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OIST Mini-Symposia & COLOSS ASIA Okinawa

ABSTRACT BOOK

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

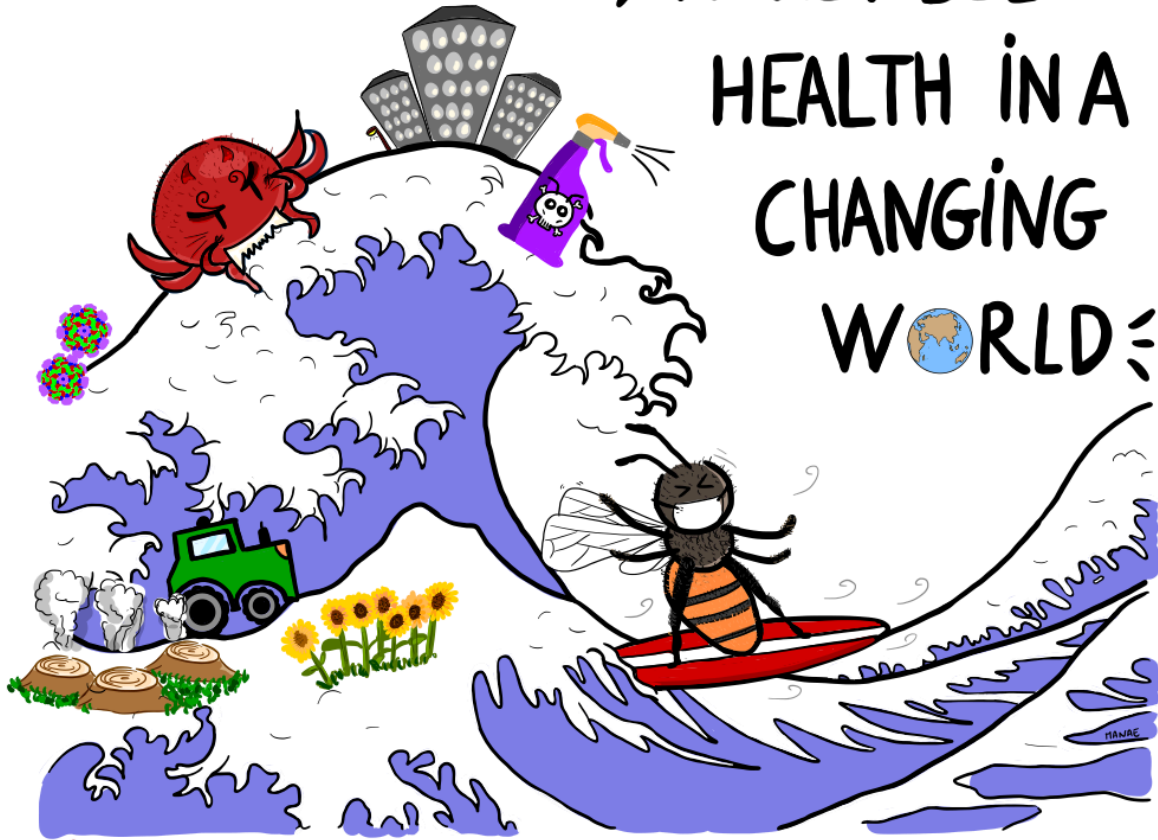


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OIST Mini-Symposia 22-24 March 2021

≡ HONEY BEE HEALTH IN A CHANGING WORLD ≡



OIST MINI-SYMPOSIA BY THE ECOLOGY & EVOLUTION UNIT
22-24 MARCH 2021

Drawing by M. Techer

ABSTRACT OIST Mini-Symposia 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 Eliash, 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 Eliash*, Miyuki Suenaga and Alexander Mikheyev

*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 Iflavirus 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.

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

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

Keywords: apiculture, evolution, host-pathogen, social insects, xenobiotics

A holistic view of global honey bee health

It is evident that the uncertain health of managed honey bees, *Apis mellifera*, requires global attention. For over a decade, beekeepers are noticing unsustainably high colony losses in many areas globally. Coordinated surveys (www.coloss.org) revealed that these losses are variable in both space and time for so far unknown reasons. Even though there is agreement that ectoparasitic mites and their associated viruses are key players, sustainable solutions are still not at hand. Moreover, the roles of other pathogens, agrochemicals, nutrition and beekeeping management (incl. breeding, import/export of bees and hive products) are debated, but a consensus has not been reached. This is probably due to complex interactions between (too) many known and unknown factors as well as variation in both space and time for any given mechanism. It is further apparent that not a single, but instead multiple possibly interacting mechanisms are governing the overall health of any honeybee colony ranging from individual bee, over caste to colony and population level. Accordingly, individual laboratories just cannot cover all aspects and snap-shots in space and time may provide false negative evidence. In light of the apparent complexity of the issues to be tackled, I herewith suggest going globally beyond agreed protocols (aka BEEBOOK) into joint concerted large-scale experiments ranging from cell cultures to the landscape level. Focus should probably be given to a more systematic comparison of areas and *Apis* populations / species with and without health issues and the apparent major gaps in knowledge of social immunity / detoxification.

Brock A. Harpur, PhD
Assistant Professor, Purdue University

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 Donnelley 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*, Yani Meibord, Gus MacFarlane, and Gregor 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_plea_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 manageable with inexpensive modifications such as robbing screens.

Alexander (Sasha) Mikheyev, PhD
Adjunct Professor, Okinawa Institute of Science and Technology, Japan
Senior Lecturer, Australian National University, Canberra, Australia

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.

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

Bees facing Varroa and viruses over space and time: a historical study

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

Kiyoshi Kimura, PhD

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

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

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

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

Beekeeping is not a large industry in Japan. Only about 5,000 beekeepers register with local governments, and most of them keep less than 100 hives. In addition, most honey consumed in Japan is imported from abroad. Nonetheless, in Japan beekeeping is thought to be very important in agriculture due to the role of honeybee as pollinator in horticulture. Honey bees are used as pollinators for production of fruit such as apple and plum, and more than 200,000 bee hives have been introduced into greenhouse production of crops such as strawberry. Even though domestic honeys are traded in the market at higher prices than imported honey, and the demand for honey bees as pollinator remains steady, beekeeping still faces many problems in Japan, e.g. damage due to insecticides, bee diseases, parasitic mites, malnutrition, lack of flowers, as it does in other countries. In this symposium, I am going to introduce Japanese beekeeping, focusing on the problem of bee diseases, which is thought to be the most important issue confronting Japanese beekeeping.

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 surveillances of *Varroa* damage in the Japanese apiaries to understand actual situation of Japanese apiaries. 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 apiaries. 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 apiaries 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 apiaries. Fluvalinate is one of the permitted acaricides for *V. destructor* control in Japan. We found some of *Varroa* mites in the Japanese apiaries has a specific amino acid substitution in a fluvalinate-targeted sodium channel, which substitution has been known to confer fluvalinate resistance. These results reveal actual situation of *Varroa* infestation in Japanese apiaries and indicate the need of more effective *Varroa* control for Japanese apiaries.

Helene Delatte, PhD, HDR

Researcher at CIRAD – Reunion Island (based in the moment at Madagascar)

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

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

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

Co-authors on this work: Delatte H el ene*, Lebreton G erard, Esnault Olivier, Ma eva Techer, Henriette Rasolofoarivao, Tsiory Rasoloarijao, Julien Galateau, Benoit Jobart, Nicolas Blot, Johanna Cl emencet

The South West Indian Ocean (SWIO) archipelagos and Madagascar constitute a hotspot of biodiversity with high rate of endemism where the endemic subspecies *Apis mellifera unicolor* (African lineage) has ancestrally been described as the honeybee of Madagascar. Our recent genetic (mitochondrial and nuclear) and morphometric analyses supported the differentiation of this subspecies in the surrounding islands before the Human colonisation, proving its indigenous status in the Mascarenes, Seychelles and Comoros archipelagos. Further ecological studies (interaction with the native flora) made in La R eunion suggested that *A. m. unicolor* significantly preferred native flora and could play a major role in its pollination. In 2010, the invasion of *Varroa destructor* was reported in Madagascar, then in Mauritius (2015), and most recently in La R eunion (2017). Since its arrival we had conducted several epidemiological and mortality surveys to evaluate the evolution of *V. destructor* invasion such as the diversity of other pathogens before and after its invasion (whenever possible). The pathogens surveyed included 3 ectoparasites (*V. destructor*, *Tropilaelaps sp.* and *Braula sp.*), 2 bacteria (*Melissococcus plutonius* and *Paenibacillus* larvae), 2 Nosema species (*Nosema apis* and *N. ceranae*) and 6 viruses (ABPV, BQCV, CBPV, DWV, KBV and SBV). Different pathogen patterns were observed within the different islands. *Nosema ceranae*, BQCV, CBPV and DWV were detected in each country, no P. larvae were detected in the SWIO area. Despite the high prevalence of DWV observed in the Seychelles, not colony mortality were registered, the only territory where we found high mortalities were in Madagascar, Mauritius and La R eunion after *V. destructor* invasion. More precise observations on the dynamic of colonisation of this ectoparasite, such as its interaction with other pathogens on the indigenous honeybee are on going in La R eunion.

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 USA. 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 *Varroa destructor*, one of the most striking observations has been the concurrent increase in virus levels in honeybee colonies. *Varroa* 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 *Varroa* as a vector, we investigated if honeybee viruses actively replicate in *Varroa* by determining whether they trigger the mite's antiviral immune response. Using deep sequencing of small RNA in honeybees and *Varroa*, 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 *Varroa*, 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 *Varroa* 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 Sangyo 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 for *Apis mellifera* to overcome these problems, particularly our method is to inject 30uL 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 Sangyo University. This study is published in *Applied Entomology and Zoology* (2020) 55: 351-354, introducing as a new method for preparing tissue specimens for histopathological examinations.

Philipp Engel, Prof.

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 crosstalk between the microbiota and the host. Social bees are our favorite study system as their gut microbiota is evolutionarily conserved, functionally relevant, and experimentally tractable. This allows us to combine bioinformatics and genomics with experimental approaches to further our understanding of microbiota-host interactions.

Keywords: Microbiome, honey bee host

The gut microbiota affects the social network of honey bees

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

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

Panuwan Chantawannakul, Prof. Dr.

Department of Biology, Faculty of Science, Chiang Mai University, Thailand

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).

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

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

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

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

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

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

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

The BeeBiome consortium established a publicly available online resource, namely the BeeBiome data portal (www.beebiome.org), which provides an overview of and easy access to all currently available metagenomic datasets involving bee-associated microbes. Currently the data portal contains 11019 Sequence Read Archive (SRA) experiments for 140 *Apoidea* hosts. During this presentation challenges to finance, develop and maintain such a data portal for the bee research community will be presented.

COLOSS ASIA Okinawa 25-26 March 2021

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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 *scarlet (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-blastoderm embryos. More than 50% of G₀ 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 G₀ queens developed in the colony when *melt* and *st* were targeted. After allowing oviposition to these mosaic virgin queens, the *st* gene-knockout G₁ 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 *V. destructor*.

The *V. 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 *V. 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 to the non-shifted native mites, thereby setting the stage for speciation of the invasive lineage of *V. 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 *V. destructor*. Hybridization between shifted and non-shifted mites also indicate a potential threat to the original host of *V. 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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In the last decades, Western honeybee populations all over the world are in continuous battle against introduced parasitic *Varroa* mites. Since *Varroa destructor* switched from its original host the Eastern honeybee to the Western honeybee, it has successfully spread almost worldwide and is considered as the most damaging pest in beekeeping. The high inbreeding in combination with an important loss of genetic diversity following host switch led to the assumption that *V. destructor* invasive populations originated from two quasi-clonal strains. Yet, *Varroa* mites are persisting after 80 years of invasion and independently develop novel traits such as acaricide resistance. How can this specialist parasite evolve new traits despite an initial loss of genetic diversity?

To identify the key demographic and genetic mechanisms behind *Varroa* invasive success, we took advantage of our Vdes3.0 reference genome and the unique *Varroa* mite collection that we steadily build in the past five years. The sampling participation network covers most of *V. destructor* invasive and native range and involved honey bee researchers, beekeepers as well as veterinary services worldwide. To date, the collection gathers 1,660 mites from 61 countries - including all known *Varroa* species on different hosts - and with a sampling period spanning from 1989 to 2021. Using whole-genome resequencing of 1,350 *V. destructor* (including 32 mites from *A. cerana*), we found ~3.3 million variable sites throughout the genome. The identity of *Varroa* present on *A. mellifera* populations was confirmed and compared to genomes of sister species and non-switching mites (*V. jacobsoni*, *V. rindereri*, *V. underwoodi* and *Varroa sp.* type "Luzon" and type "Mindanao"). In my talk, I will briefly present how from a single mite library, we were able to 1) infer host information (metagenomics), 2) reveal previously undetected host switches (mitogenomes) and 3) show that at least 10 genetic populations co-exist worldwide (nuclear genomes). I will explain how these results will help us to further elucidate the origin and evolution of *V. destructor* on its new host. As parasitic mites are continuously spilling-over from Asian *Apis* to *A. mellifera*, our knowledge on *V. destructor* success could help us in predicting future outbreaks.

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

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

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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 pathogen. 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 (25g ai/ha) and double of the recommended dose (50 g ai/ha) on *Brassica napus* (GSC 7) following the standard guidelines mentioned in COLOSS Beebook. 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 8th 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 unripe honey hoarding in a colony. These parameters started increasing on 7th day of application (6.95 bee-frames & 2787.41 g, respectively) and became significantly higher than pre-treatment on 14th day of application (8.19 bee-frames & 3780.96 g, respectively). The mean ripe honey hoarding did not decrease below pre-treatment. The mean unsealed worker brood also showed similar trend i.e. reduction upto 7th day of application (612.09 cm²/colony) which became statistically at par with pre-treatment on 14th day of application (688.20 cm²/colony) but the pollen hoarding decreased upto 14 days (290.30 cm²/colony) and on 21st day of application (372.79 cm²/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 queen caging and trapping comb, 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 (queen caging and trapping comb 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): Queen Caging + treatment with oxalic acid (OA).** **Group 2 (G2): Trapping comb + 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 comb 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 organics 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 comb" 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: varroase, American foulbrood, noseosis 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 noseosis 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

Removal of veterinary treatments and other residues from beeswax

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

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

Keywords : Decontamination, residues, veterinary treatments, bees

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

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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*, *Trachyspermum 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*: 3.5% 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 α -terpinene were found to be the main constituents in the oils. In the toxicity assay, three oils (*T. ammi*: 4h-LC50=48.75 $\mu\text{g/ml}$ (2.44 mg oil/L Air volume); *T. schimperi*: 4h-LC50=109.16 $\mu\text{g/ml}$ (5.46 mg oil/L Air volume); *C. citratus*: 4h-LC50= 586.49 $\mu\text{g/ml}$ (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 $\mu\text{g/ml}$ (3.54 mg thymol/L Air volume)) and carvacrol (4h-LC50= 106.10 $\mu\text{g/ml}$ (5.31 mg carvacrol/L Air volume)) induced high toxicity to *Varroa* mites. And citral (4h-LC50= 322.51 $\mu\text{g/ml}$ (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 $\mu\text{g/ml}$). The results showed that thymol and carvacrol-bearing essential oils were highly acaricidal and can be a useful tool in controlling *Varroa* mite infestations.

Beekeeping industry in Iran: Current status and future directions

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Modern beekeeping 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 beekeepers organized in more than 200 co-operatives throughout the country. Most of beekeepers 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 beekeeping more efficient. As a result, other products such as pollen, royal jelly, beeswax and propolis took bigger parts in the beekeeping economy. Despite the presence of many young beekeepers, Vast areas of rich floral and favorable climate which has made Beekeeping as an ideal activity, there are many challenges facing Iranian beekeeping 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 beekeeping industry in Iran, focusing on the challenges and future directions.

Keywords: Iran, beekeeping industry, Honey bee

THERE AND BACK AGAIN – Deciphering the host specificity of *Varroa spp.* with population genetics

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

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

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

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

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

Polymorphism of *COI* gene in honey bees from different regions of Ukraine

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The rapidly evolving mitochondrial *COI* 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 *COI* 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 aboriginal breeds of honey bees.

Keywords: biodiversity, molecular markers, cytochrome oxidase

Observation of monthly metabolic changes in honey bee workers using ^1H 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 (^1H 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 500 MHz 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_3 singlet signal at δ 2.83 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 tagmas 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 tagma-specific increase in the level of TBARS. This indicates an increase in the oxidative processes in bees.

Keywords: honey bee, carbohydrate diet, temperature

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

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The project's goal was to develop a scientifically substantiated best practice apiculturist citizen science protocol for using honeybee colonies to bio-monitor the environment. The specific focus was to monitor pesticides and pollen diversity via utilization of the honeybee colony as a well-functioning biosampling tool. It has a forager's cohort of thousands of workers that forage in a large area. Unintentionally, contaminants present in flowers are collected along with nectar and pollen and accumulate in the hive. The basic principle of the honeybee colony as a biosampler is to apply efficient matrices to obtain information from the colony. To select the best pesticide matrix in terms of efficiency and practicality, two bee matrices, beebread and trapped pollen, and two non-biological passive samplers, the Beehold tube and the APIStrip, were tested in 2019. Our results found that the APIStrip and beebread were the best pesticide matrices, while trapped pollen was the most practical matrix for pollen identification. Since beebread is, compared to the APIStrip, less reliable and requires a time-consuming sampling practice, the APIStrip was selected for testing in 2020.

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

Keywords: Citizen-science, honeybee colony, biosampling, pesticides, pollen

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

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

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

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 jemenatica*. 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) Asir (6.222) Jazan (6.205) Al-Baha(2.088). In the honey samples, the highest Fe concentrations were found in Asir (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 COLOSS 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.

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

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

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

Chemosensing of the *Varroa* mite

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The ectoparasitic mite *Varroa destructor* Anderson and Trueman (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 abilities 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 (Vd40090, Vd25130 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

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

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Israeli Acute Paralysis Virus (IAPV) is emerging as a model virus since its association with severe colony losses. Using worker pupae, we studied the transcriptomic and methylomic consequences of IAPV infection over three distinct time points after inoculation. Contrasts of gene expression and 5 mC DNA methylation profiles between IAPV-infected and control individuals at these time points – corresponding to the pre-replicative (5 h), replicative (20 h), and terminal (48 h) phase of infection – indicate that profound immune responses and distinct manipulation of host molecular processes accompany the lethal progression of this virus. We identify the temporal dynamics of the transcriptomic response to with more genes differentially expressed in the replicative and terminal phases than in the pre-replicative phase. However, the number of differentially methylated regions decreased dramatically from the pre-replicative to the replicative and terminal phase. Several cellular pathways experienced hyper- and hypo-methylation in the pre-replicative phase and later dramatically increased in gene expression at the terminal phase, including the MAPK, Jak-STAT, Hippo, mTOR, TGF-beta signaling pathways, ubiquitin mediated proteolysis, and spliceosome. These affected biological functions suggest that adaptive host responses to combat the virus are mixed with viral manipulations of the host to increase its own reproduction, all of which are involved in anti-viral immune response, cell growth, and proliferation. Comparative genomic analyses with other studies of viral infections of honey bees and fruit flies indicated that similar immune pathways are shared. Our results further suggest that dynamic DNA methylation responds to viral infections quickly, regulating subsequent gene activities. Our study provides new insights of molecular mechanisms involved in epigenetic that can serve as foundation for the long-term goal to develop anti-viral strategies for honey bees, the most important commercial pollinator.

Keywords: DNA-methylation, alternative-splicing, gene expression, temporal, immunity

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 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

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

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Much attention has been paid to honey bee health due to dramatic changes of climate and environment, which resulted in honey bee colony collapse. Here, we isolated and identified gut bacteria from the Taiwan bumble bee *Bombus eximius* and one of them, TBE-8, was confirmed as *Leuconostoc 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 *Paenibacillus larvae*. Regarding carbohydrate utilization, the TBE-8 can metabolize 22 of 49 carbohydrates, including the toxic sugars to bees, such as arabinose, xylose, galactose, mannose, melibiose and raffinose. The TBE-8 has good tolerance of sugar osmotic stress, that is, the viable cell number of TBE-8 in 72 h incubation still has 10⁸ CFU/ mL, starting from 4.9×10⁸ CFU/mL. Additionally, the TBE-8 can increase nearly 2-fold of cell number in MRS pH 5.0, suggesting that it can survive well in bee hind gut environment. Furthermore, we investigated immunomodulatory and nutrition-modulatory effect of the TBE-8 fermented broth on honey bees. We found that the TBE-8 broth can increase the expression of the nutrition-related genes major royal jelly protein 1 (*mrjpl1*) in the head and vitellogenin (*vg*) in the abdomen by about 1400-fold and 20-fold, respectively, in comparison with the group of MRS medium. Additionally, the expression of gene encoding two antibacterial peptides, hymenoptaecin and apidaecin in bee abdomen was significantly induced by 17-fold and 7-fold, respectively, after bees ingesting TBE-8 fermented culture. These findings suggest that the bacteria strain *L. mesenteroides* TBE-8 has high potential as bee probiotic and hold opportunities to enhance the fitness of the colony.

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

Evaluation of hygienic behaviour of *Apis mellifera* Linnaeus colonies by pin-killed brood method

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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' apiaries 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, 44.00-92.67 and 55.67-95.00 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 86.44 - 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.

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

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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: Honeybees¹, Neonicotinoids², Pollinators³, Orientation⁴, Sugar Responsiveness⁵, post-consumption behavior⁶

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 caged 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 Nux vomica 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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Report

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