucose, (3) 50% fructose and (4) mixture of 25% glucose + 25% fructose. Half of the bees were exposed to low-temperature (+14 °C) stress for 7 days. The level of TBARS was measured in tissues of the head, thorax and abdomen of bees.

It was shown that the fructose diet led to an increase and glucose diet led to a decrease of TBARS levels in tissues of the tarsi compared to the another diets when the bees were kept at +28 °C. However, in glucose - and glucose + fructose-fed bees kept at +14 °C significant increase in the level of TBARS in thorax and head tissues compared to the bees kept at optimal temperature was observed. Sucrose-fed bees kept at low temperatures showed a significant increase of TBARS levels in thorax tissues but a significant decrease in abdominal tissues compared to the bees kept at optimal temperature. At the same time fructose-fed bees kept at +14 °C was a significant increase this value only in head tissues compared to the bees kept at +28 °C. Our data show that the prolonged combined temperature and nutritional stress leads to tarsi-specific increase in the level of TBARS. This indicates an increase in the oxidative processes in bees.

**Keywords:** honey bee, carbohydrate diet, temperature

**Keywords:** Citizen-science, honeybee colony, bioassaying, pesticides, pollen

study produced an overview of pesticides collected by honeybee colonies, an overview of available biomonitoring in a beekeeper's citizen science study including laboratory protocols. Furthermore, this of this study was a scientifically substantiated guideline for the application of honeybee colonies for diversity in Latvia, Denmark, UK, Ireland, Belgium, France, Austria, Italy, and Greece. The outcome complemented with the INSIGNIA data. In 2019 we detected over 70 pesticide residues in Denmark, Austria, and Greece. In 2020 we re-tested the APIstrip for pesticides and trapped pollen for pollen metabarcoding and classic palynology are comparable. The European pollen ITS2 library will be The metabarcoding ITS2 technique was applied to determine the origin of the pollen. The results of 2020.

reusable and requires a time-consuming sampling practice, the APIstrip was selected for testing in most practical matrix for pollen identification. Since beehives are compared to the APIstrip, less found that the APIstrip and beehive were the best pesticide matrices, while trapped pollen was the biological passive samplers, the Beehive tube and the APIstrip, were tested in 2019. Our results of efficiency and practicality, two bee matrices, beehive and trapped pollen, and two non-efficient matrices to obtain information from the colony. To select the best pesticide matrix in terms accumulate in the hive. The basic principle of the honeybee colony as a biosampler is to apply areas. Unintentionally, contaminants present in flowers are collected along with nectar and pollen and functioning bioassaying tool. It has a forager's cohort of thousands of workers that forage in a large to monitor pesticides and pollen diversity via utilization of the honeybee colony as a well-science protocol for using honeybee colonies to bio-monitor the environment. The specific focus was The project's goal was to develop a scientifically substantiated best practice apicultural citizen

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**sampler for pesticides**

**The INSIGNIA project: A citizen science protocol for honey bee colony as bio-**

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**Key words:** Pollinator health, Sulfoximine, butenolid insecticide, Deformed wing virus, Black queen cell virus gene expression, virulence.

co-exposure to these novel ACHR insecticides does not significantly exacerbate viral impacts on gene expression. We conclude that common viral pathogens pose a major threat to honey bees whilst viral load, the combined treatment SULF and DWV-B led to a synergistic up-regulation of dicer-like we found no evidence of synergistic interactions among stressors on either honey bee survival or harmful effects of FPF and pathogens on honey bee survival and immune gene expression. Through in which treatments were administered singly or in combination to individual insects, we recorded viruses (BQCV) and Deformed wing virus (DWV) genotypes A and B. Through laboratory experiments fully crossed experimental design with three common viral honey bee pathogens, Black queen cell (nACHRs), flupyradifurone (FPF) and sulfoxiafor (SULF), at sublethal and field realistic doses in a insect, the honey bee, we used two new nicotinic acetylcholine receptor agonist insecticides pesticides and pathogens. To explore pesticide-pathogen interactions in an important pollinator considered to be the interaction between environmental stressors, particularly between exposure to The decline of insect pollinators threatens global food security. A major potential cause of decline is

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## Do novel insecticides and viral pathogens interact to harm honey bees?

# Determination of some heavy metals and elements in honeybee and honey samples from Saudi Arabia

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Honeybees and honey have become important tools for ecotoxicity because of their extraordinary ability to bioaccumulate of mineral element and toxic metals from the environment. The present study aimed to evaluate and detect the mineral element and pollution levels of honeybees and their product, honey, by heavy metals. These minerals include heavy metals: cadmium, chromium, copper, iron, manganese, nickel, lead, zinc, and elements (calcium, potassium, magnesium, sodium). The levels of heavy metal pollution are collected from different regions in Saudi Arabia using foraging bees of *Apis mellifera jemenensis*. For this purpose, bee and honey samples were collected directly from different geographic regions in Saudi Arabia. The results of this study showed that the highest contamination level among the heavy metals in question was the iron (Fe). In the honeybee samples, the highest Fe concentrations were in Makkah region (8.794) Air (6.222) Jazan (6.202) Al-Baha (2.088). In the honey samples, the highest Fe concentrations were found in Air (1.904) Jazan (1.843) Al-Baha (1.340) and Makkah (0.907). In addition, we found that the most concentrated mineral element is potassium (K) in all four agricultural areas from which the samples were collected in this study. The results showed that metal levels are within the limits of international standards in bee and honey in Saudi Arabia.

**Keywords:** Honeybees; Honey; heavy metals ; elements ; foragers honeybee workers.

## Bridging bee research and Asian beekeeping practice: how do beekeepers gain knowledge about good beekeeping practices?

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- The ongoing colony losses in many regions of the world led to a significant increase of research on honeybees and on beekeeping techniques. However, to achieve an impact of the honeybee research onto the actual bee health, a bidirectional knowledge exchange between research and beekeeping practice is crucial. Beekeepers must be aware of the newly gained knowledge to be able to put it into practice. On the other hand, researchers need to have access to the beekeeper's knowledge and view to focus their research onto topics relevant to the beekeeping practice.
- Bridging Research and Practice (B-RAP) was established as a core project within the COL022 association with the specific intention of supporting beekeepers and beekeeping by connecting science and beekeeping through the work of the beekeeping advisory services. Our latest project aimed to understand the way beekeepers keep themselves informed about beekeeping techniques and research. We performed an online survey in 23 languages, receiving answers from over 11.000 beekeepers from 99 countries. This information will help advisors, beekeeping organizations and researchers to adapt their communication plans to the needs and habits of the beekeepers.
- However, the survey was centered on beekeeping with the Western honeybee *Apis mellifera*. Therefore, the dataset does not allow to draw conclusion about the needs and information habits of the whole of Asian beekeeping community as it excludes beekeepers, who work with other *Apis* species especially *Apis cerana*. Therefore, we plan to use our experience from the world-wide survey to design a follow-up survey for Asian beekeepers. This new survey will be specially adapted to the Asian beekeeping conditions. In our presentation, we give an insight into our plans for the Asian beekeeper survey and invites all Asian countries to participate.

**Keywords:** *Apis* spp.; citizen-science; monitoring; wild colonies

sustainable beekeeping around the globe.

underlying colony health and population declines in the *Apis* genus, thereby fostering more our project will form a solid basis for future research aiming to understand the mechanisms Red List status for honey bees. Moreover, as a first global effort to study wild honey bee colonies, this monitoring, the Honey Bee Watch project ([www.HoneyBeeWatch.com](http://www.HoneyBeeWatch.com)) aims to amend the IUCN generate a comprehensive database by mapping and monitoring free-living colonies globally. With conservation status of these species. Here, we propose to take advantage of citizen science to *Apis* spp. At present, a severe lack of data on free-living colonies hampers our understanding of the In general wild pollinators are in decline globally, which probably also holds true for honey bees.

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## Free-living Colonies of all *Apis* Species Honey Bee Watch: Using Citizen Science for the First Global Monitoring of



# Chemosensing of the Varroa mite

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The ectoparasitic mite *Varroa destructor* Anderson and Trewman (Acari: Varroidae) is a major threat for the honey bee, *Apis mellifera* L. It is well known that chemical cues from adult bees and brood play a crucial role in the parasite's life cycle, by guiding host finding, selection and regulating its reproduction. These cues are perceived by sensory receptors mainly localized in the forelegs and gnathosoma, but disclosure of their chemosensing functions and structure remains elusive. By mechanically blocking the main olfactory organ of *Varroa* forelegs by varnishing with nail polish, we were able to show that other sensory organs cannot significantly compensate chemosensory aptitudes required for mite's host selection, identification as well as reproduction. Moreover, we analyzed the role of twelve putative odorant carrier proteins in *Varroa* using two approaches: 1. Exclusion of the chemosensory appendages (forelegs and gnathosoma), 2. Silencing a putative odorant carrier protein, Niemann-Pick disease protein, type C2 (NPC2) transcript (Vd40090) and 3. Evaluating the impact of silencing on the expression of other putative odorant carrier proteins and reproduction related transcripts on mite's behavior and survival. We found that transcripts belonging to the NPC2 group (Vd440090, Vd252130 and Vd74517) and other group of odorant binding proteins - OBP (Vd22214, Vd15084) are mainly expressed in the forelegs. Only one of the NPC2 transcript (Vd74517) was expressed similarly in both the foreleg and gnathosoma. Furthermore, silencing of NPC2 gene transcript (Vd40090) had no effect on the expressions of the other putative binding proteins but significantly reduced the expression of vitellogenin 2 and vitellogenin receptor. Moreover, silencing of this gene transcript switched the commonly known host preference from a nurse to a pollen forager bee, and reduced mites' survival and reproduction in worker brood cells. The implications of these findings are discussed.

**Keywords:** Gustation, Olfaction, Reproduction, Silencing, Odorant binding proteins

**Keywords:** DNA-methylation, alternative-spllicing, gene expression, temporal, immunity

anti-viral strategies for honey bees, the most important commercial pollinator. mechanisms involved in epigenetic that can serve as foundation for the long-term goal to develop quickly, regulating subsequent gene activities. Our study provides new insights of molecular are shared. Our results further suggest that dynamic DNA methylation responds to viral infections other studies of viral infections of honey bees and fruit flies indicated that similar immune pathways in anti-viral immune response, cell growth, and proliferation. Comparative genomic analyses with mixed with viral manipulations of the host to increase its own reproduction, all of which are involved These affected biological functions suggest that adaptive host responses to combat the virus are Hiipo, mTOR, TGF-beta signaling pathways, lipidation mediated proteolysis, and spliceosome. dramatically increased in gene expression at the terminal phase, including the MAPK, Jak-STAT, pathways experienced hyper- and hypo-methylation in the pre-replicative phase and later decreased dramatically from the pre-replicative to the replicative and terminal phase. Several cellular phases than in the pre-replicative phase. However, the number of differentially methylated regions transcriptomic response to with more genes differentially expressed in the replicative and terminal accompany the lethal progression of this virus. We identify the temporal dynamics of the indicate that profound immune responses and distinct manipulation of host molecular processes corresponding to the pre-replicative (2 h), replicative (20 h), and terminal (48 h) phase of infection – mc DNA methylation profiles between IAPV-infected and control individuals at these time points – IAPV infection over three distinct time points after inoculation. Contrasts of gene expression and 2 colony losses. Using worker pupae, we studied the transcriptomic and methylomic consequences of Israeli Acute Paralysis Virus (IAPV) is emerging as a model virus since its association with severe

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## Viral Infection Dynamics of Temporal Transcriptomic and Epigenomic in Response to Lethal

# Draft genome sequences of the Japanese honey bee, *Apis cerana japonica* and comparative genomic analysis for honey bee health

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The Eastern honeybee (*Apis cerana*) carries unique characteristics compared to the Western honeybee (*Apis mellifera*). Especially, they are more resistant to several pathogens. *A. cerana japonica* is one of the subspecies of *A. cerana* that shows many unique features and therefore it is important to study the molecular biological and genetic aspects these features. To accelerate genetic research on *A. cerana japonica*, we sequenced the genome of this subspecies. The draft genome sequence of *A. cerana japonica* is of high quality in terms of basic genome status (e.g. N50 is 180 kbp, total length is 211 Mbp, and largest contig length is 1.31 Mbp) and BUSCO results.

The use of this draft genome sequence of *A. cerana japonica* provided herein, combined with those of the other *Apis* (sub) species will help to accelerate comparative research on the genome of honey bees. *A. cerana* (northern (Korea) and southern (China)) strains that have been sequenced. In addition, the full-genomic sequencing of *A. florea* and *A. dorsata* are available. By comparing the genomes of four species of *Apis*, we can identify unique or similar genetic characteristics of the honey bees. To start with, we annotated the genes related to innate immunity. We searched for 41 orthologs related to the IMD and Toll pathways, which have key roles in the immune reaction to invading pathogens. This indicates that the Toll and IMD pathways function in the same way as in *A. mellifera* and *Drosophila melanogaster*.

At the symposium, we will introduce some results of the on-going comparative genomics research on *Apis* species, including the re-sequencing of the *A. cerana japonica* genome.

**Keywords:** draft genome, *Apis cerana*, comparative genomic analysis, innate immunity, re-sequencing

**Keywords:** *Leucospota* sp., honey bee, probiotic, nutrition and immune genes

opportunities to enhance the fitness of the colony.

suggest that the bacteria strain *L. mesenteroides* TBE-8 has high potential as bee probiotic and hold 17-fold and 7-fold, respectively, after bees ingesting TBE-8 fermented culture. These findings antibacterial peptides, hymenoptaecin and apidacacin in bee abdomen was significantly induced by comparison with the group of MRS medium. Additionally, the expression of gene encoding two the head and vitellogenin (vg) in the abdomen by about 1400-fold and 20-fold, respectively, in broth can increase the expression of the nutrition-related genes major royal jelly protein 1 (mjpr1) in nutrition-modulatory effect of the TBE-8 fermented broth on honey bees. We found that the TBE-8 can survive well in bee hind gut environment. Furthermore, we investigated immunomodulatory and Additionally, the TBE-8 can increase nearly 2-fold of cell number in MRS pH 5.0, suggesting that it viable cell number of TBE-8 in 72 h incubation still has  $10^8$  CFU/mL, starting from  $4.9 \times 10^8$  CFU/mL. mannose, melipiose and raffinose. The TBE-8 has good tolerance of sugar osmotic stress, that is, the of 49 carbohydrates, including the toxic sugars to bees, such as arabinose, xylose, galactose, *Leucospota mesenteroides* based on the sequences of 16S rDNA and 16S-23S rDNA intergenic spacer region. The *L. mesenteroides* TBE-8 strain was further characterized for probiotic properties and shown to have wide spectra of carbohydrate utilization, high sucrose, acid tolerance and anti-bee pathogen *Pseudaerans* larvae. Regarding carbohydrate utilization, the TBE-8 can metabolize 22 bee pathogen *Pseudaerans* larvae. Regarding carbohydrate utilization, the TBE-8 can metabolize 22

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## A potential probiotic, *Leucospota* sp. TBE-8 for honey bee, *Apis mellifera*

# Killed brood method Evaluation of hygienic behaviour of Apis mellifera Linnaeus colonies by pin-

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Hygienic behaviour is an important behavioural mechanism of disease resistance and as a behavioural defence against the Varroa destructor Anderson & Trueman, a serious pest damaging bee colonies. The utilization of this will minimize chemical contamination of hive products. This trait can be used as a selection criterion in queen breeders' aparies for achieving multiple benefits. It is usually assessed by measuring the rate at which a colony removes dead pupae that have been deliberately killed using either a pin or by freezing. In the present study, 100 honey bee colonies were assessed for hygienic behaviour by the pin-killed brood method. Each colony was screened thrice, each at 72 h interval. Three such tests were conducted for three alternate brood cycles i.e. at every six weeks interval between each of the two consecutive test brood cycles, to account for any variability w.r.t. the sub-families in the colony. The percentage of brood removal in each colony was recorded after 24 h, 48 h and 72 h. The hives that had removed 80 per cent of dead offspring after 24 h were considered to be hygienic and those that had removed less than 80 per cent were considered non-hygienic. In the test colonies, after 24 h, the mean brood removal ranged between 31.33-91.33, 44.00-92.57 and 52.57-92.00 per cent, respectively in brood cycle I, II and III, the mean of 46.78-91.00 per cent. Among these colonies, seven most hygienic and three most non-hygienic colonies were selected and re-evaluated for hygienic behaviour, to assess their consistency for hygienic traits carried by them. The hygienic behaviour in seven most hygienic colonies ranged between 88.44 - 91.00 per cent and in non-hygienic colonies, it ranged between 46.78-47.33 per cent. The seven most hygienic colonies are being maintained for selective bee breeding programme.

**Key words:** Apis mellifera, disease resistance, hygienic behaviour, mite resistance, pin killed brood method.

# Condition Orientation Behavior of Honey Bees (Apis mellifera) Under Laboratory Formulated Neonicotinoid Insecticides influence the Sugar Responsiveness and

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The honeybee is at the top and plays a crucial role as a crop pollinator, providing food and honey that are important to human nutrition. The decline in pollinators, which has increased over the last decades, may contribute to a simultaneous decline in food production and plant species as well. Neonicotinoid insecticides are used to protect crops but with documented secondary effects on bees at sublethal doses. Honeybees use their sense of smell to locate queens and nestmates, to signal danger, and to distinguish flowers during foraging. Few behavioral experiments to date have been examined that how neonicotinoid insecticides impact the ability of bees to differentiate odors from contaminated flowers. For this reason, we used a separate behavioral evaluation test to determine how neonicotinoid toxicity influences the learning, memory, and olfactory recognition of honeybees. In order to include sublethal toxicity evidence and possible risks of neonicotinoids, we simulated a laboratory test and evaluated the toxicity of six widely used formulations of neonicotinoids to honey bees viz., clothianidin, imidacloprid, Thiamethoxam, acetamiprid, dinotefuron and nitenpyram @ 50 ppm for sugar responsiveness by proboscis extension response (PER) and three of them for orientation behavior (clothianidin, imidacloprid, and Thiamethoxam @ 50 ppm) by modified Y maze apparatus. Our data revealed that when bees fed neonicotinoids in sugar syrup were unable to discriminate odors during early days of exposure but started to distinguish and discriminate after 10 days of exposure to contaminated flowers and not to sugar syrup relative to control groups. Our research has shown that honey bees cannot regulate their exposure to neonicotinoids in sugar syrup, but avoid contaminated flowers if they have already been exposed to and survived.

**Keywords:** Honeybees<sup>1</sup>, Neonicotinoids<sup>2</sup>, Pollinators<sup>3</sup>, Orientation<sup>4</sup>, Sugar Responsiveness<sup>5</sup>, post-consumption behavior<sup>6</sup>

# The effect of dynamic diluted compounds in controlling Varroa mite population in honey bee colonies (Apis mellifera)

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A wide array of chemotherapeutics are available for the control of the Varroa mites within honey bee colonies. In this project, the efficacies of various dynamic diluted compounds were measured in laboratory cages to control the Varroa mite of honey bees, minimize the pesticide residues in the colonies and hive products, and thwart the acaricide resistance. Varroa mites are believed to feed on the bees' fat bodies and weaken the bees' metabolism. After the dynamic diluted compounds treatments, the Varroa infestation levels were determined directly by counting the dead mites on the hive bottom inserts cage. The study was performed on 24 cages in an incubator at 37 °C and 50% humidity. Those were divided into four different treatment groups of six replicated cages. These four groups were consisted of the following: (1) treated with Pulsatilla C30; (2) treated with Silicea C30; (3) treated with Nuxvomica C30; (4) a control group, which received no drug treatment. Varroa mite mortality was recorded in each caged-bee group at intervals of 24 and 48 h. All of the tested compounds increased the overall Varroa mortality in the laboratory experiment. The results showed significant differences between the treatment groups and control in the bees' protein and fat body.

**Keywords:** Bees, Varroa mites, dynamic diluted compounds.

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