

# **5<sup>th</sup> EUROPEAN CONFERENCE OF APIDOLOGY**

**3-7<sup>th</sup> September 2012**  
Halle an der Saale, Germany

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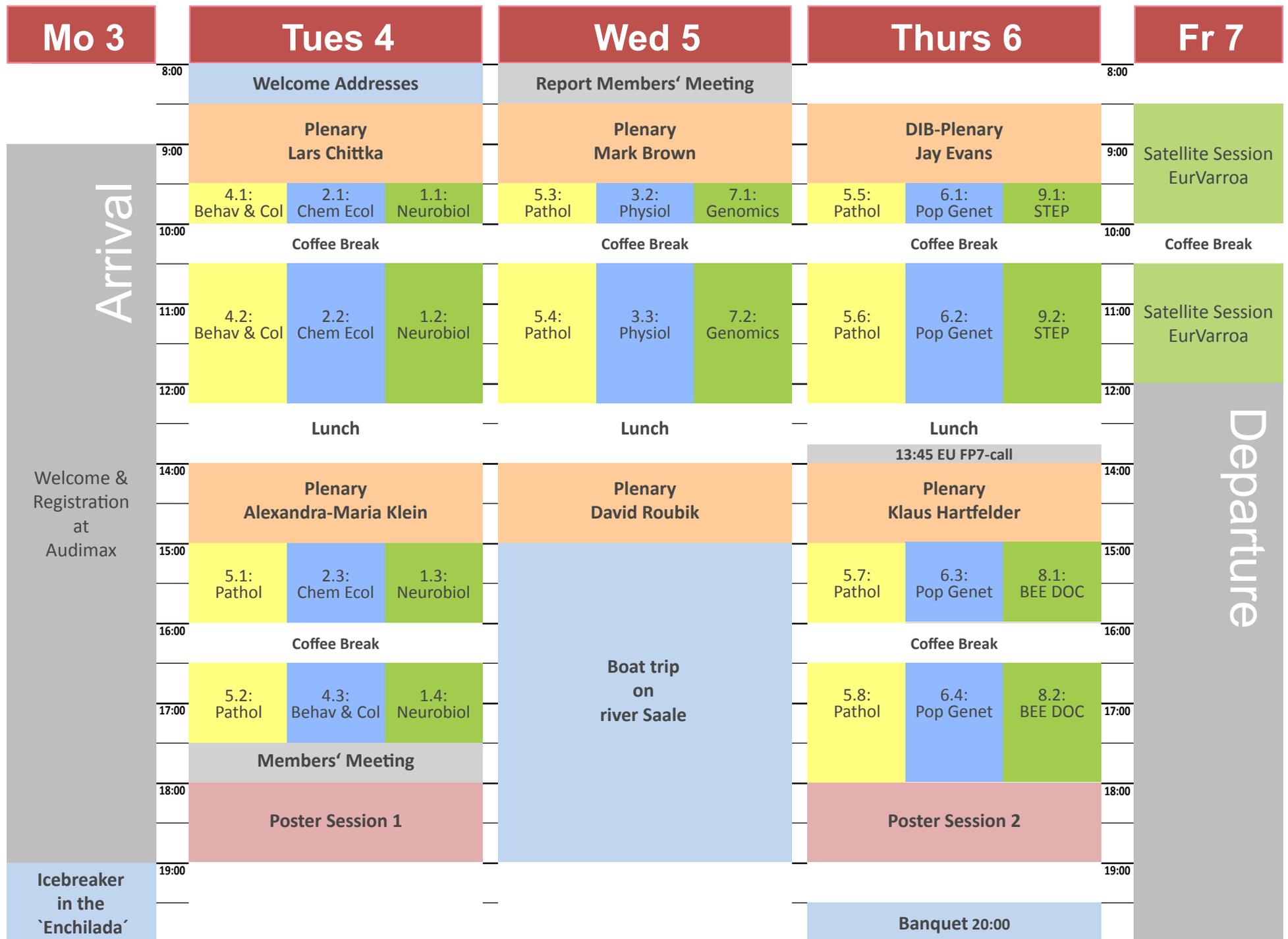
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## Preface

The 5<sup>th</sup> congress of the European Society for Apidology (EURBEE) has brought you to the Martin-Luther-University of Halle-Wittenberg. Founded in 1502, it is one of the oldest universities in Europe, and only a few kilometres distant from where Martin Luther, teaching at the University of Wittenberg, started the reformation that was to change Europe in a most fundamental way. I was not able to assess Martin Luther's H-index but clearly his impact was profound. The old philosophers in Wittenberg allowed policy-makers across Europe to cast the continent into extremely fierce and controversial conflicts, some of which remain a source of conflict till today.

But we are clearly neither here to comment on history and ponder about the past nor to start religious revolutions. We meet for the seemingly moderate aim to be updated on the achievements in research on bees to enable us to develop new directions for the future of apidology. The goal is only seemingly moderate. In fact, bees are essential for life on this globe as such, and this has been increasingly recognised by today's European policy-makers that luckily see the benefits of not casting the continent into despair. Indeed support for bee research has never been more generous than in recent years because public awareness for the fundamental role of bees as essential pollinators has risen exceptionally (but probably still not appropriately enough). Bees have made it into the headlines of public media that express concern about their low abundance, low diversity and deteriorating well being. Whereas in the past bees were used as a model tool for studying fundamental biological functions and processes, now research focuses more and more on their ecological role to maintain ecosystems and provide pollination services. Clearly, in order to understand these processes, we will need the full breadth of biological disciplines to better comprehend and unravel the functional basis of how bees negotiate with their environment, starting from the molecular within-gene level up to the level of entire landscapes.

I am extremely happy to say that this is not a novel insight, but it was the very reason why we established EURBEE 10 years ago in Udine. EURBEE was to bring together the direly needed experts across all disciplines necessary to tackle the broad problems resulting from human impact on nature. Today the EURBEE 5 hosts two EU research networks specifically dealing with these issues as well as the cost action COLOSS. For the first time the EU Commission has launched a call for a large integrated research project on honeybee health, and preparations for appropriate funding for bee research in the forthcoming EU Horizon programme are under way. In addition numerous national grant schemes in many European countries have dedicated support schemes for research on bees. Yes, this is a success story! It was only possible because of a sustained, active, dynamic and forceful research community and so I feel privileged and honoured as president of EURBEE to thank you for this great contribution to advancing our field of research.

EURBEE 5 has attracted researchers from all over the world, not only within Europe. With well over 300 members, the society has established itself as a major international driver of bee research of both wild and managed bees. EURBEE has developed into a success story, and I am glad that you as a participant of the 5<sup>th</sup> congress can join in celebrating its 10<sup>th</sup> anniversary. Enjoy the academic diversity of the congress. Use this opportunity to seek the companions you need to fulfil your own research goals. Do not forget to engage in the academic debate whenever necessary. It is only through a well-balanced mix of constructive critique and support that we will advance in developing the future of the discipline. And do not forget to reconcile your academic polemic with a cold drink after a heated debate. We have tried our best to provide you with the appropriate environment.

Halle an der Saale, August 2012

Robin F.A. Moritz  
President EURBEE

## Acknowledgements

We are grateful for financial support by our sponsors: BASF, Bayer, Deutsche Forschungsgemeinschaft, Deutscher Imkerbund, Diagonal, EU Commission, Martin-Luther-Universität Halle-Wittenberg, Stadtmarketing Halle, Ministry for Wissenschaft und Wirtschaft Saxony-Anhalt, Qiagen.



# Plenary Lectures

**Mark Brown**  
(London, UK)

**Lars Chittka**  
(London, UK)

**Jay Evans**  
(Beltsville, USA)

**Klaus Hartfelder**  
(São Paulo, Brazil)

**Alexandra-Maria Klein**  
(Lüneburg, Germany)

**David Roubik**  
(Balboa, Republic of Panama)

PL

## Large societies and small brains: insects as minimal models of social cognition.

Chittka LUA

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The social brain hypothesis holds that the cognitive demands that come with living in societies have shaped brain evolution, and that social group size might in turn be linked to brain size. This hypothesis is controversial even within the primate world, but more complications arise when one inspects the social insects. Ants, bees and wasps build cohesive societies with small brains and 10s of thousands to millions of individuals. Just like in humans, these societies are not (only) held together by individual recognition, but by learnt cues that indicate the location of society, and the place of the individual within it.

However, it would be incorrect to view social insects as anonymous societies, since individual recognition determines dominance hierarchies in several species. The facial recognition of some social wasp species is one example, and indeed some insects can assemble configural representations of facial cues, and identify faces even when rotated. There are also various forms of social learning in the insects, with the consensus building process in honeybee swarms as one example that is unique in the animal kingdom. Since insects' nervous systems are comparatively small, this raises the question of what the minimal neural circuitry is that is required to achieve these feats. Neural network analyses show that many 'advanced' cognitive feats are possible with very limited neuron numbers (i.e. 100s or 1000s, rather than the billions in some vertebrate brains).

PL

## Do honeybees substitute biodiversity to almond pollination in California?

Klein A-M

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Global declines in honeybees have led to concerns about negative impacts on food production due to low levels of pollination. This is exemplified in California where the demand for honeybees *Apis mellifera* to pollinate almond *Prunus dulcis* is increasing, but problems with honeybee health suggest it may not be sustainable to rely solely on the pollination service of a single species. I will discuss the potential of landscape and organic management to restore wild pollinators in intensive almond production landscapes of California, USA.

We found species richness and flower visitation frequency of wild pollinators, but not of honeybees, being related to fruit set. I will show various methods and results exploring the mechanisms of this unexpected finding. This will include spatial complementarity in different environmental conditions and species interactions.

Although my talk will mainly present results of a case study system, I will discuss and highlight some results for wider implications across countries and agricultural systems.

I will conclude that honeybees, although by far the most efficient pollinator taxa in many agricultural systems - and currently the only productive livestock for producing almond in intensive landscapes of California - cannot fully substitute the functional performance of diverse pollinator communities. Restoring semi-natural perennial habitat strips implemented in intensive agricultural landscapes have potential to promote sustainable agro-pollination services.

for your remarks:

PL

**Not one, but many: complex host-parasite systems in bees.****Brown MJF**

Email: Mark.Brown@rhul.ac.uk

Parasites play a key role in the dynamics of their hosts, and in bees are responsible for and implicated as the causes of dramatic declines in managed and wild populations. However, while most theory and empirical studies focus on understanding the interactions between single parasite species and single host species, most parasites are shared by multiple host species and all hosts have multiple parasite species. This complexity and the interactions that result from it dramatically change how we view host-parasite dynamics. Here I will discuss our current understanding of these complex systems, with a focus on our work in bumblebees, and the implications that these systems have for management of wild and commercial bee populations.

PL

**Almost social: a natural history of bee success.****Roubik DW**

Email: roubikd@si.edu

Bee natural history suggests social behaviour, like parasitism, is an evolutionary end arrived at due to both physical and social traits. Advanced social behaviour is founded in soft parasitism that became cooperation. Both parasitism and sociality deviate along similar lines from the solitary life cycle of nesting and mating favored for most bees. Extreme sociality became possible with the novel adaptations of honey and presumably recruitment, which maintain colonies by efficiently hoarding food. Stingless bees invented honey in tropical habitats during the Cretaceous, aided with natural antibiotics in resins used as building material. Sociality encompasses over 8% of bee species, while parasitism includes roughly three times this number. Evolution of sociality may be thwarted by climatic adversity, and hindered in final evolution by the advantages of parasitism over sociality.

for your remarks:

PL

**Molecular forensics and honeybee host-parasite interactions.**

Evans JD

Email: Jay.Evans@ars.usda.gov

Honeybee colonies decline from the paper cuts of increased worker mortality caused by biological agents, poor nutrition, and exposure to chemicals, along with other minor threats. Host-pathogen and host-parasite outcomes are affected by the virulence of single and multiple infections, and the abilities of bees to both avoid infection and to resist or tolerate active infections. We are using genetic tools and experimental methods to determine the major biological causes of honeybee mortality, in hopes that these approaches can identify the primary threats to bee health and lead to better breeding or management. With sequenced genomes for honeybees and their major threats, this system is emerging as an important one for general understanding of disease and resistance in a social context.

I will review collaborative projects including country-wide survey data from the U.S., longitudinal surveys of threats and colony fates, and experimental results from dual exposure of bees to microbes. *Varroa* mites and their associated viruses continue to present themselves as chief factors in bee health, with supporting roles from dicistroviruses and microsporidia in some environments. COLOSS-led efforts to standardise methodologies, along with a generally increased interest in bee health, have enabled an unprecedented view of how honeybees deal with or succumb to their contingent of pathogens and parasites.

PL

**Royal jelly, signalling pathways and genes – how far are we along in understanding caste development?**

Hartfelder KH

Email: klaus@fmrp.usp.br

Caste polyphenism is the morphological manifestation of the highly efficient division of labour in social insects, but understanding how a single genotype can give rise to two very distinct phenotypes is still a long, yet fascinating journey for developmental biologists. In the honeybee, the trigger is differential nutrition of the larva, with queens receiving copious amounts of royal jelly throughout larval development, while workers are fed less frequently and receive a diet less rich in sugar during the third and fourth instar. Furthermore, royal jelly contains a labile protein, royalactin, with developmental effects through activation of the EGF receptor pathway. Other factors, such as juvenile hormone, insulin and TOR signalling are equally ancient modulators of insect development, and are all known to play important roles in caste development. Genomics now also brings new insights into the role of the respiratory metabolism in caste development, upon revealing overexpression of hypoxia core genes in worker larvae. But how do all these signalling pathways integrate so as to generate divergent developmental patterns and what are their targets? The latter can best be addressed through investigating differential gene expression, for instance in the larval ovary, and this has brought to light new players, long noncoding RNAs, two of these mapping to a QTL for ovariole number variation in workers. Long non-coding RNAs may directly regulate the expression of specific patterning or effector genes, or they may interact with epigenetic regulators, thus building a bridge towards the findings on differential DNA methylation and its wide ranging effects on caste morphology.

for your remarks:

# Session One

## Neurobiology

**Chairs:**

**Dorothea Eisenhardt**  
(Berlin, Germany)

**Jean Christophe Sandoz**  
(Toulouse, France)

### **Symposium description**

Honeybees show a wide range of individual and social behaviours as well as impressive cognitive abilities that have long been thought to characterise only vertebrates. For this reason, the honeybee has become in the last century a standard model system for studying the neurobiology of behaviour, perception, learning and memory. Starting with the discovery of the bee dance by Karl von Frisch and the establishment of behavioural analysis on free flying bees in the middle of the last century, the study of bees' behaviour strongly progressed with the establishment of learning paradigms that allowed studying the behaviour of single animals in the laboratory.

In addition, the development of electrophysiological and optophysiological methods together with the recent deciphering of the honeybee genome have provided a rich array of scientific tools for understanding the neurobiological basis of bees' behaviour and sensory physiology. This symposium will present recent advances in the field of honeybee neurobiology based on this diversity of experimental approaches. The work presented by the selected speakers will demonstrate that honeybee neurobiology is not only a fascinating field in itself but helps to elucidate major questions of the neuroscience of behaviour.

1.1

### Neuronal mechanisms underlying behavioural plasticity in the honeybee.

Rössler W\*, Groh C, Muenz TS, Scholl C

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Division of labour is a crucial component of insect societies and an attractive model to study fundamental mechanisms of behavioural plasticity. Honeybee workers exhibit a pronounced age-related polyethism. Young individuals take over in-hive duties, whereas bees around the third week of adult life start to forage. This brief transition is associated with structural synaptic reorganisation in neuronal microcircuits in the mushroom bodies (MBs), brain centres associated with sensory integration, learning and memory. We investigate mechanisms underlying this plasticity by combining high-resolution imaging with ultrastructural and molecular tools. Our results show that massive rewiring of MB input synapses (microglomeruli, MG) is induced by sensory exposure and associative learning rather than an internal programme. At the ultrastructural level, presynaptic specialisations become modified, and numbers of associated dendritic profiles increase resulting in drastic changes in synaptic divergence from the level of olfactory and visual projection neurons to MB intrinsic neurons (Kenyon cells, KCs). CaMKII, a protein associated with synaptic plasticity, and f-actin are highly enriched in KC dendritic spines and are likely to link activity with the massive changes in dendritic morphology. The results of various experimental lines are fed into a model aimed to understand the regulation and behavioural consequences of this remarkable neuronal plasticity. Support by HFSP; DFG SPP 1392.

1.2

### Influence of nutritional state on appetitive and aversive olfactory learning in honeybees.

Wright GA

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Learning how to predict where to find food allows foraging animals to be more efficient. The mechanisms that regulate feeding, therefore, are also likely to influence learning and memory processes that are required during foraging. In the ontogeny of feeding, animals undergo cyclic changes in nutritional state, largely defined by levels of blood nutrients or other nutrient reserves. Here, I will present evidence to show that both appetitive and aversive learning in adult forager honeybees is modulated by nutritional state. Our data indicate that present nutritional state, as well as the flux in hemolymph nutrients that occurs during foraging, both have a profound influence on both the acquisition and the consolidation of olfactory memories in honeybees.

for your remarks:

1.3

**Colour vision and learning in honeybees.****Hempel de Ibarra N\*, Niggebrügge C, Leboulle G, Nicholls E, Balamurali GS**

Email: N.Hempel@exeter.ac.uk

Foraging honeybees largely rely on colour information to select and recognise floral food sources. They quickly associate a coloured stimulus with a reward in free flight. However when restrained they seem to learn with much greater difficulty which limits the possibilities to the study the mechanisms underlying colour perception and learning. Using modified conditioning procedures, we were able to demonstrate that restrained bees can learn colours after just a few training trials. Bees discriminated between colours, but showed also broad generalisation. This result is in strong contrast to the known ability of bees to finely discriminate colours under free-flight conditions. It raises new questions about the temporal and perceptual processes underlying the ability of bees to discriminate and learn colours in different behavioural contexts.

1.4

**Comparative evaluations of reward dimensions in honeybees – evidence from 2AFC PER conditioning.****Shafir S\*, Yehonatan L**

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The objective of the present research was to test whether bees perform absolute or comparative evaluations of reward dimensions, relying on Weber's law of relative perception. We performed six experiments, each having four treatments, using 2-alternative forced choice (2AFC) proboscis extension response (PER) conditioning. The dimensions controlled were 1) the delay until delivery of reward, 2) the amount and 3) the concentration of the sucrose reward. Every subject was tested in only one condition of one experiment. Within a treatment, the high and low profitability rewards varied in only one dimension. Between treatments of one experiment, the values of only one dimension increased progressively. Monotonic variation in choice proportions between the treatments of each experiment would support absolute evaluations. According to the comparative model, dimensions are assessed independently of each other. Hence, since in the different treatments of any one experiment the values of only one dimension differed between the high and low profitability options, and they remained identical between treatments, choice proportions would remain constant between the treatments of each experiment. Comparison of the choice proportions between the four treatments of each experiment revealed that the choice proportions were not statistically different from each other in any of the experiments, thus providing further support for comparative evaluations by honeybees of cost and gain dimensions.

for your remarks:

1.5

### Does exposure to sublethal pesticide doses affect the development and behaviour of bees?

Gill R, Raine NE\*

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Bees play a critical role in the pollination of both food crops and wild flowers. Therefore, understanding the causes of widespread declines in bee populations has important consequences for the ecology, economy and food security of our environment. Our reliance on agricultural chemicals, such as pesticides, to boost crop yields is one factor which could have a significant detrimental impact on both wild and managed bees. Although pesticides are generally applied at concentrations considered to be below the lethal dose we know little about the potential effects that exposure to sublethal doses of multiple pesticides has on bee behaviour and overall colony health. Here, we present results from an experimental study investigating the separate and combined effect of sublethal doses of two pesticides on bumblebee (*Bombus terrestris*) behaviour. This study is novel because we studied the possible developmental and behavioural effects of pesticide exposure at both the individual and colony level. Furthermore, pesticide concentrations used in this experiment were analogous to those that bees are likely to experience foraging on crops in the UK, thus providing an ecologically realistic scenario. Using Radio Frequency Identification (RFID) tagging technology we examined detailed foraging patterns of over 1000 individuals from 40 colonies under natural conditions in real time.

1.6

### Functions of the mushroom body in the honeybee brain.

Menzel R

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The mushroom body (MB) of the honeybee is known to be involved in memory processing. We used intracellular and extracellular recordings from MB intrinsic and extrinsic neurons as well as Ca imaging to elucidate the locations and processes of neural plasticity as related to olfactory learning. We found that MB intrinsic neurons, the Kenyon cells, undergo associative and non-associative plasticity leading to an olfactory memory trace that is best conceptualised as “matrix memory” because the content of memory appears to be represented in the pattern of synaptic changes in an associative matrix. Multiple forms of learning related plasticity was found in MB extrinsic neurons. Examples will be described. In some cases as in the identified neurons PE1 a mechanistic model can be developed which tracks the associative changes to presynaptic inhibitory neurons, the A3 neurons. These neurons have also been studied in visual context dependent forms of olfactory learning. Subsets of A3 neurons enhance or reduce their responses to the learned compounds of stimuli. A model of neural interactions will be presented that captures part of the described neural plasticity. The available data will be used to derive a model of MB function. The MB is interpreted to recode the highly specific and diverse sensory input to a small number of value based dimensions.

for your remarks:

1.7

### Age-related and molecular correlates of changes in responsiveness to alarm pheromone in bees.

Urlacher E\*, Tarr I, Devaud JM, Mercer A

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Pheromones are important signals in the life of honeybees which not only impact their physiology and behaviour but also their cognitive abilities: young bees exposed to the queen mandibular pheromone (QMP) do not show aversive learning, and foragers exposed to sting alarm pheromone (SAP) have impaired appetitive learning performances. We aimed to study the interplay of QMP and SAP in the modulation of appetitive learning as bees age.

We show here that SAP fails to impair such learning in young bees. As young bees are more into contact with the queen and her pheromones than older bees, we examined whether QMP inhibits responses of young bees to SAP. We will also examine the effects of QMP on the levels of expression of a recently identified allatostatin receptor that appears to be intimately involved in mediating the effects of SAP. We will present age-related changes in level of expression of the allatostatin pathway, both in bees raised with/without QMP, in parallel to the behavioural effect of SAP on appetitive learning.

for your remarks:

1.8

### How are reward experiences translated into memory?



Martert K\*, Eisenhardt D

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Here we ask about the impact of the reward magnitude on learning and memory formation during classical olfactory conditioning of the proboscis extension response (PER) in harnessed honeybees (*Apis mellifera*). A recent study in honeybees demonstrates a correlation between the reward duration during classical conditioning and the susceptibility of a long-term extinction memory for protein synthesis inhibition (Stollhoff & Eisenhardt, 2009). However, a correlation between learning performance and the reward duration was not observed. This might be due to the fact, that only the occurrence of the PER was recorded, resulting in dichotomous data. We study the behaviour of the animals, quantify the activity of the proboscis extending muscle M17 and interfere with long-term memory (LTM) by protein synthesis inhibition in order to understand the reward duration's impact on learning and memory formation.

Our experiments demonstrate that the reward duration has no effect on learning. Nevertheless, it affects the sensitivity of the LTM to protein synthesis inhibition and thus suggests different molecular mechanisms underlying memory formation after classical conditioning with different reward durations.

1.9

**Tyramine and honeybee behaviour.****Scheiner R\*, Toteva A, Bode V, Reim T, Thamm M, Chatterjee P, Yi SV**

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Biogenic amines regulate a multitude of behaviours in vertebrates and insects. Whereas the functions of most biogenic amines have been studied in depth, tyramine has long been neglected. It was assumed that it only acted as a precursor of octopamine. Now that specific tyramine receptors have been described in different insects, we wanted to know how this amine functions in honeybee behaviour.

Tyramine is most likely involved in the regulation of division of labour. Workers performing different foraging roles differ significantly in the methylation of the only characterised tyramine receptor in the honeybee, but not in that of the characterised octopamine receptor. We have first indications that gene regulation occurs via splicing. Expression of the tyramine receptor gene does not differ between pollen and nectar foragers.

The effects of tyramine on behaviour are diverse and complex. (1) Tyramine increases sensory responsiveness to sucrose in different age groups. (2) Tyramine increases locomotion, but does not affect responsiveness to light. (3) Olfactory learning performance is improved by tyramine in young bees but not in older bees. These findings suggest that tyramine has a decisive regulatory function in honeybees and other insects.

1.10

**The serotonin 5-HT<sub>2</sub> receptor system of the honeybee (*Apis mellifera*).****Blenau W\*, Thamm M, Rolke D, Jordan N, Baumann A**

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Serotonin (5-HT) plays a key role in regulating and modulating physiological and behavioural processes in both protostomes and deuterostomes. In the honeybee *Apis mellifera*, serotonin has been implicated in division of labour, visual processing, and learning processes. Most 5-HT receptors belong to the superfamily of G-protein-coupled receptors. In the honeybee, a 5-HT<sub>1A</sub> receptor and a 5-HT<sub>7</sub> receptor have been molecularly and functionally characterised. However, bioinformatics indicated the presence of two additional 5-HT receptor genes, Am5-ht2a and Am5-ht2b, for which we have recently cloned the full length cDNAs. In addition, truncated transcripts representing alternative splice variants were detected for both 5-HT<sub>2</sub> receptors. HEK 293 cells were stably transfected with either the Am5-ht2a gene or the Am5-ht2b gene. Activation of both receptors with serotonin causes an increase in the intracellular Ca<sup>2+</sup> concentration with Ca<sup>2+</sup> being released from the endoplasmic reticulum in an IP<sub>3</sub> dependent manner. Full pharmacological profiles were established for both receptors. Am5-HT<sub>2a</sub>-tr, the truncated splice variant of Am5-HT<sub>2a</sub>, was also heterologously expressed but did not evoke any cellular response. Receptor mRNA expression patterns were investigated by quantitative real-time PCR. Interestingly, the expression of the full length variants of both honeybee 5-HT<sub>2</sub> receptors is highest in the hypopharyngeal gland, whereas expression in the nervous system is very low for both receptors.

for your remarks:

1.11

**A cellular alphabet of honeybee learning: ionic current modulations and effects of insecticides.**

Grünewald B\*, Ziegler-Himmelreich S, Fischer J, Triltsch M

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The antennal lobes and the mushroom bodies within the honeybee brain are differentially involved in odour learning, memory formation and recall. We assume in both brain areas a crucial role of cholinergic synaptic transmission during learning-dependent plasticity. Therefore, we investigate the modulation of currents through nicotinic acetylcholine receptors (nAChR) by biogenic amines. In addition we study the cell physiological and behavioural effects of the commercially used neonicotinoid insecticides, clothianidin, and imidacloprid. Our results show that octopamine or serotonin applications reduce nAChR currents of cultured bee neurons probably via a cAMP-dependent pathway. Using  $Ca^{2+}$ -imaging techniques we observed octopamine-evoked  $Ca^{2+}$ -transients. These findings indicate that learning-related plasticity may occur both in the AL and the MB via cAMP and calcium-dependent signalling cascades. Neonicotinoids are agonists of the insect nAChR. Feeding sublethal doses of these substances to adult bees affect their foraging activity. They also have distinct effects on honeybee motor pattern. Brain applications of clothianidin increase whereas imidacloprid or thiacloprid applications decrease activity in the muscle M17 and movements of the whole bee. We are currently investigating the effects of insecticides on cell physiology and on olfactory learning.

for your remarks:

# Session Two

## Chemical Ecology

**Chairs:**

**Manfred Ayasse**  
(Ulm, Germany)

**Thomas Eitz**  
(Bochum, Germany)

### **Symposium description**

Bees live in a world of complex sensory impressions amongst which chemical stimuli are of foremost importance. Bees use a broad range of endogenous pheromonal chemicals for intraspecific communication, e.g. for identifying colony membership, location of food sources, mate recognition etc. Furthermore, they use information from host plant odours to locate resources, and to enhance foraging efficiency. The speakers of our symposium will present various examples of intra- and interspecific chemical communication in different groups of solitary and social bees.

2.1

### Chemical recruitment communication and “pheromone” learning in stingless bees.

Jarau S

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Foragers of several stingless bee species accurately recruit their nestmates to specific food sites by depositing attractive pheromones on a certain stretch from the food towards the nest. The pheromones originate from the bees' labial glands and are composed of species-, as well as nest specific blends of esters. Interestingly, recruited workers are mainly attracted by their nestmates' pheromone, while the pheromones deposited by foreign workers are largely ignored. The preference for pheromones deposited by members of a worker's mother colony probably helps to avoid competition for resources. In the species *Scaptotrigona pectoralis* and *S. subobscuripennis* we investigated whether recognition of colony specific pheromones is based on a fixed, innate mechanism or whether the bees have to learn their specific pheromone blend. We placed brood combs of the two species into nests of the respective congeneric species and tested whether workers, once they have reached forager age, are attracted by the pheromone of their genetic sisters from their mother colony or by the pheromone of workers from the foster colony in which they had hatched. A significant majority of bees chose the pheromone from workers of the nest they lived in, even if it was the pheromone of the other species. Apparently, stingless bees do not innately recognise their nest specific pheromone blend, but learn it within the colony. The underlying mechanism may be similar to learning of food odours during recruitment.

2.2

### Nest wax signals trigger worker reproduction in bumblebee colonies.



Rottler A\*, Schulz S, Ayasse M

Email: ann-marie.rottler@uni-ulm.de

Workers of the primitively eusocial bumblebee *Bombus terrestris* are capable of producing male offspring in the presence of the queen. Nonetheless, they refrain from reproduction in favour of their mother's progeny during the social phase (sp) of colony development. Workers only compete for reproduction at the end of the breeding season, when the colony starts to produce reproductives (competition phase cp). Timing the onset of worker reproduction is crucial as it is not in the workers' interest to impair the colony performance. In this study we examined the influence of nest wax signals on the competition over male production.

Analysing the chemistry of nest wax at different stages of colony development we found a complex blend of hydrocarbons, aldehydes, and wax esters. On the cuticle surface of female bumblebees, the same substances serve various inter-individual communication purposes, e.g. signalling fertility. In wax extracts the substance patterns differed between sp and cp colonies and allow workers to determine the status of their colony. Behavioural assays showed that queenright workers started to compete for reproduction when facing an artificial nest made of cp wax. Additionally, the ovaries of these workers were further developed than in workers exposed to sp wax. The effects of nest wax on the reproductive behaviour and physiology of workers suggest that wax signals play a crucial role in the social communication of bumblebees. (Funded by the DFG AY 12/3-1)

for your remarks:

2.3

### How the social parasitic bumblebee *Bombus bohemicus* sneaks into power of reproduction.

Kreuter K, Bunk E, Lückemeyer A, Twele R, Francke W, Ayasse M\*

Email: manfred.ayasse@uni-ulm.de

Social parasitism is widespread in many groups of social living hymenopteran species and has also evolved in the genus *Bombus*. Cuckoo bumblebees (subgenus *Psithyrus*) are obligate brood parasites in nests of other bumblebee species. After nest usurpation and the killing of the host queen, the parasite female has to control worker reproduction in order to accomplish and maintain reproductive dominance and to ensure her reproductive success. The aim of our study was to examine whether the generalist parasitic bumblebee *Bombus bohemicus* monopolises and prevents worker reproduction by physical or chemical means and to identify possible odour compounds involved therein. We performed bioassays with callow workers of the host *Bombus terrestris* and have shown that *Bombus bohemicus* females are able to suppress host worker ovarian development, when these host workers are under the direct influence of the parasite female. Furthermore, by chemical analyses, we have demonstrated that the parasite females adjust to the odour profiles of their host queens in order to maintain the level of fertility signalling inside the host colony although the host queen is absent. We also found that host workers change their odour profile after nest usurpation by the parasite female and consequently, we suggest that the host and parasite are caught up in a chemical arms race. (Funded by the DFG AY 12/2-2)

2.4

### Silenced by the regime: under virgin queens honeybee workers develop ovaries, but fail to advertise it.



Orlova M\*, Malka O, Hefetz A

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Queen mating status in social insects is a matter of crucial importance for workers because of its influence on the queen's productivity and consequently their fitness. Behavioural and physiological reactions of workers to the queens mating status have been studied as a proxy to mechanisms maintaining insect sociality. Here we show that unmated honeybee queens have considerably impaired capacity to trigger worker sterility in comparison to mated (and thus more productive) queens. Together with this it is shown that honeybee workers exposed to unmated queens despite being active reproductively display an impaired ability to advertise their fertility compared to queenless workers. These findings suggest that reproductive development and production of fertility signals are differentially regulated and differently influenced by the queen's presence.

for your remarks:

2.5

### Three African honeybee subspecies and their mandibular gland pheromone composition.

Pirk CWW\*, Yusuf AA, Crewe RM

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The mandibular pheromones produced by the queen in part regulate worker reproduction. Under queen right conditions, non-laying workers secrete from the mandibular gland are dominated by fatty acids that are incorporated into the food given to the brood and to nestmates via trophallaxis. Queen loss can result in the onset of reproductive activity in workers, in which they synthesise fatty acids, which are normally produced by queens and that contribute to their reproductive status and success. We compared the mandibular gland pheromones of three sub-saharan subspecies, *Apis mellifera capensis*, *A. m. scutellata* and *A. m. adansonii*. *A. m. capensis* workers produce the precursor of the major component of the queen pheromone even in the presence of their own queen and can therefore dominate reproduction, outcompeting the host queens in colonies of other subspecies. In comparison workers of *A.m. scutellata* show the standard worker profiles of mandibular gland pheromones while *A.m. adansonii* workers show an intermediate between the two with significantly higher proportions of 9-HDA and HOB than *A. m. scutellata* and lower than in *A. m. capensis*. The implications for these variations to worker reproduction and the regulation thereof are discussed.

2.6

### On how bees recognise friend from foe.

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Discrimination against non-nestmates is crucial in the lives of social insects as it helps to prevent robbery and parasitism, and directs helpful behaviour towards related individuals. The exact proximate mechanism of how guards use cues for this discrimination is still somewhat elusive. Recent advances have shown that several species of ants and bees respond aggressively to unknown cues on the cuticles of encountered individuals. In contrast, individuals that have beforehand learned these new cues do not respond aggressively to the lack of them, suggesting an elemental habituation-type of learning involved. Here, we test this same prediction with guards of *Apis mellifera*, but instead of adding an unknown cue, we increase the concentration of a known cue, which better simulates natural differences between colonies. In addition we investigate the speed at which these cues can be learned and how other proximate factors influence the guards' discriminatory behaviour. These results may have important implications for both the physiological/neurobiological understanding of kin discrimination and the evolution of recognition systems.

for your remarks:

2.7

### Parasite-induced changes in chemical signals in the three castes of *Apis mellifera*.



Dussaubat C\*, Maisonnasse A, Alaux C, Beslay D, McDonnell C, Brunnette JL, Le Conte Y

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In social insects chemical signals play an important role in the regulation of group homeostasis, but unfortunately this balance can be altered by parasite infections. For example, in honeybees the parasite *Varroa destructor* can modify a group of cuticular chemicals involved in social recognition. While the modification of host chemical signals is well-known in other organisms, in social insects the impact of parasites in social communication has not been deeply studied. Thus we ask if the honeybee pathogen *Nosema ceranae*, known to induce oxidative and energetic stress among other effects, has the potential to disturb social regulation through the modification of individual chemical signals. First, we measured in parasitised workers the production of ethyl oleate, a primer pheromone involved in the regulation of division of labour. Then, we analysed in parasitised queens the levels of mandibular pheromones which allow the queen to control colony organisation by monopolising the reproduction and regulating the cohesion of the society. Finally, we studied in infected drones the variations of cuticular hydrocarbons used in social recognition. In workers and queens infection significantly altered pheromone levels, while in drones cuticular hydrocarbon profiles were also modified. We discuss the consequences of chemical signal modifications in the three honeybee castes for colony survival.

2.8

### Avoidance of contaminated flowers by foraging bumblebees.



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Bumblebee colonies are founded by a single-mated queen. They are more susceptible to parasites and diseases than polyandrous and/or polygynous social insects. A greater resistance towards parasites occurs with increased genetic variability within a colony. The first barrier against a parasite is to avoid it. We investigate the parasite avoidance behaviour of bumblebees by observing them foraging on two artificial flowers. Four experiments were performed. The first choice experiment consisted of colonies choosing between uncontaminated (UCF) and contaminated flowers (CF); in these experiments the flowers were contaminated by either *Escherichia coli* or *Crithidia bombi*. In the other two experiments, we investigated the role of scent-marks by their removal in the test experiment. Observation of individuals foraging between a scented and rewarding flower, and unscented and low-rewarding flower; while in the other experiment there were the UCF and CF containing *C. bombi*. Firstly, bumblebees show an aversion to CF, which is stronger in the presence of a specific parasite, since they visited the UCF proportionally more often when the CF contained *C. bombi*. Secondly, presence of a parasite in the flower reduces its reward; they spent less time feeding on the CF. Thirdly, avoidance of CF is innate and possibly originates from the odour of the parasite. Avoidance behaviour efficiency increases over time due to social cues, probably from copying behaviour, since scent-marks do not interfere with the choice of the bees.

for your remarks:

2.9

**Host plant finding in oligolectic bees: from behaviour to molecules.**

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Many bee species are specialised and collect pollen for their offspring only from a few closely related plant species. Such bees need to recognise their specific host plants and discriminate them from others available on the floral market. Visual and olfactory flower cues are known to play important roles in host finding of bees, however, little is known about i) the relative importance of these two cue modalities and ii) the specific visual (e.g. colour) and olfactory cues (scent compounds) oligolectes use to find their specific host plants. We used a multifaceted approach combining behavioural, chemical, and electrophysiological techniques to gain a deeper insight into bee-plant interactions. The relative importance of visual and olfactory cues in locating potential host flowers varied among oligolectes, with some species relying more on visual others on olfactory cues; however, a combination of both cues was generally most attractive. Though attractive, visual cues did not allow all the bee species to recognise their host plants. Instead, olfactory cues were most important for oligolectes to discriminate host from non-host plants. Bees relied on highly specific, uncommon floral scents, which are typically emitted in trace amounts from their host flowers. These compounds are often host specific and therefore reliable cues for oligolectic bees in search for pollen hosts.

2.10

**Chemical profiles of stingless bees: to be or not to be sticky.**

Leonhardt SD\*, Blüthgen N, Drescher N, Wallace HM, Schmitt T

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Stingless bees (Meliponini) collect large amounts of plant resins which they use for nest construction, defence and to transfer resin-derived compounds to their body surfaces. We compared the chemical profiles of stingless bees of Asia, Australia and America, and found that resin-derived compounds were a predominant characteristic of paleotropical stingless bees, where eleven out of twelve species had significant amounts of these compounds. By contrast, only 56% of the neotropical species had resinous compounds. To better understand the role of resin-derived compounds in stingless bees, we compared resin foraging, the prevalence of resin compounds in nest material, and defensive properties of resin compounds in the bees' cuticle for two Australian species. Australia is unique in comprising stingless bees of two genera with different geographic origins: *Tetragonula* from Asia and *Austroplebeia* from Africa. Whereas *Tetragonula* has a high proportion of resin-derived compounds in the chemical profile, *Austroplebeia* lacks such compounds. The two species strongly differed in their resin collection behaviour, and *Austroplebeia* was more vulnerable to predation than *Tetragonula*. However, *Austroplebeia* had higher amounts of alkenes in their nest material and protected their nests by narrowing the entrances. The usage of resin and resin-derived compounds may hence represent an alternative, yet highly efficient chemical and behavioural strategy of bees to protect colonies.

for your remarks:

2.11

**What is repellent in chemical footprints of flower-visiting bumblebees?**

Eltz T\*, Witjes S, Brunsbach H, Tries K, Radermacher L

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Foraging bumblebees are repelled by flowers recently visited by conspecifics or other bees. This repellency is based on olfactory cues deposited by the previous visitor (footprints), and not by direct cues linked to the floral reward. The chemical nature of the behaviourally active odour stimuli has remained unclear. Bumblebee footprints consist mostly of long chain hydrocarbons (CHCs), alkanes and alkenes, ranging from 21 to 31 C atoms. Although tricosene (C23:1) and other synthetic CHCs have been found to repel foragers in bioassays (Goulson et al. 2000), the biological relevance of this result is uncertain: long chain CHCs have very low volatility, remaining on visited flowers for up to 48 hours, whereas the behavioural repellency of footprints usually vanishes after an hour or less. Therefore we searched for volatile trace compounds in bumblebee footprints/tarsal extracts. Combining different 'fractioning' techniques, including (i) partial evaporation of footprints on glass surfaces and (ii) preparative GC of tarsal extracts, with behavioural experiments, we found evidence for the existence of highly volatile compounds that mediate repellency. However, such compounds were not detectable by GC, presumably due to very low concentration. In contrast, the massive low-volatility CHC fraction, comprising nearly 100% of analytes in tarsal extracts, proved behaviourally inactive. Ongoing research will hopefully allow to further elucidate the chemical nature of repellent bee footprints.

for your remarks:

# Session Three

## Physiology

**Chair:**

**Karl Crailsheim**  
(Graz, Austria)

### **Symposium description**

Classical disciplines have received more attention in honeybee science during the last years. This is due to new results and insights into hormonal, nutritional and behavioural physiology, and of course all metabolic aspects regarding immune defense. Especially nutrition went into the focus as not only being responsible for maintenance of the colony but also influencing the development of single bees from the larval stage on and having strong impacts on various parameters during the whole life of the bees. Knowledge about the genome of the honeybee also pushed physiological research forward. Colony losses during the last years have led to more intensive research of basic mechanisms. Still reasons for these losses are mostly unknown and that has led to the assumption that multiple factors might accumulate and lead to the death of bees. Nutrition, immune defense, temperature, endocrinological and neurobiological reasons are discussed as those factors.

3.1

### Insulin signalling in honeybees – social life history accompanied by changes in an ancient signalling module.

Hartfelder KH\*, Azevedo SV, Wang Y, Amdam GV

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Insulin/insulin-like signalling (IIS) is an ancient module adjusting growth and reproduction with nutrient availability. In fruit flies, IIS determines the onset of metamorphosis and regulates fertility and lifespan. In honeybees, social evolution resulted in a rewiring of certain endocrine circuitries, as insulin receptor expression is down-regulated in queen larvae. Here we asked whether the knockdown of insulin-like peptide (AmILP-1 and AmILP-2) gene functions affects the hemolymph JH titre and sugar levels, as well as body mass and ovary size. AmILP-2 but not AmILP-1 mRNA levels were significantly reduced in larvae fed dsRNAs and there was a significant interaction in double knockdowns. While sugar levels were not affected, the JH titre was significantly decreased in dsAmILP-1-treated larvae, and ovariole numbers were so in AmILP-2 knockdowns. Thus, in honeybee caste development, the ILPs may play a more target-specific modulatory role than TOR. Standing in contrast with current paradigms of IIS function in insects, future studies will require ligand-binding analyses with InRs and other growth factor receptors to reveal alternative roles of IIS in the social life history of honeybees.

3.2

### Hallmarks of accelerated and negligible senescence in different worker phenotypes of the honeybee.

Münch D\*, Amdam GV

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The honeybee (*Apis mellifera*) is the best-studied social invertebrate to date; it has a long tradition in neurobiological research, and now has also gained popularity as a model in biogerontology. Its recognition as an aging model is founded upon a stunning diversity of longevity patterns that outrivals natural as well as experimentally induced life span differences in more traditional aging models. What's more, phenotypes with short and extremely long lifespan can be transformed into one another, providing the unique opportunity to study how negligible senescence and the reversal of aging symptoms can emerge.

To address this, research in our group studies aging – defined as intrinsic functional decline – in short lived forager bees and their long-lived counterparts (diutinus, winter bees). Aging in foragers is associated with a rapid decline in diverse brain functions, and accompanying changes in the brain's protein matrix. However, aging is not homogenous among individuals, functions or tissues. For winter bees we show how largely slowed (negligible) senescence can emerge as a function of social factors, including hive demography. The impact of social factors on aging is further illustrated by the rapid accumulation of a marker for cellular senescence after winter bees changed to foraging, and by brain protein level changes, when short lived foragers revert back to a longer lived worker type.

for your remarks:

## 3.3

**Physiological basis of social immunity.**

Alaux C\*, Le Conte Y

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Honeybees have evolved a social immunity consisting of the cooperation of individuals for preventing the entry and spread of parasitic infection between the colony members. Such social immunity includes, for example, grooming, the use of antimicrobial materials, social fever, nest hygiene and social removal. Since their description, many studies have explored the behavioural mechanisms of these collective immune defences against pathogens but the physiological basis remains largely unknown. We first identified a set of genes involved in hygienic behaviour by analysing the brain transcriptome of bees, who efficiently detect and remove brood infected with the *Varroa destructor* mite. Comparing their genomic profile with those from other behaviours suggests a link with brood care and the highly varroa-hygienic Africanised honeybees. Second, we tested the role of the immune system in social removal by treating bees with a non-pathogenic immune-activator (lipopolysaccharides). The immune challenge induced the development of a forager profile, which suggests that the interplay between the brain and immune system may be an important regulatory factor of social immunity in insects. As a result, the identification of the physiological basis that influences social immunity promises to not only improve our understanding of its mechanisms but also provide new insights into the evolution of collective defence in insect societies.

## 3.4

**Molecular analysis of immune system and aging gene expression in long-lived winter honeybees.**

Aurori CM, Dezmirean DS, Mărghitaş LA, Moritz RFA, Erler S\*

Email: silvio.erler@zoologie.uni-halle.de

Honeybees (*Apis mellifera*) are excellent models for the study of aging because the female castes (workers and queens) show extreme variance in life span. Also within the worker caste the bees have an extreme polymorphism concerning life span. Whereas workers in summer live only for a few weeks, workers in the overwintering colony can live for many months. Here we tested the effect of aging on winter-honeybees (long-lived workers) in relation to pathogen infection by using honeybees from different stages of overwintering and measuring gene expression of several immune system and aging related genes. Immune gene (e.g., relish, apidaecin and hymenoptaecin) expression did not differ between immune challenged and non-challenged bees, but was up-regulated in the late over-wintering bees, that might be explained by the immunity / longevity trade-off. Genes that had been identified in other organisms to be aging-related did not show any consistent pattern related to the age of the workers. Vitellogenin was not significantly different within and between groups (challenge and months) and not linked to immune gene expression.

for your remarks:

3.5

**Dietary regulation of adult honeybees.****Paoli P\*, Stabler D, Donley D, Wright GA**

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The aim of this study was to compare the nutritional intake targets between nurse bees and foragers, and how diet influences lifespan. In previous studies that investigated the optimal nutritional intake of adult worker honeybees have used solid diets including casein, pollen, and royal jelly to replicate protein sources. In this study, liquid diets composed of the ten essential amino acids and sucrose were used. Using the Geometric Framework, a model-based approach to identify nutritional optima that uses two limiting dietary factors to calculate a target intake, we presented newly emerged and mixed aged foragers with different concentrations of amino acids in sucrose. From this, we were able to determine how dietary intake of amino acids affects lifespan and development.

We found that honeybees regulate their intake according to their carbohydrate needs, and that newly emerged workers (nurses) are more tolerant of higher protein concentrations than older, mixed age foragers. The survival of both nurses and foragers depended on the proportion of protein in the diet. Working foragers hold a dietary bias towards carbohydrates as they may no longer need protein as they do not feed larva, but also the energy demand for constant flying is higher and is more readily filled by carbohydrates.

3.6

**Colony heterothermy in stingless bees (*Melipona scutellaris*).****Hrncir M\*, Roldão YS**

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Despite large variations in ambient temperature, honeybees (Apidae, Apini) are capable of maintaining the temperature of their nests within narrow boundaries. Active thermoregulatory mechanisms underlying such colony endothermy are considered key to the ecological success of these animals, which can be found in many terrestrial environments ranging from cold temperate climates to hot desert-like habitats. The eusocial stingless bees (Apidae, Meliponini), by contrast, inhabit predominantly tropical and subtropical habitats, which might be due to their limited thermoregulatory capacity. Here, we show that nest temperatures of *Melipona scutellaris* vary in accordance with changes in ambient temperature in the course of the year. The temperature in peripheral nest areas was the same as the temperature of the surrounding environment. Brood-area temperatures were, on average, 5° higher than environmental temperatures and oscillated little during a day. In the course of the year, however, brood-area temperature varied between 22°C and 32°C and was strongly correlated with ambient temperature. Our results indicate that colonies of *M. scutellaris* behave like heterothermic endotherms that, although generating heat through metabolic processes, nonetheless do not maintain a constant body temperature. The observed dependency of brood temperature on ambient temperature might be the key factor restraining the geographic distribution of these bees. [CNPq 481256/2010-5, 304722/2010-3]

for your remarks:

3.7

### The role of brood pheromone as a signal in failing queen health in the honeybee *Apis mellifera*.

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Honeybee colonies rear new queens when the queen is killed (emergency queen rearing) and to supersede or swarm. When the queen presence pheromone, queen mandibular gland pheromone (QMP), levels drop emergency queen replacement begins. With the other two queen rearing events the queen is still present and it is unknown what triggers either supercedure or swarm cell production. It has been proposed that QMP is limited in failing queens but an alternate hypothesis is that a decline in brood pheromone may trigger supercedure.

In the current study we test this hypothesis that brood pheromone plays an important role in signaling queen health. We tested synthetic QMP and brood pheromone (Brood Boost) alone and in combination on the suppression of emergency queen rearing. It had previously been shown that QMP alone could suppress emergency queen rearing but only for three days. The current studies looked at queen rearing over a 10 day period and suppression of emergency queen cell production was achieved. The results will be discussed and the role of brood pheromone in queen maintenance elaborated relative to how the colony perceives a failing queen.

for your remarks:

3.8

### Trophallaxis as an important feature within the complex honeybee society.

Enzinger S, Crailsheim K\*

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Trophallaxis (food transfer from mouth to mouth) between honeybee workers is an essential feature of communication and nutrition. Carbohydrates, lipids and proteins are shared this way. Different members of the colony receive different amounts and different quality of food. The present study – focusing on the imaginal members of the colony – demonstrates how the composition of the transferred food can be affected. In *in vitro* experiments two to four bees were caged in one cage. A nurse-aged bee that was fed a 3H-polyethyleneglycol labeled carbohydrate solution and was injected 14C-phenylalanine before the experiment had the function of a donor. The labeled amino acid was incorporated into proteinaceous food by the nurse bee. One day old nurse-aged and forager bees were the recipients. However only the donor had access to food. Using these tracers, the dissemination of food from donors to recipients could be tracked. Factors as begging behaviour and the age of the recipient influenced the pattern of food distribution. Donors that had more recipients to provide did not incorporate more of the amino acid into proteinaceous food, but distributed more. The amount of proteinaceous food given to a recipient did not correlate linearly with the amount of carbohydrates that were transferred during trophallactic contacts.

3.9

**Pheromones and social regulations in the honeybee *Apis mellifera*.****Le Conte Y\*, Castillo C, Maisonnasse A, Plettner E**

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Division of labour in honeybee colonies is a fascinating area of research. The workers perform all non-reproductive tasks, based on an important plasticity. Young workers perform non-risky tasks inside the nest (cleaning, nursing) and older bees perform risky tasks (guarding and foraging). An important issue is to understand how the ratio between foragers and nurses is regulated providing optimal development of the colony. The ratio is controlled by a primer pheromone, ethyl oleate (EO) produced by the foragers. The pheromone signal inhibits the progression of younger workers from nursing to foraging tasks. We present our study on EO in the control of normal work distribution to understand the molecular, behavioural, physical and physiological underpinnings of the pheromone-controlled social regulation in the nurse/forager ratio.

We made significant progress in the knowledge of the effects of EO in social regulation mechanisms. Different steps were investigated, such as the genes involved in the biosynthesis of the compounds, the mode of transmission of the pheromone between the workers and the targets of EO.

Moreover, we will present data on the discovery of a volatile component of the brood pheromone which is also involved in social regulations of the workers.

HFSP Program Grant team (Award Year 2007)

for your remarks:

# Session Four

## Behaviour & Colony Function

**Chairs:**

**Matthias Becher**  
(Harpenden, UK)

**Volker Grimm**  
(Leipzig, Germany)

**Thomas Schmickl**  
(Graz, Austria)

### **Symposium description**

*Honeybee colony organisation: from empirical studies to modelling approaches and technical applications.*

Honeybee societies are among the best researched complex societies. The abilities of these societies concerning flexibility, robustness and scalability is impressive. Natural selection has favoured a magnitude of behaviours that allow these societies to integrate thousands of individual agents (bees) into a well functioning self-regulating collective. On the one hand, due to the complexity of the regulating network of feedbacks in such colonies, mathematical models and computer simulation are a valuable tool to further improve understanding of empirical studies and to develop novel (empirically) testable hypotheses. On the other hand, the observed regulatory mechanisms have already inspired several engineering applications, ranging from honeybee-inspired swarm-intelligent algorithms to physically embodied bee-inspired robotic swarms.

## 4.1

**The origins and maintenance of eusociality in bumblebees.****Bryden J\*, Jansen VAA**

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Bumblebee colonies show division of labour between a queen that lays eggs for the brood and workers that forage for food and care for the brood. This unselfish worker behaviour characterises eusociality and is, on the face of it, open to invasion by a worker bee, which lays its own eggs. It has been argued that this behaviour may result from diploid workers being more related to each other than their own offspring which are produced with haploid males. There has been considerable debate about this perspective.

Here we present an alternative model, which focuses on the population structure of the bees. Inspired by work modelling the evolution of sociality in aphids, the model is based on the life cycle of a typical bumblebee queen and includes a trade-off between investing in social behaviour and investing in reproduction. We incorporate drift of bees between colonies and derive a variant of Hamilton's rule.

This result paints a plausible picture of the origin of eusociality which does not need haplodiploidy. Since recent phylogenetic analysis has found that eusociality evolved only once in the corbiculate group of bees, which includes both honey and bumblebees, understanding the evolution of eusociality in the more primitive bumblebees may well shed light on the origin of this fascinating behaviour.

## 4.2

**Individual differences in honeybee social network connectivity.****Gernat T\*, Robinson GE**

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Division of labour is thought to be one reason for the ecological success of social insects. In the honeybee, division of labour appears to be age-based: as a worker bee grows up she progresses through a series of jobs until finally becoming a forager. Most honeybees turn to foraging when they are 3 weeks old, but if environmental conditions or the needs of the colony change, an individual bee may begin to forage much earlier or later. Although the basic mechanism underlying this flexibility has been described, experimentally studied, and modelled, fundamental pieces of its workings are still poorly understood. For example, it is unclear how often bees communicate division of labour related signals and how the interaction network is structured through which this information is transmitted.

To gain insights into these questions we implemented a novel method to automatically track all bees living inside a small glass-walled observation hive. This method produces trajectory logs from which we infer which bees are interacting at which point in time. Testing this method in a week-long experiment resulted in the first interaction network of a full honeybee colony. This network shows striking differences in connectivity between bees. We intend to study whether this diversity has functional consequences, perhaps with respect to the remarkable ability of honeybees to maintain stable behaviour at the level of the colony while being faced with environmental changes.

for your remarks:

4.3

### Blending into the hive: a novel biomimetic honeybee robot for the analysis of the dance communication system.



Landgraf T

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The honeybee dance “language“ is one of the most intriguing examples of information transfer in the animal world. After returning from a valuable food source honeybee foragers move vigorously, in a highly stereotypical pattern, on the comb surface conveying polar coordinates of the field site to a human observer. An amazing amount of knowledge on navigation, memory and communication in honeybees has been gathered and we can rely on compelling evidence indicating that honeybees actually use the information encoded in the dance.

However, today, more than 60 years after its discovery, it still remains unknown how follower bees decode the information contained in the dance. In order to investigate the characteristics of the communication process we are building a robotic honeybee that is able to reproduce all known stimuli, that can blend into the honeybee society and that enables us to finally resolve many questions that remained unanswered for a long time. As of now, we have sent bees out to previously visited and also to entirely new locations by means of the robotic dance. We were able to excite the highly stereotypical following behaviour. An essential signal in this process seems to be the wing oscillations. We have also been able to track the flight of recruits in the field using a harmonic radar system provided by the group of Professor Menzel. However, what determines the follower bees to pursue the robotic dance remains unknown and will be investigated in further experiments.

4.4

### Modelling collective decision making in honeybees.



Szopek M\*, Bodi M, Radspieler G, Schmickl T, Crailsheim K

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In one-dimensional temperature gradients young honeybees locate themselves at their preferred temperature of 36°C. Single bees in a complex temperature gradient in a circular arena show a different behaviour and the majority does not stop at 36°C but moves around randomly. However, groups of bees in the same gradient collectively aggregate at their preferred temperature and are also able to distinguish between a global and a local optimum. We found, that when a bee stops after meeting another bee, the waiting-time correlates with the local temperature. The warmer it is where the bee stops, the longer she waits. The longer the bees wait the higher is the probability of other bees joining. This simple emergent phenomenon leads to bigger clusters in warmer areas and, in the end, an aggregation of the majority of the bees at 36°C. Using parameters derived from the biological experiments (e.g., waiting time of the bees, temperature distribution in the arena), this aggregation behaviour was implemented in a simple top-down model (Vensim).

We compared the results obtained from the model with the results from real honeybee experiments. This tool allows us to predict the behaviour of honeybees in complex temperature gradients and will lead to a better understanding of the underlying mechanisms of collective thermotaxis in honeybees.

for your remarks:

4.5

**Collective decision making in robot swarms without communication.****Kernbach S**

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We investigate spatial collective decision-making in a swarm of microrobots, inspired by the thermotactic aggregation behaviour of honeybees. The sensing and navigation capabilities of these robots are limited; no digital sensor data processing and no direct communication are allowed. In this way, we can demonstrate that even such a limited swarm is none the less able to exhibit simple forms of intelligent and adaptive collective behaviour.

for your remarks:

4.6

**Honeybee aggregation with a mobile robot swarm.****Arvin F\*, Yue S**

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In this study, we implement a cue-based aggregation algorithm with a mobile robot swarm. The algorithm takes inspiration from the aggregation behaviour of honeybees. The scenario was performed with different swarm sizes using real-robot (AMiR: Autonomous Miniature Robot) and simulation-based experiments. In addition, we proposed two modifications on the original honeybee aggregation algorithm with increasing the sensing ability of the robots. The proposed modifications are: i) different velocities in robot's motion based on the thermal clues and ii) resting time depends on the size of the aggregate. We compared the performance of our proposed algorithms with the state-of-the-art cue based aggregation strategy BEECLUST. We are interested in having a fast aggregation around the thermal cue. Therefore, we use aggregation time as one of our metrics. Since BEECLUST aggregation depends on the collisions between the robots, we define the number of collision as a second metric. The data obtained from the experiments was statistically analysed to examine the influence of two independent variables (population and strategies) on each of the dependent variables (aggregation time and number of collisions) to determine the main effect of each independent variable. The results showed that, the proposed strategies outperform BEECLUST employing the additional sensing ability of the agents.

4.7

### ASSISI and REBODIMENT - Novel experimentation paradigms to investigate social behaviour in social life forms.

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ASSISI is the name of an upcoming project investigating novel methods for automated behavioural experimentation with (eu-)social life forms. The main focus within this project is laid on two species: the honeybee *Apis mellifera* and the zebrafish *Danio rerio*. One aspect of the proposed novel experimentation paradigm is to use reactive and learning coupled actuator-sensor units (CASUs), which allow to build self-adjusting, reactive experimental setups. The CASUs are equipped with a big variety of sensors and actuators, allowing to interact with the respective lifeform using only local physical cues. By programming self-adapting swarm algorithms into the CASUs, it is possible to generate two interacting (e.g., cooperating) social entities (animal and CASU), and to analyse the resulting behaviour based on the observations of the whole interacting system, as well as on the sensory inputs and actuator outputs of the CASUs. This will not only lead to a deeper understanding of social lifeforms, but will also promote robotic projects, e.g., the project REBODIMENT, which uses bioinspired algorithms to organise a robotic swarm in an complex temperature gradient.

4.8

### ECOBEE: bee colony monitoring in agrosystems.

Odoux J-F\*, Bretagnolle V, Aupinel P, Gateff S, Requier F, Henry M

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Traditional apicultural practices have been replaced by intensive ones in order to compensate colony losses and yield decreases. The choice of the best locations to set up beehives in farming plains for honey production is largely empiric today. ECOBEE is a monitoring design allowing to an ecological approach to honeybee biology which focus on three topics of interest: i) the impact of land use on colonies development; ii) the impact of farming practices on colonies development; iii) landscape structures effect analyse on colonies behaviour. The design contains data acquisition concerning environmental variables in land use, floral resources or agricultural features in one hand, and colony responses variables concerning colony parameters, life history and collection in the other hand.

In an intensive cereal cropping system, our study concluded to a food shortage for the honeybees in the end of spring, between the rapeseed and sunflower blooming. We described the kinetic cycle for several honeybee parameters, and a general pattern over several years can now be used to test different environments all along a landscape gradient. We showed a gradient of foraged diversity of pollen within a short distance during a period where floral diversity is expected to play a role. The couple land use - colony monitoring data on a large scale is a reference to test different environmental factors of food resources as well as some aggressors like diseases, predators or anthropic constraints.

for your remarks:

4.9

**Food selection dynamic by honeybees in agricultural landscape.****Requier F\*, Odoux J-F, Tamic T, Guillier F, Pierrick A, Henry M, Decourtye A, Bretagnolle V**

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Agricultural landscapes have been strongly changed because of farming intensification for several centuries. These landscape changes are mainly related to a decrease in semi-natural habitats, standardisation of land use and an increase of field area, which have created some intensive cereal farming systems. Honeybee (*Apis mellifera* L.) is the main crop pollinator. Nectar constitutes the energy resource and is transformed in honey for storage whereas pollen is the main protein resource for the physiology development. Pollen is little stored and the supply must follow a flow tended between need and collection. However, during the breeding season, the succession of flowering crops leads to strong spatio-temporal dynamics of food resources. In such an environmental context, which food choices make honeybees and which influences will they have on the dynamics of pollen harvest during these seasons?

Thanks to the large spatial scale of our experimental design on a 200 colonies monitoring, set up in an intensive cereal farming system, we clearly assume that a bimodal temporal pattern of pollen harvest exists. The first peak is linked to the blooming of spring plants (in May) and the second one to the simultaneous flowering of sunflower and maize (in July).

We observe that species which are present in hedgerows and forest edges were strongly selected as well as weeds such as the poppy. On the contrary, the rapeseed is little selected for its pollen resource while the maize which is anemophilous pollinated, is strongly chosen.

4.10

**The combined effects of *Varroa* and forage availability on honeybee colony development and survival.****Kennedy PJ\*, Swain JL, Becher MA, Chandler D, Grimm V, Pell JK, Thorbek P, Osborne JL**

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Over recent decades there has been increasing concern about the health of honeybees following reports of substantial losses of managed colonies in several countries, especially in Europe and U.S.A., and there has been growing support for the idea that the losses may be driven by multiple and interacting factors. Two factors that are known to independently affect colony development and survival – the parasitic mite, *Varroa destructor*, and forage availability – were investigated together in an experiment to determine which has the greater effect on colony development and survival, and whether there is a measurable interaction between the two. Four similar-sized honeybee nucleus colonies were set up at each of five apiary sites within 1.6km of one another and hence within a similar foraging landscape. Two experimental factors: – 1) high or low *Varroa* infestations, and 2) normal or restricted access to forage – were combined to impose four treatments on the experimental colonies. Data were collected on colony development, forage returns and colony survival over 10 months in response to *Varroa* levels, disease prevalence and external environmental factors. Preliminary analyses suggest the experimental factors have an interacting and seasonal effect on colony growth and survival. The results are tested against simulated output from BEEHAVE, the honeybee – *Varroa* model, with an agent-based foraging module, that will be presented separately (Becher et al.).

for your remarks:

4.11

**BEEHAVE: a honeybee model integrating colony dynamics, foraging and parasites.**

Becher MA\*, Grimm V, Kennedy PJ, Pell JK, Chandler D, Thorbek P, Osborne JL

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There have been substantial losses of managed honeybee colonies in Europe and North America. So far, no single driver has been identified that accounts for this phenomenon. Instead, it is believed that interactions between several stressors result in the decline of colonies. Empirical studies addressing this phenomenon are costly in time and resources whereas an in silico approach might offer a cost effective way to tackle this problem. We have hence developed a computer model, simulating the dynamics of a single honeybee colony.

In order to keep computational time low but allow for flexibility in decision-making, we combined a cohort-based population model with an agent-based foraging module. We included the *Varroa* mite as a bee parasite, which acts as vector for two different bee viruses: deformed wing virus (DWV) and acute paralysis virus (APV or ABPV). Crop maps defining the availability of food sources can be used in a separate, spatially explicit landscape model, to allow the application of realistic foraging scenarios with specifically defined nectar and pollen flows over time. In this talk we will describe and explain the model's design and present output for colony dynamics under scarce and abundant forage scenarios, with and without *Varroa*.

4.12

**A stage-structured model of honeybee colony population dynamics assessing impacts of pesticides and other stressors.**

Zhu W

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A healthy honeybee colony is a population of closely interacting individuals that form a highly complex society. As an aid to testing hypotheses for the causes of recent colony failure and providing suggestions for management actions to promote the recovery of honeybee population, we developed a worker-based, stage-structured model of honeybee population dynamics. This model was formulated with difference equations consisting of six discrete stages based on the temporal polytheism: egg, larva, pupa, nurse, house bee and forager stage. Numerical simulation of a healthy colony exhibited seasonal patterns similar to published field data.

Sensitivity analysis suggested critical thresholds of stage-based survival rates beneath which colony size decrease gradually. Also, if the social factor (brood care, transition rate and foraging), particularly precocious foraging, is interrupted beyond the critical threshold a rapid population decline is predicted and colony failure is inevitable. This model suggested that a disrupted colony by varying social regulation factor in the colony might be able to produce sudden collapse symptoms similar to colony collapse disorder.

for your remarks:

4.13

**Self-organising task allocation in middle age honeybees:  
balancing localisation and diffusion mechanisms.****Johnson BR**

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Task allocation is the study of how colonies allocate the appropriate number of workers to tasks that change in location and demand. The best understood case of task allocation is that of honeybee foragers who use complex signals such as the waggle dance to coordinate their collective foraging efforts over many square miles. Middle age honeybees (MABs,) in contrast, allocate themselves over a smaller area (the nest surface), but this space is still quite large compared to the size and sensory range of a single bee. Further, MABs do not produce signals. Based on a series of experiments a model of MAB task allocation is presented that shows that at the group level, task allocation in MABs is based on balancing the effects of localising and diffusing with respect to spatial variation in task demand. The relationship between this self-organising mechanism and specialisation effects due to spatial structure and genotype will also be discussed.

for your remarks:

# Session Five

## Pathology

**Chairs:**

**Dirk de Graaf**  
(Ghent, Belgium)

**Joachim de Miranda**  
(Uppsala, Sweden)

### **Symposium description**

This symposium covers a broad spectrum of factors that affect bee health at individual, colony or population level. These include the classical biological pathogens and parasites (viruses, bacteria, fungi, microsporidia and mites), genetic pathologies, pesticides-poisoning, predators and pests, as well as various syndromes and conditions. Also included as part of bee health are beneficial (micro-)organisms, genetics, chemicals and management strategies that enhance the health status of bees at individual, colony and population levels. Since much of the focus will naturally be on honeybees, the symposium particularly encourages contributions of the pathologies of non-*Apis* species as well as of innovative and sustainable health-management solutions for all bee species.

## 5.1

**Lactic acid bacteria - symbionts of honeybees.**

Vásquez A\*, Olofsson TC

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Lactic acid bacteria (LAB) are well recognised beneficial host-associated members of the microbiota of humans and animals. A few years ago, we discovered a LAB microbiota in honeybees that plays a key role in honeybee food production of honey and bee bread. Our studies on *Apis mellifera* subspecies showed that the LAB microbiota is constant within subspecies and added in high amount to their food. Honeybees possess this abundant, diverse and ancient LAB microbiota in their honey crop with beneficial effects for bee health, defending them against microbial threats and bee pathogens. Our research of LAB in all extant honeybee species plus related apid bees revealed one of the largest collections of novel species from the genera *Lactobacillus* and *Bifidobacterium* ever discovered within a single insect and suggest a long (>80mya) history of association. Bee associated microbiotas highlight *Lactobacillus kunkeei* as the dominant LAB member. Every LAB member show potent antimicrobial properties and are acquired by callow honeybee workers from nestmates and maintained within the crop in biofilms, though beekeeping management practices negatively impact this microbiota. We anticipate this microbiota will become central to studies on honeybee health, including colony collapse disorder, and act as an exemplar case of insect-microbe symbiosis.

## 5.2

**Epidemiology of honeybee pathogens in Uruguay.**

Anido M\*, Branchiccela B, Castelli L, Harriet J, Campá J, Zunino P, Antúnez K

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In Uruguay (South America), beekeeping has experienced a strong development during the last decades, being honey production and exportation, the most important activity. Honeybees are infected by a wide range of pathogens, being the most important the mites *Varroa destructor*, RNA viruses like ABPV, BQCV, SBV, DWV, KBV and IAPV; microsporidia *Nosema apis* and *Nosema ceranae* and the bacterium *Paenibacillus larvae*. During the last years a high incidence of these pathogens has been reported in our country, affecting beekeeping and agricultural production. The aim of the present work was to analyse the prevalence and distribution of these pathogens in Uruguay.

A representative sampling was designed, and in autumn 2011, 103 apiaries were sampled, nurse and forager bees and honey were collected and analysed. The obtained results showed that *V. destructor* is the most prevalent pathogen (78%), several provinces presented dangerous infestation values. Viruses ABPV, BQCV, DWV and SBV were detected with 13, 78, 29 and 18% of prevalence respectively. BQCV shows a high prevalence and wide distribution, while the other viruses are found in the main productive regions. KBV and IAPV were absent. *N. ceranae* is the only microsporidia found in the country (15%) which could suggest a replacement of *N. apis*. *larvae* is almost absent in the territory (2%) as a result of the severe measures to control it. These results are useful for the design of control strategies and prevention of colony losses.

for your remarks:

5.3

**Deformed wing virus infection in honeybee queens.**

Francis RM\*, Nielsen SL, Kryger P

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The queen is the longest living member of a honeybee colony. A healthy queen is critical to the well-being of a colony and replenishment of the workers. The current understanding of viral infection in queens is limited. We collected 86 honeybee queens from beekeepers in Denmark. The queens were in general healthy queens, but a few queens not performing well. Each queen was dissected to yield five tissue samples: head, thorax, ovary, intestines and spermatheca. Samples were separately tested by real-time PCR for the presence of DWV. The worker bees accompanying each queen on shipment to the lab were similarly tested, as a single pooled sample in the analysis. A set of drones from heavily infected colonies were dissected into head, thorax, abdomen, endophallus and sperm samples to quantify viral load across these tissues. By quantifying viral loads across tissues and amongst queens and workers, we seek to identify sites of viral replication among tissues. The study provides insight into horizontal and vertical transmission of DWV.

for your remarks:

5.4

**Molecular pathogenesis of *Paenibacillus larvae* infections.**

Genersch E\*, Fünfhaus A, Garcia-Gonzalez F, Poppinga L

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The etiological agent of the globally occurring epizootic American foulbrood (AFB) is the gram-positive bacterium *Paenibacillus larvae* (*P. larvae*). Despite being one of the most important honeybee pathogens, the pathogenesis of *P. larvae* infections largely remains elusive hampering the development of sustainable control or curative measures. The existence of different genotypes of *P. larvae* which differ in virulence opened the possibility to explore the virulence mechanisms by simply comparing these genotypes using different -omics approaches. Comparative genomics using suppression subtractive hybridisation (SSH) and comparative proteomics via 2D-SDS-PAGE analysis led to the identification of several putative virulence factors including various toxins, secondary metabolites, proteases, and an S-layer protein. The functional analysis of some of these putative virulence factors will be presented and their role in pathogenesis and their impact on virulence will be discussed.

5.5

### Interactions between two flavonoid groups affect growth inhibition of *Paenibacillus larvae*.

Mihai CM, Mărghitaş LA, Dezmirean DS, Chirilă F, Moritz RFA, Schlüns H\*

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Propolis is well known for its antibacterial properties and used by honeybees (*Apis mellifera*) to protect their hives. We investigated antimicrobial effects of ethanolic extracts of propolis from different Romanian origins on the American foulbrood pathogen (*Paenibacillus larvae*) that can cause a dangerous larval disease in honeybee colonies. A large variation in the content of the total polyphenols and in the content of the total flavonoids, which include i) flavones and flavonols, and ii) flavanones and dihydroflavonols was found among our propolis samples. The *in vitro* growth of *P. larvae* was substantially reduced by all examined samples of propolis. The growth inhibition effects of i) the flavones/ flavonols and ii) the flavanones/ dihydroflavonols as well as the interaction effects between these two groups were statistically significant. In contrast, we did not find significant inhibitory effects of other phenols. Our finding of significant interaction effects between the two groups of flavonoids of our propolis extracts in inhibiting the growth of *P. larvae* indicates that these effects should generally be taken into consideration when antimicrobial effects of different chemical compounds are investigated.

5.6

### Bees and probiotics: a new solution to contain the American foulbrood disease.

Hamdi C, Sansonno L, Crotti E, Balloi A\*, Gonella E, Essanaa J, Marzorati M, Manino A, Alma A, Daffonchio D, Cherif A

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Honeybee colonies can be affected by several factors, including bacterial and fungal pathogens, microsporidia, parasites and several viruses. Among them, *Paenibacillus larvae* is known to be the causative agent of the American foulbrood disease (AFB). The aim of this work was to identify a possible alternative strategy to control AFB.

Our strategy relied on the identification of honeybee symbionts that could be applied as probiotic (i.e. feed additive). By means of an ecological survey based on culture-based and culture-independent techniques on the gut microbial community of healthy and symptomatic adults and larvae, we identified and isolated 40 Acetic Acid Bacteria (AAB), 55 Lactic Acid Bacteria (LAB), and 63 Spore Forming Bacteria (SFB). The most active bacteria have been used for *in vitro* inhibition assays, determining the ability of the isolates to inhibit *P. larvae*. The best performers (1LAB, 1AAB, 2SFB), were used in *in vivo* rearing assay to test the susceptibility of young larvae to the pathogen infection. The treated larvae were exposed through the diet to the single symbionts or a mix of them and survival rates were compared with those of the control group (larvae not exposed to symbionts). The proposed mechanism of action relies on the synergistic action of: i) modification of the pH in the gastrointestinal tract; ii) competitive exclusion against the pathogen; iii) immune system stimulation; and iv) production of specific antagonistic factors against the pathogen.

for your remarks:

5.7

**The honeybee colony losses: the case of a Mediterranean country.****Higes M\*, Martín-Hernández R**

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Bee colonies have been monitored in Spain to identify agents related to colony losses. The presence of pathogens, beekeeping practices and pesticides were evaluated. A national cross-sectional study designed according to the census of beekeepers in every region was conducted during the years 2006, 2007, 2008, 2010 and 2011. In the first period of study (2006-2008), the high prevalence of *Varroa destructor* and *Nosema ceranae* in all regions is highlighting, verifying the close connection between *N. ceranae* and warmer climate areas. Viral infection and co-infection rates resulted surprisingly low. An increasing trend in the prevalence of *N. ceranae*, *V. destructor* and *A. apis* in 2010 and 2011 has been observed. In the regional study developed in 2008, a clear causal relationship between the presence of *N. ceranae* and loss of honeybee colonies was established. Regarding the pesticides residues in pollen, the results found in all the studied years (2006-2011) were very similar. The most frequently detected were those ones mainly used in the treatment for varroosis. The presence of residues of neonicotinoids or phenylpyrazoles was very low from some specific areas of Spain.

All results confirm that *N. ceranae* is a major sanitary threaten in Spain, the higher honey and pollen producer in Europe (and with the higher census of colonies and professional beekeepers). This situation could be similar in other temperate areas where the professional beekeeping is developed.

5.8

**Behavioural effects of the parasite *Nosema ceranae* on honeybee foragers revealed by harmonic radar.****Wolf S\*, McMahon DP, Lim KS, Pull CD, Paxton RJ, Osborne JL**

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Honeybees (*Apis mellifera*) are one of the most economically and ecologically important pollinators. Recent declines in honeybee populations have, therefore, received intensive scientific attention. Among the numerous putative causes for colony losses, emergent diseases like the microsporidian gut parasite *Nosema ceranae* has been proposed to play a prime role in colony collapse. However, little research has been conducted on sub-lethal effects these diseases may have on the behaviour of individual bees. Honeybee foragers infected with *N. ceranae* have been shown to have reduced homing times and homing success. Both impaired orientation abilities and energetic stress in infected bees have been hypothesised to be the underlying causes. Applying harmonic radar technology in a homing experiment allowed us for the first time to characterise flight performance and orientation abilities of healthy and experimentally nosema-infected honeybee foragers on landscape scale. Additionally, directionality of homing flights and use of landmarks as functions of a bees orientation ability were assessed. Based on these data we tested the hypothesised causes for increased forager losses in nosema-infected colonies. Using modelling we translated the foraging performance into colony level effects. This provides the most comprehensive high-resolution analysis of sub-lethal effects of *Nosema ceranae* on honeybee foraging flight behaviour and its effects on colony level.

for your remarks:

5.9

**The effect of seminal fluid on spores of *Nosema apis*.****Peng Y\*, Baer B, Lee-Pullen T, Heel K**

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Seminal fluid of honeybees is a complex mixture of molecules that have a wide range of effects. For example, seminal fluid proteins are keeping own sperm alive, but kill sperm of potential rival males. Seminal fluid components have also been found to affect the reproductive behaviour and physiology of the queen. A proteomic analysis of honeybee seminal fluid revealed the presence of a number of antifungal proteins but no experimental studies have so far been conducted to confirm their biological activity against potentially sexually transmittable diseases. We used microsporidia parasite *Nosema apis* of honeybees, and tested for effects of seminal fluid on *N. apis* spores. To do this, we incubated purified spores in seminal fluid at 1:2, 1:5, 1:10, 1:100, and 1:1000 dilutions for 0, 2, 4, 8, and 24 hrs. We then measured the survival of spores using two fluorescent dyes and flow cytometry. We found that drone seminal fluid significantly decreases the survival of *N. apis* spores. Given earlier observations of *N. apis* spores being present in honeybee ejaculates, we here provide first experimental data to show seminal fluid of honeybees has antimicrobial properties and seems capable of reducing possible infection risks of queens.

for your remarks:

5.10

**Experimental evolution in *Nosema ceranae*.****Müller MY\*, Moritz RFA**

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The honeybee *Apis mellifera* and its Microsporidian parasite, *Nosema ceranae*, were used to test the short-sighted evolution hypothesis in an infection experiment. We studied if transmission of *N. ceranae* can show a response to selection, and whether virulence trades off against transmission. *N. ceranae* spores were selected for survival outside the host organism as a fundamental attribute for transmission. After several weeks of exposure to drought, spore survival and infectivity were heavily reduced in infection of workers. In a subsequent infection with freshly produced spores from the previous infection, the selected *Nosema* strains showed a highly significant increase in virulence within newly infected honeybee workers. We discuss this result in light of the evolutionary theory.

5.11

### Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee.



Aufauvre J\*, Biron DG, Vidau C, Fontbonne R, Roudel M, Diogon M, Viguès B, Belzunces LP, Delbac F, Blot N

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In ecosystems, a variety of biological, chemical and physical stressors may act in combination to induce illness in populations of living organisms. While recent surveys reported that parasite-insecticide interactions can synergistically and negatively affect honeybee survival, the importance of sequence in exposure to stressors has hardly received any attention. In this work, Western honeybees (*Apis mellifera*) were sequentially or simultaneously infected by the microsporidian parasite *Nosema ceranae* and chronically exposed to a sublethal dose of the insecticide fipronil, respectively chosen as biological and chemical stressors. Interestingly, every combination tested led to a synergistic effect on honeybee survival, with the most significant impacts when stressors were applied at the emergence of honeybees. Our study presents significant outcomes on beekeeping management but also points out the potential risks incurred by any living organism frequently exposed to both pathogens and insecticides in their habitat.

5.12

### Oxalic acid treatments decrease *Nosema ceranae* infections in honeybees.

Higes M, Nanetti A\*, Martín-Hernández R

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*Nosema ceranae* (Microsporidia) is an obligate, spore-forming, intracellular parasite infecting European and Asian honeybees. It attacks and destroys the ventricular epithelial cells, harming individual adults and impairing the entire colony. However, the role of this parasite on the colony losses is rather controversial. Indeed, connections between *N. ceranae* infections and colony collapses could be found in warm regions, but experiments conducted under other environmental conditions (e.g.: non-temperate areas) led to different conclusions. Our knowledge is insufficient to draw a complete picture of the environmental factors involved in the evolution of *N. ceranae* infections, beekeeping practices included.

However, in this study we demonstrate for the first time that oxalic acid applications, often used in the control of *Varroa* infestations, may reduce *N. ceranae* infections in *Apis mellifera iberiensis*. In more details, Autumn administrations of oxalic acid solutions by trickling led to a significant decrease of the parasitisation rate in old and young adult bees in comparison to untreated controls, where increases were recorded. Further investigations are needed to establish the influence of natural acaricides (like organic acids) on the long term development of *Nosema* infections. Possible relationships between the use of these substances and epidemiologic aspects of nosemosis type C must be studied in depth.

for your remarks:

5.13

### ***Crithidia mellificae* is widespread in Europe and can be used as a predictive marker of honeybee colony losses.**

Dainat B\*, Evans JD, Gauthier L, Neumann P

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Honeybee, *Apis mellifera*, colonies are commonly parasitised by various microorganisms which, according to the presence of triggering factors, may or may not produce clinical symptoms. Among these, the trypanosome *Crithidia mellificae* has recently been isolated during an US survey, even though its pathogenicity for honeybee colonies is still uncertain. Here, we show that this parasite can also be highly prevalent in Europe. In Switzerland and France, *C. mellificae* was detected in most the colonies tested (N = 97 in France, N = 29 in Switzerland) throughout the year (three seasons sampled). So far, the detection of *C. mellificae* in colonies cannot be directly linked with obvious clinical symptoms. Nevertheless, a Swiss survey of 29 colonies differentially infested by the mite *Varroa destructor* suggest that this trypanosome can also be a significant predictive marker for winter losses (Linear model:  $p < 0.001$ ), with higher prevalence in colonies prone to collapse during winter (Mann Whitney U test  $p < 0.01$ ). These results support that honeybee parasites, which may not harm reasonable healthy colonies, can be able to take advantage of other initial infections/infestations thereby constituting secondary infections.

5.14

### **Honeybee immunity and colony collapse.**

Nazzi F\*, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E, Pennacchio F

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Recent descriptive studies suggest that the collapse of honeybee colonies is related to an interaction between pathogens and other stress factors including parasites. We carried out a multilevel study combining molecular, field-longitudinal and theoretical approaches to describe the mechanistic basis and dynamical properties of collapse-causing interactions. We found that the parasitic mite *Varroa destructor* can de-stabilise the within-host dynamics of deformed wing virus (DWV). The de-stabilisation of DWV results from a widespread immunosuppression characterised by a strong downregulation of the transcription factor NF- $\kappa$ B. The centrality of NF- $\kappa$ B to host responses to a range of environmental challenges suggests that this transcription factor acts as a common currency underlying colony collapse that may be triggered by different causes.

for your remarks:

5.15

### The functionality of the bacterial community of the social pollinator *Bombus terrestris*.

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Social pollinators, like honeybees and bumblebees, have a specific microbiota at species level yet overlapping phylotypes. In contrast solitary bees harbour other bacterial phylotypes in their gut and other non-social insects show another and more variable gut microbiota. The consistent microflora in social pollinators let to the hypothesis that these symbionts serve a specific functional role. On the other hand the characteristic microbiota of social pollinators could also be a consequence of their uniform diet and intimate contact between different generations. Therefore we tested the fitness impact of different disrupted microbiota in microcolonies of *Bombus terrestris*. We demonstrate that, under pathogen free and ad libitum nutrient availability, most bacterial phylotypes are redundant and bumblebee fitness is maintained. Yet, this does not rule out that certain bacterial species are specifically beneficial. Indeed, impairing the microbiota with the broad range antibiotic streptomycin resulted in a microbiota dominated by *Lactobacillus* sp. (GenBank HM534867). This shift had a positive effect on the bumblebees fitness, increasing the offspring number and mass by 19.5% and 17.7%, respectively, opening promising applications for commercial pollinator rearing.

5.16

### Pharmacophagy by a specialist bee protects it from a common fungal brood disease.

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The diet of solitary herbivorous bees consists of pollen and nectar collected from a broad range of plant taxa or from only a single plant family or genus. Bees with a narrow host-plant diet, also known as oligolectic bees, have been shown to develop normally when reared on pollen collected from plants unrelated to their normal host-plant. Thus, specialist bees are not necessarily nutritionally constrained to their specific host-plant. What is the adaptive advantage of a narrow diet? Through rearing and inoculation experiments we demonstrate that the narrow diet of the solitary bee *Chelostoma florissomme* provides significant protective benefits against a widespread and common bee brood pathogen, the fungus *Ascospaera aggregata* (Ascomycota, Onygenales). Chalkbrood, the disease caused by this fungus and other members of the genus, has never been observed in *C. florissomme* in nature. The active plant compound against this fungus *in vitro* is also one of the primary chemical cues used by the bee for host-plant pollen recognition. This interaction represents a form of pharmacophagy, a practice not well documented in solitary bees.

The results of our study suggest that the evolution and maintenance of oligolecty, especially on those plants whose pollen exhibits antimicrobial activity, may be driven by prophylaxis against specialised bee brood pathogens.

for your remarks:

5.17

### First record of mantisflies (*Plega hagenella*) parasitising eusocial bee colonies (*Melipona subnitida*).



Maia-Silva C\*, Hrcir M, Koedam D, Machado RJP, Imperatriz-Fonseca VL

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The mantisfly *Plega hagenella* (Neuroptera, Mantispidae) was found parasitising nests of the stingless bee *Melipona subnitida* (Apidae, Meliponini) between April and June of 2012 in the semi-arid region of Northeast Brazil. In two experimental meliponaries (distance between locations: 50km), approximately half of the 64 colonies had been infested by the parasite. The mantisfly larvae (second and third instars) developed inside closed brood cells, feeding on the larvae or pupae of the bees. This resulted in a dramatic decrease in the production lony's population. Still inside the brood cells, the *Plega* larvae spun a cocoon of resistant silk that could neither be opened nor destroyed by the worker bees, who removed the wax surrounding the cocoon. *P. hagenella* emerged from the cells as pupa, using its mandibles to cut through the silk, and quickly left the cocoon. After eclosion of the parasite, the empty cocoons frequently dropped to the floor of the meliponine nests, and, subsequently, were removed by nest-cleaning bees. Recently, *P. hagenella* has been described parasitising nests of solitary bees (Colletidae) in Trinidad. Our observations are the first report on the occurrence of this mantisfly species in the semi-arid region of Northeast Brazil. In addition, the present study is the first record of this parasite in colonies of the stingless bee *M. subnitida*, demonstrating the devastating effect of an infestation for eusocial bees.

5.18

### How disease-ridden are our pollinators? Honeybee pathogens in bumblebees (*Bombus sp.*) across the UK.

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It appears the main beneficiaries of globalisation, after humans, are invasive species and infectious agents such as parasites and pathogens. Pathogen pollution and spillover events from traded livestock are, at a global scale, the major source for emergent infectious diseases that impact native species. Trade in honeybees has been the key driver of emerging diseases in managed honeybee populations. However, the parasites and pathogens behind these diseases pose a further threat, as they are increasingly being recognised as multi-host, rather than Apis-specific parasites. In particular, a range of these parasites have recently been detected in bumblebees (*Bombus sp.*). Even though potentially detrimental to the native bee populations, next to nothing is known about the basic epidemiology of the newly detected parasites in these vital and charismatic pollinators. We conducted a structured geographic survey across 26 sites in the UK, focusing on the prevalence of the microsporidian *Nosema ceranae* and the Iflavirus DWV (deformed wing virus) in bumblebees and honeybees to determine the dynamics of the parasite across the two host groups. We find the pathogens in bumblebees as well as honeybees across the UK, with strong spatial variation in prevalence. This suggests that studies are urgently needed to understand the infectivity and virulence of these pathogens in bumblebees to comprehend the threat they are facing.

for your remarks:

5.19

### Pathogen and genetic spillover from commercial to wild populations of bumblebees.

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Worldwide, 11% of bumblebee (*Bombus*) species are endangered. Recent studies from North America suggest that disease transmission from commercially reared bumblebees to wild populations, pathogen spillover, has led to marked range contractions in some species. In Europe, data on the incidence of pathogen spillover is lacking, despite the widespread production and transport of hives within the EU since the early 1980s. We determined the permeability of cropping systems to commercial bumblebees and quantified the prevalence of four pathogens in commercial *B. terrestris* hives and adjacent wild conspecific populations at increasing distances from greenhouses in Ireland. In parallel, the population genetic structure of commercial and wild populations was investigated to estimate rates of introgression and potential establishment of commercial *B. terrestris*. Commercial bees collected up to 97% of non-crop pollen, and hives had markedly higher frequencies of two gut parasites, *Crithidia* spp. and *Nosema bombi*, compared to adjacent wild populations. The highest prevalence of *Crithidia* was observed within 2km of greenhouses and the probability of infection declined in a caste- and pathogen-specific manner up to 10km. We suggest implementing measures that prevent the interaction of commercially reared and wild bumblebees by integrating local enforcement with national and international legislation that regulates the sanitation of commercial hives in production facilities.

5.20

### Does a pathogen-infected individual blow up the social network in bumblebees?



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Pathogens and diseases represent a strong threat reducing survival, or the fitness of an organism; a strong evolutionary force. The life history traits of social insects (high density, low genetic variability) provide a rich and stable environment to pathogens. With this social life, other mechanisms have evolved to resist and reduce transmission compared to solitary life. In order to resist against a parasite infestation inside the colony, a prophylactic behaviour seems to be the better strategy.

Recently new methods have been established to investigate the organisation and the behaviour of social insects' colonies as a whole entity. Combining with new discoveries on social behaviour towards disease transmission and resistance, it becomes feasible and is important to investigate the reaction of a social group facing a pathogen threat.

Given these new methods and recent discoveries, we would like to investigate the disturbance of a contact network of bumblebee workers generated by infection with a specific gut parasite (one individual per group), *Crithidia bombi*. We record the contact network of a group of bumblebees, before and after infection of one of them. The parasite is cultivated in cell media, and then inoculated to bumblebees. Afterwards, the bumblebees are killed and the infection level is measured.

for your remarks:

5.21

### The health and status of the feral honeybee (*Apis mellifera*) population of England.



Thompson CE\*, Budge G, Pietravalle S, Biesmeijer JC

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Feral or unmanaged honeybee colonies were considered to have been wiped out by the arrival of the parasitic mite *Varroa destructor*. However a number of sites have been identified where colonies appear to be persisting. Managed honeybee health is monitored in England and Wales by the National Bee Unit based at the Food and Environment Research Agency, York, yet the health of feral colonies is unknown. Samples of foraging bees were collected from the entrance feral colonies and the nearest managed colonies at varying sites across England. The intensity of management and the frequency and type of *Varroa* control was recorded for all managed samples. Pooled samples of foragers from each colony were screened for the presence of deformed wing virus, black queen cell virus, chronic bee paralysis virus, sacbrood virus, acute bee paralysis virus, Kashmir bee virus, Israel acute paralysis virus, chalkbrood, acarapis, *Nosema apis*, *Nosema ceranae* and European foulbrood using RT-PCR. The number and quantity of pests and pathogens were calculated and compared between feral and managed colonies. The results will be discussed in the context of feral bee colony health and implications on the health of managed honeybee colonies with different levels of intervention.

5.22

### *Varroa* and viruses in Norwegian apiaries in relation to *Varroa* treatment strategies.

Dahle B\*, Rimstad E

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*Varroa destructor* is not recognised as an important factor in colony losses by Norwegian beekeepers, even though losses reported by Norwegian beekeepers are significantly higher in areas with established *Varroa* populations than in areas where *Varroa* mites are still not established. We analysed bee samples from 80 beekeepers distributed throughout Norway. Samples were collected in September/October prior to the late fall *Varroa* treatment with oxalic acid used by most beekeepers. *Varroa* infestation levels were low in most colonies ( $X = 1.2 \pm 3.0$  (SD) mites/100 adult bees (n=316)). Hundred and eighty nine samples were analysed for eight viruses (DWV, BQCV, SBV, SBPV, IAPV, ABPV KBV and VdMLV) using Real Time PCR. BQCV and DWV were the most common viruses, found in 34 and 10% of the colonies, respectively and they were significantly more common within the areas with established populations of *Varroa* than outside these areas. The relationships between *Varroa* infestation level *Varroa* treatment strategies, viruses and winter mortality will be discussed.

for your remarks:

5.23

**The key for success in reducing bee colony losses to a minimum.****Boecking O\*, Aumeier P, Liebig G**

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Bee colony losses are on one hand the most critical actual limits in apiculture with negative consequences for other fields involved. On the other hand success in reducing bee colony losses to a minimum is one of the most challenges of the 21<sup>st</sup> century. In our research project we found the key for success in reducing bee colony losses to a minimum. Practical beekeepers involved showed that our approach can help to reduce winter losses significantly.

Also a honeybee colony is under the influence of many positive and negative factors, the most critical are *Varroa* mites and virus infections. However, the management of the bee colony is the starting point for a beekeeper to interfere with the imbalance of the host parasite relationship. We first tested two classical beekeeping management techniques. Based on these data a new modular concept was developed. It allows some flexibility for the practical use within a narrow frame to hinder it from its realisation within the beekeeping community. Our concept comprises five components: treatment of nucleus colonies with lactic acid, use of drone brood capture combs in full size colonies, the use of formic acid 85% before the production of winter bees, monitoring the natural mite downfall, and oxalic acid treatment into the tight winter cluster. A consequent use of this modular concept finally revealed a significant reduction of winter losses to a minimum of one third of the high average winter losses recorded over and over the last years.

5.24

**A comparison of different summer control strategies against the ectoparasite *Varroa destructor* in a monitoring programme in North and Central Italy.****Lodesani M\*, Besana A, Costa C, Dall'Olio R, Tesoriero D, Vaccari G**

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To evaluate the applicability of four different control strategies against varroaosis, a trial in four different Italian regions (Piedmont, Veneto, Tuscany, Lazio) was carried out on 180 colonies from summer 2010 until spring 2011, in collaboration with beekeepers associations. Two chemical treatments (thymol with Api Life var and two different formulation of formic acid) were compared to two technical practices (brood interruption and capped brood removal).

In each colony samples of adult bees and brood were collected before and after treatment to assess the presence and / or quantity of the following parasites: *Varroa destructor*, *Nosema ceranae* and the main honeybee viruses (DWV, ABPV, BQCV, SBV). Parameters of colony strength (number of bees or brood cells) were assessed at several points during the trial, and status of colony (dead or alive) was also recorded. Results showed that the most effective for maintaining low *Varroa* infestation levels and higher colony survival in spring was, in all regions, brood interruption. Threshold levels for colony survival in the winter and spring were determined. Analyses for virus presence showed that in spring 2011 the most prevalent virus was DWV, followed by ABPV. Analyses for quantification of *N. ceranae* spores showed regional differences in infection levels.

The trials were performed in the context of "APENET: monitoring and research in beekeeping", research project funded by the Italian Ministry of the Agriculture.

for your remarks:

5.25

### HopGuard® - a new medication to control varroosis in colonies of *Apis mellifera*.

Rademacher E, Harz M\*

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Varroosis is known to be the cause for large scale honeybee colony losses in Europe and other parts of the world. Synthetic acaricides in different drugs were successful in the short term control of *Varroa destructor* but in the long term can lead to resistances and a problematic residue situation in honeybee products. The organic acids and ethereal oil products registered in many countries provide alternatives for the control of *V. destructor* but the efficacy depends also on the outside conditions. In spite of these possibilities to control varroosis bee colony losses occur every year. Therefore, it is important to continue to develop new substances in order to provide a variety of effective and easy to handle drugs to beekeepers. Over recent years we have worked on the development of hop beta acids in an effective formulation to combat varroosis. HopGuard® has now been tested on bee colonies in Germany and is approved for use in honeybee colonies under Section 18 emergency use exemptions in selected states of the USA. We will present our results covering the development and application of HopGuard®, data on efficacy against *V. destructor*, bee tolerance, residue situation in bee products as well as user and consumer safety. We recommend the use of HopGuard® as an alternative winter treatment within an Integrated *Varroa* Control Concept.

for your remarks:

5.26

### The female sex pheromone of *Varroa destructor* – prospects for a biological control?



Ziegelmann B\*, Rosenkranz P

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Mating of *Varroa destructor* occurs exclusively within the sealed honeybee brood cell between the mature male offspring and usually the youngest of the 1-3 daughter mites as soon as they reach maturity. By extracting young attractive females we could prove in a laboratory bioassay that the male mating behaviour is elicited by a volatile sex pheromone that is released by the female shortly after the adult molt. Within the cooperation project “FIT-BEE” we now aim for developing a biological control of *V. destructor* based on the female sex pheromone. After fractionating the total extracts of attractive females we reduced the number of potential active compounds to six substances and finally identified the qualitative composition of the *Varroa* sex pheromone as long chain fatty acids and the corresponding ethyl esters. Bioassays with pure substances revealed that all six compounds elicit the male mating behaviour. In further experiments we tested the pure substances in different dosages and performed first trials to evaluate the potential of the *Varroa* sex pheromone for disturbance of the mating process. So far, an “overdose” of synthetic pheromone did not reveal a repellent effect. However, males were quite confused finding a suitable mate when exposed to the pheromone blend in the presence of an entire “*Varroa* family”. This result is promising with regard to the further development of a mating disruption technique.

5.27

**A product platform for honeybee health with targeted control.****Paldi N\*, Hayes J**

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Ribonucleic Acid interference (RNAi) applications for invertebrates have been on the scientific agenda since RNAi was initially discovered. The work done on *c-elegans* not only was awarded with the Nobel Prize in 2006, but also triggered many initiatives that focused on how the RNAi mechanism can be used for pest management. Utilising that discovery and its technology, Beeologics and now Monsanto, have been developing product concepts to control honeybee viruses and parasites. The first and most advanced product in the development pipeline is called Remebee® – an anti-viral agent fed to the bees protecting them from infection caused by the Israeli Acute Paralysis Virus (IAPV). In induced trials this virus has been associated with honeybee mortality and in 2007 was identified as one of the potential participating factors of colony collapse disorder (CCD). The technology platform built in the creation of honeybee antivirals is now supporting the R&D efforts to target the *Varroa* / virus complex implicated in decreased honeybee health, vitality and vigor.

5.28

**The EU Reference Laboratory: work programme and its role in an EU wide surveillance programme.****Chauzat M-P\*, Ribière M**

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The European Commission designated the ANSES Sophia-Antipolis laboratory as the European Union Reference Laboratory (EU RL) for bee health by Regulation No. 87/2011. The EU Reference Laboratory covers the main parasitic, bacterial and viral bee diseases, as well as the invasive species (insects *Aethina tumida* and *Tropilaelaps* mites) threatening the honeybee population in Europe. The laboratory addresses also the aspect of colony poisoning through research on the most dangerous pesticide residues for honeybees.

In 2011, the European Commission started to set up and co-finance a standardised and Europe wide voluntary surveillance programme to obtain reliable and accurate measure of honeybee colony losses and information on honeybee health. This programme was based on the technical document 'Basis for a pilot surveillance project on honeybee colony losses' that the EU RL has elaborated in June 2011. The surveillance procedure foresees to study randomly selected apiaries in order to quantify colony losses (both mortality and weakening) and to investigate the possible causes for such losses. Sampling methods will be consistently implemented in each of the 17 selected Member States in order to provide comparable data. Preparatory work and training for this project is already underway and visits to apiaries and sampling starts in September 2012.

for your remarks:

5.29

**Optimised spray application in oilseed rape on nectar and pollen contamination.****Wallner K\*, Maus C, Tritschler M, Friessleben R**

Email: Klaus.Wallner@uni-hohenheim.de

Oilseed rape is one of the most important agricultural crops in Germany (cropping area >1.5 mio ha), and likewise of key importance in beekeeping as early-season bee forage. To prevent losses of yield, oilseed rape has to be treated with different pesticides during blooming season. The application of pesticides into the blossoms has led to residue findings in honey which caused concerns by beekeepers. We present a concept to optimise pesticide application technology, towards minimisation of exposure of bees to pesticide residues in treated blossoms. The under-leaf application technology is a modified system (Dropleg) that has been used in vegetable crops for several years. In semi-field and field trials in 2011 and 2012 it was tested in how far under-leaf spray with the Dropleg system in flowering oilseed rape can reduce pesticide residues in nectar and pollen collected by exposed bee colonies in comparison to conventional application technology. Pollen loads and stored nectar of experimental bee colonies were sampled at different points of time after application and analysed for pesticide residues. As model substances, a systemic fungicide and a non-systemic insecticide were applied, in 2012 additionally a systemic insecticide. First results from 2011 suggest that Dropleg application can significantly reduce pesticide exposure of bee colonies. The project is conducted in the scope of the FitBee Project supported by German BMELV and will be continued until 2014.

5.30

**Toxicity of neonicotinoids used for maize seed coating.****Medrzycki P\*, Lodesani M, Bortolotti L, Porrini C, Pochi D, Maccagnani B, Balconi C, Girolami V**

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“APENET: monitoring and research in beekeeping”, was a two years research project funded by the Italian Ministry of Agriculture. One out of six research topics involved the study of the effects on honeybees of neonicotinoids (imidacloprid, clothianidin and thiamethoxam) and fipronil used for maize seed coating. The results showed that the dust emitted in the field by the pneumatic seed drill, deposited on the ground with concentrations that varied between 0.5 and 5 µg/m<sup>2</sup>. Damages in the exposed hives were also detected.

The lethal and sublethal effects of the active ingredients on honeybees were studied in laboratory and semi field tests. The active substances had negative effects on the recovery of medium-term and long-term memory (PER test) and on the foraging behaviour and homing ability (field trials), even at much lower doses (roughly 1/5) than the LD<sub>50</sub>. A.i. concentration in guttation droplets was very high, but no bee foraging activity on the droplets was observed in field. Agronomic tests have been carried out in 20 locations distributed mainly in maize-growing areas, to evaluate the agronomic usefulness of maize seed coating. From two years of trials, using two different experimental designs (randomised plots and strip test), no statistically significant differences in the yield between dressed and undressed maize seeds emerged.

In conclusion, it seems clear that the active ingredients used in maize seed coating may be hazardous towards bees.

for your remarks:

5.31

### Effects of genotype and environmental factors on the survival of European honeybee colonies.

Büchler R, Berg S, Bienkowska M, Panasiuk B, Le Conte Y, Costa C\*, Dyrba W, Bouga M, Hatjina E, Charistos L, Petrov P, Ivanova EN, Kezić N, Korpela S, Kryger P, Pechhacker H, Uzunov A, Wilde J  
Email: cecilia.costa@entecra.it

To better understand the role of bee genetics in the Europe-wide occurrence of colony losses an experiment on genotype – environment interactions (GEI) was started by Coloss working group 4 in July 2009.

621 honeybee colonies, representing 18 different genotypes, were comparatively tested in 16 apiaries across Europe. The colonies were kept without any chemical treatments against *Varroa destructor* and other diseases. Colony and queen survival were registered continuously, besides bee population development, productivity, feed balance, swarming, gentleness, hygienic behaviour and the infestation with *Varroa*, *Nosema* and viruses.

The tested genotypes clearly differed in their honey productivity, gentleness and swarming tendency which can at least partially be explained as a consequence of different breeding intensity for these traditional selection characters. However, it is important to note that even for these traits highly significant genotype – environment interactions were observed.

To sum up our primary results, we can state a high relevance of interactions between honeybee genotypes and different environmental conditions within Europe. Obviously, the genetic adaptation of honeybees to a specific environment influences its population dynamics, health status, and productivity. Consequently, the conservation of European honeybee diversity and the support of local breeding activities should be encouraged.

5.32

### The Bee Informed Partnership: determining best management techniques through cross sectional surveys.

vanEngelsdorp D

Email: dennis.vanengelsdorp@gmail.com

Managed honeybee colonies have been in decline in the US for at least the last 60 years. The causes for past and continuing colony losses are multiple and interrelated. It is precisely the complex nature of disease causation in honeybee colonies that makes the application of an epidemiological method to this system desirable. Indeed, the success of epidemiological methods to elevate suffering in human populations is undeniable. It is therefore not surprising that researchers in other systems, such as veterinarians and plant crop scientists, have adopted the epidemiological method in the past. This talk will focus on results from various retrospective and prospective epidemiological surveys of honeybee colony health and management. In addition to introducing various epidemiological terms predictors of colony health as well as management techniques which are associated with higher or lower winter loss we be highlighted.

for your remarks:

# Session Six

## Population Genetics

**Chair:**

**Pilar de la Rúa**  
(Murcia, Spain)

### **Symposium description**

Population genetic studies have grown since the development of molecular markers such as microsatellites and SNPs, coupled with increasingly sophisticated analytical tools. In particular, in Hymenoptera (not only honeybees, but also bumblebees and solitary bees), an important effort in relation to this subject is being achieved due to the concern about population declines of both wild and managed bees. In this symposium, some of Europe's leading experts will bring together current knowledge on the present status of bees and their biodiversity and conservation, and on factors such as population size and inbreeding.

## 6.1

**The population genetics of Europe's wild bees.****Paxton RJ**

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Wild bees are thought to be important pollinators of both crops and wild flowers in Europe, yet many species are in decline or are placed on national IUCN red data lists. There is therefore growing interest in understanding the population genetics of Europe's wild bees because such knowledge can guide conservation action that aims to enhance their genetic diversity, gene flow and adaptive potential. In reviewing the current status of the field, I shall highlight some of the approaches that can now be used to interpret population genetic data whilst also pointing out potential pitfalls, including ascertainment bias and the analogous issue of the interpretation of Wright's F-statistics for genetic markers varying widely in heterozygosity. In comparison to honeybees and bumblebees, there are few studies addressing the population genetics of Europe's other social and solitary bees, in part due to a lack of appropriate genetic markers. Markers such as microsatellites are, however, now available for major groups of bees, and next generation sequencing can provide microsatellites and SNP markers at much reduced costs for any species. These need to be employed on a wider diversity of species before we can draw generic conclusions about the population genetics of Europe's wild bees.

## 6.2

**Sexual selection in social insects: males fight but queens rule.****Jaffé R\*, Garcia-Gonzalez F, den Boer SPA, Simmons LW, Baer B**

Email: rojaff@gmail.com

Multiple mating by social insect queens (polyandry) can reduce offspring relatedness and thus undermine the inclusive fitness gains that promote eusociality. Relatedness and inclusive fitness benefits are, however, not only influenced by queen mating frequency but also by paternity biases among offspring (paternity skew). We performed a large scale phylogenetic analysis of paternity skew in polyandrous social insects, and found that across ants, bees and wasps, the evolution of increased paternity frequency is associated with a decrease in paternity skew. Species with low paternity frequencies showed high paternity skew, which increases inclusive fitness gains. At the other end of the spectrum, in species with highly polyandrous queens, paternity was equalised among fathers and genetic diversity among offspring was thus maximised. Our findings highlight the importance of genetic diversity benefits and suggest that sexual selection and sexual conflict may have played key, yet overlooked roles in social evolution.

for your remarks:

6.3

**DWV quasispecies and the honeybee drone mating flight.**

Yañez O\*, Jaffé R, Fries I, Moritz RFA, Paxton RJ, de Miranda JR

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Deformed wing virus (DWV) is one of the most studied of the 18 viruses of honeybees (*Apis mellifera*). This virus has a worldwide distribution and is associated with malformed wings, shortened abdomens and severely reduced life span in highly infected bees. DWV is a single-strand RNA virus, the high mutation rate and lack of proofreading mechanisms of this type of virus produces high levels of genetic variability that confers adaptability to rapid environment changes as different transmission routes. Four stable DWV strains were characterised from the highly variable DWV-Lp gene at two honeybee drone populations. Two DWV strains were found in Sweden and were denominated Ultuna and Gotland. Other two strains were found in Ireland and were denominated Kilkenny and Tipperary. Previous studies demonstrate that the vertical transmission of DWV is a possible event in nature. Additionally, it has been shown that DWV do not hinder the mating flight of asymptomatic infected drones caught in drone congregation areas (DCAs) and emphasis the epidemiological implications for the virus to be vertically transmitted. To test whether some of these strains could be favour during vertical transmission, this study compares the distribution of these DWV strains in sexually mature drones collected from DCAs with similar drones collected from the contributing hives.

6.4

**Genetic variation in Greek honeybees: molecular and morphometrics approach.**

Bouga M

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Honeybee populations from various areas of Greece are studied using isoenzymic analysis, PCR-RFLP's and sequencing analysis of mtDNA gene segments, classical and geometric morphometry. The aim of this research is to investigate the gene flow existence as a result of beekeeping practice and to find out if these populations can be still discriminated.

MDH\*-1, EST\*-3 SOD\* ACPH\* ALP\* and ME\* loci are polymorphic in almost all populations studied in isoenzymic approach. PCR-RFLP's analysis on 16srDNA, COI and ND5 gene segments shows that Nco I and Sty I for COI and Alu I for ND5 are diagnostic for *A.m. macedonica*. Sequencing analysis of ND5 and COI gene segments shows that the highest genetic divergence values observed in COI, but there is mixture of honeybee populations in Greece. The morphometric characters analysed that are length and width of fore and hind wing, cubital index, number of hamuli, length of femur and tibia, length and width of basitarsus and length of proboscis and geometric morphometry based on coordinates of 19 landmarks located at vein intersections of the wing, show that inter population variability exists and honeybees from Aegean Sea islands seem to maintain their pure characteristics.

These results are useful for the conservation of local honeybee populations, as well as, for the accredited bodies that exist for the certifications of honeybees' queens' quality and genetic origin.

for your remarks:

6.5

### The Carpathian mountain ridge does not separate *Apis mellifera carnica* and *Apis mellifera macedonica*.

Coroian CO\*, Muñoz I, Schlüns E, Mărghitaş LA, Dezmirean DS, De la Rúa P, Cauia E, Furdui E, Paniti-Teleky O, Schlüns H, Moritz RFA

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The Carpathian mountain ridge has been suggested as a natural divide between the two honeybee subspecies *Apis mellifera carnica* and *Apis mellifera macedonica* (Ruttner, 1988). Here we analyse genetically honeybee workers sampled at over 200 locations at the sequence of the rRNAleu-Cox2 intergenic region and twelve microsatellite loci. The results show that the Carpathian mountain ridge is not a natural divide between the two endemic honeybee subspecies but rather the “temperature zones” generate a pattern in natural distribution of *A. m. carnica* and *A. m. macedonica* honeybee subspecies. *A. m. carnica* is distributed on both sides of the Carpathians in regions where the mean average temperature drops below 10°C whereas *A. m. macedonica* honeybees is found in regions with mean temperatures above 10°C.

for your remarks:

6.6

### Microsatellite DNA polymorphism in *Apis mellifera* L. from Bulgaria.

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In this investigation genetic polymorphism in honeybee populations of *A. mellifera* from 26 different regions in Bulgaria was characterised by usage of microsatellite DNA analysis. All honeybee samples were analysed for nine microsatellite loci: Ac011; A024; A043; A088; Ap226; Ap238; Ap243; Ap249 and Ap256 which were found to be polymorphic in all of the populations with the presence of a total 121 alleles. The mean number of alleles per locus was found to vary between 3.899 and 6.444. Average observed and expected heterozygosity values were calculated as 0.586 and 0.566, respectively. Thirty five private alleles were observed in 19 of the studied populations. The largest number of private alleles was observed for Ap256 locus. There was given information about allele frequencies,  $F_{ST}$  and  $N_m$  values and genetic differentiation. Nei's genetic distance between studied populations was calculated and UPGMA and neighbor-joining phylogenetic trees were constructed. The populations from Bulgaria were grouped in two main clusters with six branches for the first cluster and three – for the second one. The populations from western Bulgaria were clearly grouped in a different cluster. The populations from central and eastern Bulgaria were clustered together but in different branches.

6.7

**Genetic Structure and Diversity of *Apis mellifera* from Near East.**

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The genetic structure and distinctness of *Apis mellifera* subspecies from Turkey, Iran, Iraq, Syria and Cyprus has been assessed by using 25 microsatellite markers. A total of 400 apiaries were sampled for microsatellite analysis. The genotypes were identified on an ABI 3130 Genetic Analyser using fragment analysis. The mean number of alleles ( $N_a$ ), effective number of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), gene flow ( $N_m$ ) and  $F_{is}$  values were estimated for all populations. According to microsatellite allele frequencies, Turkey, Iran, Iraq, Syria and Cyprus honeybee populations are clustered into different groups by NJ analysis which is compatible with their geographical locations. The present study suggests that honeybee populations from Near East have high level of genetic diversity. The results of this study may be useful for designing future investigations of genetic variation and developing conservation strategies in honeybees from Near East.

for your remarks:

6.8

**Confirmation of the fifth mitochondrial DNA lineage of the western honeybee from Sudan.**

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Using mtDNA variability, the *Apis mellifera* subspecies could be classified into five major lineages: 1) lineage A including *A. m. intermissa*, *A. m. adansonii*, *A. m. scutellata*, *A. m. capensis* and *A. m. monticola* subspecies; 2) lineage M composed of the western European subspecies *A. m. mellifera* and *A. m. iberiensis*; 3) lineage C composed of *A. m. ligustica*, *A. m. carnica* and *A. m. caucasica*; 4) lineage O comprising *A. m. caucasica* and *A. m. lamarckii*; 5) lineage Y with *A. m. jemenitica*. *A. m. jemenitica* occupied a large area extend from Mali in west Africa through Sudan to the Arabian peninsula in Asia. However its classification is still controversial in morphometrical as well as genetic studies. The Y lineage which had recently been classified in *A. m. jemenitica* of Ethiopia, has never been detected elsewhere even in the same subspecies from the Neighboring countries. Moreover very recent morphometrical analyses described honeybee of Ethiopia as a single new subspecies, *A. m. simensis*.

In this paper, we aim to resolve this contradiction of classifying *A. m. jemenitica*. Using Dra I analyses and the partial sequence of the tRNA<sup>Leu</sup> COII region of 30 sampled colonies along the river Nile in Sudan, we detected three different mtDNA lineage: A, O, and Y, each lineage compose of suit of haplotyps. We found the Y-lineage in 10% of the sampled colonies. This result report the existence of Y lineage in *A. m. jemenitica* of Sudan for the first time and gives further confirmation to the Y lineage in central and east Africa.

6.9

### Fine-scale population genetics of East European *Apis mellifera* reveal differentiation and introgression events.

Muñoz I, De la Rúa P\*

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The East European evolutionary lineage of the honeybee *Apis mellifera* expands its distribution area from the Italian and Balkan Peninsulas to the Carpathian Mountains. Subspecies widely propagated as *A. m. carnica* and *A. m. ligustica*, and others as *A. m. macedonica* are included in this lineage. Our objectives were to test an earlier hypothesis of local *A. m. carnica* morphometric varieties (Alpine and Pannonic) described according to zoogeographic zones, and to evaluate the genetic structure of the subspecies distributed in this vast area. Using a panel of twelve microsatellite loci and honeybees collected from 14 countries, we confirmed the existence of two genetic clusters in *A. m. carnica* corresponding to the described Alpine and Pannonic ecotypes, plus another two within *A. m. macedonica* that we have named as continental and Mediterranean *macedonica* ecotypes. Introgression between neighbor subspecies and ecotypes has been detected in the contact zones and also from the Italian honeybee (*A. m. ligustica*). Our results provide a high resolution analysis of the genetic structure of the eastern honeybee subspecies and argue that successive introductions may be a disruption for the conservation of local varieties.

6.10

### Microsatellite analysis in museum samples reveals inbreeding before the regression of *Bombus veteranus*.



Maebe K\*, Meeus I, Maharramov J, Grootaert P, Rasmont P, Smaghe G

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Essential pollinators in natural and managed ecosystems, like bumblebees, are undergoing decline. Several hypotheses have been proposed to explain the observed declines. Also genetic factors could play a role. Population genetics try to correlate genetic parameters with bumblebee decline. In order to do so historical data are needed. In this project, we used eight microsatellite loci to genotype pinned museum specimens of the declining bumblebee *Bombus veteranus* (Fabricius, 1793). This bumblebee is a good example of a species in decline as it was one of the most abundant bumblebees in Belgium one century ago but after starting to decline in 1950 the species is now almost totally vanished. Bumblebee samples were collected spanning a period of three decades (1895-1923) and their genetic parameters were measured. In all time periods we detected low genetic diversity and high inbreeding coefficients (even when corrected for null alleles). Inbreeding was also confirmed by the occurrence of sterile diploid males, and all this while the population remained stable as the actual decline of *B. veteranus* occurred after 1950. Based on these results, we speculate that low genetic diversity and inbreeding did not directly result in the collapse of *B. veteranus* in Belgium. However, inbreeding still plays an indirect role in the decline of bumblebee populations because of the appearance of diploid males and because a low HE reduces the capacity to react to the drivers of bumblebee decline.

for your remarks:

### 6.11 Identification of commercial and Turkish native *Bombus terrestris* populations by using microsatellite markers.



Meydan H\*, Karşlı BA, Yıldız MA, Gürel F, Balcıoğlu MS

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*Bombus terrestris* L. is the most common bumblebee species found in Turkey's natural fauna. A total of 863 worker bee samples were collected from 14 native *B. terrestris* populations and four commercial companies. The genetic structure of the native *B. terrestris* populations in Turkey and the introduction of imported foreign genotype into the native populations were investigated by using ten microsatellite markers.

The mean number of alleles ( $N_a$ ), effective number of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), gene flow ( $N_m$ ) and  $F_{is}$  values over all population were estimated. In consideration of observed and expected heterozygosity, high genetic variation in commercial and Turkish native *B. terrestris* with regard to these microsatellite loci was found. There was not significant deviation from Hardy-Weinberg equilibrium in the population. Most of the genetic variation was present within populations rather than among populations. Our results showed that all the *B. terrestris* samples from the native populations and commercial companies have common ancestors and belong to same subspecies *B. t. dalmatinus*. It seems that additional molecular techniques would be necessary to identify and distinguish ecotypes of *B. terrestris*.

### 6.12 Adaptive evolution of genes with direct and indirect fitness effects in social insects.



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Eusocial insects are characterised by overlapping generations, cooperation for brood care and the existence of a reproductive caste. Kin selection theory allows to integrate this altruistic behaviour into the framework of the Darwinian evolution theory. Briefly, an altruist individual will increase his fitness indirectly by helping related individuals (called indirect or inclusive fitness). The haplo-diploid sex determination in Hymenoptera might have favoured the establishment of eusocial structures, due to a high relatedness between sisters. In these societies, social interactions might have impact on the expression of genes, so on the phenotype of individuals (known as indirect genetic effects).

From a population genetics perspective, genes with indirect fitness effects, e.g. social effect genes, are expected to harbour higher levels of sequence polymorphism within populations than direct fitness genes (reproductive effects genes). This is a result of a less effective selection on them, due to the indirect fitness (which is always less than the direct fitness).

To test this hypothesis, we will sequence genes involved in direct (directly related to oocytes production) and indirect fitness (genes expressed only in worker caste) in eusocial bumblebees and in socially parasitic bumblebees. Bumblebees represent an excellent model system, since socially parasitic bumblebees possess no worker caste and show high host specificity, offering a unique opportunity for paired comparisons of sequence variation for direct and indirect-effect genes.

for your remarks:

6.13

### Population structural changes of an intestinal parasite of bumblebees (*Bombus* spp.) in the course of one season.



Popp M\*, Erler S, Lattorff HMG

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The population dynamic of annual social insects might influence horizontally transmitted parasites by driving temporal variation in transmission rates and inducing seasonal fluctuations in the virulence of the parasites. In order to analyse these seasonal fluctuations in prevalence, we use an established model system in evolutionary biology – bumblebees (*Bombus* spp.) and their gut parasite *Crithidia bombi*. The prevalence of the parasite in field caught bumblebees was analysed during the course of one season. Parasites were genotyped to determine the occurrence of multiple infections and changes in genotype frequencies. Parasite prevalence varied throughout the year, reaching its maximum in July in two different species, *B. terrestris* and *B. lapidarius*. The intensity of infection decreased throughout the season being highest in the beginning and steadily decreasing. Significant changes in allele frequencies were observed potentially as a result of within host competition between parasite genotypes, leading to selection of specific alleles and increasing the frequency of homozygote genotypes in the middle of the season. Similarly, as competition declines later in the season, negative frequency dependent selection may reduce the frequency of these alleles. In addition, recovery of heterozygosity and disappearance of linkage disequilibrium also gives strong evidence for genetic exchange in these trypanosomes in natural populations.

6.14

### New threats for honeybee conservation on the Island of Læsø.

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The population of honeybees (*Apis mellifera mellifera*) on the Læsø Island has received much attention, since the first enforcement of a conservation Law in 1993. Not all local beekeepers were willing to give up their introduced bees, and challenged the Law in Court. Further years of introgression resulted, before the Court case was settled. However, a small population remained in situ with as few as fifty relatively pure colonies remaining by 2005. A plan was drawn up to use the purest of these colonies for increasing both population size and purity. A controlled breeding area was established and respected by all beekeepers on the Island. By 2007 a core population of 123 colonies was designated for further breeding, based on 250 examined samples.

In 2010 a new sampling of the population was performed to see if the conservation effort was functional and in order to measure genetic drift in the closed population. The population remains pure, but show signs of genetic drift resulting from the loss of rare alleles. The threats from genetic drift and various risks associated with introducing genes from neighbouring *A. m. mellifera* populations will be discussed.

for your remarks:

6.15

### The impact of apiculture on the genetic structure of Atlantic island honeybee populations (*Apis mellifera* L.).



Muñoz I\*, Pinto MA, De la Rúa P

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Beekeeping practices such as importation of non-native honeybee queens may interact with the conservation of honeybee subspecies and ecotypes biodiversity. Island honeybee populations are especially appropriate to test the impact of the introduction of foreign subspecies into their genetic diversity and structure. Here we used microsatellite markers to test whether genetic introgression from introduced honeybee queens is taken place in the honeybee populations from the Canary Islands, Azores and Madeira (Macaronesian region). We found introgression signals from foreign honeybee populations on these Atlantic islands based on Bayesian structure analysis and population parameters. Fst pairwise comparisons with Iberian, North African and other European populations and structure analyses suggest different sources of honeybee queens into the islands: while honeybees from the Canary Islands showed introgression from European honeybees, the honeybees from Azores and Madeira showed a closer relationship with those from the Iberian Peninsula. On the other hand, the existence of endemic honeybee populations on the Canary Islands prone to be conserved can still be depicted from the aforementioned analyses.

6.16

### APESLOW: a collaborative project to save the autochthonous Sicilian bee *A. m. siciliana*.

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Efforts for *A. m. siciliana* conservation on Sicily began in the late '80s, when three colonies were isolated on the small Ustica island to avoid introgression with the Italian yellow bee *A. m. ligustica*. A professional beekeeper, Carlo Amodeo, and CRA-API (Honeybee and Silkworm Unit of the Council for Research and Experimentation in Agriculture) cooperated over 30 years to preserve a pure population.

The special biological traits shown by *A. m. siciliana* have recently fuelled interest in this race on behalf of Sicilian beekeepers. Moreover, Slow Food International Foundation for Biodiversity places a spotlight on the Sicilian bee. In Dec. 2011 a three year reintroduction project (APESLOW: "Reintroduction and conservation of endangered subspecies *Apis mellifera siciliana*, Dalla Torre 1896: a tribute to Prof. Genduso") has started, with the aim to establish protected breeding areas for *A. m. siciliana* and to obtain a better knowledge of its biological and productive traits.

Here we present an overview of the abovementioned project and also molecular, morphometric and enzymatic data from past and recent monitoring activity on *A. m. siciliana* populations on both conservation islands and on Sicily main island.

for your remarks:

6.17

**Candidate loci for selection in Iberian honeybees: a genome-wide scan using SNP genotyping.**

Pinto MA\*, Chávez-Galarza J, Henriques D, Muñoz I, De la Rúa P, Azevedo J, Patton JC, Johnston JS

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Over the last 20 years Iberian honeybees (IHB) have been scrutinised with a variety of genetic markers including morphology, allozymes, mtDNA, and microsatellites. The complex patterns and processes revealed have yet to be fully understood. While mtDNA is useful to reveal the maternal component, biparental markers capture genome-wide effects (admixture, expansions, and contractions) and locus-specific effects (selection). Accordingly, we expect to dissect out, through genome-wide scans, neutral and selection effects. In this study, the Illumina GoldenGate Assay was used to genotype SNPs across the complete genome of IHBs. The SNP data set was analysed for evidence of selection using a frequentist, FDIST, and a Bayesian method, BayeScan. Additionally, a spatial analysis method (MatSAM) was used to test for associations between SNPs and climatic variables. With these approaches we detected eight loci with a strong signal of directional selection. The genome positions and putative gene functions, given in the HB genomic resources, suggest that these putatively selected loci are strong candidates for selection. The spatial representation of these same loci reveals a clinal pattern of variation, further supporting selection. Our approach allows for a rigorous consideration of selection as the underlying cause of observed patterns of diversity and contrast with previous studies where evocation of selection was merely speculative.

6.18

**SNP (Single Nucleotide Polymorphism) analysis on the honeybees of Turkey.**

Tunca RI, Kence M, Galindo A, Giray T, Kence A\*

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Ruttner has said that Anatolian bees were present in Thrace and they were separated from the rest of the honeybees of Europe along the borders of Turkey. He also stated that it's hard to explain this observation. We have studied 50 samples from Turkey at 994 SNP loci to answer this question. After structure analysis of 50 samples we have recognised Thracian honeybees and Anatolian honeybees with different genetic combinations. Besides according to our previous microsatellite analysis between Thracian and Anatolian honeybee populations there were minimum gene flow. A phylogenetic tree was constructed using distance matrix based on SNP results also revealed that Thracian honeybees of Edirne and Kırklareli region form a separate cluster compared to samples from other Anatolia regions. Therefore Thracian honeybees are completely different from Anatolian honeybees and they must be closer to European honeybees.

for your remarks:

6.19

**Rural avenues can sustain a viable feral population of honeybees.**

Tofilski A\*, Oleksa A, Gawroński R

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Some honeybee (*Apis mellifera*) subspecies are endangered with extinction because their feral population almost completely disappeared. The major threat to the feral populations of bees is habitat loss and replacement of woodlands with agricultural fields. In many places the only habitat suitable for honeybee nesting are rows of trees along road sides. We studied a feral population of honeybees inhabiting avenues in northern Poland. We inspected 142km of avenues in 201 sites and we examined 15.115 trees. Hollows were noticed in 23% of the trees. Feral colonies occupied 1.2% of the hollow trees. In total 43 feral colonies were found. The estimated density of feral population inhabiting the avenues was 0.09 nests per square kilometre. Honeybees preferred to build their nests in trees with a thick trunk and somewhat weak state of health. There was no strong preference of bees to any species of trees. Rural avenues provide suitable nesting habitat for honeybees and deserve to be protected. New avenues should be created along rural roads in order to improve pollination of surrounding crops. They can provide suitable habitat not only for honeybees but also other pollinators.

for your remarks:

# Session Seven

## Genomics

**Chair:**

**Martin Hasselmann**  
(Cologne, Germany)

### **Symposium description**

The number of genome sequences available for social insects have been substantially increased. Given the ongoing improvements of sequencing and analysis tools, we are still faced with the challenging task to decipher the relevant information to answer our scientific questions. Whole genome comparisons and a genome scale comparative analysis of protein-coding regions can reveal patterns of genetic change that coincided with the evolution of the differing social systems. Alternatively or in addition to that, candidate gene approaches may shed light on evolutionary novelties within distinct social insect lineages. Investigating levels of gene expression using RNAseq between different social insect castes can provide novel insights into the complexity of genetic regulations within organism. Despite certain challenges, genomic studies in social insects hold promises to elucidate more fundamental questions in evolutionary biology in the future.

7.1

**Genetic changes associated with eusocial evolution in bees.****Fischman BJ\*, Woodard SH, Pitts-Singer T, Hudson ME, Sadkhin B, Clark AG, Robinson GE**

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Identifying genetic changes that have occurred during transitions from solitary to social living is important for understanding how complex eusocial societies evolve. Here, I present two studies that took advantage of next-generation sequencing technologies to identify genetic changes associated with eusocial evolution in bees. First, I will discuss results of a molecular evolution study of ~3,600 protein-coding sequences among ten socially diverse bee species. This study identified a core set of genes showing a molecular signature of accelerated evolution in eusocial relative to solitary lineages across three independent origins of eusociality suggesting a prominent role for these genes in eusocial evolution. We also identified distinct sets of genes evolving rapidly only in particular types of eusocial lifestyles indicating that there may be multiple molecular routes to the evolution of eusociality. A higher level analysis of gene function highlighted several biological processes, including carbohydrate metabolism and gland development, that exhibit a disproportionate amount of genetic changes associated with bee eusocial evolution. I will also present recent findings from our analyses of the first available genome of a solitary bee, the alfalfa leafcutting bee, *Megachile rotundata*. Results of genomic comparisons of *M. rotundata* and recently sequenced social insect genomes will be discussed.

7.2

**A Y-like social chromosome causes alternative colony organisation in fire ants.****Wurm Y\*, Wang J, Riba-Grognuz O, Nipitwattanaphon M, Keller L**

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Intraspecific variability in social organisation is common, yet the underlying causes are rarely known<sup>1-4</sup>. In the fire ant *Solenopsis invicta*, the existence of two divergent forms of social organisation is under the control of a single Mendelian genomic element marked by two variants of an odourant binding protein (OBP) gene<sup>5-9</sup>. Here we characterise the genomic region responsible for this important social polymorphism and show that it is part of a pair of heteromorphic chromosomes having many of the key properties of sex chromosomes. The two variants, hereafter referred to as the Social B and Social b (SB and Sb) chromosomes, are characterised by a large region (ca. 13 Mb) where recombination is completely suppressed between SB and Sb. Recombination appears to occur normally between the SB chromosomes but is impossible between Sb chromosomes because Sb/Sb individuals are non-viable. Genomic comparisons revealed limited differentiation between SB and Sb, with all or at least the vast majority of the 616 genes identified in the non-recombining region present in the two variants. Inversions on the Sb chromosome explain the lack of recombination with SB. Importantly, the non-recombining region comprises most of the genes with demonstrated expression differences between individuals of the two social forms. These findings highlight how genomic rearrangements can maintain divergent adaptive social phenotypes involving many genes acting in concert by locally limiting recombination.

for your remarks:

7.3

**Molecular evolution of immune genes in socially diverse bees.****Viljakainen L\*, Fischman BJ, Woodard SH, Robinson GE, Clark AG**

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The evolution of sociality in ants, bees, wasps and termites has been substantially influenced by the elevated pathogen pressure in densely inhabited colonies. Social insects have evolved many collective defences, i.e. social immunity, to complement the physiological individual-level immune responses. These include the use of antimicrobial compounds in the nest building material and collective recognition and removal of infected individuals. The effect pathogens have had on the evolution of genes involved in physiological immune system genes in social insects can be studied by comparing evolutionary patterns in immune genes of closely related social and solitary organisms. We have done this by studying sequence evolution of a large number of immune genes in bees with varying levels of sociality (highly eusocial, primitively eusocial and non-eusocial) using codon-based likelihood models of nucleotide substitution. There is surprisingly little evidence for positive selection in the contrasts done to date. This may suggest that social immunity acts as a buffering mechanism and reduces selection pressure on immune genes.

7.4

**Prospects for a comparative honeybee neuroethology in the era of “omic”-technologies.****Brockmann A**

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The European honeybee species, *Apis mellifera*, has been one of the most important and successful models in the study of animal behaviour. Increasingly, it is also becoming a model for studying disease ecology, aging, phenotypic plasticity, and social evolution. However, there are at least seven other honeybee species, all of which are native to Asia. For many of these species, we have a rich description of their ecology and behaviour. Comparative studies indicate a high level of diversity in many important social traits, e.g. division of labour, dance communication, foraging activity and worker longevity. So far, molecular and neuroethological studies on Asian honeybees are still very rare. But there is hope that this might change in the near future, as the sequencing of three Asian honeybee genomes, *Apis florea*, *Apis dorsata* and *Apis cerana*, are underway. I recently started a honeybee neurobiology lab at the National Centre for Biological Sciences in Bangalore, India. The lab will focus on comparative behavioural and molecular studies using *A. florea*, *A. dorsata*, *A. cerana* and *A. mellifera*. In my talk, I will present current projects on sex-pheromone communication and dance language and will discuss the prospects of honeybee neuroethology in the era of “omic” technologies.

for your remarks:

7.5

### Conservation of microsatellites – the rate of genome evolution, evolution of chromosomal structures and genome assembly improvements.



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Microsatellites are common genomic elements. They are thought to evolve mostly neutrally, with a high turnover due to high mutation rates. We aimed to study the conservation and distribution of these repetitive DNA elements along evolutionary time scales in the genomes of related insect species. We show that microsatellites can be retained for several hundred million years, although different types and motifs exhibit specific patterns. Comparing Diptera and Hymenoptera in relation to divergence time, the flies show a loss of microsatellites which is twice as fast as in the Hymenoptera, suggesting a slower genome evolution in the latter - consistent with previous studies based on substitution rates in protein coding sequences. However, if the number of generations produced per year (generation time) is considered, the Diptera show an 8.5-fold slower rate than the Hymenoptera. This suggests a faster genome evolution, which might be due to smaller effective population sizes and genetic drift in the social and parasitic Hymenoptera species.

The high number of conserved microsatellite loci between genomes related species allows also for genome-wide analyses of synteny with high resolution. We use this to compare genome assemblies and detect potential assembly errors, as in *Bombus impatiens*. Furthermore it allows for mapping unplaced scaffolds into assembly-gaps. Finally whole chromosomes or genomes can be analysed and the evolution of their organisation can be investigated.

7.6

### Molecular evolution of antiviral genes in bumblebees.

Lattorff HMG\*, Helbing S

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Social insects are prone to parasites. High density of closely related individuals enhances pathogen transmission. Adaptations and counter adaptations between hosts and parasites left signatures in the genome, especially on antiviral RNAi genes indicated by high rates of adaptive evolution in *Drosophila*. In order to quantify the impact of host-parasite conflict effects on the evolution of RNAi genes in social insects, eight genes were sequenced and compared across different species of the genus *Bombus*. Additionally, we test for the impact of sociality on molecular evolution by directly comparing social species and their respective socially parasitic cuckoo bumblebees.

We show that RNAi genes exhibit an elevated rate of adaptive evolution compared to non-immune genes due to a co-evolutionary interplay between RNAi genes and viruses, as the latter evade hosts defence mechanisms by production of suppressor-proteins interfering with the RNAi- pathway. Furthermore, RNAi genes exhibit vary in their rates of adaptive evolution closely linked to their pathway-specific position, as proteins directly interacting with viral components evolving most rapidly. We compared the evolutionary rates of RNAi genes between cuckoo bumblebees (non-social) and their respective host lineages to infer the impact of sociality on molecular evolution. Here, we show that RNAi genes evolved significantly faster in social species due to their larger population sizes.

for your remarks:

7.7

**From where did *Apis mellifera* originate?****Webster MT\*, Wallberg A, Han F**

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The native range of the honeybee *Apis mellifera* encompasses Europe, Africa and the Middle East, whereas the nine other species of *Apis* are found exclusively in Asia. It is therefore commonly assumed that *A. mellifera* arose in Asia and expanded into Europe and Africa. However, other hypotheses for the origin of *A. mellifera* have also been proposed based on phylogenetic trees constructed from genetic markers. In particular, an analysis based on >1000 single-nucleotide polymorphism markers placed the root of the tree of *A. mellifera* subspecies among samples from Africa, suggestive of an out-of-Africa expansion. Here we re-evaluate the evidence for this and other hypotheses by testing the robustness of the tree topology to different tree-building methods and by removing specimens with a potentially hybrid background. These analyses do not unequivocally place the root of the tree of *A. mellifera* subspecies within Africa, and are potentially consistent with a variety of hypotheses for honeybee evolution, including an expansion out of Asia. Our analyses also support high divergence between western and eastern European populations of *A. mellifera*, suggesting they are likely derived from two distinct colonisation routes, although the sources of these expansions are still unclear.

for your remarks:

# Session Eight

## Bee Doc EU Network

**Chair:**

**Bernhard Kraus**  
(Halle, Germany)

### **Symposium description**

BEE DOC is a European research network of eleven partners from honeybee pathology, chemistry, genetics and apicultural extension aiming to improve colony health of honeybees. The aim of BEE DOC is to empirically, experimentally fill knowledge gaps in honeybee pests and diseases, including 'colony collapse disorder' and quantify the impact of interactions between parasites, pathogens and pesticides on honeybee mortality. Parasites and pathogens in focus include *Nosema* microsporidia and *Varroa* mites and three model viruses (deformed wing virus, black queen cell virus, Israel acute paralysis virus). BEE DOC aims to identify novel genes for disease resistance by using transcriptome analyses to explore host-pathogen-pesticide interactions. Final goal of BEE DOC will be to develop novel diagnostic screening methods and sustainable concepts for disease prevention using novel treatments and selection tools for breeding and identifying resistant honeybee stock. The BEE DOC is linked to various national and international ongoing European, North- and South-American colony health monitoring and research programmes, to ensure pan-European but also global visibility and the transfer of results to apicultural practice in the world community of beekeepers. The Eurbee 2012 BEE DOC session will give an overview of the current state of research and the results achieved so far.

8.1

### **Bees in Europe and the Decline Of honeybee Colonies - an European research network.**

Moritz RFA

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The BEE DOC comprises a network of eleven partners from honeybee pathology, chemistry, genetics and apicultural extension aiming to improve colony health of honeybees. The BEE DOC will empirically and experimentally fill knowledge gaps in honeybee pests and diseases, including the 'colony collapse disorder' and quantify the impact of interactions between parasites, pathogens and pesticides on honeybee mortality. Specifically BEE DOC will show for two model parasites (*Nosema* and *Varroa* mites), three model viruses (deformed wing virus, black queen cell virus, Israel acute paralysis virus) and two model pesticides (thiacloprid,  $\tau$ -fluvalinate) how interactions affect individual bees and colonies in different European areas. The BEE DOC will use transcriptome analyses to explore host-pathogen-pesticide interactions and identify novel genes for disease resistance.

The BEE DOC will specifically address sublethal and chronic exposure to pesticides and screen how apicultural practices affect colony health. The BEE DOC will develop novel diagnostic screening methods and develop sustainable concepts for disease prevention using novel treatments and selection tools for resistant stock. The BEE DOC will be linked to various national and international ongoing European, North- and South-American colony health monitoring and research programmes, which will not only ensure pan-European but also global visibility and the transfer of results to apicultural practice in the world community of beekeepers.

8.2

### **Interactions between *Nosema* and virus infections and their impact on individual honeybees.**

Doublet V\*, Zschiesche L, McMahon DP, Labarussias M, de Miranda JR, Paxton RJ

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Many drivers of honeybee decline have been identified and, within these, diseases caused by pathogens, alone or in combination, are thought to be of major importance. Indeed, when a pathogen first enters a honeybee colony, the disease organism can potentially spread rapidly into many individuals, impacting their health and thus make individuals and the colony more susceptible to an invasion by a second pathogen. Here we report our results on experimental co-infection of individual adult honeybees by the microsporidian *Nosema ceranae* and two common honeybee viruses; deformed wing virus (DWV) and/or black queen cell virus (BQCV), widespread pathogens that all infect the honeybee ventricular tissue. The responses to these multiple infections were recorded as honeybee mortality and changes of the bees' immune system. The impact of one pathogen on the course of infection of other pathogens has also been tested by differential timing of infection, e.g. by experimentally administering both *Nosema* and virus at the same time, or one after the other. These experiments at the level of the individual bee complement those of other BEE DOC working groups investigating colony-level down to gene-level responses to co-infection of honeybees by multiple pathogens.

for your remarks:

8.3

### Synergistic effects of honeybee pathogens and pesticides on the longevity and behaviour of bees on the colony level.

Rosenkranz P\*, Odemer R, Larue A, Tanner G, McDonnell C, Crauser D, Williams GR, Mehmman M, Le Conte Y, Neumann P

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It is supposed that honeybee colonies are weakened by chronic contaminations with sublethal doses of certain pesticides thereby resulting in a higher susceptibility to honeybee pathogens. We exposed honeybee colonies over a two month period with Fluvalinate, an acaricide of the group of synthetic pyrethroids and Thiacloprid, an insecticide of the neonicotinoid group. We used sublethal doses matching a worst case scenario under beekeeping and agricultural field conditions. Additionally, we separated these colonies in two groups with high and low infections with BQCV. These colonies served together with non-treated control colonies as source for bees that were used for observation hive experiments. From all experimental groups of colonies we took freshly hatched bees and fed half of them with approximately 50.000 spores of *Nosema ceranae* per bee. By this procedure we created eight experimental groups of bees according to previous pesticide exposure on the colony level and individual infections with pathogens. About 100 individually marked bees per group were then introduced into observation hives with approximately 2.000 healthy bees. We here present the results on the social behaviour, flight activity and longevity of the differently treated bees recorded over a three week period.

Supported by the EU project "BEE DOC". (244956 CP-FP)

8.4

### Brain transcriptomic analysis of honeybees (*Apis mellifera*) parasitised by *Varroa destructor* or *Nosema ceranae*.

McDonnell C\*, Alaux C, Parrinello H, Desvignes JP, Serot M, Le Conte Y

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Precocious foraging is a natural mechanism by which the hive can respond to environmental cues but it can also signal that a colony is on the verge of collapse. Honeybees infected by either *Varroa destructor* or *Nosema* species share the behaviour of leaving the hive early, but are they really precocious foragers? We compared the transcriptomic profiles of brains from honeybees infected by either *Varroa* or *Nosema*. In 10 day-old bee brains, we found that varroa-infested bees showed a greater number of genes that changed compared to *Nosema ceranae*-infected bees. Despite the difference in developmental stage at which the bee is parasitised, *Nosema* and *Varroa* infected bees shared more gene changes with each other than with other available honeybee brain expression gene sets, such as those of forager, nurse and scout castes. In addition, we identified significantly higher viral loads in the brains varroa- and nosema-infected bees than control bees. Thus, the brain expression profiles are most likely indicative of sick bees than of precocious foragers but with significant differences depending on infection type.

for your remarks:

8.5

### Quantitative trait locus and selective sweep analysis for *Nosema ceranae* tolerance in the honeybee *Apis mellifera*.



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*Nosema ceranae* has been recently introduced into the honeybee *Apis mellifera* as a novel microsporidian gut parasite. We combined the quantitative genetic and population genetic analysis to locate the genetic region involved in *N. ceranae* infection tolerance. Four QTLs were identified significantly reducing the *N. ceranae* spore load and explaining 20.4% of total spore load variance including a major QTL, an additive QTL and a paired epistatic QTL. Moreover a selective sweep was revealed within the major QTL region. The genetic variability of the swept loci was not only reduced in relation to the flanking markers within the selected strain, but also significantly reduced compared to the unselected honeybee strain. The consistent results indicated the existence of a genetic basis of *N. ceranae* infection resistance in the honeybee *A. mellifera* and suggested the positive selection driving the success of the selective breeding.

for your remarks:

8.6

### BeeDoctor and BeeClinic, two new tools for bee health diagnosis.

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In the 'diagnostic' department of the BEE DOC project two new tools for bee health diagnosis have been developed. BeeDoctor is an 'extension grade' tool based on the Multiplex Ligation-dependent Probe Amplification (MLPA) technology. It is a highly versatile technique permitting to multiplex up to 45 target in one reaction. The technology was used in order to diagnose the most important honeybee viruses. Within the BEE DOC project intensive training sessions have been organised for the different partners in order to allow the utilisation of this tool in different Working Packages. BeeClinic is based on a colourimetric DNA chip for gene-expression studies and has been categorised as a 'research grade' tool. The switch to colourimetric development permits the 'in house' application of DNA chip technology. Genes were selected for the detection of immunological, nutritional and detoxification stress and are now being screened using bees with a known stress history. BeeClinic has a great potential in research related topics but could also become a valuable tool in diagnosis of bee health, for instance to determine the cause of bee colony mortality.

8.7

**Pathogen prevalence in varroa-free, varroa-infested and varroa-surviving Scandinavian honeybee populations.****de Miranda JR\*, Locke B, Semberg E, Forsgren E, Fries I**

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*Varroa destructor* is the major parasite of the European honeybee (*Apis mellifera*), and the most common biological cause of honeybee colony death. This is large part due to its association with many secondary pathogens, particularly a number of honeybee virus diseases for which it is an active vector. In order to assess the extent and nature of the relationship between mite infestation and secondary pathogens, we determined the prevalence of honeybee pathogens, including viruses, bacteria, microsporidia, fungi and tracheal mites, in a large Scandinavian survey covering areas with different histories of *Varroa* infestation, including areas beyond the *Varroa* expansion front that have never had *Varroa* infestation, recently invaded areas, areas with long-standing chronic *Varroa* prevalence and populations that have developed natural tolerance to *Varroa* infestation, allowing long-term survival without *Varroa* population control. The associations between the different pathogens will be presented as well as their relationship to the presence of *Varroa* in the area over different lengths of time.

8.8

**Is there any relationship between propolis chemical composition and bee colony health?****Bankova V\*, Popova M, Fries I, Reyes M**

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Propolis is well known as the chemical defensive weapon of honeybees against infections and parasites. It is also known that propolis chemical composition is highly variable and depends on the geographic location and on the specificity of the flora at that location. However, there are no data concerning the relationship between the chemical composition of propolis and the health of the respective bee colonies. We tried to answer some aspects of this question, studying the chemical composition of propolis (by GC-MS) of bee colonies which possess some level of tolerance to *Varroa* infestation from France and Sweden and compared it with colonies which are non-tolerant to *Varroa* from the same regions. Preliminary results indicated that non-tolerant colonies tended to collect more resin than the tolerant ones. In addition, the percentage of some biologically active compounds (esters of caffeic acid) was higher in propolis balsam from tolerant colonies (difference demonstrated to be statistically significant). Further studies are needed to clarify the role of these compounds as anti-*Varroa* agents.

for your remarks:

8.9

### Profiling of cytokine release stimulated by proteins of honeybee nutrition.

Bilikova K\*, Simuth J, Zakostelska Z

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Profiling expression of cytokines stimulated by RJ proteins on the global scale will have significant impact not only in human immunotherapy but also in characterisation of immune potential of honeybee nutrition. The presence of specific cytokines, chemokines, interleukins and other proteins captured by an antibody chip was detected using Streptavidin-conjugated dye as a reporter.

RJ-proteins at concentration of 50-500 µg/ml stimulated mouse macrophage to release: granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-12 (IL-12), tumor necrosis factor alpha (TNF-alpha), monocyte chemoattractant protein-1 (MCP-1), serum platelet factor-4 (PF4), matrix metalloproteinase inhibitors (TIMP1 and TIMP2) approximately in the same level as by application of lipopolysaccharide (LPS), as a positive control. The vascular endothelial growth factor (VEGF) - the key regulator of physiological angiogenesis during embryogenesis, skeletal growth and reproductive functions were detected at concentrations two times higher.

Presented simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for evaluation of honeybee nutrition quality and it is a contribution to nutritional immunology.

for your remarks:

8.10

### BEE DOC - transfer of results (WP8).

Neumann P\*, Dietemann V, Williams GR

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If we are aiming at sustainable solutions for honeybee health, an efficient transfer of results to all involved stakeholders and an enhanced public awareness are required. This is achieved through the active participation of the BEE DOC partners in "COLOSS" (= prevention of honeybee COLony LOSSes), a global network with currently 306 members from 61 countries. COLOSS aims at coordinating international research efforts to mitigate honeybee colony losses not only in Europe, but also in other regions in the world, where apiculture is well established. For that purpose, the "BEEBOOK: standard methodologies for *Apis mellifera* research" is currently in preparation as a way to improve scientific output via standardisation. Direct contacts between BEE DOC members and stakeholders are facilitated by the network as well as publications in beekeeping journals and popular articles are the main extension tools. In particular, implementation of Good Apicultural Practice will be facilitated by the publication of guidelines for the maintenance of colony health in local beekeeping magazines as well as online at [www.coloss.org](http://www.coloss.org). Only a global network on bee health can drive the large scale international collaborative efforts necessary to limit honeybee decline.

# Session Nine

## STEP EU Network

**Chair:**

**Simon Potts**  
(Reading, UK)

### **Symposium description**

The STEP project (Status and Trends of European Pollinators, [www.STEP-project.net](http://www.STEP-project.net)) is a major new European project addressing critical questions about pollinators and the services they provide, and includes more than 50 researchers from 20 countries. STEP is assessing the current status and trends of pollinators in Europe, quantifying the relative importance of various drivers and impacts of change, identifying relevant mitigation strategies and policy instruments, and disseminating this to a wide range of stakeholders. STEP is a very inclusive project by considering all pollinator taxa from wild bees, bumblebees and honeybees to hoverflies, flies and other insects; it also addresses both crop and wild flower pollination and the wider values of pollinators to society.

This symposium will deliver key findings from the STEP project relevant to researchers, farmers, conservationists, beekeepers, policy makers and the general public.

## 9.1

**Status and trends of European pollinators.**

Potts SG

Email: s.g.potts@reading.ac.uk

The STEP project (Status and Trends of European Pollinators, [www.STEP-project.net](http://www.STEP-project.net)) is a major new European project addressing critical questions about pollinators and the services they provide, and includes more than 50 researchers from 20 countries. STEP is assessing the current status and trends of pollinators in Europe, quantifying the relative importance of various drivers and impacts of change, identifying relevant mitigation strategies and policy instruments, and disseminating this to a wide range of stakeholders. STEP is a very inclusive project by considering all pollinator taxa from wild bees, bumblebees and honeybees to hoverflies, flies and other insects; it also addresses both crop and wild flower pollination and the wider values of pollinators to society.

for your remarks:

## 9.2

**How has diversity of bees and plants that depend on pollinators changed in the past 60 years?**

Carvalho LG\*, Biesmeijer JC, Kunin WE

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Bees are important pollinators of a large number of wild plants. Concern on the consequences of pollinator loss has led to an increase of investment in pollinator-friendly management measures in the past years. The existent extensive databases of historical records of species occurrence provide a unique opportunity for the study of biodiversity dynamics. Our current research applies novel analytical methods that cope with the non-standardised nature these databases, to evaluate how bees and plants that depend on them for pollination are changing through time and space. The results show how species loss and homogenisation processes have evolved in the past 60 years in three European countries (UK, the Netherlands and Belgium) at local, regional and country levels.

9.3

**Sublethal insecticide exposure reduces pollinator reproductive success.****Sandrock C\*, Tanadini LG, Pettis JS, Biesmeijer JC, Potts SG, Neumann P**

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Pollinating insects provide crucial and economically important ecosystem service to crops and wild plants. Therefore their recent global declines are alarming. Various drivers are suspected to affect pollinator health, including prevalent pesticide applications for crop protection. Sublethal pesticide exposure negatively impacts numerous pollinator life history traits, but its influence on reproductive success remains largely unknown. Such information is pivotal, however, to understand long-term effects on population dynamics. We show that realistic concentrations of systemic neonicotinoid insecticides in nectar decrease the reproductive performance of the Red Mason bee, *Osmia bicornis*. Monitoring of experimental populations revealed that although adult mortality was not affected by chronic exposure to neonicotinoids it resulted in severely reduced total offspring production, and further in a significantly male biased offspring sex ratio. Results strongly suggest that sublethal exposure to neonicotinoids has long-term detrimental effects on solitary bee populations. Consequently, current agricultural practices may directly contribute to recent declines in wild pollinators.

9.4

**Large scale distribution patterns of solitary bees and bumblebees.****Franzén M**

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In this presentation I present preliminary results from the first analysis (within the STEP-project) of large scale distribution data across Europe for solitary bees and bumblebees. How climatic conditions, land use and other pressures influence the distribution of the species are presented. Distribution of life history traits show that body size increases with increasing altitude. Other traits that have been analysed are sociality and dietary niche breadth. Future scenarios predict that high altitude species are threatened because of future changes in climate.

for your remarks:

9.5

### STEP WP 5: effects of mass-flowering crops on pollinator communities in agricultural landscapes.



Riedinger V\*, Holzschuh A, Steffan-Dewenter I

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Pollinator communities are negatively affected by multiple environmental pressures, but combined effects of these pressures and consequences for pollination functions across Europe are not well understood. One important factor that influences pollinators is the current expansion of mass-flowering crops. Spill-over effects between these crops and semi-natural habitats can enhance pollinators. On the other hand wild plants may compete with mass-flowering crops for pollination services. To study these effects a network of 96 study sites was established in six countries in 2011 in the framework of the EU-project STEP. We focus on local, landscape and temporal scale effects of mass-flowering crops on pollinator visitation rates and diversity. In a common study design we survey pollinator communities in semi-natural habitats and different mass-flowering crops. Additionally, population dynamics and reproductive success of bee-populations and crop pollination services are assessed. With the establishment of 32 wild flower strips in four countries we extended the basic design to evaluate the effects of a mitigation strategy on flower visiting insects. The overall aim of the work package is to perform a comprehensive and comparable study on a European scale and by this fill the gaps of knowledge that were identified by other work packages. We will give an overview of the structure in work package 5 in the STEP project and present first results from the German study region.

9.6

### First European Red List for bees.

Roberts SPM\*, Rasmont P, Biesmeijer JC, Potts SG et al.

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Assessing the status of the several thousand species of European pollinators is a major task that requires a coordinated large-scale effort involving specialists from across Europe as well as a standardised framework of assessment. The STEP project is using the IUCN Red list procedures to guide the development of a Red Data Book for European bees (and a roadmap for European hoverflies) while at the same time realising that the knowledge base for this assessment is both taxonomically and geographically incomplete. We are involving taxonomic specialists and national/regional experts in a series of intensive workshops, which will aim at: (i) agreement on standardised assessment criteria relevant for pollinators; (ii) training of experts to improve consistency of the assessment; (iii) identifying leaders for assessment of specific taxonomic groups; (iv) conducting initial broad assessments of the status of European pollinator species; (v) identifying knowledge gaps and ways to overcome them; and (vi) developing a process towards accurate assessment of the status of European pollinators as a basis for policy decisions concerning their conservation.

A Red Data Book for European bees will be published as both an open access e-book and in print version, and a roadmap for developing a Red Data Book for hoverflies will also be drafted.

for your remarks:

9.7

### A preliminary analysis of the fate of European bumblebees.

Rasmont P\*, Smet J, Iserbyt S, Roberts SPM, Schweiger O, Biesmeijer JC, Castro L, Cederberg B, Dvorak L, Fitzpatrick U, Gonseth Y, Laget D, Mahé G, Manino A, Neumayer J, Odegaard E, Paukkunen J, Pawlikowski T, Reemer M, Sima P, Straka J, Potts SG

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The authors gathered all the distribution data on European bumblebees (*Bombus*). The resulting data base includes information on nearly 900.000 specimens from all parts of the West Palaearctic. There are 77 bumblebee species in the studied region, including twelve social obligate parasites (ten from subg. *Psithyrus*, and two others). Species have been mapped using a 50km UTM grid. Three time intervals have been considered: (i) before 1950, (ii) from 1950 until 1989, and (iii) since 1990. All these maps are available at: <http://www.zoologie.umh.ac.be/hymenoptera/page.asp?id=169>.

Based on these maps, it is possible to present a preliminary assessment of the fate of most species using an analysis of the number of squares occupied by each species across the three time periods. The species showing the greatest contraction in range are bumblebees from step-pic areas and dry grasslands, and include *Bombus armeniacus*, *B. confusus*, *B. cullumanus*, *B. fragrans*, *B. laesus* and *B. mocsaryi*. Some species from cold areas in high latitudes or altitudes are also at risk, e.g. *B. monticola*, *B. mesomelas* and *B. polaris*. There is an impoverished area in Western and Central Europe that is roughly defined between the 45° and 55° latitudes. In contrast, there are several species (e.g. *B. haematurus*, *B. hypnorum* and *B. semenoviellus*) that show a recent and conspicuous range expansion. The observed shifts in many cases are related to the known life history traits of this genus, and the changing environmental conditions in Europe.

9.8

### Economic consequences of pollination service declines - advances from the STEP project.

Breeze TD\*, Gallai N, Bommarco R

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Pollination is widely recognised as an economically significant ecosystem service, influencing the productivity of agriculture and the aesthetic quality of the wider landscape. Past studies have used a range of simple bio-economic methods to assess the economic benefits of pollination services, however many of these studies are limited by data and methodology, resulting in vague and limited value estimates of these benefits to only a core handful of outcomes. A core objective of the STEP project is to augment and expand upon these methods to provide more comprehensive analyses of the impacts of pollination services on human societies. In particular the project has undertaken several studies into the value of pollination services beyond the crop market, including the benefits to small holders and seed producers in France and the non-market benefits of landscape aesthetics and local food availability in the general public of the UK.

for your remarks:

9.9

**Mitigating pollinator loss in Europe:  
what strategies are most effective?**

Kleijn D\*, Scheper J

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Flower-visiting insects provide vital pollination services to crops and wild plants. Accumulating evidence for declining populations of pollinators has increased the urgency to identify and implement measures that effectively mitigate pollinator loss. Europe has a long tradition of mitigating biodiversity loss on farmland, for example, through agri-environment schemes. Although such schemes rarely target pollinators directly, the management prescribed by these schemes can be expected to benefit pollinators. Here we present the results of a review of the effectiveness of different measures to mitigate pollinator loss, using a meta-analysis approach.

All investigated mitigation measures effectively enhanced species richness and abundance of pollinators. Supplementing pollinator food resources through the establishment of sown flower strips produced the most pronounced beneficial effect and appears to be a particularly effective mitigation measure. The effects of mitigation measures were more pronounced in arable landscapes than in grassland systems. Plant species richness produced by mitigation measures furthermore influenced the effectiveness of measures. Although population-level positive effects of flower strips may be expected, the species richness and abundance data analysed in this study do not merit unambiguous conclusions about effects of flower strips on landscape-wide populations of pollinators.

for your remarks:

**P1.1 Effects of neonicotinoids on the electrophysiology of cultured bee neurons.**



Fischer J\*, Grünewald B

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Honeybees may encounter a variety of harmful chemicals during foraging. Among them are neonicotinoid insecticides that are widely used in modern agriculture.

Neonicotinoids were developed for the use against pest-insects. They act as agonists on the insect nicotinic acetylcholine receptor and are supposed to have no effect on the mammalian acetylcholine receptor, rendering it harmless to humans and farm animals. Although bees are not a target of these chemicals, they are also affected by them. Bees that were exposed to non-lethal doses of different neonicotinoids (clothianidin, imidacloprid or thiacloprid) show various influences on behaviour or motor activity, depending on the different kind of neonicotinoid they were exposed to.

Cell physiological investigations of the action of these chemicals directly on the nerve cells of bees have just started. Here we want to show the effects of clothianidin and thiacloprid on cultured neurons from honeybee brain. For this we use whole-cell patch-clamp recordings with pressure application of the neonicotinoids. We hope to shed light on the various effects of the different neonicotinoids on bees that are evoked by binding to the same receptor.

**P1.2 Neuroanatomical correlations to social organisation and floral preference in closely related wild-bee species.**

Kelber C\*, Rössler W

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Among Hymenoptera, many ecologically successful species evolved a eusocial lifestyle. Several studies emphasise that eusocial Hymenoptera - like honeybees and ants - possess a complex brain including a high number of functional units (glomeruli) in the antennal lobe. Brain complexity may have evolved as the result of eusociality or in response to other selective pressures and, therefore, be a precondition for the evolution of eusociality. We analysed specific neuroanatomical traits in halictid bee species (Halictidae) with different grades of sociality, but polylectic floral preference and in solitary polylectic and oligolectic mason bee species (*Osmia*). We employed confocal microscopy scanning and 3D-reconstruction for quantitative analyses and focused on the antennal-lobe structure. We found no significant difference in glomerular number between solitary and eusocial species (*Lasioglossum*: 157-172 glomeruli, *Halictus*: 175-192 glomeruli). The investigated *Osmia* species showed a significantly lower number of glomeruli (100-146 glomeruli) and a large interspecies variance. The strictly oligolectic species *Osmia adunca* showed the lowest number of glomeruli, while polylectic *Osmia* species showed larger glomerular numbers. Our results give first hints that a more complex antennal-lobe structure is not the result of a eusocial lifestyle. Furthermore, floral preference seems to have a larger impact on antennal-lobe complexity than social lifestyle. Funding: DFG KE-1701 1/1.

**P1.3 Expression patterns of serotonin receptor subtypes in the honeybee, *Apis mellifera*.**



Rolke D\*, Blenau W

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The biogenic amine serotonin (5-HT) controls and modulates a great variety of physiological and behavioural processes by interacting with various 5-HT receptor subtypes. The honeybee, *Apis mellifera*, is known to express at least four 5-HT receptor subtypes: Am5-HT1A, Am5-HT2 $\alpha$ , Am5-HT2 $\beta$ , and Am5-HT7. Interestingly, m5-HT2 receptors are expressed both as a full length transcript and as a truncated splice variant, as it is known for certain dopamine receptors in *Caenorhabditis elegans* and humans. To provide a basis for further investigations and to develop testable hypotheses, e.g. on the physiological and behavioural functions of 5-HT receptors, the tissue specific expression patterns of both Am5-HT2 receptors were investigated using quantitative real-time PCR.

Both full length and truncated transcript variants of Am5-HT2 $\alpha$  could be detected in all tissues tested. In case of Am5-HT2 $\beta$ , the presence of the full length transcript could be verified in all tissues except the head salivary gland. In the nervous system and in the hypopharyngeal gland the expression of the full length Am5-HT2 $\alpha$  transcript was higher than that of the truncated version, whereas the opposite holds true for the Am5-HT2 $\beta$ . The hypopharyngeal gland showed considerably higher expression of Am5-HT2 $\alpha$ -full length than any other tissue. This led us to the assumption, that the hypopharyngeal gland activity is modulated by serotonin - although we did not find any evidence for serotonergic innervation of these glands.

**P1.4 Effects of chronic neonicotinoid exposition on the development of individual honeybee larvae and colonies.**



Schild H-A\*, Fischer J, Ross A, Grünewald B

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Neonicotinoids are insecticides that are widely used in agriculture. Honeybees can be exposed to those chemicals during foraging. We investigate the influence of non lethal doses of three common neonicotinoids, clothianidin, imidacloprid and thiacloprid, with two different test methods. Colonies were artificially fed with the insecticides and honeybee larvae were *in vitro* reared under influence of the insecticides. The treatment of the colonies revealed an influence on the amount of foraged contaminated sugar water, containing the different neonicotinoids, and the weight development of the colonies over time. Clothianidin led to an increased foraging activity and thereby also an increasing weight. Imidacloprid and thiacloprid had the opposed effect, reducing the amount of collected sugar water accompanied by a loss of weight. While the concentrations of thiacloprid tested in this study had no influence on the mortality rate of artificial reared honeybee larvae, clothianidin had complex effects on the development of larvae. The two lowest tested concentrations (0.23ppb, 2.25ppb) seemed to have a positive effect on the survival time, but the two highest tested concentrations had negative effects. To monitor the food uptake of the larva regarding the different treatments we are developing a new method to determine larvae gut contents.

Our long-term goal is to relate the effects on colony development to the effects on brood development and *vice versa*.

P1.5

**The honeybees gustatory sensitivity to amino acids.**

Simcock NK\*, Wright GA

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The honeybee gustatory system is comparatively simple and relatively little is known about what these insects are able to taste. Adult worker honeybees spend a large proportion of their time foraging for nutritional resources and amino acids used both by themselves and colony members. Amino acids, the second most concentrated components of floral nectar, could provide a good source of protein to the honeybee diet. This study explored the gustatory sensitivity of honeybees to a range of different amino acids using behavioural assays, with a future aim of linking in gustatory receptors to their ligands in bees. We found that whilst honeybees are highly tuned to the detection of carbohydrates, they are also sensitive to essential and a few non-essential amino acid compounds. We also investigated how nutritional state influenced gustatory sensitivity towards amino acids in honeybees. There is no clear split between the essential and non-essential amino acid groups but it is clear that pre-feeding history and existing nutritional state influences how bees respond to the presence of amino acids in reward solutions.

P1.6

**Olfactory learning abilities of bumblebees using pavlovian PER conditioning.**

Sommerlandt FMJ\*, Rössler W, Spaethe J

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Learning olfactory cues is essential in bees for orientation and recognition of nest sites and food sources. The proboscis extension response (PER) is a well-established method for a quantitative evaluation of olfactory learning abilities in bees under controlled environmental conditions. Experiments using the PER paradigm have shown that honeybees perform well on simple association tasks and learn complex non-elemental configural associations. In contrast, cognitive abilities of eusocial bumblebees have been poorly investigated so far. In the present work, *Bombus terrestris* workers were trained in different olfactory learning tasks using classical PER conditioning. We compared learning performance of four different odours. Individuals were able to solve absolute (A+) and differential (A+ vs. B-) conditioning tasks, and no differences were found between odours and odour combinations, respectively. Moreover, bumblebees performed well on a positive pattern discrimination task (A-, B- vs. AB+), but failed to solve negative pattern discrimination (A+, B+ vs. AB). These results indicate that workers of *B. terrestris* possess elemental olfactory learning abilities but fail in more complex tasks, such as negative pattern discrimination that requires processing of configural associations. Comparing the learning capabilities of different bee species using the PER paradigm may thus allow us to better understand the evolution of complex olfactory learning within the Apidae.

P1.7

**Caste-specific adaptations of the visual system in Asian honeybee species.**

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Caste-specific selection pressures in eusocial insects have resulted in the evolution of caste-specific morphological and physiological adaptations. Workers, for example, have become adapted for foraging and colony maintenance, queens for fecundity and males for mating. The interplay of natural and sexual selection may also have affected the evolution of caste-specific adaptations in the sensory systems. The Western honeybee *Apis mellifera* serves as important model organism for the study of sensory systems and its visual system has been studied in great detail. In contrast, little is known about its Asian congeners. We investigated the peripheral visual system of the three castes of four Asian species (*A. andreniformis*, *A. florea*, *A. cerana*, *A. dorsata*) and compare it to *A. mellifera*. We measured eye size, ommatidia size and determined ommatidia number.

We found relatively smaller eyes with fewer but larger facets in queens vs. workers in all species. Drones show extremely enlarged eyes with a distinct dorso-ventral regionalisation with greatly enlarged facets in the dorsal eye region, which serve in detection of the queen against the sky. Further, we detected a significant correlation between male ommatidia size and published records of mating times. We conclude that spatial vision may be poorer in queens and higher in males compared to workers and that the large eyes and their distinct regionalisation in drones can be considered a synapomorphy of honeybees.

P1.8

**PKG and honeybee behaviour.**

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The honeybee foraging gene (*Amfor*) codes for a cGMP dependent protein kinase (PKG). An orthologue of this gene occurs in *Drosophila melanogaster* (*Dmfor*), where it is involved in regulating sucrose responsiveness, learning and food searching behaviour. In honeybees, expression of *Amfor* was earlier shown to be involved in the transition from hive bee to forager, probably by mediating visual responsiveness through PKG.

We show for the first time that *Amfor* occurs in two splice variants which differ considerably in their mRNA expression patterns and in the distribution of the respective PKG protein. The *Amfor-α*-PKG protein occurs nearly in all examined tissues, excepting the flight muscles. In contrast, high amounts of *Amfor-β*-PKG protein only occur in Malpighian tubule system and fat body. The two isoforms also differ in their behavioural functions. *Amfor-α*-PKG appears to be involved in the regulation of age-independent division of labour. *Amfor-β*-PKG presumably mediates sucrose responsiveness and associative learning processes. These findings pave the way for a deeper understanding of the functions of PKG in honeybees and other insects.

P1.9

**Effects of neonicotinoid insecticides on the muscle activity of the honeybee.**

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Neonicotinoid insecticides act as agonists of the insect nicotinic acetylcholine receptor. Applications of sublethal insecticide doses lead to various motor abnormalities in honeybees. Here, we investigated the effects of clothianidin, imidacloprid, or thiacloprid on the motor activity of bees. In a first approach we monitored their movement in a small arena before and after being fed with sugar water containing clothianidin or thiacloprid. We observed increased motor activity after clothianidin and a decreased activity after thiacloprid-treatment.

To analyse the effects on motor activity we recorded electromyograms of the muscle M17 before and during insecticide perfusion. Muscle spikes and proboscis extension reactions were elicited by stimulating the antennae and the proboscis with sugar solution. We investigated neonicotinoid-induced changes by determining spikes at different intervals after substance application. Insecticide or phosphate-buffered saline (1 $\mu$ L) was pipetted onto the brain. Our results showed that clothianidin (1 $\mu$ M) increased the muscle activity and in some animals even led to continuous spiking without sugar stimulation. By contrast, imidacloprid and thiacloprid (both 1 $\mu$ M) reduced the spike activity during the whole recording period (1h). Our results, although preliminary, indicate distinct effects of the various insecticides on honeybee motor activity. One of the next steps will be to study, whether neonicotinoids act on neurons, muscles or both.

P1.10

**Surveying the levels of pesticides within UK apiaries.**

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In its role as a pollinator the honeybee has become an important vector in world agriculture contributing to an increased crop output production. However honeybee numbers have been in decline over recent years and this is considered to be due to factors such as disease, climate change and pesticide exposure. Pesticides originating from either agricultural sources or apicultural practices are thought to have a negative effect on a colony's health and survival. Previously documented studies, most notably from America, France and Germany, have looked at the levels of residual pesticides contained within comb wax. Comb wax can be seen as a good indicator of a honeybee's exposure to pesticides, as lipophilic chemicals are known to be very soluble and stable within wax with a half-life of around five years. Currently no published data exists on the levels of pesticides contained within wax from the United Kingdom (UK). In our study we collected 150-plus wax samples from across the UK and analysed these with various chromatographic techniques i.e. gas and liquid chromatography-mass spectrometry (GC-MS and LC-MS) in addition to gas chromatography-electron capture detection (GC-ECD). In the poster we hope to present a general picture of any pesticides contained within these wax samples.

P2.1

**The male sexual marking pheromones of the *Bombus laesus* group.**

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Since its description by Krüger, there is so much confusion in the taxonomy of the *Bombus laesus*-group and this one has always raised discussion within the scientific community. Previously considered as a separate subgenus (*Laesobombus* Skorikov), now included into the *Thoracobombus* Dalla Torre, *Bombus mocsaryi* and *Bombus laesus* were merged into a single taxon called *Bombus laesus*. In this paper we attempt to shed some light in this confusion by studying the male cephalic labial secretion. This chemical composition is reported for the first time. The bumblebee male cephalic labial secretions act as sexual attracting pheromones and they are known as generally very species-specific. Therefore, the strong differences between the secretion on *B. mocsaryi* and *B. laesus* lead to take these taxa as good separated species.

P2.2

**The isolation and identification of phagostimulants within honeybee pollens.**

Bridgett R\*, Drijfhout F

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Previous approximations have suggested that pollination via animals contributes to the reproduction of around 225.000 angiosperm species globally. Indeed, estimates indicate that as much as 33% of all our food could be derived from primarily bee pollinated crops. In financial terms, this could be as much as €153bn worth of agricultural produce annually. One theory for the decline of honeybee populations currently being is that bees become more susceptible to disease through reduced colony strength over the winter period. Commercial beekeepers may feed high protein supplemental diets to colonies to both increase nutrient diversity, and stimulate brood production. The intended result is an increase in both bee numbers, and colony strength. Typically though, these diets are not as acceptable to bees as natural pollen, and previous studies have documented that the addition of some natural pollen (or pollen extract) to supplemental diets improves their uptake. It is therefore believed that pollens contain naturally occurring phagostimulatory compounds which increase honeybee feeding. Isolating and identifying these stimulants may assist in maintaining good levels of honeybee nutrition, through enhancing the palatability of food supplements which beekeepers may provide. This is being attempted through a series of pollen extractions fractionations, coupled with bioassay feeding trials, with UK colonies.

P2.3

**Chemical signatures of nurses and foragers influence the transmission of the parasitic mite *Varroa destructor* among beehives.**

Cervo R, Bruschini C, Cappa F, Meconcelli S, Pieraccini G, Pradella D, Turillazzi S\*

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The factors which induce *Varroa destructor* to abandon a collapsing bee colony to infest other hives are not completely known. Previous researches have shown that mites prefer to ride on nurse bees, recognising them from foragers via cuticular hydrocarbons; but in highly infested or close to collapse colonies, we would expect mites to adopt a strategy that allows them to move onto foragers. Using binary choice tests, we explored if mites change their preference for hosts with different tasks when colony infestation increases. Our results shows that the mite behaviour depends on the level of mite infestation. We found that, at low infestation rate, mites remain within the hive and promote their reproduction by riding nurse bees that have a distinct cuticular chemical signature with respect to foragers. However, GC-MS analyses show that the chemical signatures of nurses and foragers overlap when the level of infestation increases. This chemical homogenisation between bees with different tasks does not provide mites with discrimination cues, promoting a rise in the percentage of mites departing from infested hives by riding foragers.

P2.4

**Variation in oral acute toxicity of thiamethoxam according to the volume administered in Algerian honeybees.**

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Thiamethoxam is a neurotoxic systemic insecticide belonging to the neonicotinoid family. Approved under the trade Algeria Actara WG 25%, the plant protection product is recognised toxic to the bees after acute exposure. However, this product is persistent, has a significant residual activity and accumulates in plants. It is therefore necessary to completely reassess its toxicity. To do this, we determined the sensitivity of the Saharan and Tellian honeybee, *Apis mellifera sahariensis* and *Apis mellifera intermissa*, to thiamethoxam by testing the acute oral toxicity on worker bees in the laboratory. The study is based on determining the LD<sub>50</sub> according to the volume administered. Each batch of bees were fed 100, 200, 500 and 1000µl (5, 10, 25 and 50µl per bee) 55,5% (w/v) sucrose solution with increasing doses (1, 10, 20, 50, 70 and 90 ng per bee) of thiamethoxam dissolved in acetone, for trial treatments, and 55,5% (w/v) sucrose solution supplemented with acetone, control treatments. The results showed that the toxicity is manifested by acute symptoms of early neurotoxicity and cumulative mortalities that occur 24 hours after treatment. The LD<sub>50</sub> varies with the volume administered. Indeed, the LD<sub>50</sub> values decrease with increasing volume, and there is an inverse relationship between the LD<sub>50</sub> obtained and administered volumes.

P2.5

**Manual application of insecticidal dust in semi-field trials – effects on honeybees (*Apis mellifera* L.)**

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In order to assess the risk of insecticidal dusts for honeybees, next to several field trials with sowing of maize and winter oilseed rape and drift of dusts on neighbouring flowering crops, semi-field trials with manual application of fractionated maize dust on flowering *Phacelia* and winter oilseed rape were conducted in order to assess the effects on bees after manual application of different rates of active substance (0.1, 0.25, 0.5, 1.0 and 2.0g a.i. / ha Clothianidin) or different particle sizes of dust ( $x \leq 160\mu\text{m}$ ,  $250 < x > 450\mu\text{m}$ ,  $x > 500\mu\text{m}$ ; application rate: 2.0g a.i. / ha).

In the experiments, twelve gauze-covered tents (10x4 m) with bee colonies were set up on the flowering crop. During full bee flight activity a mixture of dusts and soil (seed treatment dusts and standard soil LUFA 2.2) was manually applied on the flowering crop inside the tents. Foraging intensity and mortality of the colonies were assessed for at least 7 days after application and samples of dead bees taken for residue analysis. In contrast to other dust fractions applied at the same rate of a.i. per ha significantly increased mortality was detected for fine dust particles " $x \leq 160\mu\text{m}$ ", which is the particle size range of pollen (2 – 250 $\mu\text{m}$ ). The comparison of application rates showed that the "no observed effect rate" application is below 1.0g a.i. / ha. Further trials in 2012 are expected to allow a further specification of the NOER.

P2.6

**Propolis chemistry and resin provisioning in Australian stingless bees (*Tetragonula carbonaria*).**

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Propolis is a mixture of beeswax, salivary secretions and plant resins. Bees use propolis to build their nests, for chemical defence and social immunity. The chemical variability of propolis depends on the botanical resins foraged by bees. Differently than honeybees, stingless bees store deposits of plant resins inside their nests, likely for incorporation into propolis (bee cerumen). This study investigated the chemistries of propolis and hive-resins in Australian stingless bees (*Tetragonula carbonaria*). Beehives were located in surveyed botanical sites in 2011. Four different resin deposits were observed, and labelled as 'creamy', 'white', 'orange' and 'red' hive-resins. Ethanolic extracts of propolis and hive-resins were subjected to gas and liquid chromatography mass spectrometry (GC-MS, LC-UV-MS) for the identification of individual Natural Products. Methylated flavonoids, isoprenoids and polar unknowns were found across samples. Comparative profiling of hive-resins indicated a diversity of chemical composition with some compounds found in common across deposits. Chemotaxonomy of the 'creamy' hive-resin confirmed that *Corymbia torelliana* was the botanical source for fruit resins. Molecular fingerprinting of the hive-resins indicated characteristic compounds as the chemical markers of the different propolis types. As propolis is valuable for medicinal preparations, these findings will enable next dereplication work on these chemotypes of Australian stingless bee propolis.

P2.7

**Cuticular profiles of the robber stingless bee *Lestrimelitta* and its potential hosts (Hymenoptera: Meliponini).**

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The Neotropical stingless bee *Lestrimelitta* is an obligate cleptobiont that obtains food and nest resources by raiding other stingless bee colonies. Cleptobionts may enter a target host colony by deception, force or possibly a combination of both. In various taxa of social insects, the blend of surface cuticular compounds is responsible for nest mate recognition. We compared the cuticular profiles of *L. nitkib* from the Yucatan Peninsula in Mexico and its potential hosts to determine if chemical mimicry may be used by this cleptobiont. The results showed that adult *L. nitkib* had few compounds (n=6) that represented more than 1% of the total peak area of analyses, and that it shared four out of those six with worker foragers of its preferred hosts *Nannotrigona perilampoides* and three out of six with *Plebeia* sp.. However, *L. nitkib* shared only two and three compounds of the 14 and 15 most abundant in the seldom raided species *M. beecheii* and *S. pectoralis*, respectively. Our results suggest that chemical deception may be a first step to enter some host species, leading to eventual mass raids by *L. nitkib*.

P2.8

**The fate of *Bombus cullumanus*: regression and species status.**

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*B. cullumanus* is known as a strongly regressing species in West-Europe. While the species once had a large distribution from S-Sweden to N-Spain, it now seems to be extinct. The last specimen was collected in 2004 in the Massif Central (France). There are two other taxa from disjunct areas that are very close relatives: *B. serratissima*, from Spanish, Central- and East-European steppes, and *B. apollineus*, from the Caucasian mountain steppes. Despite their conspicuously different colour patterns and differences in ecology, these three taxa share a very similar morphology. Based on a study of CO1 barcodes, Williams et al. (in press) showed that these taxa appear conspecific. Most bumblebee species can also be accurately identified from the secretions of the male labial glands, which they use as species-specific recognition cues. We sampled and analysed these secretions. For *B. cullumanus*, *B. apollineus*, and *B. serratissima*, we found that the male cephalic labial secretion are almost identical, sharing all of their components with similar relative abundances. As they do not show any difference in their species-specific recognition blend, we confirm that *B. cullumanus*, *B. apollineus* and *B. serratissima* are best seen as conspecific. While the ssp. *cullumanus* is extinct, the ssp. *apollineus* and *serratissima* remain more or less abundant in some parts of the original distribution area. However, the general regression of the whole species is so drastic that its long-term survival could be questioned.

P2.9

**Toxicity of abraded seed coating particles to honeybees.****Wallner K\*, Mühlhäuser H**

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A new technology (Retsch CAMSIZER) was used to characterise the size of abraded dust particles of maize seeds used in the Rhine Valley in 2008. These particles are created during handling and transport of seeds in packages and during the sowing process in pneumatic drilling machines. Noticeable amounts were generated, when the coating was done neglecting the instructions for a correct use of the product. Abraded dust is under further mechanical influence in the drilling machine, which may lead to an additional fragmentation of crude particles to fine dust. In 2008 dust particles of the Poncho pro® (Clothianidin) coatings reached flowering plants and caused damage to bee colonies, collecting contaminated nectar and pollen. Microscopically, dust particles were detected in the mid gut of poisoned bees and in pollen load from returning forager bees. Their size was in the range of pollen grains. Abraded Poncho pro® dust particles of different size (<100µm) were fed to caged single bees to determine their size dependant toxicity.

Due to the extreme insecticidal potential of Clothianidin already very small particles, which are expected to be more influenced by wind and thermal lift than by gravity, showed poisoning effects on the bees. With regard to these results it is important, to improve seed dressing not only with regard to the reduction of abraded dust but also to avoid the release of weightless fine dust particles during the seeding process.

**P3.1** **Trap-nesting bee communities in two different agricultural scenarios: Mediterranean polycrops vs. monocrops systems.**

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At the landscape level, the diversity of crops together with the spatial heterogeneity, connectivity and the proportion of natural elements are important factors in the quality of the agricultural matrix. The many resources derived from the high productivity associated with crop fields, within agricultural landscapes formed by mosaics of small elements and systems of low agricultural intensity, could favour the populations of certain species. Using trap-nesting solitary bees, we evaluate the effect of two Mediterranean crop systems: monocrops (vineyard and cereal) and polycrops (vegetable crop areas) in two levels of farming intensity, on nest colonisation and population structure.

In the comparative study carried out in two Mediterranean scenarios with marked differences as regards crop richness, the polycrop proves to be an important system, offering additional resources which benefit bee populations, especially the offspring production of those belonging to the genus *Osmia*. The dominant farming intensity is a determinant factor, especially in monocrop systems, where low farming intensity maintains essential semi-natural habitats for their persistence and for the maintenance of species-rich communities. A matrix occupied by crops rich in flowers, such as in traditional Mediterranean polycrop system, together with low farming intensity would provide heterogeneous landscapes with a variety of natural and cultivated resources that benefit trap-nesting bee populations.

**P3.2** **Comparison of sperm from drone (*Apis mellifera*) reared in laboratory and semi-field conditions.**

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The queen longevity is related to its oviposition capacity and to its egg-laying activity. Since the queen mates one time in her life with many drones, the drone semen seems involved in the reproductive potential of the queen. Therefore, the poor quality of drone semen can be the cause of the queen fertility decrease. Based in this hypothesis and in order to study the semen quality, we conducted a drone rearing in laboratory and under semi-field conditions for 20 days. Then, semen was collected by manual eversion of the endophallus and the number, the viability, the energetic state of spermatozoa (ATP), the activity of superoxide dismutase (SOD) and level of proteins in semen were determined. Compared to drones reared in laboratory conditions, drones reared under semi-field conditions exhibit a higher number of spermatozoa and higher protein content and lower values of the spermatozoa viability, ATP content and SOD activity.



**P3.3** **Effect of semen dose and application on the performance of instrumentally inseminated honeybee queens kept with 25 worker bees.**

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The study was conducted in 2008 and 2009 in Poland. Carniolan sister queens were inseminated at the age of 7 days. They were either inseminated: once with one of the following doses: 1, 2, 3, 4, 6 or 8µl, or they were inseminated two times with one of the following doses: 2, 3 or 4µl, or they were inseminated three times and four times with doses 1 and 2µl of semen. The queens were kept after insemination with 25 bees. Of the 951 queens, only 2.6% died two days after insemination. Nearly 87% of the queens had completely cleared the oviducts, and 10.7% had some residue of semen in the oviducts. Queens that were inseminated with very small doses of semen (1-2µl) cleared the oviducts completely. Queens inseminated with bigger doses had some semen residue in their oviducts. The significantly highest number of spermatozoa in the spermatheca had queens inseminated four times with 2µl of semen. Small, single doses of 1 and 2µl caused around 2-3 million spermatozoa entering the spermatheca. More than 4mln spermatozoa in the spermatheca ranged in queens inseminated 1, 2, 3, or 4 times from 32.5 to 63.4%, from 56.3 to 91.4%, from 94 to 97.7% and from 87.7 to 100% respectively. The highest percentage of queens (76.7%) with 4 to 6 million spermatozoa in their spermatheca had been inseminated three times with a dose of 1µl of semen.

**P3.4** **Are honeybee development and physiological performance influenced by electro-magnetic radiation?**

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Among the suspects in the lost colonies mantra, Electromagnetic Fields (EMF) often figure, but seldom pertain as a subject of study. Surveying the literature, very much is obscure and suspect, showed that since early studies on the effects of steady EMF and around high voltage power lines, not many convincing studies have been performed. In the meantime new EMF types have increased strongly worldwide, with the use of mobile phones and more. Effects cannot be ruled out, and many people are concerned about possible effects on bees as well as other organisms including man. An experiment was conducted in 2011. Colonies were subjected to the radiation of a nearby antenna, shielded (control) in a Faraday cage, or exposed (cage with plastic netting). The experiment has been carried out with a blind, and where possible double blind, set up.

Parameters measured:

- the developmental success(egg → larva → adult)
- the flight performance of adult bees, developed from egg to pupa + or - EMF
- morphometric and physiological parameters (body weight & size, fluctuating asymmetry)
- the longevity of bees developed + or - EMF
- the development of the colonies, and winter survival

**P3.5 Influence of pupal temperature on longevity, temporal polyethism and flight performance of honeybees.**



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Honeybees maintain the brood nest within a narrow temperature. To study the impact of pupal temperature we placed capped worker brood from five different colonies in incubators at 32.0°C, 34.5°C and 36°C. Per temperature treatment and colony 124-310 bees were individually marked and added to three different colonies housed in observation hives. Longevity and behaviour of these bees were noted in a scan sampling approach in the fore- and afternoon during at least 20 of the following 30 days. In four out of the five colonies, as well as in the pooled sample, we found higher mortality in bees raised at 36°C compared to those raised at 32.0°C or 34.5°C (Kaplan Meier logrank test,  $p < 0.0001$ ). Median adult longevity (LT50) decreased with higher pupal temperatures ( $p < 0.05$ , Pearson). We attribute this earlier mortality to an accelerated temporal polyethism in bees raised at 36°C. Indeed, behavioural observations showed that bees raised at 36°C did nurse brood at an earlier age than the other two groups ( $p < 0.01$ , Mann-Whitney) and foraged earlier than bees raised at 32°C ( $p < 0.01$ , Mann-Whitney). We confirmed reduced longevity of bees raised at 36°C also in caged bee experiments (Kaplan Meier logrank test,  $p < 0.0001$ ) what excludes homing deficiencies as a single cause. Additionally, we measured flight performance in a flight mill and found no differences in flight duration, distance covered or average and maximum speed of bees reared at the different temperatures ( $n = 22-28$ , Anova,  $p > 0.05$ ).

**P3.6 All the royal makings of a queen.**

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Queen larvae of the western honeybee *Apis mellifera* are fed exclusively with royal jelly (RJ), a secretion of the hypopharyngeal gland of young worker bees that nurse the brood. Therefore, it is widely accepted, that the determination of a female larva into a queen is governed by nutrition. Nevertheless, it is still not known how a RJ diet can cause differential gene expression in the larvae initiating queen differentiation. In spite of considerable research efforts, there are surprisingly few and only incomplete studies that address the complete biochemical characterisation of RJ components. Here, we embark on a comprehensive functional characterisation of the major RJ proteins (MRJPs) that constitute up to 90% of total protein content of RJ. All MRJPs of *A. mellifera* were cloned and expressed as insoluble inclusion bodies in *Escherichia coli*. The following steps including protein refolding and purification form the basis for homogenous preparations of all MRJPs to allow for subsequent biochemical characterisation and testing of supplemental feeding to *in vitro* reared larvae. Only by including a comprehensive testing of all MRJPs it will be possible to resolve their role as potential caste determinant compounds in RJ.

**P3.7 Effects of pollen nutritional quality on bee health and tolerance to stressors.**



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The development and the maintenance of the honeybee colonies are tightly linked to the floral resources that contain bees nutrients. Among these nutrients, there is the pollen, which is the main source of protein, lipids and essential amino acids, that is essential to the colony growth and bee physiological development. In the absence of pollen bees are more susceptible to biotic and abiotic stressors. However, bees are rarely facing a total absence of pollen. They are rather confronted to pollen resources whose quality and diversity are highly variable in time and space. Since all pollens are not equal regarding of their composition and nutritional value, we assessed the importance of quality and diversity of pollen on bee health and tolerance to biotic and abiotic stressors. This was achieved by measuring the impact of the nutritional quality of pollen on bee longevity and on molecular markers of physiological integrity.

**P3.8 An evaluation of the efficiency of different antibiotics for control of the American foulbrood disease of honeybees (*Apis mellifera* L.).**

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Twenty antibiotics belong to ten antibiotic groups were evaluated to control the American foulbrood disease of honeybees. Four isolates of *Paenibacillus larvae* (P.I.1.1, P.I.1.2, P.I.1.3, and P.I.1.4) were used in the present study. Present results indicated that P.I.1.3 was the most sensitive isolate to the antibiotics with an average of inhibition zones of 9.35mm in diameter followed by P.I.1.4 (8.92mm), P.I.1.1 (6.09mm) and P.I.1.2 (5.21mm). Oxytetracycline was used as a reference to calculate the relative efficiency (R.E.) percentage for each tested antibiotics. The most effective antibiotic against P.I.1.1 was Penicillin with 202.55% R.E. Doxycycline and Chlortetracycline were found to be more effective on P.I.1.2 isolate with 176.32 and 159.56% R.E, respectively. There was a strong synergism between Penicillin and Streptomycin when mixed together. Doxycycline was the most effective antibiotic against both of P.I.1.3 and P.I.1.4 isolates with 298.72 and 294.35% R.E., respectively.

**P3.9 The age dependent variation in reproductive organs of honeybee drones.**



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We investigated the variation in weights and sizes of reproductive organs of drones during and after sexual maturation period. The drones emerging within 6 hours in three colonies were collected and then marked with paint marker. After marking, the drones were introduced into their colonies. The testes (TE), seminal vesicles (SV) and mucus glands (MG) of marked drones were taken out and weighed when they were 0, 3, 6, 9, 12, 18, 30 and 40 days old. Other marked drones were dissected for length measurements of TE, SV and MG at the age of 0, 3, 6, 9, 12, 18 and 30 days. The images of these organs were taken with a digital camera attached to a microscope for the length measurements. The weight and length of TE decreased continuously from emergence through 30<sup>th</sup> day. The TE lost most of their weight and length during sexual maturation period. The weight and length of SV increased from emergence through 6<sup>th</sup> day. A gradual decrease in the weight and length of SV also happened between 6<sup>th</sup> and 30<sup>th</sup> days. The weight and length of MG started to gain weight and length from emergence through 9<sup>th</sup> day. The weight and length of MG decreased gradually during the period between 9<sup>th</sup> and 30<sup>th</sup> days. The weights of TE, SV and MG did not significantly change during the period between 30<sup>th</sup> and 40<sup>th</sup> days. These results suggest that the weight and size of SV and MG change not only during sexual maturation period but also after sexual maturation.

**P3.10 The effect of drone type on sperm competition in honeybees (*Apis mellifera*).**

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According to several research results small drones cannot compete with large drones to mate with queens in the mating arena. In this research we tested whether small drones (SD) from laying worker colonies (LWC) could compete with large drones (LD) from queenright colonies (QRC) when the queens were instrumentally inseminated with their semen mixture (1:1). Four sister queens were inseminated with semen from 6 LD and 6 SD. Each queen was inseminated with 7.2µl semen collected sequentially from twelve drones each contributing equal volumes (0.6µl) of semen. Three months after the queens started to oviposit, the newly emerged workers of these queens were collected from their colonies in order to determine paternities. Paternity analyses for five polymorphic microsatellite loci (A079, A113, A024, Ap226 and A43) were performed on 300 daughters and 48 drones that were used to inseminate four experimental queens. Competitiveness of LD and SD was inferred from the proportion of worker offspring of a particular drone relative to the total number of workers analysed of a specific queen. All workers genotyped could be unequivocally assigned to one of the potential fathers. The patriline frequencies of LD and SD were 67.0% and 33.0%, respectively. Drone type (LD and SD) had a significant effect on the patriline frequency ( $p < 0.001$ ). These results revealed that SD reared in worker cells in LWC remained behind LD reared in QRC in sperm competition.

**P3.11 Honeybee queen quality control in Croatia.**



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Honeybee queen quality control is estimated by monitoring of spermatheca morphological characteristics. Three queens were taken from 28 Croatian queen breeders. The quality control was carried out on Croatian native honeybee queens (*Apis mellifera carnica*). Diameter of spermatheca and number of spermatozoa were analysed. After dissection bronchial net was removed from spermatheca. Calibrated Dino Capture camera was used to measure diameter of spermatheca. The average measured value of diameter was  $1.0233 + 0.056$  (min. 0.934mm, max. 1.197mm). Content of spermatheca was dispersed in 0.5ml saline solution. The number of spermatozoa was counted in Neubauer hemocytometer. The average measured value of spermatozoa number in 1ml was  $3\ 856\ 000 + 1\ 976$  (min. 700 000, max. 11 600 000). One unfertilised queen was determined in 64 observed samples.

**P3.12 Toxic effect and biochemical study of spinosad on honeybees (*Apis mellifera intermissa*).**

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Bees are pollinators that occur naturally in agroecosystems and susceptible to pesticides used in these environments. Spinosad is an insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa*. This biopesticide is used on lawns, vegetables and fruit trees, to control caterpillars, thrips, leafminers, borers, fruit flies, spidermites, aphids, and more. Spinosad is known to affect honeybees through mortality and inhibition of physiological enzymes. Under laboratory conditions, honeybee (*Apis mellifera intermissa*) workers were exposed via oral exposure in supplemented sugar syrup to different concentrations of spinosad. Lethal concentration that caused 50% mortality ( $LC_{50}$ ) was estimated and the enzyme acetylcholinesterase (AChE) as biochemical indicator was determined after 24h in head, thorax and abdomen in bees surviving exposure to this biopesticide. Results indicated that exposure to spinosad showed toxicity to honeybees with  $LC_{50}$  value of  $0.053\ \mu\text{g}\ \mu\text{l}^{-1}$  and spinosad significantly inhibited AChE activity in different organs of honeybee workers.

**P3.13** **Reproductive anatomy of the small hive beetle *Aethina tumida*,  
Coleoptera: Nitidulidae.**

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The small hive beetle is a parasite and scavenger of social bee colonies native to sub-Saharan Africa. It has become an invasive species with well-established populations in the USA and in Australia. Despite its impact in the new ranges, many aspects of the general small hive beetle biology are still poorly understood, in particular related to reproduction. Earlier studies suggest a high reproductive potential of the female beetles and a female-biased sex ratio. Here, we therefore investigated the general morphology of the female reproductive system by histological thin cut series. The dissections clearly show that each ovary of *A. tumida* is formed of teleotrophic ovarioles and that the females also possess a specialised sperm storage organ (receptaculum seminis), which is a common feature in the Coleoptera.



**P3.14** **Impact of different light conditions on the foraging behaviour of  
bumblebees.**

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In bumblebee colonies workers of different size can be found, a phenomenon known as allometry. Bees that forage early in the morning can gather more nectar and pollen as many flowers accumulate nectar and pollen overnight. By allocating large workers to forage, especially at dawn and dusk, a colony might be able to significantly increase its overall food intake rate. However, whether large bumblebees are indeed better able to forage under significantly poorer light conditions than small nestmates is unknown. Improved vision in low light conditions could also increase the pollination services delivered by bumblebees in greenhouses.

Here we investigated if colonies of *Bombus terrestris* have different foraging behaviour in changing light condition and if this can be explained by improved vision of the workers. To study both questions the foraging capacity of bumblebees was determined on colony level by scoring the number of bumblebees who went foraging for 1h under high and low light conditions. This parameter was compared with the individual light sensitivity of 20 workers from each colony. Colonies with a higher foraging capacity consisted of workers with a better individual light sensitivity. Bumblebee body size, weight and morphological parameters of the eye correlated with the measured light sensitivity of the workers for only 20-40%, indicating that morphological differences between workers couldn't fully explain the observed differences in foraging capacities.



**P3.15** **Variation of wing nervature angles in honeybees from the territory of  
Toplica region in Serbia.**

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Wing nervature of the honeybee (*Apis mellifera carnica* Poll) although very complex is not random and presents a significant taxonomic character. The study of variations in the values of quantitative parameters of the wing nervature allows the determination of racial and population origin of honeybees. Preservation of natural populations and genotypes of the honeybee is one of the most important tasks in modern beekeeping from the selection and ecological standpoint. In this paper eleven angles of the front wing were observed: A4, B4, D7, E9, G18, J10, J16, K19, L13, N23 and O26 and three angles of the rear wing: W1, W2 and W3. Native bees were sampled from three sites in the Toplica region: Prokuplje, Rgaje and Jastrebac. The total variability, the minimum and maximum values of tested parameters were determined. The highest variation coefficient was found in the front wing angle L13 (0.1634), and the lowest in the angle D7 (0.0336). Considering angles of the rear wing, the highest variation was determined in the angle W2 (0.0910), and the lowest in angle W3 (0.0098).

**P3.16** **Protein levels and colony development in honey Africanised and  
European bees fed natural and artificial diets.**

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The feeding with protein rich diets is essential to bees in times of pollen scarcity. Under this perspective, we tested two artificial protein diets in laboratory and the field to be used as pollen substitutes when there is lack of pollen in nature. In the lab, groups of 100 newly-emerged workers were confined in cages and fed Bee Bread, soy milk powder/albumin (T1), soy milk powder/beer yeast/ rice bran (T2) or 70% sucrose syrup (T-) for seven days. The protein content in the hemolymph determined the efficiency of these diets tested with Africanised bees in Brazil in the USA with European honeybees. We found the two diets to be as efficient as Bee Bread and showed no differences ( $p > 0.05$ ). These diets were significantly higher than the sucrose solution (T-). We tested these diets in the field, in a period of low pollen availability. We used six colonies of *A. mellifera* in two different Brazilian regions. Four colonies received artificial supplementation with T1 and T2 diets and several parameters were evaluated to determine the diets efficiency.

In general, the results indicated that the colonies artificially fed presented an expressive improvement in all analysed parameters. In relation to the colonies left as control, we documented the decrease of all parameters during the experimental period. Through these results, we were able to demonstrate the importance of supplementing colonies with artificial protein rich diets in times of low pollen availability.

**P3.17 Bumblebees: cold tolerance and impacts of diet.**

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Bumblebees (*Bombus terrestris audax*) are economically important crop pollinators with extensive commercial usage. Despite this, their thermal biology is largely unknown. Experiments examining cold survivorship and freezing temperatures were used to investigate the cold tolerance of *B. t. audax*, and the influence of dietary components on cold hardiness. Results illustrated the bees' ability to tolerate temperatures as low as -5°C for 10h without significant mortality. Below this temperature, death was caused by a combination of freezing and cold shock mortality. When fed pollen and nectar, SCP ranged from -5°C to -10.9°C (mean -7.1°C ±0.22). After removal of pollen from the diet, the SCP was decreased to a minimum of -17.9°C (with a mean of -12.5°C ±0.52) after 14 days on a nectar-only diet. Therefore, pollen increases bumblebees' freezing temperature, making them less cold tolerant. These results contribute to a cold tolerance profile of *B. t. audax* and can be used to optimise the commercial usage and aid conservation of the species.

**P3.18 Honeybee nutrition and the importance of a balanced protein to carbohydrate ratio.**

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The nutritional needs of honeybees for carbohydrates are supplied by nectar, whereas proteins and other nutrients are found in pollen. However, little is known of what the bees actually need, how they achieve nutritional balance and the effects of an unbalanced diet. Using newly emerged workers (*A. m. scutellata*) and a geometric framework approach, we investigated whether bees maintain their intake target when confined to pairs of unbalanced complementary diets varying in protein to carbohydrate (P:C) ratio. Additionally we tested for the effect of different protein sources (casein, royal jelly and Feed-Bee®) and sucrose on both survival and ovarian activation. Bees switched between diets and converged on the same P:C intake target, although this target differed between protein types (1:12 on casein, 1:14 on royal jelly and 1:11 on Feed-Bee®). Survival was highest on casein and lowest on Feed-Bee® diets but ovarian activation was highest on royal jelly and lowest on casein diets. In an additional set of no-choice experiments we tested the effect of P:C ratio and protein source (casein, pollen or royal jelly) in an imbalanced diet. Again bees survived best on a low P:C ratio and ovarian activation was highest on royal jelly.

Our results suggest that the nutrient intake of broodless workers is directly related to their own physiological requirements, and the strong carbohydrate bias may reflect the high metabolic rate of honeybees even under resting conditions.

**P3.19 Experiments on the water foraging of *Apis mellifera* L. in relation to the relevance of guttation.**

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Guttation fluid as such poses no environmental risk. However, pesticides deriving from soil residues or seed dressings (e.g. neonicotinoids) might change the environmental risk and honeybees could be exposed to toxic substances when collecting guttation drops. Thus, the major task was to examine the honeybee's water foraging behaviour in relation to the process of guttation. Due to the wide foraging range it is difficult to record the activity of water foragers under field conditions. Therefore, samples of returning bees were collected in front of the hive entrances at different hours of the day during summer and autumn 2010. In the laboratory, bees with pollen or propolis were removed from the sample and only bees with a honey sac weight of  $\geq 0.005$ g (considered "successful foragers") were used for further analysis. Based on the sugar concentration in the honey sac and according to literature data these bees were grouped into "nectar foragers" (25-65% sugar) and "water foragers" (<15% sugar). The activity of water foragers is not restricted to certain times of the day, water seems to be gathered all-day. The data further indicate that the water demand in autumn is almost exclusively covered by water, whereas in summer nectar is the main source for water. In view of the current discussion about the relevance of guttation as a water source for honeybees, this research illustrates a further step in the risk assessment of pesticide application in the form of seed dressing.

**P3.20 Local crop and landscape heterogeneity affect offspring characteristics in the solitary bee *Osmia caerulea* L.**

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Rosenheim et al. (1996) proposed a parental investment model based on the assumption that individual females, to maximise reproductive success, determine their sex investment according to resource availability. Population size, in terms of the offspring production by an animal living in a complex landscape, is determined by the characteristics of the habitat patches where its nest is located, including both the local resources and their distribution and abundance across the landscape. In this study we analyse how landscape heterogeneity and local resources influence parental investment in the polylectic and widely distributed solitary bee *Osmia caerulea* L.

Standardised trap-nests were located in three types of Mediterranean crops (cereal, vineyard and vegetable crop areas) in two levels of farming intensity. We examined the effects that local habitat type in which trap-nest is located, and the percent cover of seminatural habitats, had on offspring weights, offspring size (number of individuals), and progeny sex ratio.

Offspring characteristics are determined by percent cover of seminatural habitats in different ways, as a function of the local crop type where the trap-nest was located. The maintenance of agricultural landscape heterogeneity, with high crop diversity and the presence of seminatural habitats is essential for conservation of abundant and efficient *Osmia caerulea* populations.

**P3.21 Adult worker bumblebees regulate their intake of carbohydrates in preference to protein.**

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Pollinating bee species meet nutritional needs by acquiring carbohydrates and proteins from floral nectar and pollen. Surprisingly little is known about the optimal nutrition of bee species other than honeybees. Here, we have applied the Geometric Framework for nutrition to bumblebees (*Bombus terrestris*) to investigate their ability to meet and maintain an optimal intake of protein and carbohydrate using liquid diets. Two protein sources were used: sodium caseinate or a synthesised amino acid diet composed of the ten essential amino acids in equal concentrations. Sucrose was provided as carbohydrate. A single diet or a choice between two solutions was provided. When fed sodium caseinate paired with sucrose-only, bumblebees defend an intake of carbohydrate in bias of protein, over ingesting protein to maintain a stable intake of carbohydrate, depending on treatment. However, in amino acid diets, bumblebees consume less of solutions containing amino acids and predominantly feed on sucrose-only solutions. We suggest that diet taste may influence feeding choices such that amino acid diets of high concentration are repellent, whereas proteins may be regulated post-ingestively. Survival is greater on sodium caseinate diets over amino acid diets further suggesting that amino acids and proteins are regulated independently of one another. Carbohydrate bias in bumblebees is likely due to the energetic cost of flight and as adult workers are sterile, protein requirements are minimal.

**P3.22 Surviving of *Osmia cornuta* (Hymenoptera: Megachilidae) larvae on different pollen diets in experimental conditions.**

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The solitary bee *Osmia cornuta* Latr. is known as excellent orchards pollinator across Europe. The biology of this species is relatively known, as well as approaches for augmentation under natural conditions. One of the basic steps to find new technology for its successful breeding under controlled conditions is to test the survival of their larvae on diverse pollen-nectar nutrition.

The source of tested pollen was obtained from different plants, harvested at the entrance of the honeybee hives, freshly frozen and stored at -20°C. We used four kinds of monofloral pollen (>80% of a single plant) for larvae rearing: willow, rapeseed, acacia and linden. As a control, we used pollen provisions from *O. cornuta* nests in the orchard with dominant *Rosaceae* cultivars. Rearing of the larvae was conducted in artificial drilled "cells" made of dry wood, provided with fermented mixture of pollen and water solution of honey.

We found significant difference in the larval survival reared on the willow and rapeseed (survival rate 89% and 94%) compared to the other sources of pollen (survival rate within 9-21%). No significant difference among control, willow, and rapeseed pollen diet. We suggest that *O. cornuta* larvae have certain physiological adaptations for successful development on pollen diet comes from early flowering plants when this bee is usually active. In contrast, larvae reared on the late flowering plants pollen diet died in period of 10-26 days.

**P3.23 Direct and delayed effects of *Bt* maize pollen consumption on honeybee larvae (*Apis mellifera*).**

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The honeybee is the most comprehensively studied insect species, and more importantly, it provides a vital ecosystem service by pollinating both wild plants and crops. This makes the honeybee an excellent model species for Environmental Risk Assessments (ERA) evaluating risks on non-target arthropods. Several ERA studies on transgene crops have addressed their effects on adult bees; however most of these crops have been designed to affect the larval stage of pest insects, as this is the development stage that negatively impacts the crop. Therefore we tested the effects of transgene crops on honeybee larvae. We used a stacked *Bt* maize variety and compared it with several controls; its near-isogenic line, another (non-transgene) maize variety, multi-floral pollen and toxic pollen (positive control). *In vitro* reared larvae were fed a range pollen-dosages between 0 and 10mg. We measured the larval mortality, weight and developmental time, and quantified the digestibility of the different pollen types at the 5<sup>th</sup> larval instar (direct effects). Also we compared emergence rate, emergence weight and longevity of adult bees that were reared with different pollen diets (delayed effects). The results of this study will contribute to the safety of transgenic crops for honeybees.

**P3.24 An infrared insight into honeybee (*Apis mellifera* L.) hemolymph: analytical potentials.**

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As functional equivalent to blood and lymph of vertebrates, insect hemolymph represents equally complex multifunctional system. Most of the records on honeybee hemolymph composition are focused on individual analysis of selected biochemical compounds, but it has not yet been explored on comprehensive molecular level. As opposed to standard biochemical methods, midium-infrared spectroscopy (MIR) provides a unique fingerprint of a sample based on overall biochemical composition (molecular structure) therefore enabling low-cost and multi-component measurement. Presence of unique features in IR spectrum that correspond to the specific functional groups in organic molecules is a major reason for broad acceptance of IR spectroscopy as analytical tool. Fourier transformed infrared (FT-IR) spectroscopy is a rapid and powerful method utilised nowadays for analysis of various biological specimens (blood, tissues, microorganisms), which indicates on the possibility to detect alterations that occur in the hemolymph due to both physiological differences and pathological changes. However, this method has never been applied on any insect species in this regard. The feasibility study of FTIR-method for hemolymph analysis was based on 88 individual hemolymph samples of workers that were recorded using FT-IR attenuated total reflectance (ATR) technique. This study provides the first infrared insight into honeybee hemolymph by biochemical interpretation of the obtained FTIR spectra.



**P3.25** **Role of pollen nutrition on honeybee susceptibility to stressors.**

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The recent honeybee colony losses related to the syndrome named CCD (colony collapse disorder) are often not caused by a single factor: it is commonly agreed that the phenomenon is multi-factored. One of the factors, since the beginning suspected to have an important role in these losses, is the nutritional status of the colonies. Honeybees need to eat pollen to ensure their development and growth. The nutrient content of pollen can vary widely basing on the botanical origin. In fact, honeybees tend to collect pollen from plants of different species. Instead, if the colony is located in an area characterised by low biodiversity (e.g. intensive agricultural areas), the bees will mainly collect pollen from only few plants species, according to the season.

The aim of this study is to investigate how different pollen diets can influence the susceptibility of bees to stressors. Newly emerged bees were fed ad libitum with water, honey and diets containing pollens of different botanical origins. Then, adult bees reared with different pollen diets were subject to one additional stressor (i.e. pesticide or pathogen) to investigate the presence of any influence, e.g. synergistic effects.

**P4.1** **Trap-nesting bee communities in agricultural Mediterranean landscapes subjected to farming intensification.**

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Agriculture has traditionally been considered detrimental to natural communities of pollinators, but even in agroecosystems, when subjected to moderate levels of farming intensity, some bee communities could benefit from certain crops in heterogeneous landscapes.

To evaluate the effects of local crop on bee communities, in landscapes with two different levels of farming intensity, standardised trap-nests were used. The study was developed in a vegetable Mediterranean crop region in Northern Spain, with three traditional vegetable crops: potato, artichoke and garden.

The trap-nests bee community is dominated by bees of the genus *Osmia*. On the one hand, the most generalist species, *O. caerulea* and *O. bicornis*, could benefit at a local level, from potato crops, which might not offer adequate resources to other bees. Additionally, other oligolectic *Osmia* species could, in more intensified areas, take advantage of artichoke crops. On the other hand, gardens offer many different resources that benefit other bee species, independently of farming intensity. An agricultural landscape with moderate intensification, that offers both natural and cultivated flower resources, would be useful for generalist bees, highly adapted to agricultural matrices, that could provide an important pollinating service, being able to exploit the local available resources.

**P4.2** **The return of the bumblebees on the post-flood areas in the 2011.**

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The monitoring of bumblebees in the post-flood area near the Wilków village (Poland; near Puławy) in 2011 was conducted. The community of Wilków has been flooded twice in 2010 (May and June). Up to 90% of the total areas of the community was under the water. The objective of this study was to assess the taxonomic composition and density of bumblebees in the post-flood areas near the Wilków village. In the research 6 research plots were chosen: three located on borders of flooded areas and three in the flooded areas.

In the study nine species of bumblebees were found, most common species was *Bombus terrestris*. The largest number of species and density of bumblebees were observed on the river dike Chodelka near Wilków village (all of the species found). This is probably due to migration of the bumblebees from the near, non-flooded, above lying areas. The number of species and their density was much lower on the research plots situated in the flooded areas.

**P4.3** **Modelling aggregation behaviour in bees and robots.**

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Groups of young honeybees aggregate at their preferred temperature of 36°C in a temperature arena, whereas only a few single bees stay there. In a gradient with an optimum (36°C) and a sub-optimum (32°C) groups choose their preferred temperature collaboratively. Inspired by this interesting swarm intelligent behaviour we developed a robot control algorithm called BEECLUST. A robot swarm controlled by BEECLUST shows, similar to young honeybees, behaviour that fulfils all requirements to be classified as swarm intelligent. This behaviour emerges by following a few simple rules: whenever an individual meets another individual it stops, measures a local cue (bees measure temperature, robots measure light) and 'calculates' a waiting time. The higher the temperature (light), the longer the waiting time. This leads to aggregation clusters in the optimum which are able to react dynamically on changes in the environment.

Using Vensim, we developed simple Stock-and-Flow models for bee aggregation as well as for robot aggregation and implemented the parameters derived from real experiments. We compared the result from the real robot experiments to results from the models. Furthermore these models allow us a better understanding of the fundamentals which lead to this decentralised aggregation behaviour.

**P4.4** **Prediction of emergence of brood rearing during wintering period of honeybee colonies.**

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European honeybee (*Apis mellifera* L.) has adapted to survive cold winters in northern climate. Nevertheless success of wintering depends on different biological and physical factors. Duration of broodless period is one of biological factors which influence quality of wintering. Brood rearing through winter or too short broodless period during wintering can result in starvation because of too high food consumption rate and overburden of intestine part with residues which can cause rise of intestine diseases. Considering the importance of duration of broodless period to wintering quality, we carried out research work with an aim to analyse automatic detection of broodless period and emergence of brood rearing. In total 30 bee colonies were involved in a research. Twenty colonies were wintered in a special wintering building with a possibility to control microclimate environment while remaining ten were wintered outdoor. All colonies were equipped with wired temperature sensors. Sensors were placed on top of the nest right above the top bars of the frames. Validation of data revealed that if average weekly temperature measured by sensor exceeds +13.5 °C then we could predict that cluster of the colony is ruined and brood rearing expanded. Whereas cluster of the colony is certain if average weekly temperature measured by sensor is lower than +10.3 °C. Temperature interval between +10.3 °C and +13.5 °C was common for both traits.

**P4.5 Effects of the instrumental insemination of honeybee queens with the semen of drones of different age.**

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The aim of this study was comparison of percentage of offspring of two drones in age 15 or 25 days. Sister queen bees of *Apis mellifera carnica* were kept in mating nuclei. In the 8<sup>th</sup> day of life queens were instrumentally inseminated with a single dose of semen collected from two drones. One of the drones was *A. m. ligustica* and the other was *A. m. carnica*. From each of the drones 4µL of semen was collected. Age of the drones differed between groups:

(1) DL15/C25 – 15-day-old *A. m. ligustica* and 25-day-old *A. m. carnica*, (2) DL25/C15 – 25-day-old *A. m. ligustica* and 15-day-old *A. m. carnica*, (3) DL15/C15 – 15-day-old *A. m. ligustica* and 15-day-old *A. m. carnica*, (4) DL25/C25 – 25-day-old *A. m. ligustica* and 25-day-old *A. m. carnica*.

In total, 64 queens were used. The percentage of *A. m. ligustica* workers in offspring of the queens from groups DL15/C25, DL25/C15, DL15/C15 and DL25/C25 was 41.7%, 42.3%, 49.5% and 46.6%, respectively, and did not differ significantly. Percentage of queens that started oviposition in groups DL15/C25, DL25/C15, DL15/C15 and DL25/C25 was 94.1%, 86.7%, 88.2% and 100%, respectively. The queens started egg laying on average ( $\pm$ SD) after 4.9 $\pm$ 2.58, 5.3 $\pm$ 2.01, 3.7 $\pm$ 2.09 and 5.7 $\pm$ 2.35 days, respectively. The differences between the groups were not significant. The age of drones used for instrumental insemination did not have any effect on the number of queens starting oviposition and the onset of egg laying by them either on the percentage of worker bees after drones of two subspecies.

**P4.6 Reproductive value of honeybee (*Apis mellifera*) drones incubated at suboptimal temperature.**

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We compared the size of paired reproductive organs, the readiness to ejaculate semen, the amount of ejaculated semen and spermatozoa viability of honeybee (*Apis mellifera*) drones incubated at 32°C and 35°C in the capped brood stage.

One drone comb with sealed brood was cut into two pieces and placed in two incubators. Temperature in the incubators was 32°C and 35°C. One day before the expected time of drone emergence, the combs were placed in separate cages and transferred into a bee colony. Drones that emerged during 24 hours were examined. The length and width of testes, seminal vesicles and mucous glands were measured on days 2, 5, 10 and 15 of drone's life. The percentage of drones everting their copulatory apparatus and ejaculating semen was examined on day 15 of life. Sperm volume collected from each drone was evaluated. Spermatozoa viability was determined by the percentage of living spermatozoa in the collected semen.

Drones incubated at 32°C had larger reproductive organs and higher viability of spermatozoa. On the other hand drones incubated at 35°C were more likely to evert copulatory apparatus and they ejaculated a larger volume of semen. To collect a semen dose of 8µl, on average 16.2 drones incubated at lower temperature were required, but 10.9 drones incubated at higher temperature produced the same amount.

**P4.7 Development of *Apis cerana* beekeeping with ethnic minorities and small farmers in rural areas of Vietnam, 2009-2012.**

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The main income generating activity in the rural areas of Northern Vietnam is agriculture and forestry. There are small plots of horticulture and fruit production and a few small fish ponds. The average acreage per family is very small and provides only the most basic needs. There is a significant need to increase options for income generating activities among the ethnic minorities as well as other groups, whose income is based on small-scale farming.

The development objectives in the project are to contribute to income generation opportunities by supporting capacity building and sustainable development among low income groups/ethnic minorities in rural areas of Northern Vietnam. The objectives were:

(1) Capacity building for the local trainers as well as the primary target group to ensure technical knowledge/ability to further develop income generating activities related to honey hunting/beekeeping. (2) Establishment of test apiaries where sustainable methods of beekeeping based on local resources and knowledge is tested and developed. To collect and disseminate knowledge of local natural resources, e.g. how the use of forest trees as nectar and pollen sources can support productive beekeeping. (3) To facilitate the formation of local beekeeping clubs which can ensure advisory services as well as continued development of appropriate methods for the local beekeeping. (4) To give local beekeeping clubs the incentive to take up advocacy activities for the benefit of the local communities.

**P4.8 Relation between flowering phenology, honeybee population dynamic, and honey production in a Mediterranean sylvo-pastoral ecosystem.**

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Mediterranean sylvo-pastoral systems supply several ecosystem services. The goal of the research was to assess the contribution of different vegetation types to honeybee colony development, foraging activity and honey production. The richest vegetation types in plant species providing pollen, nectar or both were *Rhamnus* hedges and *Olea europea* open woods. Phenological distances between species, based on the individual distributions of flowering frequencies, revealed a strong gradient, ensuring the supply of nectar and pollen throughout the entire season. Cross analysis of pollen spectra over time performed by comparing sediments in honey and plant phenological flowering, allowed to identify species or species groups with major role in providing forage to honeybees in each considered time interval. Pollen from the *Trifolium repens* group was foraged from twelve *Trifolium* species with different phenological behaviour. *Salix purpurea* and *Rhamnus alaternus* were relevant at the beginning of the season, followed by *Lavandula stoechas*, *Echium plantagineum* and *E. italicum*. This analytical approach generates information that can be exploited to increase the market value proposition of honey by including a specific botanical denomination in the label.

**P4.9 Residues in guttation droplets of maize – potential risk for honeybees (*Apis mellifera* L.).**



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In two semi-field studies the possible risk of guttation for honeybees (*Apis mellifera* L.) was investigated. The study was performed in maize treated with Clothianidin and untreated maize. The tents in the semi-field trial (four treatment and two control, each covering 96m<sup>2</sup> of maize) were covered with a gauze impenetrable for bees. In the first study (BBCH 13-15) two variants were used, one with and one without an artificial water source containing uncontaminated tap water. In the second study (BBCH 15-19) all variants had an artificial water source. The mortality of bees was assessed and sampled daily for residue analyses. During the whole exposure period (10 days in each study) the occurrence of guttation was documented and sampled daily for residue analyses. The observation of brood development was conducted once before and up to four weeks after set-up of the colonies.

In the first study guttation was only observed once, which caused in the artificial situation without any additional water supplies a high impact on mortality and also on the brood development. This is indicating the sensitivity of the semi-field test system but representing an unrealistic worst-case scenario. In variants with treated maize and additional water supply, no effects on adult mortality and brood were observed. In the second run guttation of the corn occurred on 6 days. The mortality in treated and control variants was on a similar level and within normal range.

**P4.10 Foraging for health: do honeybee workers adapt choice behaviour in response to infections?**



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The defence strategies of the honeybee can be prophylactic, such as the intake of propolis by worker bees to prevent fungal and bacterial growth on the hive, or to cover the hive's imperfections, or curative, once the bee family is being infected, the workers will try to eliminate the sick individuals in order not to spread the disease in the whole hive.

Following a trail of clues from all over the world, there is a race to solve the mystery of the diseases and to solve it in a different, more efficient way. Without the effort of science, the existence of honeybee could be in danger, because mass disappearing has been reported from around the globe. In this paper we will try to prove that bees can have self-curative behaviour depending on a few simple conditions. The odour recognition behaviour can play a major role in self-treatment of honeybees, together with their incredible foraging behaviour due to special anatomic particularities.

**P4.11 Change level of hygienic behaviour in the Caucasian honeybee (*A. m. caucasica* G.) subspecies.**

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In this study dead pupae removal of total 229 selected honeybee colonies that represent Caucasian honeybee subspecies was determined. Liquid nitrogen was applied to the total of 165 pupae in each colony. The application was repeated four times in different dates during beekeeping season. Colonies cleaned significantly ( $p < 0.01$ ) different number of dead pupae. While three colonies cleaned 100% of 165 dead pupae, one colony cleaned only 30.9% of dead pupae. In the population 44 colonies cleaned 95% and over dead pupae. Thus, the hygienic behaviour of colonies that represent *A. m. caucasica* determined as nearly as 18.41% percent. These 44 colonies were selected as the parent of the future generation. Eight queens were reared from each of these selected colonies, and total of 352 queens were instrumentally inseminated.

**P4.12 Observations on *Apis florea* in the Middle East and North Africa region.**

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The dwarf honeybee *Apis florea* is originally native to South East Asia, but in recent years its geographic range has been steadily expanding westwards, both naturally and accidentally, and continues to be reported from new locations including Sudan, Egypt, Saudi Arabia and Iraq during the past half century. In 2007 it was discovered in an isolated area at the gulf of Aqaba, Eilat Jordan and Israel respectively, raising questions about its origin, its population development, its acceptability and possible environmental impact. We here present our research in this site on population monitoring, thermal behaviour, drone mating flight timing, predators and discuss prospects of potential further spreading.

**P4.13** Studies on certain factors effect on aggressive behaviour of the honeybee *Apis mellifera* L. colonies.

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Four factors (stored food, type of brood, state of wax combs, and the time of the day hours) affecting the aggressive behaviour of the honeybee colonies were studied. The obtained results showed that defence behaviour of the honeybee colonies is a complex character controlled by many factors. Among these factors the quantity and type of the stored food. The honeybee colonies housed in hives having storage of pollen showed the highest level of defence behaviour either in speed action or in density of chasing or pursuing. On the other hand colonies having brood combs showed the lowest level of aggressiveness. With concerning the condition of the wax combs of the experimental colonies the collected data indicated that the colonies that occupied hives having only empty wax combs showed the highest aggressiveness level towards stranger. With relation to behaviour of the bees during the day hours, the honeybee colonies revealed different levels of aggressiveness over the day hours, reaching to the speed action on 2.00pm and maximum pursuing on 4.00pm.

**P4.14** Monitoring the effect of imidacloprid under semi-field conditions using electronic bee counters.

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Honeybees exposed to very small doses of neurotoxic substances such as imidacloprid, don't die but present dysfunction or disorder of their nervous system, their glandular system as well as their cardiac and respiratory rhythms. Laboratory assays provide valuable basic knowledge on the effect of pesticides on honeybees. However, applied research on the field is necessary since this is a reliable way to assess and confirm the laboratory findings. The objective of this work was to determine the impact of imidacloprid on flight activity of honeybees, brood temperature, and *Nosema* prevalence in semi-field conditions.

Flight tunnels of 12x2x2 m were used and two colonies were introduced in each tunnel. Imidacloprid was administered in syrup and pollen in dosages of 2 and 3ppb respectively. Pollen pastries were applied inside the colonies, while syrup was placed in feeders at the opposite end of the tunnels. Electronic bee counters were used at the entrance of the colonies in order to monitor the daily activity of the honeybees. The results have shown that treated colonies were keeping significantly lower brood temperature compared to controls, they had higher loads of *Nosema* spores at the end of the experiments and they presented higher losses of foragers after about 10 days of imidacloprid administration. The importance and the implications of the findings are discussed.

**P4.15** Is the distance of bee colonies to a treated crop a necessary and a useful risk mitigation measure?

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Findings of high concentrations of bee-toxic compounds in guttation drops from crop plants seed-treated with systemic insecticides triggered concerns about a potential risk for water collecting bees and honeybee colonies.

As bee colonies seem to prefer water sources in the near surroundings of their hives, a field trial was set up to assess the potential risk of guttation droplets containing residues towards bee colonies at different distances to a seed treated crop in field realistic conditions.

The experimental field consisted of two plots; one planted with seed-treated oilseed rape (Elado®, a.s. Clothianidin) and one adjacent plot with untreated oilseed rape. The bee hives were placed in the untreated plot with a distance of 0m (field border), 10m, 30m and 75m distance to the adjacent treated plot, before emergence of the plants. At each distance, six bee colonies were set up with the entrance pointing towards the treated plot. During the whole experiment (August 2011 until April 2012) the climatic conditions, the growth stage of the crop plants and the presence of guttation, rain or dew drops were recorded. If guttation occurred, droplets were sampled for residue analyses. Population development of the colonies and bee mortality in dead bee traps were assessed throughout the experiment, with exception of the overwintering time. After completion of the field experiment residue analyses of guttation drops and dead bees were conducted.

**P4.16** How do social bees respond to differences in resource diversity of landscapes?

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Worldwide, wild and managed *Apis* bees as the main pollinators of many plants are at a serious decline. Habitat loss is thought to play a major role in this decline, with diverse landscapes (comprising a variety of resources) most likely providing bees with a nutritional advantage over intensively farmed monocultures. However, little is known about how resource availability and diversity affect the ecology of bees.

We studied the foraging behaviour of the Australian stingless bee *Tetragonula carbonaria* (Apidae: Meliponini), including pollen, nectar and resin intake to unravel influences of landscape, season and resource quality on colony fitness. We set up hives in different landscapes (macadamia plantations, natural forests and suburban gardens), representing different levels of resource diversity, and monitored foraging patterns and weight gain across the season.

We found that the diversity of resources collected was reduced in plantations, and that seasonal shortages in food availability strongly affected colony weight, although foraging patterns were largely similar across different landscapes. Moreover, pollen and sugar intake was smaller in plantations than in gardens and forests, whereas resin intake was similar across landscapes. Colonies in plantations additionally faced a higher pest and predation pressure. Consequently, even eusocial bees that can forage at long distances from their nest are unlikely to cope with the reduced resource diversity and availability caused by intensive agriculture.



**P4.17** Effect of Imidacloprid on the locomotor activity of honeybees.

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The level of locomotor activity (LMA) of honeybees is important for the health of colonies. We have tested the effect of imidacloprid on the LMA of worker honeybees representing *A. m. anatoliaca* (two ecotypes), *A. m. caucasica* and *A. m. carnica* races in Turkey. In order to quantify LMA, we used an activity monitoring system that can supply high resolution data (at 1 minute periodic intervals). Our main purpose was to show the effect of imidacloprid at low doses (5ppb, 10ppb and 50ppb). Our results indicate that the LMA of honeybees is significantly reduced even at the lowest dose (5ppb) applied. Results based on control bees also indicated a variation in natural LMA for different races and ecotypes. The reaction of different races and ecotypes against imidacloprid is also found to be varied.

**P4.18** The regulation of cell sealing in the stingless bee *Melipona subnitida* (Meliponini).

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In stingless bees queens lay all or the majority of eggs, and from every single cell a single individual emerges. In several species workers contribute to male production by the laying of fertile eggs. At times their activity therefore causes direct competition for egg-laying opportunities at cells filled with liquid food for the future larva. Such competition becomes visible by the way workers of some species sneak their eggs into cells already oviposited in by the queen. In others, reproductive workers physically impede queens to oviposit; at a specific moment a worker blocks the cell mouth, deposits her egg and consecutively seals off the cell with a waxen cap. In this way, workers can be successful in safe guarding their eggs from being destroyed by the queen. After being deposited, the queen's eggs receive similar protection but in these cases are the workers not reproductively active that provide the cell cap. This study aims to clarify how cell sealing is regulated and what the role of a queen is. For this, we tested the effect of the various elements of an oviposited cell, like the egg, larval food and its wax structure, on worker conduct in *Melipona subnitida*.

**P4.19** Artificial light affects honeybee activity.

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To determine whether artificial light has an impact on honeybee activity, we exposed bees in an observation hive to invisible infra-red and visible white light, perceived by the bees as darkness and light respectively. Each time the perceived light intensity changed from dark to light, a sudden increase in bee activity was observed. This increased activity, which appeared to be a 'nervousness' or 'runniness' behaviour, lasted for less than a minute before returning to normal levels. During exposure of low intensity light followed by high-intensity light, sudden increased bee activity was again seen at every shift. The duration of the increased activity was shorter during the second time phase indicating possible habituation in response by the bees after the first light intensity stimulus. The data suggests that observation hive experiments can be conducted in white light but that caution should be taken to record behaviour during the initial period of light exposure.

**P4.20** Effect of honeybee (*Apis mellifera*) pollination on the response of cotton.

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Field experiments were conducted in special farm at Diarb negm, Sharkia gover norate, Egypt, to study the effect of honeybee (*Apis mellifera* L.) as a pollinator on bee products (broods, honey and pollen) as well as (abscission and productivity) of Egyptian cotton (Giza 86), *Gossypium barbadense* L. during 2010 and 2011 seasons. Other field experiments were conducted with eight of cotton varieties (Aleppo 33; McNair 308; Tashkent 3; McNair 307; McNair 235; Tam Cot CAMD; McNair 220 as imported var. and Giza 86 (*G. barbadense* L.) as local var. The results indicate that honeybee pollination significantly increased the total amount of extracted honey, mean areas of pollen and broods increased in hives put in cotton cultivars compared with those away from cotton. Also, increased the values of number bolls/plant, yield and its components (number of open bolls/plant, and seed cotton yield per plant and fed), but decreased the total abscission percentage compared to the unpollinated plants. The population of cotton whitefly, *B. tabaci* had three main peaks in growing season (July, August and September) with Temp. (26.2-26.6oc), R. H. (68.3-69.0%), wind speed (0.77-0.90m/sec.) and sunshine duration (12.4-13.9hr). There were high significant difference between infestation of cotton varieties with *B. tabaci*. The local variety (a 86) was the most tolerant against infestation with *B. tabaci*. It was found that the rates of cotton yield were decreased with increasing infestation rates of white fly.

**P4.21 Behavioural defence of honeybees against *Nosema ceranae*.**

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Honeybees, as eusocial organisms, possess high interaction rates. To avoid the expected increased parasite infestation honeybees evolved several behavioural and organisational defence mechanisms. One group of their diverse parasites is the Microsporidian *Nosema ceranae*. In this study we demonstrate that the interaction frequency and the behaviour towards *N. ceranae* infected honeybees are distinct to healthy nest mates. A behaviour assay was designed in which groups of honeybee workers were recorded several days and contacts of *N. ceranae* infected honeybees and non-infected controls with their nest mates were counted. In every experimental replicate the infected bee received more contacts than the uninfected control bee. This interaction ratio increased for the infected bee over time. A high proportion of the infected bees died as a consequence of continuous attacks by nest mates. Additionally no evidence for parasite transmission was found within in the group. Hence, behavioural modulation of healthy bees towards infected ones seems to benefit the honeybee at the group level rather than the parasite.

**P4.22 Susceptibility to chalkbrood and hygienic behaviour of worker bees in colonies of chosen races and lines of honeybee.**

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The aim of the study was to evaluate the susceptibility of larvae infected with *Ascosphaera apis* spores and hygienic behaviour of adult bees in relation to brood killed with a low temperature. The field observations were conducted in the apiary belonging to Research Institute of Horticulture, Apiculture Division in Puławy, Poland.

Three races of bees were used in the research: *Apis mellifera carnica* (lines car GR1 and car Mr), *Apis mellifera caucasica* (line cau P) and *Apis mellifera mellifera* (line mel Asta). Mummies of brood were observed in the comb cells and on hive boards up to 6<sup>th</sup> day of observation in colonies of car GR1 and mel Asta but up to 8-10<sup>th</sup> day in car Mr and cau P. The percentage of dead brood in relation to sealed cells in colonies, calculated as a sum of mummies collected from each colony, was below 10% in car GR1, about 20% in car Mr and 25% and 30% in mel Asta and cau P, respectively. Bees cleaned within 48 hours almost 100% of freeze-killed brood in mel Asta and car GR1 colonies and 70-80% in car Mr and cau P.

**P4.23 The composition of agricultural landscapes influences life history traits of honeybee workers.**

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Honeybee workers play a major role within the colony by taking care of the breeding of larvae until the supply in food of the entire colony, by division of tasks. The age polyethism offers to the worker the capacity to carry out successively the totality of these tasks, following its age. It is known that the worker switches the tasks following a gradient internal-outside the hive during its imago life, the oldest workers making the foraging tasks. However, the available food in agricultural landscape is managed by the entomophilous crop phenology. To insure the food resource income in the colony, the workers have to adapt their life histories according to the available food. The originality of this work concerns the use of the RFID technology (Radio Frequency Identification), recently adapted to the honeybee. This tool allows to follow continuously income/outcome of the bees during all their lifespan. Emergent workers are tagged with a transponder just before being introduced in the colony. 1640 workers life history were followed in this study, born in various periods of the season, during the bloom of the entomophilous crops versus not. For the first time the results allowed to characterise exactly the bee's life history by behavioural analysis. This characterisation shows that the temporal available food dynamics of the resources in agricultural landscape influences strongly the life history of bees at the individual level.

**P4.24 Foraging behaviour of *Apis m. adansonii* and other bee species on four plant species in Dang, Cameroun.**

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Flowers of four plant species were observed in Dang (Ngaoundéré) during rainy and dry season for at least two weeks from August to December 2010, between 6am and 4pm, for the registration of the specific nectar and/or pollen foraging behaviour of bees. *Cajanus cajan* (Fabaceae), *Bracharia brizantha* (Poaceae), *Waltheria indica* (Sterculiaceae), and *Vigna unguiculata* (Fabaceae) flowers were observed to study the most frequent bee species and their activities. The results show that *Cajanus cajan* flowers opened at 8am and insect activities began at around 9:30am, the activities lasted all day; bees harvested pollen and nectar and reached their activity peak between 10am and 11am. For *Bracharia brizantha*, flowers opened at 6am; insect activities began at around 6am; bees harvested pollen and reached their activity peak between 7am and 8am. *Waltheria indica* flowers opened at 7am; insect activities began at around 7am, activities lasted all day; bees harvested pollen and nectar, and reached their activity peak between 12am and 1pm. *Vigna unguiculata* flowers opened at 6am and closed at 12am; insect activities began at around 7am; bees harvested pollen and nectar, and reached their activity peak between 7am and 8am. A total of eleven bee species were observed as pollinators in this study; *Apis m. adansonii* was the most frequent.

**P4.25** **Beekeeping in Poland – the current state.**

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In recent years economical conditions of Polish beekeeping were changed. Mainly due to integration with the European Union in 2004. Poland became a member of this community, and beekeeping received support from the Common Agricultural Policy of EU. From this reason the aim of the study was to determine the current economical situation of Polish beekeeping. The study was conducted in 2011. Materials and data used in the study come from different sources: Ministry of Agriculture and Rural Development, Agricultural Market Agency, Veterinary Inspection, Polish Beekeeping Association and own analysis and estimates.

According data from registries kept by Veterinary Inspection there are 49 432 beekeepers, who own 1 246 633 bee colonies in Poland. Honey production in 2011 was valued about 23 thousand tonnes. The average production of honey was valued at 18.4 kg per bee colony. Prices of honey in wholesale market were very low and different, depended also on varieties of honey (for example: 2 EUR – rape honey, 6 EUR – heather honey). The costs of production in the small apiaries were estimated at 64 EUR per bee colony, but in professional bee farms costs were higher – 80 EUR. The value of the balance in foreign trade during the 2011 year was minus 17 thousand EUR (preliminary data). Honey import was much higher than export. The average losses of bee colonies during the winter 2010/2011 was 13,5%.

**P4.26** **Interaction between disease and social differentiation in the honeybee, *Apis mellifera*.**

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Division of labour is an integral component of insect eusociality. In honeybees, the worker caste performs different social tasks in an age-related manner (temporal polyethism). The mechanism responsible for the shift from nurse to forager is linked to differential expression of key reproductive genes such as vitellogenin and is accompanied by significant changes in nutritional (insulin/insulin-like) signalling. Variation in the reproductive ground plan of workers is also linked to whether workers specialise in collecting pollen or nectar. For example, workers with many ovarioles tend to forage earlier and collect pollen instead of nectar. Pathogenic organisms also influence honeybee behaviour: *Nosema ceranae* is a microsporidian gut parasite that reduces life span, affects flying ability, and causes energetic stress. In this study we explore the interaction between disease and social differentiation. Due to the central role of energy status in both honeybee caste differentiation and gut infection, we test the hypothesis that *Nosema* infection influences honeybees differently depending on the reproductive status of workers.

**P4.27** **Monitoring the longevity and activity levels of four *Apis mellifera* subspecies infected with *Nosema apis*.**

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In this study two hoarding cage experiments and two activity monitoring experiments were conducted with *Nosema apis* infected honeybees with control bees in spring and fall season. *A. m. caucasica*, *A. m. carnica*, Muğla and Yığılca ecotypes of *A. m. anatolica* subspecies were used for the infection experiments. Two colonies for each of the subspecies and 60 infected and control bee samples from each colony were used for the hoarding cage experiments. The activity of a total of 64 honeybees, 32 of which were controls, were recorded by a monitor system for twelve days monitoring experiments. These hoarding cage experiments showed the differences between the longevity of bee races in spring and fall period. When the bees were infected with *N. apis*, survival percentages of all races decreased. The mortality results were supported by the activity monitoring data. The mean activity difference between infected and control honeybees were statistically significant for both spring and fall samples of monitoring results. The results of the hoarding cage trials showed that, dosing with *N. apis* spores significantly reduced the lifespan of bees for each of the four races in spring and fall period.

**P4.28** **The ability of honeybee foragers (*Apis mellifera*) to discriminate between pollens according to their fatty acid composition.**

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Honeybee foragers, *Apis mellifera*, collect pollen from flowers to fulfill the colony nutritional needs. The aim of the present study was to test the ability of foragers to discriminate between pollens according to their fatty acid composition. In a first set of experiments, colonies were deprived of pollen for a week to create a pollen shortage. They were then allowed to forage for a week from a feeder containing soyflour enriched with 10% pollen mixture and a vegetable oil. The oil was rich in one of two essential fatty acids, linoleic or linolenic acids. They were then provided a choice between three feeders that were identical except for the type of vegetable oil enrichment: the same as in the previous week, a different oil but of similar fatty acid composition, or a different oil complementing the lacking fatty acid. Foragers preferred the feeder that was different and that complemented their fatty acid deficiency. These results support the geometric approach to nutrition hypothesis that foragers can discriminate between fatty acid sources and balance the colony's nutritional needs. A second similar set of experiments is in progress, in which floral pollens of known fatty acid composition are used. In addition, we are analysing the dances of foragers to test their subjective evaluation of pollens that complement or do not complement their fatty acid needs.

**P5.1 Occurrence of nosemosis in honeybee colonies *Apis mellifera intermissa* in the Mid-North of Algeria during 2011.**



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Nosemosis (*Nosema* disease) is one of the most serious and prevalent adult honeybee diseases worldwide and is caused by intracellular microsporidian parasites from genus of *Nosema*. The aim of the present study was to determine the prevalence of *Nosema* sp. in the Mid-North of Algeria (one of the important beekeeping centre in Algeria). The sampling was conducted on the beekeeping areas located in six regions in the season of spring and summer during the year 2011. In the laboratory the spores of *Nosema* sp. are detected and counted separately for each sample according to the protocols proposed by the OIE (Office International des Epizooties, 2008). The rate of the infection was reached through counting the numbers of the *Nosema* spores on the body of the bees.

The infection of the honeybee colonies was of its utmost level in the spring (31,7%), however the amount was considered to be low in the summer (5,5%). In spring, regions of Tipaza and Boumerdes area is the most infested with diseases (35,5 and 30,5%). Other areas have an infection rate of 20% (Alger, Blida, Bouira and Tizi ouzou). This high prevalence of *Nosema* in two regions is linked to climatic conditions (high humidity).

**P5.2 Percentage of infection of varroasis in four ecozones in Iran.**

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Varroasis is an external parasitic disease in honeybee. Samples were collected from 2273 colonies (1130 Apiaries) in four ecozones (Caspian sea, mountain, warm and dry zones, and warm and wet zones) from selected provinces as follows:

1) Mountainous zone: West Azarbayejan, Lorestan, Ardebil; 2) Caspian zone: North Khorasan, Golestan; 3) warm and dry zone: Kerman, Isfahan, Yazd and 4) warm and wet zone: Fars.

Tests had been conducted according to OIE terrestrial Code Manual. When presence of even one mite, colonies were considered as positive. In this study, the percentage of infection was of 23, where the highest and lowest percentages of infection were found in Yazd & Kerman, with 6% and 48%, respectively. Regarding to the fact that this study is conducted in four ecozones and the movement of honeybees because of environmental conditions, we can suggest that the results of this study are representative of *Varroa* frequency in Iran provinces.

**P5.3 Efficacy evaluation of organic treatments against *Varroa* mite in different climatic zones of Chile.**

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In Chile as in other countries, *Varroa destructor* has a major economic impact in beekeeping. This mite can be found in at least 93% of the Chilean apiaries. Between 2009 and 2012 the effect of temperature (T°) and humidity (H°) on the efficacy of five organic treatments for the control of *Varroa* was evaluated in five apiaries located in the four Chilean climatic zones with major beekeeping activity. Thymol, formic acid and oxalic acid were use in different presentations and concentrations. In every apiary, six beehives for each treatment were employed and six beehives were kept as control group. T° and H° were monitored during the entire experimentation period. The efficacy (Ef) was determined regarding the initial and final infestation rates and the percentage of mite mortality. The efficacy of formic acid treatments was greater than 70% when the T° was higher than 19° C and the H° less than 50%. In the case of thymol treatments, the efficacy was greater than 68% when the T° was above 11°C and the H° greater than 65%, in more temperate conditions the efficacy went up to 93%. The oxalic acid syrup treatments weren't affected by environmental factors, their efficacy ranged between 42-60%. Nevertheless, the efficacy was closer to 82% in the apiaries that were affected by a low nectar flow in conjunction with bad weather conditions (low T° and rain). It can be concluded that environmental variables such as temperature and humidity affect the efficacy of some organic treatments.

**P5.4 Effectiveness of some plants oils against *Paenibacillus larvae*, the causal agent of American foulbrood.**

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*Paenibacillus larvae* ssp. *larvae* is a Gram-positive and spore forming bacteria which represents a major bacterial pathogen of honeybee brood and the causative agent of American foulbrood disease. *In vitro* antibacterial activity of 28 plant essential oils against *P. larvae* (ATCC 9545) was evaluated. Oxytetracycline hydrochloride was used as a positive control. 20 out of 28 plant essential oils tested were found effective. Based on their MBCs values (Minimum Bactericidal Concentration), effective oils were placed into three categories: highly effective, moderately effective and least effective. Cinnamon oil, lemon grass oil, thyme oil, corn mint oil, rosemary oil, funnel seed oil, clove bud oil and Eucalyptus oil were highly effective; MBCs values ranged between 162.0µg/mL and 375.0µg/mL. Cinnamon oil was the most effective in all the essential oils with MBCs value 162.0µg/mL. Results of time-response effect showed that no viable cells of *P. larvae* 9545 were observed after 24 hrs treatment with cinnamon oil (162.0µg/mL), 36 hrs treatment with lemongrass oil (186.0µg/mL), 48 hrs treatment with thyme oil (224.8µg/mL) and 48 hrs treatment with oxytetracycline (5.89µg/mL). The essential oils tested showed significant antimicrobial activities against *P. larvae* 9545, and they might contain new materials that will play a considerable role in the treatment or prevention of American foulbrood disease.

P5.5

**Prevalence of *Paenibacillus larvae* genotype ERIC I and ERIC II in two Italian regions.**

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The Gram-positive spore-forming bacterium *Paenibacillus larvae* is the causative agent of American foulbrood (AFB). The genotypic characterisation of *P. larvae* strains based on rep-PCR using Enterobacterial Repetitive Intergenic Consensus (ERIC) primers has identified four different ERIC patterns designated ERIC I-IV. These four genotypes have different phenotypic characteristics. They have also different virulence and this seems to influence the spread of the disease and some clinical aspects. The identification of the *P. larvae* genotype, circulating in a determinate geographical area or in a single apiary, assumes then a relevant practical importance. *P. larvae* strains, isolated in our laboratory in the years 2008-2011 and coming from 49 apiaries in nine province of Lombardia and Emilia Romagna Regions, were genotyped by ERIC-PCR to evaluate the prevalence of ERIC genotypes in this territory. Strains were isolated from winter debris sampled from individual hives of 17 apiaries during an AFB infection monitoring plan and from brood coombs coming from 32 outbreaks of AFB. Results showed the presence in our two regions, in addition to the "classic" ERIC I genotype, of the pigmented ERIC II genotype of *P. larvae*. This genotype was isolated both in colony with sub-clinical infections and in brood with clinical signs of AFB. Co-infections by both genotypes were frequently observed in apiaries examined but also in individual colonies. Possible diagnostics implications are discussed.

P5.6

**Biochemical changes of the worker brood, *Apis mellifera intermissa*, parasitised by *Varroa destructor*.**

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In order to study the impact of *Varroa destructor* on the biochemical composition of carbohydrates in the hemolymph of worker bee, *Apis mellifera intermissa* at different developmental stages, three stages are chosen: white-eyed nymphs, brown eyes nymphs in the early stage of pigmentation and bees pre-emerging provided from the North of Algeria. These categories were classified according to the intensity of parasitisation: bees with 1-3 mites, 4-6 mites and bees without mites (control).

The results show that at a parasitisation of 1-3 mites, the carbohydrates concentration of pupae white eyes and pupae with brown eyes was reduced by 50%. This concentration decreased by about 69.9% in the heavily infested pupae with brown eyes (4-6 mites). The hemolymph carbohydrates content of the slightly and heavily infested pre-emerging stage in comparison to control pre-emerging was reduced by 33.8% and 49.1%, respectively.

P5.7

***Nosema apis* and *Nosema ceranae* in honeybee (*Apis mellifera* L.) colonies in Lithuania.**

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Despite the global spread of *Nosema*, little is known about the current distribution and presence of *Nosema* species in the Baltic countries. In this study we investigated the occurrence and incidence of *Nosema* parasites in honeybees, *Apis mellifera*, in Lithuania. Honeybee samples were collected from 129 colonies in 46 apiaries throughout the country. Two to five hives per apiary were sampled. A microscopic examination revealed 100 bee samples positive for *Nosema* spp. Molecular methods based on the polymerase chain reaction (PCR) of the 16s rRNA were applied to distinguish *Nosema ceranae* and *N. apis* species. DNA was extracted applying the DNeasy Plant Mini Extraction Kit (Qiagen). Species-specific primers 321 APIS-FOR/REV and 218 MIROC-FOR/REV were used for the detection of *N. apis* and *N. ceranae*. Both *Nosema* species were detected in the samples originating from all regions of Lithuania. Of the 100 bee samples infected with *Nosema* (revealed by microscopic examination), sole infection with *N. ceranae* was detected in 32 samples, sole infection with *N. apis* in 36 samples, and co-infection with both species in 32 samples. *N. ceranae* was most common in bees from southern and western Lithuania, with *N. apis* most common in northern and eastern Lithuania. Based on a distribution pattern, we conclude that the new invasive parasite *N. ceranae* is spreading within the country from Southwest to Northeast. *N. ceranae* was recorded for the first time in honeybee colonies in Lithuania in 2011.

P5.8

**Apibioxal, a new drug for the control of *Varroa* mite.**

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Until the year 2010 the Italian beekeepers were using organic acids (formic and oxalic acid) to fight *Varroa*. Above all, the oxalic acid had proved indispensable for the treatment to be carried out in the absence of brood in winter period because of the resistance developed from the mite against drugs (Perizin) and the lack of alternative products. With a circular, the Italian Ministry of Health confirmed the prohibition of the use of organic acids if not sold as veterinary drugs. The collaboration between Veterinarians, Researchers, Health Ministry, Beekeepers and a Pharmaceutical company allowed the authorisation of a drug containing oxalic acid in a very short time, overcoming all difficulties. To allow the immediate use of the product before its approval, the Ministry authorised a double trial, one conducted by a research center, the other by beekeepers. An example of collaboration between institutions, companies and beekeepers to imitate.

P5.9

**The beekeeping industry in Europe – data from 2010.**

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Over the last few years, high rates of troubles have been reported affecting honeybee colonies (*Apis mellifera*) in many European and North American countries. However, the beekeeping industry is poorly documented in Europe. The European Reference Laboratory for Honeybee Health has sent to the National Reference Laboratories for honeybee diseases of each member states of the European Union (plus Kosovo and Norway) a detailed questionnaire targeted on beekeeping activity. Collected data allowed generating a European database on the structure of the beekeeping industry, the beekeeping production, the economic aspects of this industry and health aspects. The total number of beekeepers in Europe was estimated at 600 000. The European honey production was evaluated around 200 000 tons in 2010. The price of honey varied within a range of 1.50 and 40.0 €/kg depending on the country and on the network of distribution. The colony mortality varied from 7 to 28% depending on the country and the origin of the data. The overall conclusion of this survey underlines the high heterogeneity of figures coming from each country. These data will be communicated to the beekeepers and to the stakeholders from the European member states and the European authorities. In addition, these results give some outlines of the global honeybee colony declines observed in Europe that will have to be further investigated through the implementation of appropriate surveillance systems.

P5.10

**Diversity of microbes in Thai honeybees.**

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Insects are one of the unique habitats of microbes. Most studies have been focused on the microbial pathogens. However, in fact the microbes causing diseases in insects are in small proportion when compared to the non-pathogens, the latter of which greatly benefits to insect host in various ways (e.g. food digestion and growth inhibition of some insect pathogens). In Thailand, we have domesticated at least two species of honeybees (European honeybee (*Apis mellifera*) and cavity nesting Asian honeybee (*Apis cerana*)) for honey production. Our study was to study the bacteria colonising in the mid-gut of these two species of honeybees so as to apply this basic knowledge to beekeeping industry in the near future.

The bacterial species in the group of Gammaproteobacteria were found to be common in all stages of both species of honeybees, but in addition, the Firmicutes group was found in the adult stage. Different location did not affect the bacterial communities found in the mid guts of *A. mellifera*. However, the bacterial community in *A. cerana* differed between locations, even though the hives in each location were allocated in a similar niche, Longan orchards. During our study of microbial diversity of honeybees, food sources and hive materials, we proposed one new species of Actinomycetes, and three species of yeast which were found in raw honeys. Some of isolated bacteria showed high antagonistic effects against honeybee pathogens.

P5.11

**Survival of *Melissococcus plutonius*, the causative agent of European foulbrood, on wood and in honey.**

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To improve our knowledge about the risk of spreading European foulbrood by movement of contaminated material, we artificially applied two strains of *Melissococcus plutonius* (the type strain and a wild strain) to spruce wood, as well as nectar and honeydew honeys. We maintained infected material at different temperatures (room, and 4 and -20°C) over two years, and frequently quantified surviving bacteria by media cultivation.

Whereas the number of surviving type strain *M. plutonius* decreased significantly faster on spruce wood compared to the wild strain, the wild strain survived also better than the type strain in honeydew honey, and both strains survived better in nectar honey than in honeydew honey.

On wood, temperature had a large influence on bacterial load. No change was seen over time when materials were maintained at -20°C; however, decreases were observed when kept at room temperature and 4°C. For example, after one year of storage at room temperature, wood initially exposed to the type strain was free of infectious bacteria and a reduction of 10<sup>2</sup> was observed in materials exposed to the wild strain. These results will have an impact on sanitation measures for European foulbrood.

P5.12

**The surveillance of honeybee diseases in Romania.**

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Romania is one of the most important honey producers from Europe and it has many melliferous zones of acacia, lime and sun flower plantations, all over the country. In this way, in Romania, in 2010, there were registered 963.342 bee colonies of *Apis mellifera carpatica* species. There were obtained 22.224 tons of honey and 100 tons of pollen. The surveillance of notifiable diseases is carrying out by laboratory investigations, at the Institute for Diagnosis and Animal Health, in the National Reference Laboratory for honeybee diseases and in the other 41 county laboratories. The bee diseases are surveyed by laboratory exams in accordance with a National Programme, financed from the state budget. There are presented the laboratory tests which are performed in our Institute, statistic data regarding the number of samples worked between 2007-2011, notifiable diseases, and the other major causes of bee mortality encountered in colonies, in this period.

Dynamic evolution of outbreaks in Romania is determined by: 1) deficiencies in enforcement against specific treatments, or their omission; 2) absence of integrated control of parasitic diseases by beekeepers; 3) incorrect application of compulsory measures of hygiene and sanitary - veterinary. Also, the pastoral practice of beekeeping in temporary apiaries clusters with different epidemiological situations, helps to keep disease outbreaks and offers the permanent possibility of reinfection in varroosis and chronic evolution of nosemosis.

**P5.13 Amber inclusions of spiders (Araneae), ancestors of nowadays predators of bees (Apoidea).**

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The aim of this study is presentation of fossil spider collection as contribution for further research on these relics of the past. They were based on author's amber collection of selected and comparatively transparent raw amber pieces and jeweller products. They were look over under stereoscopic microscope from the point of presence of inclusions, with particular reference to spiders. Fossilised specimens were identified, pictured and compared with data in literature of the subject. Comparison of fossilised spiders found in amber of past epochs with nowadays species show their good adaptation for predatory mode of life, e.g. strong legs, massive mouth parts (pedipalps, chelicerae with venomous claws), glands producing neurotoxins causing paralysis of attacked preys, simultaneously introducing digestive enzymes, which dissolve internal body tissues (histolysis) of them and make possible absorption of such prepared food by predators. Some species (e.g. Araneidae) have special glands (spinnerets) producing substance for spider-web construction as effective traps for their pray (mainly flying insects). Representatives of other spider-groups (Salticidae, Thomisidae, Lycosidae, Miturgidae, Sparassidae) are equipped with spring, step or run legs. They have good developed sense organs (some pairs of eyes, chemoreceptors, sensilla, tactile setae) and they are very effective and high specialised predators, natural enemies of other arthropods including honey-, solitary- and bumblebees.

**P5.14 The efficacy and tolerability of Api-Bioxal, an oxalic based product in cool temperate climates.**

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Mite resistance to flumethrin acaricides was confirmed in Ireland during 2010. To ensure adequate control of *V. destructor*, Api-Bioxal (active ingredient oxalic acid) was registered as a follow-up winter treatment in colonies treated previously in the autumn. Although efficacy >90% have been reported for Api-Bioxal in southern Europe, its efficacy and tolerability had not been documented for cool northern temperate climates. The present study aimed to assess the efficacy and tolerability of Api-Bioxal as a winter treatment when administered using the trickling and vaporiser methods. Test colonies (n=45) were standardised based on brood area and natural mite fall. Treatments were administered according to the manufacturers' instructions using a random block design and included control colonies. Invasion pressure was monitored throughout the trial period and four weeks post treatment. All colonies were treated with a control treatment (Checkmite+; active ingredient coumaphos) and subsequently the percent efficacy of Api-Bioxal was determined. Tolerability was assessed by recording bee mortality and estimating spring build-up using brood area as the quantitative parameter. Percent efficacy was lower and bee mortality was higher for the trickling method. Neither treatment had a negative impact on spring build-up. The results will form the basis for recommendations to beekeepers for the use of Api-Bioxal in cool climates.

**P5.15 Analysis of bee antimicrobial peptides by mass spectrometry methods.**

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Bee immunity is composed of three levels of interactions with pathogens – physical barriers, cellular immunity and humoral immunity. Our research is focused on the humoral part of immunity, namely to antimicrobial peptides. They are small molecules produced by hemocytes and fat body to hemolymph and are responsible for antimicrobial activity in bee hemolymph, royal jelly and venom.

Nowadays, methods combining a mass spectrometry and a liquid chromatography are generally used for analysis of peptides and proteins in various samples. These methods serve the opportunity to identify and quantify directly the peptide related to their antimicrobial function. We are focused on specific antimicrobial peptides in hemolymph (apidaecins and abaecin). The developed method consists of several steps which combine different solid phase extraction protocols (SCX, RP) of basic peptides from bee bodies extracts and their chemical modification for more reproducible and robust detection. The final detection and quantification of desired peptides in purified samples are performed on a system consisting of nano-capillary liquid chromatography connected on-line with mass spectrometry (nanoLC-MS). Our method gives a chance to follow and quantify levels of apidaecins and abaecin in a single bee and can be used for study of immune responses to pathogens on individual and population levels.

**P5.16 Antioxidant activity of bee pollen produced in two Brazilian states.**

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The objective of this study was to determine the antioxidant activity of nine dehydrated bee pollen samples collected during November and December 2011 from four apiaries in two Brazilian South States (Santa Catarina and Paraná). Antioxidants compounds were extracted with 70% ethanol, diluted to 1mg/mL (bee pollen/70% ethanol) and aliquots were evaluated using DPPH assay system. The decrease in absorbance was determined at 517nm at 30min and the antioxidant activity was expressed as percentage of DPPH inhibition. Sample A, collected in November from Paraná, showed the higher value of the antioxidant activity with 71.7%, however, the other three samples from the same apiary, also collected in November, showed 64.5% (Sample B), 30.0% (Sample C) and 29.9% (Sample D). The percentage of DPPH inhibition by Sample E (Paraná) and Sample F (Santa Catarina), both collected in December, were 65.1% and 40.9%, respectively. Three samples (G, H and I), collected in December from Santa Catarina, showed antioxidant activity from 13.6% to 27.0%. Antioxidants compounds in pollen grains can vary depending on botanical source of the flower pollen, the main constituent of bee pollen. Pollen extracts represent a concentrated nature-derived mixture of different compounds, including antioxidants. The antioxidant activity in bee pollen ranged from 13.6% to 71.7%, lower than BHA solution (0.09mg/mL), a synthetic antioxidant, which showed 96.7% of activity. Acknowledgments: FAPESP, CNPq and CAPES.



**P5.17 Impact of pesticides on honeybee immunity and health.**

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Large-scale losses of honeybee colonies seem to have a multifactorial origin, with both biotic and abiotic stress factors being apparently involved. Among the latter, pesticides have been invoked as important agents inducing colony decline, with neonicotinoids often considered as key-players in this complex syndrome. However, the experimental data gathered so far are still fragmentary and further research efforts are therefore needed. In a previous study, colony decline was linked to viral explosion promoted by bee immunosuppression; here we investigate the impact of neonicotinoids on the immune system of the honeybee in order to understand if and how these pesticides can promote the progression of silent viral infection, with negative consequences on colony health and stability. We observed that exposure to sub-lethal doses of neonicotinoids triggers intense replication of deformed wing virus (DWV), while organophosphates did not. This effect was analysed in the framework of the model we have recently proposed, based on the central role played by NF- $\kappa$ B transcription factors in the anti-viral immunity and in the activation of physiological responses to different environmental stress factors. Besides the work that we are carrying out in honeybees, our studies on the effect of neonicotinoids on NF- $\kappa$ B activity will take advantages also from the analyses that we are currently performing using the model *Drosophila*.

**P5.18 The COLOSS BEEBOOK: facilitating worldwide honeybee research through method standardisation.**

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COLOSS is a global network made up of approx. 300 members from 60 countries that aims to understand and prevent honeybee colony losses. After four years of activity, the network has organised over 60 scientific events and contributed to over 130 publications in peer-reviewed scientific journals. During their activities, COLOSS members recognised the need for research methods to be standardised to facilitate comparison of experimental results gathered from different countries. The COLOSS "BEEBOOK: standard methods for *Apis mellifera* research" will be the definitive, yet evolving, honeybee research manual, and will be composed of 29 peer-reviewed chapters authored by more than 160 of the world's leading honeybee experts. Chapters will describe methods for studying honeybee biology, methods for understanding honeybee pests and pathogens, and methods for breeding honeybees. It is expected to be completed by late 2012, and will be published both online as an Open Access Special Issue in the Journal of Apicultural Research and as a hard copy book for use at the laboratory bench. Due to the fast evolving field, it will be kept updated via an online tool and subsequent new editions.

This poster presents the output of COLOSS and the structure and content of the BEEBOOK.

**P5.19 First report of *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) as a hives pest in Iran.**

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The infestation of Psocids, *Liposcelis bostrychophila* (Booklice) in the hives of honeybees was observed in an apiary in Isfahan province in Iran on April 2011. The Psocids size was about 0.6mm. The colonies population in the infected apiary was very weak and the production of honey was low.

Psocoptera, known as psocids, are small, soft-bodied, they have mandibulate mouthparts (biting or chewing), with large head and protruding eyes, long and threadlike antenna. Some psocids feed on algae and lichens, while others feed on moulds, cereals, pollen, fragments of dead insects and other organic materials.

The tiny insects were wandering at the top, bottom and on the frames of the hives when the hives were opened for examination. The insects were collected and capturing beautiful images and film. The insects were then examined under a compound microscope for measurement and species identification by using a taxonomy key. Result showed that the insects were *L. bostrychophila* (Psocoptera: Liposcelidae). As declared in literature several species (*Liposcelis* sp.) are partial predators, eating eggs and scale insects. It is expected that these tiny insects feeds on the eggs of honeybees.

**P5.20 Invasion rates and population growth of *Varroa destructor* in regions with high and low numbers of honeybee colonies.**

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The honeybee mite *Varroa destructor* can be distributed among colonies by horizontal transmission. Mite invasion rates usually increase in late summer when mite infestation rates are high and the limited food sources boost the robbing activities. We quantified the weekly invasion rates and the mite population growth from August till November in honeybee colonies (n=28) at apiaries with high and low numbers of honeybee colonies around. At each apiary, half of the colonies were continuously treated with acaricides in order to kill all introduced *Varroa* mites, the other group of colonies was only treated at the beginning of the experiment and then left untreated to record the mite population growth. The number of mites invading the treated colonies was highly variable among the colonies and between the study sites. The average invasion rate over the entire four month period at the location with high bee density mites was significantly higher compared to the colonies at the apiary with low bee density. As a consequence of mite invasion and mite reproduction, the *Varroa* population reached an average final infestation of more than 1.500 mites per colony (high bee density) and less than 300 mites per colony (low bee density), respectively. Our results clearly show the urgent need to treat all colonies of a certain region simultaneously to prevent re-invasion of mites in already treated colonies.



**P5.21 Genetic variability in single copy genes of *Nosema ceranae*.**

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Honeybee colonies worldwide have been hardly affected by the microsporidian parasite *Nosema ceranae*. So far, its phylogenetic classification and polymorphism studies have been based on the study of the structure and the nucleotide sequences of ribosomal RNA subunits. The interpretation of the results thus obtained might not be straight forward because these genes are present in multiple copies in the microsporidian genome. The use of single copy genes is ideal for this purpose, since it avoids the possible comparison of non-orthologous sequences.

In this work we cloned and sequenced several single copy loci - actin, alpha tubulin, beta tubulin, TATA box binding protein (TBP), largest subunit of RNA polymerase II (RPB1) and HSP70 - to estimate the level of nucleotide diversity and geographical distribution of the genetic variants present in *N. ceranae* field isolates from different parts of the world. This approach revealed the coexistence of numerous haplotypes within each sample, which would be undetected if direct sequencing was used.

The high rate of polymorphism found in all the genes probably reflects co-infection with several genotypes of *N. ceranae*. Such genotypic variation could help to trace the population history and geographic dispersal of this highly infective species out of Asia, as well as to detect variants with different levels of virulence.

**P5.22 With which sensory organ percept male mites of *Varroa destructor* the female sex pheromone?**

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*Varroa destructor* males are attracted by females via a volatile sex pheromone (Ziegelmann et al. Apidologie in review). So far, the sensory organ that empowers the males to identify the young attractive females is unknown. We supposed the sensory pit organ that is located on the front legs to be the searched sensory organ, because male mites often use their front legs like antenna while searching for females. To test this hypothesis we painted the front legs of the males with nail polish; in the control group we covered half of the idiosoma of the males with the same substance in order to see whether the nail polish has any toxic effects to the mites. The control males with the painted idiosoma showed in a laboratory bioassay the same distinct copulation behaviour as untreated males. In contrast, the males with the painted front legs did not show copulation attempts any more ( $p < 0.01$ , ANOVA). Hence, the sensory pit organ must be responsible for the perception of the female sex pheromone. Furthermore we used scanning electron microscopy to characterise the male sensory pit organ. The identification and isolation of the receptor responsible for the perception of the sex pheromone could improve approaches for the disruption of the mating behaviour of male mites as part of specific pest control.

**P5.23 Effect of an immune priming in honeybees with *Paenibacillus larvae*.**

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In recent years studies have documented that honeybees have only two thirds of the genes involved in immune defence at their disposal as compared to other invertebrates. Nevertheless, immune activation in honeybees also leads, as reported for other species of insects, to an up-regulation of genes codifying for several antimicrobial peptides that are produced during an infectious process. The implications of this genetic condition for the development of a more complex immune response as reported for other invertebrates make further investigation in honeybees worthwhile.

Here, we investigated the existence of a priming effect in honeybees upon a first bacterial exposure to *Paenibacillus larvae*, the causing agent of American foulbrood. Priming is a characteristic of the immune system that leads to a stronger immune response upon a second exposure to the pathogen as a consequence of previous immunological insult. In two independent experiments winter bees and baby bees were exposed to a dose of heat-killed bacteria of *P. larvae* or ringler solution. One week later individuals were injected with either ringler solution or a certain dose of viable bacteria and the survival rate was monitored during the next 6 days post infection. Hemolymph samples from individuals were taken and plated onto agar plates to confirm mortality due to *P. larvae*. Preliminary results indicate an increased probability of survival for previously primed bees on day 4 and 5. Further investigations are ongoing.

**P5.24 Pathogen diversity in a social context – genetic variation of *Ascosphaera apis* in chalkbrood outbreaks.**

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One of the evolutionary hypotheses for explaining multiple mating within eusocial Hymenoptera, such as the honeybee, is that high genetic diversity within a colony, among other advantages, can mitigate pathogen and parasite pressure. However, it has also been shown that disease resistance in the honeybee is influenced, in part, by the genotype of the pathogen. *Ascosphaera apis* is a common honeybee disease that infects the larvae. Infection occurs through the gut by spores ingested with larval food. *A. apis*, which only reproduces sexually, can potentially have a high level of genetic diversity in comparison to other common insect-pathogenic fungi that mainly reproduce asexually. Thus, in order to understand the disease dynamics it is important to investigate whether a disease outbreak is caused by multiple pathogen strains or a single strain. To test this we will produce single-spore fungal isolates from mummies originating from the same colonies, and look for sequence diversity using *A. apis* specific primers for three polymorphic loci.

**P5.25** Are mutations in sodium channel encoding gene responsible for pyrethroid resistance in *Varroa destructor*?

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Sodium channels (SC) in mites and insects are target sites of pesticides including pyrethroids. Some point mutations of SC gene change structural conformation of the protein affecting sensitivity to pesticides. We prepared specific primers for amplification of two fragments in SC gene according to the mRNA sequence. The primers were employed to screen different populations of *V. destructor* collected in the Czech Republic (CZ) in 2011.

The larger amplified fragment encompassed 1970 base pair (bp). In all samples, we found three point mutations G (4743)→A: silent, V1581, G (4801)→ A: conservative substitution V(1601) →I, and G (5246)→A: S(1749)→N. The last mutation has been reported previously. Comparison of the smaller fragment of 370 bp showed two different situations. A point mutation separated the population of *V. destructor* in CZ to two types. (1) Maslovice-type samples of *V. destructor* had mutation G(3156)→C: L(1052)→F (codon ttg → ttc). (2) Breclav-type samples harbored the change G(3156)→C combined with additional silencing mutation T(3154)→C in the same codon, resulting in conservation of L(1052) (codon ttg → ctc).

Maslovice-type mutation occur in *V. destructor* infesting honeybee colonies without known problems with pyrethroid treatments, while Breclav-type mutation occur in localities, which are suspicious for *V. destructor* populations resistant to pyrethroids.

Project is supported by the Ministry of Agriculture, grant No.QI111A119.

**P5.26** Changing prevalence of *Nosema apis* and *Nosema ceranae* in the Czech Republic. Who win?

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Microsporidian pathogens *Nosema* spp. in honeybees are in focus after the description and introduction of *Nosema ceranae* in the European honeybee *Apis mellifera*. Growing prevalence of *N. ceranae* is described in many countries. *Nosema apis* is almost “extinct” in regions with warmer climate. We focused on *Nosema* spp. prevalence during 3-year survey of honeybee colonies in the Czech Republic. Winter dead bees from the hive bottom of commercial queen breeders were investigated. According our previous study, we suggest that microscopical discrimination of *N. apis* and *N. ceranae* is possible for monitoring purposes. In the first season, winter 2009/10, prevalence of *Nosema* spp. was 24,9%. In the proportion of positive samples, 68,5% was *N. apis*, 21,9% *N. ceranae* and 9,6% mixed infection.

Last season, winter 2011/12, prevalence of *Nosema* spp. was 36,1%. However, proportion of positive samples showed in 18,3% *N. apis*, 65,3% *N. ceranae* and 16,0% mixed infection respectively. Our preliminary data describe fast growing prevalence of *N. ceranae* at the expense of *N. apis*.

**P5.27** Effects of Fastac 50 EC on bumblebee *Bombus terrestris* L. respiration: DGE disappearance does not lead to increasing water loss.

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Sublethal effects of pesticides in insects can be observed through physiological changes, which are commonly estimated by metabolic rate and respiratory patterns, more precisely by the patterns of discontinuous gas-exchange (DGE) cycles. The aim of the present research was to study the effect of some low concentrations of Fastac 50 EC on the cycles of CO<sub>2</sub> release and respiratory water loss rates (WLR) in bumblebee *Bombus terrestris* foragers. Bumblebees were dipped into 0.004% and 0.002% Fastac 50 EC solution. Flow-through respirometry was used to record the respiration and WLR three hours before and after the treatment. The respirometry was combined with infrared actography to enable simultaneous recording of abdominal movements. Our results show that Fastac 50 EC has adose dependent after-effect on bumblebee respiratory rhythms and muscle activity but does not affect WLR. Treatment with 0.004% Fastac 50 EC solution resulted in disappearance of the respiration cycles; also the lifespan of treated bumblebees was significantly shorter. Treatment with 0.002% Fastac 50 EC solution had no significant effect on respiration patterns or longevity. We found no evidence for the DGE cycles functioning.

**P5.28** Impact of *Spiroplasma apis* on artificially infected *Bombus terrestris*. 

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*Spiroplasma apis* is known as honeybee pathogens. They can be detected by unspecific methodologies like culturing or dark field microscopy and specifically by multiplex PCR techniques. We conducted a small survey on common European bumblebee species and found *S. apis* in *Bombus pratorum* and *Bombus pascuorum*.

Spiroplasmas are mostly known as commensals or as symbionts, however nothing is known about its nature in bumblebees. In order to study the interaction between bumblebees and spiroplasmas we needed to develop an artificial infection technique. We were able to force feed *B. terrestris*, our model species, different concentration of the *S. apis*. However no infection in the hemolymph was observed, while it was detected to reside in the gut 2 until 4 days after inoculation.

We can conclude that the lab strain of *S. apis* (ATCC-33834) is not able to colonise the gut and lost its ability in to infect the hemolymph of bumblebees.

**P5.29** Gene expression in ventriculi of bees infected by *Nosema apis* and *Nosema ceranae* Microsporidia.

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Nosemosis is the most widespread of adult bee diseases and causes economic losses to beekeepers (OIE, 2008; Giersch et al., 2009; Heintz et al., 2011). To date, two microsporidian species are known to be involved with this disease, infecting honeybees worldwide: *Nosema apis* and *Nosema ceranae*. Both are intracellular obligate parasites of epithelial cells of the honeybee ventricle. So far, the pathogenic mechanisms in this tissue have not been described in detail. In this work, RNA from ventriculi of bees experimentally infected either by *N. apis* or *N. ceranae* was extracted and the expression of ten genes related to apoptosis, six genes related to cellular cycle, six genes related to mitochondrial activity and two genes related to hormone levels was studied in cDNA. To do this, specific primers and TaqMan® probes were designed. Infection success was determined using RT-PCR in bee ampoules. Four housekeeping genes (GAPDH, EF, B-ACTINE and 18s) were evaluated to select the most appropriate ones for analysis. The efficiency of every reaction was determined to study the gene regulation. The data show an interaction of microsporidia infection and the infected ventricular cells, both in apoptosis response and cell cycle.

**P5.30** Development of one step RT- qPCR for detection of deformed wing virus from different bee products.

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Deformed wing virus (DWV), a picorna-like positive-stranded RNA virus, is a honeybee viral pathogen either persisting as an unapparent infection or resulting in wing deformity. DWV is considered one of the most serious pathogens vectored by *Varroa destructor* and plays a major role in colony collapse in the course of *Varroa* infestation. DWV is broadly distributed both in bee colonies and in *V. destructor* populations, with a much greater prevalence during autumn. A quantitative one step RT PCR with Taqman probe was developed to detect the virus in *Apis mellifera* and in different bee products such as propolis, wax, honey, royal jelly and pollen. Based on 137 partial sequences and four complete genomes available on GeneBank, primers and probe were designed within conserved regions of non-structural viral proteins.

With this experimental approach we were able to detect the virus not only in head, thorax and abdomen of symptomatic and asymptomatic bees but also in different bee products. Viral RNA was extracted from propolis, wax, honey, royal jelly and pollen stored in the hive. All samples resulted positive to DWV, while wax resulted always negative. Pollen pellets collected directly from forager bees, or directly sampled from hive resulted positive.

**P5.31** *Nosema* in Mexican bees.

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The microsporidian *Nosema apis* is a long-known pathogen of *Apis mellifera* throughout the native and introduced distribution of its host, whereas *Nosema ceranae* seems to be an exotic pathogen from East Asia that has spread throughout the range of *A. mellifera* in the last 15 years. *Nosema ceranae* has also been detected in bumblebees (*Bombus* spp.) from South America and Asia. We sampled non-native honeybees (*A. mellifera*) and native stingless bees (*Melipona beecheii*) from the same location in Yucatan, Mexico, for the presence of *Nosema* spp. using both visual detection of spores and a multiplex PCR assay based on DNA extracts that can differentiate between *N. apis* and *N. ceranae*. Out of 20 *A. mellifera* colonies sampled, 19 were clearly infected with *N. ceranae* whereas *N. apis* was not detected. Eighteen of 20 *M. beecheii* colonies from the same locality showed no evidence of *Nosema* spp. If *N. ceranae* infects species of bee other than the honeybee, then its incidence in *M. beecheii* of Yucatan is clearly low.

**P5.32** Comparison of virus loads of varroa-infested and varroa-free honeybee populations in the UK.

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The parasitic *Varroa* mite (*Varroa destructor*) is a common pest of honeybees (*Apis mellifera*) and it has been associated with the occurrence of several honeybee diseases. Its role as a vector of several bee viruses and its ability to transmit them successfully has been well documented, suggesting that *Varroa* infestation poses a serious threat to colony health. In order to investigate the role of *Varroa* mites in honeybee virus loads, samples of adult bees were taken during the summer of 2010 from managed honeybee colonies from four locations in the UK: from two mainland populations where *Varroa* infestation is well known and from two island populations in close proximity with respect to each mainland population, yet in which *Varroa* mites have never been observed. Adult bees were screened for the presence of four honeybee viruses (DWV, BQCV, ABPV, KBV) and viral loads were estimated by RT-qPCR. Comparisons between samples collected from mainland (*Varroa* present) and their close island populations (*Varroa* free) allow us to evaluate the impact of *Varroa* on virus occurrence and titre levels. Our analyses demonstrates high incidence of DWV and BQCV in the mainland populations, in contrast to the island populations where the prevalence and/or abundance of viruses seems to be significantly lower or even completely absent.

**P5.33** Efficacy of sampling period for determination of *Nosema apis* and *Nosema ceranae* prevalence.

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*Nosema apis* and *Nosema ceranae* are potentially damaging pathogen of the honeybee that has spread across the world in years. Many scientists have reported that *N. ceranae* is more virulent and possibly there is a replacement between *N. apis* and *N. ceranae* in different countries. At least 40% of honeybee colonies from different provinces of Turkey have been sampled routinely and analysed for *Nosema* spp. by both spore counting and PCR every year in bee health laboratory. In this study we investigated if there is a real replacement between *N. apis* and *N. ceranae* or if sampling period can affect the results of surveys?

By this aim, honeybee samples were collected from the same colonies in the provinces (20 different provinces) in two different sampling times in the same year. According to our results, whilst during the first sampling period (spring and autumn) we can detect *N. ceranae* and also *N. apis* in different provinces, we can not find any *N. apis* spores in the second sampling period (summer and winter), even in the same colonies and regions where *N. apis* was found before. All positive samples were recorded as *N. ceranae* in the second sampling period. To conclude, the reported high level virulence of *N. ceranae* may be the result of *N. ceranae* activity in every season contrast to *N. apis* is in only spring and autumn. Thereby sampling period must be more considered for *Nosema* surveys.

**P5.34** Disentangling the pattern of *Crithidia bombi* prevalence in *Bombus* spp.

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Animal-mediated pollination is a key ecosystem service crucial to human welfare. Bumblebees are social insects and among the most important pollinators of both wild and cultivated plants. Nowadays, they are not only threatened by human-induced environmental changes (e.g. habitat fragmentation) but also by many parasites. One of those parasites is *Crithidia bombi*, a flagellated protozoan (Trypanosomatidae) living in the gut of *Bombus* spp. Despite the challenging complexity of host-parasite interactions this study aims to identify key aspects contributing to the epidemiological process in order to understand why some individuals appear to be particularly prone to infection compared to others. Therefore natural populations of three common bumblebee species (*Bombus lapidarius*, *B. pascuorum* and *B. terrestris*) were sampled throughout the season at ten sites with three subsites each. Using microsatellite markers, workers and drones were genotyped for kinship reconstruction and scanned for *C. bombi* (prevalence, type of infection (single vs. multiple), intensity of infection, genotype). The relative contributions of host species identity, sex, sampling period, habitat quality, species assemblage and colony density of the host as well as the genetic diversity of both host and parasite towards the pattern of *C. bombi* prevalence are discussed.



**P5.35** European foulbrood control strategies in central Italy.

Pietropaoli M, Bassi S, Carra E, Cersini A, Puccica S, Antognetti V, Giacomelli A, Milito M, Pizzariello M, Palazzetti M, Micarelli G, Scholl F, Formato G\*

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It is known that *Apis mellifera* normally presents good healing abilities referring to *Melissococcus plutonius*, bacterium responsible for European foulbrood, even though in specific geographic areas, this pathogen can show an unusual pathogenicity and virulence like it happened during Spring 2011 in one outbreak in Viterbo (Central Italy) province. The apiary presented 33 hives severely infected with European foulbrood. These hives were divided into four experimental groups considering the strength of the families and the infection level: one group (nine hives) was treated with oxytetracycline, another group (eight hives) was treated with the queen removal, another group (eight hives) was treated with the shook swarm method and, finally, the last group (eight hives) did not receive any treatment (control). This study aims to compare the success of the above mentioned practices against the *M. plutonius*. Health status of the families was controlled: 1 month, 2 months, 5 months and one year after the treatment application adopting visual inspection of each single hive to search for the symptoms of the disease, field test of EFB\* (Vita Europe), cultural methods (Agar Bailey medium) and PCR techniques. Considering the relapse of the pathology and the mortality rate in each treatment group, the antibiotic (oxytetracycline) demonstrated to be the best long-term strategy for the control of the European foulbrood.

**P5.36** Neonicotinoid insecticides residues in bee collected plant materials from chemical protection rape and maize crops.

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The study was performed in 2010, in two experimental rape fields and in 2011, in two experimental maize fields. In the first field rape from seeds treated with Chinook Plus 500 FS (a.s. imidacloprid) and in the second field rape from seeds treated with Cruiser OSR 322 FS (a.s. thiamethoxam) was planted. In the first field maize from seeds treated with Gaucho 600 FS (a.s. imidacloprid) and in the second from seeds treated with 480 FS Modesto (a.s. clothianidin) was planted. The ten bee colonies were transported to each rape and maize plantations and stayed there until the end of flowering. The third group of bee colonies (control) was located in the area free of these plants. Before beginning of the experiment biological status (strength of colonies, brood area) and state of health of bee colonies was evaluated. During the whole time of the experiment bee mortality was monitored every week and parameters showing their strength and development were evaluated every 3 weeks. Residues of applied pesticides (neonicotinoids) were detected in samples of nectar collected directly from rape flowers and from deposited in comb cells, samples of rape honey, samples of rape and maize pollen from pollen traps, samples of bee bread deposited in comb cells and in bee samples. Palynological analyses were performed for to confirm the origin of investigated samples. In collected samples following neonicotinoids were detected: imidacloprid and its metabolites (M01, M06), clothianidin, thiamethoxam, thiacloprid and acetamiprid.

**P5.37** Vitality tests on bee colonies after treatment with HopGuard®.

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HopGuard® strips can be used as a treatment against *Varroa destructor*. Over recent years we have documented a high efficacy along with a good bee tolerance in large colony field trials. In the USA HopGuard® is used throughout the summer also during the breeding period. Observations by beekeepers showed an increased vitality of the treated hives. In order to investigate this subjective impression we evaluated the vitality of a honeybee colony in an observation hive after a HopGuard® treatment. Several parameters such as activity, nursing, cleaning behaviour and longevity were observed in-hive over a period of 40 days. Prior to the treatment 500 recently emerged bees were individually marked. 250 bees were then introduced in a HopGuard® treated colony placed in an observation hive on two frames. The remaining 250 bees were introduced into a non-treated control colony, equally equipped with two frames. The treatment with HopGuard® had no negative effects. Bees in the test colony even showed improved vitality with increased activity, nursing, cleaning behaviour and longevity. In addition to the results on efficacy and bee tolerance from field trials the high vitality of bees under sub-lethal dosages of HopGuard® amends the documentation needed for a registration of the product as a drug for honeybees in Europe.

**P5.38** Results of a honeybee health monitoring project in Tuscany (Italy): second year of activities.

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Tuscany Region has supported a two years project (2009-2011), named "APENET-Toscana", to monitor honeybee health. This project was coordinated by the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, in collaboration with regional beekeepers associations.

This contribution is aimed to present the results of the 2<sup>nd</sup> year (2010-2011): 200 hives selected in 20 apiaries have been monitored every 3 months to check their health status. Data on mortality, depopulation, strength, presence of diseases and anomalous behaviours were analysed. Samples of adult bees (536), wax (71) and pollen (56) were collected in order to investigate: viruses, *Nosema* spp., pollen quality and pesticides.

Average annual mortality resulted of 24,34%, with a winter mortality of 21,36%. Different viruses prevalence have been observed within all the monitored apiaries after RT-PCR analysis: DWV (87,5%), SBV (54,5%), CBPV (41,1%), ABPV (26,8%), BQCV (23,2%), IAPV (7,1%) and KBV (1,8%). *Nosema apis* could never be found and *Nosema ceranae* was detected in 40,11% of the samples, after PCR-RFLP analysis. It never could be possible to verify cases of acute toxicity due to pesticides. In wax samples, six different pesticide residues have been found: kresoxim-methyl (10%), chlorfenvinfos (5%), coumaphos (5%), endosulfan sulfate (1,5%), bifenthrin (1,5%) and procymidone (1,5%).

**P5.39** The prevalence of honeybee pathogens in the Flemish part of Belgium screened using a MLPA method.

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The European honeybee, *Apis mellifera*, is an important pollinator of many crops and fruits, and of major importance to agriculture. Since 2006 however, bee populations have shown an unusual decline in North America as well as in many European countries. Many factors have been suggested as possible causes of this decline, including pesticides and diseases. To clarify the role of specific disease agents, however, it is crucial that they can be monitored in a fast, convenient and cost-effective way. Previously, almost all past European surveys have concentrated on viruses, using reverse transcription PCR detection methods to detect specific bee viruses. Nevertheless, the downside of this method is that it is very expensive and rather laborious, since every sample should be checked separately for at least six different viruses. Hence, the sample size of these surveys was generally rather limited. Recently, a MLPA based method was developed in our laboratory which allows one to detect several honeybee viruses simultaneously in a single run at a fraction of the cost of RT PCR based methods. To validate the method we report data on the prevalence of some of the most common honeybee viruses in the Flemish part of Belgium obtained using MLPA. In addition, we also used the method to survey the presence of some emerging honeybee pathogens, including some newly discovered viruses from the USA.

**P5.40** Diversity of *Nosema ceranae*: multiplicity in unity.

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The role of *Nosema ceranae*, a highly prevalent parasite of the honeybee, in the colony losses worldwide is debated. While it has been related to colony depopulation, it has also been repeatedly detected in healthy colonies. Several hypotheses may be proposed to explain such differential virulence, including the existence of several parasitic variants. In order to evaluate the diversity of *N. ceranae*, we explored new molecular data with intriguing variation patterns.

**P5.41** Effect of time and temperature on viability of *Nosema* spp. spores determined by flow cytometry.



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Resistance or susceptibility to the temperature of *Nosema ceranae* and *Nosema apis* is an important factor in the conservation of the spores in the laboratory. On the other hand the temperature can be a key factor in the transmission and maintenance in the environment of these two parasites. In this work, flow cytometry was used to assess the effects of various temperatures and different periods of times on the viability (determined as membrane integrity) of spores of both *Nosema* spp. To do this, from an initial stock solution of fresh spores (Percoll purified) were done 300 replicates, for each species, that were distributed in four groups. Each group was maintained at 33°C, 4°C, -20°C or room temperature (mean 25°C). Dead spores were stained with Propidium Iodide, to identify dead cells, and counted using a flow cytometry (FacsCalibur, Becton Dickinson; Argon laser 488nm). During the experimental period, six months, the viability was assessed in twelve different points of time. A clear and differential effect of temperature was observed in viability of both *Nosema* spp. spores. Under our experimental conditions, along six months of exposition, *N. ceranae* spores showed more resistance to warm temperatures (33°C) than *N. apis*. However when freezing (-20°C), *N. apis* showed a much higher level of resistance than *N. ceranae* spores. Otherwise, both at room temperature and at 4°C, viability losses were the lower and similar in both species.

**P5.42** The German bee monitoring (DeBiMo): current status and results 2011/2012.

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The German bee monitoring has been established in 2004 by a board of apicultural State Institutes, beekeeper and farmer organisations and chemical companies. Since 2010 the project is exclusively financed by a governmental and State funding. Field work and laboratory analysis are supervised and performed by seven bee research institutes. The network focuses on the monitoring of colony winter losses and their correlation with bee diseases, pesticide residues and beekeeping management. The DeBiMo is supplemented by specific projects on chronic and synergistic effects of these factors conducted by the institutes with respective expertises.

We will present data on the overwintering 2011/2012 including analysis of *Nosema* ssp. infection, infection with bee viruses, *Varroa destructor* infestation and the current residue analysis. These results confirm previous analyses which consider the *Varroa* infestation in late autumn as the crucial factor for colony winter losses. Current approaches on multifactorial effects on the health of honeybee colonies are discussed.

Supported by the German Federal Office for Agriculture and Food (BLE): Ref.-No.: 2810SE001-7.

**P5.43** Dose and duration - what makes the poison? Performance of thiacloprid exposed bee colonies in a long-term trial.

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Residues of the insecticide thiacloprid can be found in nectar and pollen. Its bee toxicity is low but there are concerns related to possible long-term effects. With the goal to investigate potential chronic effects, 30 colonies were started as shook swarms in July 2011, divided in three groups (K, T1, T2) of ten colonies each and arranged in an experimental yard. From July to October control group K was fed with 25L pure syrup per colony. The treatments T1 and T2 were provided with syrup spiked with 200ppb or 2000ppb thiacloprid respectively. Colonies were weighed and number of bees were estimated in intervals of 21 days according to the Liebefeld method. Dead bees were counted regularly. Infestation with *Varroa* and *Nosema* was measured. During the feeding period 2011 the groups were statistically indifferent for the above mentioned parameters (general linear model, repeated measures). By contrast, the number of brood cells differed significantly ( $p < 0.05$ ). During winter no colony died. The number of dead winter bees on the bottom boards were similar across the groups ( $p > 0.05$ ). In 2012, T2 was the least productive group, but statistical evaluation did not identify significant differences for the parameters bees, brood, weight and queen losses. These preliminary observations do not support the hypothesis of an impact of a chronic exposure on colony vitality. However, final conclusions will be drawn in 2014, only, after two additional runs in 2012 and 2013.

**P5.44** Usage of organic preparations against *Varroa*.

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There is an organic apiary in apiculture division in Pulawy, Poland. So, due to regulation 834/2007 we can't use chemical preparations against *Varroa*. Only organic preparations are acceptable. This way in the years 2007-2011 we tested a few organic anti-*Varroa* agents: oxalic acid, formic acid and Api Life Var (based on thymol and essential oils). Every preparation was used after honey harvest. The efficacy of these substances were compared. The best anti-*Varroa* results were observed when Api Life Var in August was used (twice – for the first time two plates per colony for two weeks and then one plate for the next week) and the next oxalic acid (3,5%) in November was applied when there was no brood in the colonies. The efficacy of oxalic acid was on the level more than 95%. It is important to feed the colonies in 70% before using Api Life Var because intensive aroma of it initiated the robberies in the apiary. In 2007 5,2% oxalic acid was used and it was to high concentration of solution because colonies started to weak in the autumn and their strength after wintering was lower than usually. The worst results were during use of 65% formic acid in the brood period (efficacy only on the level 74%). Except for the fact that organic preparations must be used in every organic apiary, they could be used in conventional apiary as well and thanks to this procedure they help to reduce concentration of chemical substances in bee colonies and in bee products.

**P5.45 Colony losses and their potential causes in Israel.**

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For the last four years we evaluated and characterised colony losses in Israel through beekeepers' surveys and regular seasonal monitoring of selected hives at representative apiaries. Beekeepers' survey includes questionnaires evaluating annual and winter losses. Parallel annual monitoring of at least 100 hives at five apiaries is conducted since 2009 up to date. In 2010, the emphasis was put on the impact of *Varroa* infestation on the outbreak of diseases and colony collapse while in 2011-2012 it concentrated on the role played by *Nosema*, in treated and untreated colonies.

Our survey data represented 34-50% of total colonies, but only 9-15% of the beekeepers, and indicated that the overall level of colony losses was below 20%. However, in the last two years we experience some increase in the percentage of beekeepers experiencing losses above 20% and in frequency of CCD like losses. Most of losses occurred in summer and in autumn. *Varroa* and its associated viruses were found to be the most significant causes of colony decline. Regarding *Nosema ceranae*, the level of hive infection increases towards autumn, but its effect on the condition of the colonies varies between apiaries, research about its role in colony losses in Israel is still in progress.

**P5.46 A rapid and cost efficient assay to detect multiple honeybee pathogens.**

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The decline in densities of honeybee colonies is a major issue for pollination services in natural ecosystems and agricultural landscapes in numerous regions in the world. As there have been serious colony losses due to honeybee pathogens, the detection and monitoring of such pathogens is crucial. Apart from some invertebrate parasites, such as different mites, wax moth or small hive beetle, there are numerous microbial organisms exploiting the honeybee as a host. Although several molecular assays were published to detect those bacteria, fungi or viruses, a comprehensive, reliable and efficient test is still needed for rapid large scale screens. We developed a rapid and cost-efficient system to detect numerous pathogens in single reactions. The PCR based test has been optimised for high efficiency regarding costs, time and workload.

We aim at detecting eleven viruses in 2-4 multiplex reactions, of which one, containing some of the most common viruses, is established. Using our approach, an extracted RNA sample can be screened and analysed within 3 hours. Furthermore we investigated the potential to obtain semi-quantitative data. In addition to viruses, we also work on an assay to detect several bacterial and fungal pathogens in a similar fashion.

**P5.47 How widespread is *Nosema* in honeybees, *Apis* spp., in Thailand?**

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Beekeeping is an important occupation for Thai farmers, especially those in Northeastern Thailand where *Apis florea* is regularly harvested from the wild as a source of honey. Recently, *Nosema ceranae* infections have been found in honeybees in the North and the Center of Thailand, including in *Apis florea*. Here, we surveyed *Apis* for *Nosema* infection in 70 colonies of wild and feral honeybees in Northeast Thailand by visual inspection of crushed bee abdomens and by PCR of DNA extracts from crushed bees. Colonies were distributed across nine areas and sampled bees were stored in ethanol. There were four species of *Apis* collected: 49 colonies of *Apis florea*, 14 of *A. dorsata*, five of *A. cerana*, and two of *A. mellifera*. Multiplex PCR used three primer pairs that were designed to amplify *N. apis*, *N. bombi* and *N. ceranae*. There was no *Nosema* infection detected in honeybees from these areas. Clearly, *Nosema* infection is rare in NE Thai honeybees or the native honeybees (*A. florea*, *A. dorsata*, and *A. cerana*) are resistant to *Nosema* spp. infection.

**P5.48 Differential susceptibility of drone versus worker honeybees towards infections with *Nosema ceranae* and black queen cell virus?**

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In honeybee health research, the vast majority of studies focuses on workers, although sexuals play a key role for colony functionality. In particular, males have been neglected. Here, we evaluated the susceptibility of drones and workers to infections with the microsporidian *Nosema ceranae* and/or black queen cell virus (BQCV) in a fully-crossed hoarding cage experiment. While the association between BQCV and *N. apis* has been reported, it is not known if such a relationship also exists between this virus and *N. ceranae*. Callow individuals were inoculated with *N. ceranae* spores and with BQCV seven days later, hypothesising that *Nosema* parasitism may damage the midgut, thereby promoting virus infections. In addition to monitoring mortality for 14 days, we compared infection levels of both pathogens. The data suggest that oral inoculations of both groups with *N. ceranae* and/or BQCV was successful. Unlike workers that showed no differences in mortality among infections, *Nosema*-infected drones showed a significantly higher mortality, suggesting a higher susceptibility of males. Further results of the study will be reported and discussed.

**P5.49 Experience from the four-year investigation of winter colony losses in Poland.**

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In Poland there are about 45 thousand beekeepers and 1210 thousand honeybee colonies. Since 2008 the investigation of winter colony losses has been performed, since 2009 -using the COLOSS questionnaire. In 2009 the questionnaire was published in two most popular Polish beekeeping journals and was disseminated during different beekeeping meetings. Also internet version was accessible. Later, the questionnaire was also disseminated by the "Polish Beekeeping Association" and sent to the different regional associations and to available e-mails of professional beekeepers. In 2008 the participation of the beekeepers in the survey (anonymous participation) was 1.0%, and in the following years it was 0.8%, 0.8% and 1.6%. The dissemination of questionnaires during beekeeping meetings, caused disproportion in participation of beekeepers from different regions in monitoring. We have noted a constant increase in the activity of beekeeping associations in the survey. Only 14% of respondents participated in the survey through the internet. We conclude that in such a big country as Poland, with so many beekeepers and in the face of their low participation in internet surveys it is very difficult to obtain a satisfactory survey with non-randomised sampling, even if multi-mode data collection is used.

The estimated winter colony losses in Poland were: in 2007/2008 – 15.3%, 2008/2009 – 11.1%, 2009/2010 – 15.3%, 2010/2011 – 18.1%, although in some regions they exceeded even 30%.

**P5.50 Recommendations from the COLOSS BEEBOOK for maintaining adult workers in laboratory cages.**

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Adult honeybees are maintained *in vitro* in laboratory cages for a variety of purposes. For example, researchers may wish to perform experiments on honeybees caged in groups or individually to study aspects of parasitology, toxicology, or physiology under highly controlled conditions, or they may cage whole frames to obtain known age cohorts for future study. Regardless of purpose, researchers must control for a number of variables, ranging from selection of study subjects (e.g. parasite and host strains) to experimental environment (e.g. temperature, humidity, nutrition). Although decisions made by researchers may not necessarily jeopardise the scientific rigour of an experiment, they may profoundly affect results, and may make comparisons with similar, yet independent studies more difficult. Focusing primarily on workers, here we discuss recommendations for maintaining adults in laboratory cages and as well as knowledge gaps that require further attention. Additionally, we provide broad recommendations for experimental design and statistical analyses of experiments employing caged honeybees. The ultimate goal of this, and of all COLOSS BEEBOOK chapters, is not to stifle science with restrictions, but rather to provide researchers with the appropriate tools to perform coordinated experiments that will build upon our current understanding of honeybees.

**P5.51 Phylogenetic analysis of Polish isolates of deformed wing virus and acute bee paralysis virus.**

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Nowadays many honeybee colonies decline in Poland. There are few factors that could play a significant role in those morbidities. Honeybee viruses are one of those agents. Among honeybee viruses that has been investigated until now, deformed wing virus (DWV) and acute bee paralysis virus (ABPV) are the most common viruses in Polish apiaries and among investigated bee viruses they probably have the highest impact on winter bee mortality in Poland. The goal of this study was phylogenetic analysis of isolates of DWV and ABPV in order to obtain more details about Polish strains of these viruses. Investigated strains were isolated in the period 2008-2010 from apiaries with diverse colony losses and situated in different geographical regions of Poland. Five bees from each sample were homogenised and used for RNA extraction and afterwards for RT-PCR and electrophoresis. Specificity of the PCR products was verified by sequencing and compared to isolates of ABPV and DWV available on GenBank database. The DNA sequences were assembled, compiled and edited. Phylogenetic analysis of the most adequate sequences with three method: „Neighbor-joining”, „Maximum parsimony” and „Minimum evolution” was conducted. Three phylogenetic trees with a data set of amplified fragment for ABPV and DWV were obtained and their topology was compared. Nucleotide sequence identity of Polish and other isolates from GenBank was also compared.

P6.1

**Genetic-morphological variability of *Melipona subnitida* (Apidae, Meliponini) in northeast Brazil.**

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The *Melipona subnitida* bee is an endemic stingless bee to northeastern Brazil. In spite of its importance to local people, these bees are currently largely threatened due to the destruction of their habitat and their natural nests for honey collection. Nevertheless, population studies of stingless bee species are still very scarce, as well as the urgency in understanding the structure and dynamics of their populations for monitoring and species conservation. Under this scenario, we aimed to evaluate the variability of *M. subnitida* in different localities in northeastern Brazil through geometric morphometrics of the forewing and sequencing of COI mitochondrial gene fragment. To analyse the pattern of wing venation, we used approximately ten workers per colony, and eleven landmarks were plotted on each wing.

The results showed groups structures with separation statistically significant ( $\alpha < 0,0001$ ). The cross-validation test correctly identified 88,89% of individuals within their respective areas. Molecular analysis identified eleven haplotypes and presence of high number of exclusive haplotypes among the populations. The nucleotide diversity ( $\pi$ ) of 0,00543 and haplotype diversity (Hd) of 0,79. Both morphological and molecular results indicated that variability among the sampled populations is related both to the environment in which samples were collected as to the geographical distance between these locations, indicating the existence of locally adapted ecotypes.

P6.2

**Effect of linkage disequilibrium on inferences of population structure and introgression of Iberian and black honeybees.**

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Identification of population structure, a primary goal in population genetics, is easily performed because there is a number of methods available, implemented by user-friendly software packages. However, the user must be cautious when inferring population structure because spurious results may be obtained when there is strong linkage disequilibrium. With recent development of high-density SNPs we have now more power to interrogate the honeybee genome. However, the greater the number of loci genotyped the greater the chance of scoring loci that are linked. In addition, events such as population bottleneck, small effective population size, genetic drift, and admixture may also generate strong linkage disequilibrium. According to Kaeuffer et al. (2007), correlation rLD is the best way to deal with linkage disequilibrium. These authors recommend removing loci with rLD higher than 0.5 when inferring structure. In this study we used the GoldenGate Assay of Illumina to genotype over 1221 loci in individuals sampled from populations of *A. m. iberiensis* and *A. m. mellifera*. In this dataset we used the genetic distance between SNPs and rLD to test the effect of linkage in the number of clusters and the introgression level inferred by the clustering method implemented in the software STRUCTURE.

P6.3

**Introgression of lineage C honeybees into black honeybees: a genome-wide estimation using SNP.**

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The black honeybee, *Apis mellifera mellifera* L., is probably the honeybee subspecies more threatened by introgression from foreign subspecies, specially lineage C *A. m. carnica* and *A. m. ligustica*. In fact, in some areas of its distributional range, intensive beekeeping with foreign subspecies has driven *A. m. mellifera* populations to nearly replacement. While massive and repeated introductions may lead to loss of native genetic patrimony, a low level of gene flow can also be detrimental because it may compromise honeybee survival and local adaptation by disrupting co-evolved gene complexes. Assessing levels of introgression is an important activity in breeding programmes, especially when conservation of native races is a major concern. Previous surveys of *A. m. mellifera* populations estimated the introgression of lineage C honeybees by using mtDNA and microsatellites markers. In this presentation we used both mtDNA (sequence data of the tRNA<sub>Leu</sub>-cox2 intergenic region) and over 1245 SNPs to ascertain introgression levels of *A. m. carnica* and *A. m. ligustica* in *A. m. mellifera* populations (some included in conservation programmes) sampled in France, Switzerland, Denmark, Holland, Norway, England, and Scotland. We used different model-based approaches, implemented by different software applications (Structure, Admixture, NewHybrids). We found varying levels of introgression ranging from less than 3% (in Norway) to over 65% (in France).

P6.4

**16s rDNA diversity of *Apis mellifera* subspecies in Turkey.**

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Mitochondrial DNA variation can be used to infer honeybee evolutionary relationships. In this study, the diversity of mitochondrial 16s rDNA region was investigated in 95 honeybees from ten different populations in Turkey which mainly consist *A. m. anatoliaca*, *A. m. caucasica* and *A. m. meda*. Samples were amplified by PCR and then subjected to RFLP pattern analysis using 18 restriction enzymes. A total of 10 restriction enzymes were found to have at least one recognition site in 16s rDNA region. Nucleotide polymorphisms were revealed using restriction enzymes Bsp143I, DraI and SspI in 16s rDNA. The polymorphisms were subsequently confirmed by direct DNA sequencing with sequences thereafter deposited in NCBI Genbank Database. Molecular phylogenetic analysis revealed that Konya/Sızma and Antalya/Elmalı populations were the most distant from all the other Turkish honeybee populations surveyed.

P6.5

**Population genetics of the rare halictid bee *Patellapis doleritica* of the Succulent Karoo biome of South Africa.**

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Investigating the population genetics of a species is an essential task for appropriate conservation management. It is especially relevant for species for which other biological information is lacking or which are considered either threatened or highly endemic. *Patellapis doleritica* is a recently described halictid bee (Timmermann and Kuhlmann 2009). The species is endemic to the winter rainfall area of the Succulent Karoo of South Africa and its range is unusually small, probably due to special nesting (soil) requirements. Moreover, the dispersal ability of the species seems to be limited by inclement winter weather. Based on these facts, a low level of gene flow leading to highly differentiated populations was hypothesised for *P. doleritica* populations. In order to test this hypothesis, the genetic diversity and genetic structure of eight populations of *P. doleritica* were investigated by genotyping 258 individuals at eight microsatellite loci. This study contributes significantly to the limited knowledge of *Patellapis* species by reporting the number of alleles per locus, expected and observed heterozygosity, allelic richness, F-statistics and isolation by distance. Furthermore, the results of this study may be relevant for conservation management of other pollinators of the winter rainfall areas of South Africa.

P6.6

**Biodiversity loss in Mesoamerica: investigating the impact of habitat fragmentation on bees.**

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Worldwide, the pollinator crisis threatens ecosystem functioning in terrestrial habitats. But in hyperdiverse regions such as Mesoamerica, the conservation status for the majority of pollinators is largely unknown. In conjunction with pesticide misuse, disease transmission and pollution, habitat loss and fragmentation have been suggested as leading causes of reduced pollinator populations. In addition, the sociobiology of a species may also modulate its response to changes in land-use, whereby social bee populations may be more vulnerable to fragmentation relative to solitary taxa. To investigate the impact of fragmentation on bee populations in this region, we collected 1089 samples of *Lasioglossum* sp. (subgenus *Dialictus*) from 24 sites across a gradient of land-use intensity on the Yucatán Peninsula, Mexico. The taxonomy of *Lasioglossum* from Mesoamerica remains unresolved; therefore as a first step towards future population genetic analyses, we delineate species in our sample using an integrated taxonomic approach incorporating morphological, mitochondrial and nuclear DNA data. Our morphological data suggest eight morphospecies, but these taxa were not supported by the mitochondrial DNA barcodes. Additional morphological characters and nuclear genetic data will be integrated into future taxonomic studies on these cryptic Yucatán *Lasioglossum* taxa so as to evaluate the impact of fragmentation on the conservation genetics of individual species.

P6.7

**Microsatellite analysis supports the existence of three cryptic species within *Bombus lucorum* s.l.**

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We have earlier shown that the common and widespread European bumblebee *Bombus lucorum* s.l. contains three distinct mitochondrial DNA lineages that likely represent three species, namely: *Bombus cryptarum*, *Bombus lucorum* s.s. and *Bombus magnus*. Though these three lineages exhibit considerable interspecific DNA sequence divergence at mitochondrial cytochrome oxidase I ( $\geq 2.3\%$ ) compared to intra-taxon sequence variability ( $\leq 1.3\%$ ), the use of this or any other mitochondrial DNA marker does not permit the detection of hybrids between taxa. We have now genotyped 304 sympatric members of the *B. lucorum* s.l. group from 11 localities spread across the island of Ireland at seven (nuclear) microsatellite loci. Our samples comprise 54 *B. cryptarum* females, 170 *B. lucorum* s.s. females and 80 *B. magnus* females, as defined using mtDNA COI sequences. Our multilocus genotypes fall into three discrete clusters that conform to the three mtDNA lineages *B. cryptarum*, *B. lucorum* s.s. and *B. magnus*. That there is an excellent fit of mitochondrial (COI sequence) to nuclear (microsatellite genotypic) genetic data supports the view that these three bumblebee taxa are reproductively isolated species. These data are also a vindication of species identity using so-called DNA barcodes.

P6.8

**No evidence of interallelic non-complementation in the gene complementary sex determiner of the honeybee.**

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The honeybee, *Apis mellifera*, belongs to the group of haplodiploid organisms in which females develop from fertilised eggs, whereas males under normal circumstances develop by parthenogenesis from unfertilised eggs. Development of progeny into either males or females results from the mechanism complementary sex determination in which differentiation occurs in response to the allelic composition of a single gene, complementary sex determiner (*csd*), which is believed to act as a molecular switch, initiating development into either of the sexes. Heterozygous allelic composition of the *csd* gene leads to females, whereas males develop as a result of hemizygoty. In cases where fertilised eggs are homozygous at *csd*, diploid drones which are predated by workers shortly after hatching develop. A remarkable number of sequences of *csd* have so far been identified, and by comparison with functional complementation systems in other species, alleles of *csd* are expected to cluster into different complementation groups. In this study a hypervariable region, which is suggested to be responsible for allelic specificity, was sequenced in a total of 4508 worker bees to identify combinations of functional complementary alleles. Surprisingly, our data show no clustering of alleles implying a lack of interallelic non-complementarity between different alleles of *csd*. This study is an important step towards an understanding of the mechanism leading to sexual differentiation in honeybees.

P6.9

### Biodiversity of *Apis mellifera* subspecies from Turkey revealed by sequence analysis of three mitochondrial regions.

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Mitochondrial DNA sequence variation can be used to infer honeybee evolutionary relationships. We examined DNA sequence diversity in the COI (or Cox1), 16s rDNA and ND5 gene segments of the mitochondrial genome in samples from 15 different populations in Turkey which is mainly populated with *A. m. anatoliaca*, *A. m. caucasica* and *A. m. meda*. Six, eleven and five novel haplotypes were found for the COI, 16s rDNA and ND5 gene segments, respectively. Neighbor-Joining (NJ) trees of the three gene segments were constructed with the published sequences of *Apis mellifera* haplotypes that are available in NCBI Genbank Database. The NJ dendrogram based on the COI sequences available in Genbank showed that Eastern European races were mainly clustered together, whereas the *Mellifera* and Iberian haplotypes were clustered far apart. The NJ tree drawn with the published 16s rDNA sequences have demonstrated that the different haplotypes cannot be discriminated exactly but all the haplotypes found in this study are clustered in a big branch with some of the published haplotypes such as *Apis mellifera* from Tashkent (AF214666) and *A. m. ligustica* (EF116868). ND5 region has been studied less than the other mitochondrial regions. The NJ dendrogram of the published ND5 sequences showed that that these races cannot be discriminated since all of them belong to East European (C) lineage.

P6.10

### Bumblebee biodiversity in an Alpine environment.

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A triennial survey on the phenology of bumblebees in the Aosta Valley was carried out in the years 2009-2011. Two side valleys were chosen with different climatic conditions and orientation, placed on the left bank of the river Dora Baltea: the Valley of Saint Marcel and the Valley of Champorcher. Three sampling areas were chosen at different heights (about 1.500, 2.000 and 2.500m a.s.l.) in each valley. In each sampling area and throughout the whole period of activity of bumblebees, every two weeks, some specimens were collected along random transects together with the plants they were on.

In the three years of the research a total of 2.960 specimens of 33 species were collected, seven of which (*Bombus alpinus*, *B. barbutellus*, *B. campestris*, *B. gerstaeckeri*, *B. jonellus*, *B. norvegicus* and *B. subterraneus*) were not yet reported for the Aosta valley. *B. hortorum*, *B. humilis*, *B. lapidarius*, *B. lucorum*, *B. mesomelas*, *B. pascuorum*, *B. pratorum*, *B. pyrenaicus*, *B. ruderarius*, *B. sichelii*, *B. soroensis* are prevalent, being present each with more than 4% of specimens. Nine species belong to the subgenus Psithyrus. 1.624 specimens come from the Valley of Saint Marcel indicating a greater abundance of bumblebees in the first valley. The bumblebees visited plants belonging to 30 families with the prevalence of: Onagraceae (22,6%) Fabaceae (18,0%), Asteraceae (15,8%), Boraginaceae (15,8%); Lamiaceae (4,7%) and Campanulaceae (3,9%).

P6.11

### Morphometric and genetic diversity of honeybee population in district Khairpur, Pakistan.

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The honeybee is ecologically and economically important insect species. Recent reports of honeybee losses in different parts of the world resulting into declining diversity are alarming. Determination of present genetic diversity of honeybee populations in a specific area is important and serves as valuable information for the conservation. Morphometrics provide a relatively powerful analytical tool for the identification of different species and subspecies using various body traits of bees. Typically, morphometric analysis has been used to differentiate the groups and species within honeybee populations in this study. Random samples of 100 honeybee workers were taken from each of the seven localities from Khairpur district. Mean and the standard deviation of each character in all the samples were calculated, and multivariate analyses including principle component analysis, analysis of variance, discriminant analysis and cluster analysis were performed, analysis of variance of morphometric characters displayed statistically significant differences among populations ( $P < 0.05$ ), but the ratio of error variance was  $> 4$ . Therefore in order to find the true difference, non parametric analyses were also performed. The data analysis results for all the honeybees collected from seven different locations of Khairpur were shown to be *Apis florea*. Morphometric analysis results have been confirmed by the PCR using *A. florea* specific primers. Microsatellite markers have also been used to further examine diversity in the honeybee population from district Khairpur.

P6.12

### Mitochondrial genetic characterisation of managed honeybee population from Reunion Island.

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On Reunion Island, *Apis mellifera unicolor* is considered as the native honeybee. This subspecies is endemic to Madagascar, located about 800km from Reunion. Yet, three European lineages, *A. m. carnica*, *A. m. ligustica* and *A. m. mellifera*, had been imported by the past. Since 1982, importation of non-native queens is strictly forbidden.

To investigate the potential adaptation of these European subspecies on this tropical island, the mitochondrial genetic diversity of the managed honeybee population was characterised. A total of 1160 honeybee colonies collected from 61 different apiaries belonging to 42 beekeepers were sampled between December 2011 and February 2012. African and imported European subspecies were identified based on their differences in Dra I RFLP restriction patterns of the intergenic COI-COII mitochondrial region.

A total of four different mitotypes was observed. 94% of the colonies showed the same Dra I restriction pattern than observed for *A. mellifera unicolor*. A second mitotype, identified as *A. m. carnica*, was quite rare (3%), but present in 25% of the beekeepers apiaries. Two other mitotypes were also found but rare. Those RFLP profiles were firmly identified by sequencing. The mtDNA analyses confirmed that the managed local honeybee is *A. m. unicolor* and reflected unambiguous evidence of maintenance and adaptation of the European subspecies *A. m. carnica* on Reunion Island.

P7.1

**Presence of the Israeli acute paralysis virus in collapsing honeybee colonies.**

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Colony collapse disorder (CCD) is a special case of colony collapse that has resulted in sudden colony losses for beekeepers around the globe. CCD-affected colonies lose most of the adult bee population and are left with a live queen, abundant brood, plenty of food and no dead honeybees in the hive. Viruses and most particularly the Israeli acute paralysis virus (IAPV) were associated with CCD. RNA interference (RNAi) efficiently inhibits replication of RNA viruses by detecting dsRNA intermediates formed during their replication. To gain insight about the presence of viruses in CCD colonies we performed analysis of the small RNA populations present in CCD and IAPV-infected honeybee colonies using deep sequencing technologies. This analysis points out several characteristics of the response of the host to the viral infection that will be discussed.

P7.2

**Does bee genetics shape gut microbial communities?**

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In addition to the importance for food processing, the gut microbiota is the first barrier encountered by pathogenic microorganisms before they get access to the gut epithelial cells. Since honeybee colonies display different disease sensibility levels, we wondered how gut microbial communities could be distinct from one colony to another, and how bee genetics may influence the structure of the gut microbiota.

Queens were either mated naturally or artificially inseminated with a single drone, and subsequently introduced in colonies of the same apiary. Workers were marked upon emergence and transferred back to their mother colony or cross-fostered in a different colony. Midguts were collected at different time post emergence and 16s PCR fragments were analysed using the SSCP technique as well as high throughput DNA sequencing.

Our data suggest that factors such as bee age, nest and bee genetics could have an influence on the structure of the gut microbiota.

P7.3

**Using DNA microsatellite markers to explain the difficulty in distinguishing the Caucasian bees from the Carniolan bees based on wing venation.**

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Most of honeybees reared in Poland belong to subspecies: *A. m. carnica*, *A. m. caucasica* and *A. m. mellifera*. Affiliation to one of these subspecies is determined by testing configuration of 19 points on the workers' forewings. However, classification is not always suitable, especially in distinguishing some Caucasian and Carniolan bees. It was shown that the range of metric variability of the forewings strongly overlap, making the classification unreliable.

In this study, the origin of bees was analysed with nuclear DNA markers. Both bees with typical and atypical wing venation for their own subspecies were studied. Samples of Caucasian and Carniolan bees were taken from 19 colonies, also reference samples of *A. m. mellifera* were used. Eight unlinked microsatellite loci were used in the research. In order to assign genotypes to specific sub-species, a probabilistic method based on Bayesian statistics was used. All tested queens (bees) were clearly classified as belonging to the evolutionary branch C. A further allocation of this group into two subgroups (possibly corresponding to *A. m. carnica* and *A. m. caucasica*) indicated that all tested queens are hybrids of the two subspecies. In all genotypes admixture of genes from the other subspecies was estimated to be over a dozen of percentage.

P7.4

**The genetic basis of the solitary-eusocial transition in a socially polymorphic sweat bee.**

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Some sweat bee species are socially polymorphic, exhibiting both solitary and eusocial behaviour. We explored the genetic underpinning of this major evolutionary transition in one such species, *Halictus rubicundus*. Mt-DNA data suggest eusocial, low-elevation populations are genetically differentiated from solitary, high-elevation populations in the US. microsatellite data from European populations show subtle genetic differentiation among populations, yet suggest lack of reproductive isolation between solitary and social populations. Common garden transplant experiments indicate plasticity in social behaviour in Europe. Here we present an integrative computational and wet-lab genomics approach aimed at the identification of key genes underpinning the solitary-eusocial transition, involving the characterisation of SNPs differentiating the transcriptomes of solitary and eusocial populations in the US. We sequenced the transcriptome of one female individual from a social population as reference transcriptome for *H. rubicundus* and transcriptomes of twelve more individuals – eight of social phenotype and four of solitary phenotype – for SNP detection and subsequent association studies. We performed a hybrid assembly, annotated the assembly, and located phenotype-specific SNPs to determine non-synonymous sites differentiating the two social forms. These sites may be putative candidates of 'sociality' genes underpinning the social transition in *H. rubicundus*.

P8.1

**BEECLINIC: a colourimetric DNA chip indicating the health state of the honeybee.**

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Beekeeping is a declining industry in the past decades. One of the principal reasons for this decline in managed honeybee colonies, and of beekeepers, is extensive and unpredictable colony death. Typically, the apiculturist identifies symptoms at the colony level, and then starts diagnostic procedures to identify the disease and initiate a treatment. Yet, when clinical symptoms appear at the colony level, diagnosis often comes too late to save or cure the colony. In the frame of the EU project BEE DOC the diagnostic department developed new tools which can alert the beekeeper to potential problems before colony level symptoms appear. The research grade tool is an extension to the quantitative-PCR array BEE PATH (Evans et al., 2006) and is relying on the microarray technology. This microarray will use a colourimetric development. Different prototypes are tested to select the most useful marker genes for honeybee detoxification, the nutritional and immune status of honeybees and also a broad range of pathogen specific probes will be tested. After evaluation a compilation of the useful probes will be spotted on a single microarray, BEECLINIC, which will screen for the honeybee well-being in general.

P8.2

**RoBeeTech - Romanian apiculture research team for scientific networks.**

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RoBeetech (Romanian Centre for Bee Biotechnology) is an EU funded research project at the University of Agricultural Sciences and Veterinary Medicine (UASMV) in Cluj-Napoca. Romania has a significant apiculture potential for Europe, but suffers from the same general and economic challenges as other eastern European countries (pathologies with complex etiologies, depopulations and so on). UASMV was however able to establish very well equipped laboratories for apidologie. The state of the art infrastructure is combined with the high quality of the human resources, and therefore, the apiculture products control laboratory is approved by RENAR (Romanian Accreditation Association).

The RoBeetech project, [www.robeetech.ro](http://www.robeetech.ro), (POSCCE no. 207/20.07.2010 – 12460), promotes and greatly facilitates the integration of the Romanian research platform into European and even global research networks. RoBeeTech aims at assessing and improving honeybee health and screening the value of apicultural products for disease prevention in the colony.

P8.3

**Impact of probiotic bacteria on honeybee health.**

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Recently, a unique microbiota of lactic acid bacteria (LABs) was isolated from the honey crop of the western honeybee, *Apis mellifera*. The crop microbiota of *A. mellifera* is composed of 13 bacterial species within the genera *Lactobacillus* and *Bifidobacterium*, and a complete description of these bacteria is now available. The crop microbiota has proven to be of importance for honeybee health, since it clearly has an influence on individual larval susceptibility to infections.

Recent results demonstrate that adding the LABs to the larval food inhibit *Paenibacillus larvae*, the causative agent of American foulbrood (AFB) in larvae, and we can also report a reduced mortality rate of larvae artificially infected by the bacterial pathogen *Melissococcus plutonius*, causing European foulbrood (EFB) when larvae are fed LAB supplemented food. Results from cage experiments with adult bees infected with *Nosema ceranae* were less successful. No clear effect from LAB supplemented food on *N. ceranae* infection in adult bees could be observed..

P8.4

**Visible cause, hidden effects: a common pesticide kills more bees than previously thought.**

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We show in this study that a non-lethal exposure of honeybees to thiamethoxam, a common neonicotinoid pesticide, actually causes abnormally high mortality due to homing failure. We simulated intoxication events on free-ranging foragers using homing experiments. More than 650 foragers were captured, intoxicated (or not for control groups), and released 1km away from their colony. They were tagged with RFID microchips for automatically monitoring their flight back to the colony. Homing probabilities in intoxicated groups were significantly lower than those obtained from control groups. We estimated that intoxicated foragers had a probability to disappear away from the colony about 1.7 to 3 times greater than control foragers. Consequences for the dynamics of the colony as a whole were investigated using a population demographic model. These experiments offer new insights into the consequences of common systemic pesticides used worldwide..

**P8.5** Setting up of tests for acaricidal effect of botanical extracts against *Varroa destructor*.



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*Varroa destructor* is an *Apis mellifera* bound ectoparasite that feeds on hemolymph of honeybees and reproduces in brood cells. Infected colonies suffer direct damage caused by parasitism and indirect damage due to viral and microbial pathogens since *V. destructor* is a virus vector.

*Varroa* was first detected at the French-Spanish border in December 1985. Since then chemical acaricides have been used to control this pest. These products have caused many secondary problems, such as mite resistance and chemical residues in honey and other hive products, so that, new and less harmful treatments are being developed. Natural products such as essential oils and plant extracts are other options against *Varroa* infestations.

Here we present the set-up of the evaluation of two essential oils (*Piper nigrum* and *Schinus molle*) and one botanical extract (*Astragalus* root) as acaricidal products. Different concentrations have been tested in order to find the correct level of toxicity against the mite and at the same time, the harmless level to the honeybees. We aim to corroborate whether these substances can act as natural compounds to control the varroosis.

**P8.6** Pathogens versus pesticides in honeybee colonies: *Varroa destructor* overrides all other factors.



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Multiple factors are believed to be responsible for dramatic increases in western honeybee (*Apis mellifera*) colony losses recently experienced in many regions of the world. At the centre of attention are the potential effects of exposure to chronic, sublethal doses of pesticides commonly found in agricultural areas because they are more difficult to discern and therefore more poorly understood than acute, lethal pesticide poisonings or macro-parasites such as the mite *Varroa destructor*.

Under the framework of the FP7 project BEE DOC, we chronically exposed 16 queenright *A. mellifera* honeybee colonies for five months (May-September 2011) to sublethal doses of tau-fluvalinate (two Apistan® strips), thiacloprid (Ehrenstorfer GmbH Analytical-Standards), both or none (four colonies per treatment group) to better understand the potential role of these model pesticides on colony health. Before, during and after treatment we quantified colony strength (size of adult and brood populations, and amount of food stores), brood patchiness, and infections with pathogens (*Nosema* spp., *V. destructor*, deformed wing virus, black queen cell virus and Israel acute paralysis virus).

Our results suggest that *V. destructor* was the most debilitating agent influencing colony health (and death), regardless of pesticide exposure.

**P8.7** Results of a two-year *Nosema* monitoring in Southern Germany.



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There are contrasting reports on the prevalence and pathogenicity of Nosemosis from different parts of Europe. We therefore performed a monitoring project to follow the course of *Nosema* infestation in different apiaries in South Germany. From April 2010 - October 2011, more than 60 honeybee colonies from 14 apiaries were examined for the occurrence of *Nosema* spores in monthly intervals. In total, more than 450 samples of 60 bees collected from a peripheral honey comb were quantified for the number of spores. Throughout the whole monitoring period none of the colonies were completely free from *Nosema* spores. In both years, the highest prevalence was recorded during spring in April/May with about 90% of *Nosema* positive colonies. At the end of the season merely 50% of the colonies were infested with huge differences between the apiaries. The maximum average infestation rate of one colony was 4.700.00 spores per bee, however, only 7% of the samples revealed infestation rates of more than 1.000.000 spores per bee. During the whole sampling period no clinical symptoms and no winter losses caused by Nosemosis were reported by the beekeeper involved in the project. Molecular genetic analysis of the positive samples showed that *Nosema ceranae* was the exclusive *Nosema* species within our monitored apiaries. These confirm results of monitoring project of BEE DOC partners from other European countries and contradict reports of severe damages caused by infections with *N. ceranae*. Supported by the EU project "BEE DOC" (244956 CP-FP)“.

**P8.8** Chemical composition of dried bee pollen from state of Rio Grande do Sul, Brazil.

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Brazilian legislation, from the Ministry of Agriculture, Livestock and Supply (2001) defines pollen as a result of the agglutination of the flower pollen, made by worker bees through nectar and their salivary substances, which is collected at the hive entrance. Under this standard, the dehydrated product should have no more than 4% of moisture, minimum of 1.8% in lipids, minimum of 8% in proteins and maximum of 4% in ashes. The aim of this study was to determine the chemical composition of ten dehydrated bee pollen samples from the state of Rio Grande do Sul, Brazil, collected at spring and summer (2011) and to compare with the limits established by the Brazilian regulation. The methods used were: micro-Kjeldahl (proteins), Soxhlet (lipids), oven at 550°C (ashes) and infrared dryer (moisture). The levels ranged from 2.67% to 5.7% for moisture, 12.66% to 25.51% for proteins, 1.90% to 5.73% for lipids and 1.29% to 2.66% for ashes. It was concluded that only one of the ten samples analysed were not in accordance with the limits established by Brazilian legislation. Not many countries have specific regulation for bee pollen. For example we can see that CE, Canada, Colombia, El Salvador, EUA, Guatemala, Japan, Nepal, Romania, Czech Republic and Venezuela do not have any regulation for bee pollen.

Thanks are due to CAPES and CNPq for financial support.

P8.9

**Comparison of colony health in wild and managed honeybees.****Weise E, Müller MY\*, Moritz RFA**

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In this study we screened several honeybee populations of South Africa for prevalence of six different viruses. We used a new diagnostic MLPA tool (Multiplex Ligation-dependent Probe Amplification, “Beedoctor”, poster Lina de Smet) to test bees from nature reserves and apiaries. This allowed us to compare the health status of truly wild with that of managed honeybees. Furthermore we determined population genetic parameters to assess genetic diversity of these populations. This data provides us insight on the impact of apiculture on honeybee health.

**P9.1 Floral resources visited by *Bombus* (Apidae: Bombini) in the Guatemalan Highlands.**



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We recorded floral visits of nine species of *Bombus* to over 90 botanical species. The most represented families were Asteraceae (30 species), Fabaceae (eight species), Lamiaceae (seven species), Solanaceae (seven species), Brassicaceae (five species), Onagraceae (five species) and Verbenaceae (five species).

*Brassica campestris* L. was the most visited plant, by four of the nine species: *Bombus wilmattae* (Cockerell, 1912), *B. ephippiatus* (Say, 1837), *B. variabilis* (Cresson 1872), and *B. brachycephalus* (Handlirsch, 1888). *B. pullatus* (Franklin 1913) visited *Bixa orellana* more frequently (37%). Most of the recorded visits by *B. macgregori* (Labougle y Ayala, 1985) were made to *Brassica juncea* (86%). *Andira inermis* was the most visited by *B. mexicanus* (Cresson 1878) (50%), while *Geranium alpicola* was the most visited by *Bombus trinominatus* (Dalla Torre, 1890) (25%) and *B. weisi* (Friese, 1903) (33%).

Similarity among bumblebee species based on the visited plant species, was calculated using the Morisita index of similarity. The most similar were *B. wilmattae* - *B. ephippiatus* (0.74); and *B. ephippiatus* - *B. variabilis* (0.47). *B. pullatus*, *B. mexicanus* y *B. trinominatus*, did not show important similarities with other bumblebee species.

**P9.2 Fitness estimates of bumblebee colonies in a fully crossed ‘multiple drivers’ experiment.**



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There is clear evidence that pollinators are declining. Potential drivers include pathogens, pesticides and/or interactions amongst them. Mitigating pollinator declines critically depends on the identification of the factors affecting their fitness, as well as the demonstration of putative interactions with synergistic effects.

Here, the effects of chronic sublethal exposure to systemic neonicotinoids on the fitness of the bumblebee, *Bombus terrestris*, were investigated. Additionally, infection of the common gut parasite *Crithidia bombi* was included in a fully crossed experimental design on a colony level. The data show that sublethal pesticide exposure can significantly reduce the numbers of produced gynes and males, with a considerable proportion of colonies not investing in gynes at all. While infections with *C. bombi* alone tended to have positive effects on gyne production, lower numbers of gynes were produced in combination with neonicotinoid exposure than in all other treatments. Moreover, our data suggests that mated daughter queen deriving from the multiple driver treatment exhibit the least chances to survive hibernation.

Our results indicate that chronic sublethal exposure to a neonicotinoid, especially in combination with pathogens, can substantially reduce fitness of bumblebee colonies. Current crop management practices may therefore considerably contribute to insect pollinator declines.

**P9.3 The dying of bees as hyper collapse disorder? Procedural assembling of social and natural knowledge.**

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Up to now there has not been an adequate explanation for the phenomenon called colony collapse disorder (CCD) which has been observed in the past few years. CCD has reinforced the trend of the decline of managed honeybees that has lasted for some decades. This is a worldwide development with its core areas in the highly industrialised countries. Hence, the ecosystem and agriculture services of the bees are endangered. It is assumed that „multiple factors are at work linked with the way humans are rapidly changing the conditions and the ground rules that support life on Earth” (UNEP). However, while the “multiple factors” are intensely analysed, the corresponding “rapidly changing” has hardly been an object of social research yet. This poster presents a recently initiated (May 2012) research project which adopts a sociological perspective on the honeybee decline. The main question of this study is how the (rapid) dynamics of social change influences the coexistence of humans and bees: hyper collapse disorder? Therefore, it undertakes comparative analyses of different types of beekeeping practices, especially industrial, urban, organic and traditional beekeeping. The research relies on explorative (qualitative) methods, i.e. a combination of proved case reconstructive approaches with an ethnographic style of network analysis. Following this way promises the reconstruction of social structures of the human-bee-coexistence and its procedurally linking with apidological knowledge.

**P9.4 Protein quality of pollen used as a food resource by the stingless bee *Melipona rufiventris* (Apidae).**

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*Melipona rufiventris* “uruçu amarela” is a native species of stingless bee that is an efficient pollinator. Pollen is the only source of protein for bees. The availability and nutritional quality of pollen is fundamental for the conservation and maintenance of bee species. In this study, we examined the protein content of pollen from plant species used as a food resource by *M. rufiventris* from March to August 2010 (seasons with fewer flowering species). Pollen samples collected from corbicula were analysed using the standard acetolysis technique and Bradford dye-binding method. There was strong predominance of Myrtaceae species (*Eucalyptus* sp.). Additionally less abundant were Fabaceae (Mimosoideae), particularly *Leucaena leucocephala*, *Mimosa* sp., *Caesalpinia* sp. (Caesalpinioideae), the Anacardiaceae represented by *Schinus molle*, the Solanaceae represented by *Solanum mauritianum*, the Arecaceae and some other unidentified Myrtaceae and Anacardiaceae species. Myrtaceae species were a constant source of pollen in all months examined constituting the main protein source for native stingless bees. The pollen protein content ranged from 16.2% to 56%, with a mean content of  $29.4 \pm 17.2\%$  (mean  $\pm$ SD; n=5). In March and August (months when food resources were low) there was a predominance of pollen from species with a protein content >16%. These findings agree with other studies of *Melipona* in Brazil and show that Myrtaceae pollen is a major food resource for these bees. Financial support: CAPES - PNP

P9.5

### Differences of floral resource preferences of honeybees and wild bees in an intensive cereal farming system.



Rollin O\*, Bretagnolle V, Decourtye A, Michel N, Vaissière B, Henry M

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Bees provide an essential pollination service for crops and wild plants. But for the last fifty years, we observed substantial bee population and diversity declines in Europe and North America. Previous studies have shown that the loss of semi-natural habitats and the reduction of plant diversity in intensive agricultural landscape were one of the main causes responsible for this decline. Mass flowering crops offer a huge quantity of resources, but poorly diversified and available for a short period of time. By contrast, semi-natural habitats can offer specific nesting sites for bees and diversified and steadier pollen and nectar resources. The objective of this study was to describe the floral resource preferences among bees, between mass flowering crops and wild floral resources in a cereal farmsystem in western France. During the oilseed rape and sunflower flowering periods, we recorded foraging bees along fifty meter walking transect in different types of habitats, such as mass-flowering crops, semi-natural habitats and flowering alfalfa. We found a clearcut segregation pattern in the use of floral resources between honeybees and wild bees. This study showed the importance of semi-natural habitats, particularly the herbaceous semi-natural habitats, for sustaining wild bee populations.

P9.6

### Relative importance of landscape, farming, habitats and location on bumblebees in pastoral regions.

Santorum V, Breen J\*

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If efforts to conserve wild bees are to be effective and value for money, it is important to identify the factors that have most influence upon bee abundance and diversity. The relative importance of key environmental influences on bumblebee abundance and species richness in pastoral landscapes in Ireland was examined using methods based on information theory. The explanatory variables evaluated were: farm management intensity; landscape composition classified by proportion of highly improved grassland; structure and vegetation of grasslands and hedgerows and latitude and longitude. Principal co-ordinate analysis was used to reduce complex explanatory data to a small number of environmental variables. The importance of these predictors was compared directly using evidence ratios of sums of Akaike weights.

The most important influences upon bumblebee abundance and species richness were landscape composition and hedgerow vegetation. Increasing area of improved grassland in the landscape was associated with reductions in bumblebee abundance and species richness. Low shrub diversity hedgerows, dominated by blackthorn (*Prunus spinosa*) were associated with higher bee abundances and richness. All other tested factors were relatively unimportant to bumblebee abundance. However, some were influential upon bumblebee diversity, in particular location, grassland vegetation and presence of woodland and scrub.

# Satellite Session

## EurVarroa

**Chairs:**

**Jurek Wilde**  
(Oberursel, Germany)

**Niko Koeniger**  
(Oberursel, Germany)

### **Symposium description**

With a focus on new methods for the treatment of varroosis, a sequence of Polish German symposia on bee research started in Stettin in 1992. This initiative became one of the roots of Eurbee conferences and after a period of 20 years it seems to be worthwhile to review past developments and to discuss prospective progress of control methods of *Varroa destructor*. We invite contributions to a full range of control methods from biotechnology, breeding, biological agents, chemotherapy, diagnose to the application of organic acids.

10.1

**Half a century with *Varroa* and European honeybees.**

Fries I

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The first recorded finding of *Varroa* mites in Europe is probably from Bulgaria in 1967. Partly because of linguistic difficulties, the use of experiences on mite control in former USSR was delayed, where use of oxalic acid is a good example. Eventually, it was found that the effects from mite infestations were actually due to associated virus infections and that the species that made a host shift to European bees were in fact not *Varroa jacobsoni*, but a different species assigned the descriptive name *Varroa destructor*. Research on mite control has been crucial to maintain honeybee populations in Europe and has offered a variety of chemical solutions. This has resulted in contaminated products and mites resistant to chemicals applied. Selection for mite resistance has only been marginally effective, whereas examples demonstrate that mite resistance evolves through natural selection if beekeepers do not apply mite control. Unfortunately, most colonies would perish in a natural selection process and there is no wild honeybee population, similar to Africa or South America, where selective forces can act without severe effects for apiculture. Long-term viable solutions for European apiculture appears to rely on chemical mite control that allows some mite damage so that bad genetics can be disfavoured and good genetics can be favoured. Alternatively, for selection purposes, the mite population growth rate may also be used as selection criteria for increased mite resistance.

10.2

**Practical evaluation of a treatment concept to stabilise the varroosis parasite host system on Marmara Island/Turkey.**

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Current treatment practice confers a short term advantage of preventing colony losses but offsets any long term advantages of better adapted genotypes spreading. Not treating may increase tolerance in the varroosis parasite-host system under natural conditions as well as from “live or die” experiments. However, as a rule these developments incur intolerable bee population breakdowns. We here propose a schedule of infestation dependent treatment and requeening to be implemented in ordinary beekeeping routines, mimicking natural selection processes.

We started an experiment on Marmara Island/Turkey in 2009, where we explored in a population of about 250 colonies whether and at which infestation levels it would be possible to leave low infested colonies untreated without risking unacceptable colony losses. We further explored whether procedures of infestation diagnosis and requeening of high infested colonies can be kept on a simple level which would be acceptable for beekeepers. Two years experience indicate that diagnosis with powder sugar shaking and requeening with transfer of queen cells can be successfully performed, and stable infestation levels without apparent colony losses can be attained while leaving about one third of the colonies untreated. These results are encouraging that treatment schedules can be developed which support rather than counteract the development of varroosis tolerance.

for your remarks:

### 10.3 Alternating varroosis treatments with Coumaphos and Flumethrinon honeybee colonies: a field study.

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To study whether *Varroa destructor* infestations can sustainably be reduced by a concerted treatment using alternating therapeutics we run an area-wide long-term field study. Coumaphos (CheckMite, years 2008, 2010) and flumethrin (Bayvarol, years 2009, 2011) were used. In our study 350 to 386 beekeepers participated in a whole district in Hessen, Germany. A total of 2250–2450 hives (60–65% of all registered hives) were treated. The treatment started immediately after the foraging season (July/August) and ended almost at the same time for all colonies. In 147–188 hives in 20 apiaries the colony status and *Varroa* fall was monitored during treatment. We showed that an efficient varroosis control leads to high winter survival rates of more than 90%. Both treatments showed similar efficacies and no indications for resistances. We observed an eight fold increase in mean *Varroa* numbers from 2008 to 2010. During 2008 *Varroa* fall continuously declined within the first 3 weeks of treatment and remained low during the observation period. By contrast, 2009 to 2010 we determined substantial reinfestations 7–8 weeks after treatment onset, independent of the substance used. Our results are consistent with the hypothesis that *Varroa* infections are the main cause for colony collapses during the European winter. The increase of *Varroa* falls over the 4 years despite an area-wide treatment of approx. 2/3 of all colonies in a whole district point at the crucial role of reinfestation.

### 10.4 *Varroa*-Gate, developing a temporal quarantine of bee colonies against *Varroa destructor*.

Koeniger N\*, Koeniger G, Grünewald B, Krieger KJ

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In middle Europe, horizontal transfer of *Varroa destructor* in late summer and autumn causes major problems. The *Varroa*-Gate is developed to interrupt the transfer of mites among colonies. Fixed to the hive entrance the *Varroa*-Gate must eliminate mites attached to passing bees. Thus colonies can be quarantined and treated independently of the *Varroa* infestation levels of surrounding colonies. Under practical beekeeping conditions in Germany the main application period will be from end of July up to November.

A screening system was successfully established. Thus efficacy and tolerance levels were determined in cage tests. These tests resulted in preliminary estimations of following Gate factors: 1) Gate matrix. 2) Gate clearance. 3) Quantity (range) of active ingredient. 4) Quantity (range) of softener.

These data were established for the organophosphatic acaricide coumaphos and the pyrethroid flumethrin. In the further course of this work we will include Amitraz. As a result *Varroa*-Gates with three acaricides with different mode of action will be available. Thus a regular annual change of acaricides helps to avoid the build up of resistant mite populations. Prototypes of *Varroa*-Gates were tested in colonies without brood (caged queen). Efficacy data of *Varroa*-Gates (based on flumethrin and coumaphos) and results of residue analysis will be reported.

for your remarks:

10.5

**Sustainable *Varroa* control with regulatory approved medication.****Krieger KJ**

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After the switch over of *Varroa destructor* to the western honeybee systematic search for suitable acaricides resulted in the development and regulatory approval of amitraz, coumaphos, flumethrin and fluvalinate based medications. Two major concerns have been addressed: development of resistance and accumulation of residues. Reports about a lack of efficacy could not be confirmed in many cases and the extent of residue accumulation is subject of a continuous monitoring of honey and beeswax from apiaries which are treated annually with coumaphosor flumethrin-products and for which comb foundations are being produced exclusively from beeswax originating from respective apiaries. The results demonstrate that residues did not accumulate beyond plateau levels which amount to 0,02mg/kg and 0,05mg/kg coumaphos in honey and 8mg/kg and 40mg/kg coumaphos in beeswax after continuous PERIZIN and CHECKMITE treatment respectively, and flumethrin concentrations stayed below detection limit in honey and amount to 0,06mg/kg in beeswax after continuous use of BAYVAROL. There is no evidence for a continuous accumulation of residues. As *Varroa* continues to pose the challenge No1 for actual beekeeping, use of acaricides cannot be surrendered yet, but as any other treatment it must be based on a professional benefit risk analysis. In order to prolong their lifecycle until novel methods and/or medications may replace them, their use needs to be improved according to responsible care and integrated pest management (IPM) principles.

10.6

**Comparative analysis of tyramine and octopamine receptors of *Varroa destructor* and *Apis mellifera*.****Blenau W\*, Baumann A**

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The major problem in beekeeping worldwide is the parasitic mite *Varroa destructor*. Nowadays, products based on organic acids, essential oils, pyrethroids, organophosphates, and formamidines are used to combat the mite. Components of essential oils (e.g., thymol, eucalyptol, camphor, menthol) as well as the formamidine pesticides (e.g., amitraz) most likely unfold their effects by binding to tyramine and octopamine receptors which belong to the superfamily of G-protein-coupled receptors (GPCRs). Thus, disturbing the native function of these receptors in the mite holds promise to result in deleterious effects.

During the last decade, we have characterised various aminergic GPCRs of the honeybee. In contrast, nothing is known about the molecular properties of aminergic GPCRs of *Varroa destructor*. Therefore, we performed pilot PCR experiments and succeeded in amplifying fragments of three different GPCRs from the mite. Using RACE PCR, we have meanwhile obtained full-length clones of two receptors. We plan to heterologously express and pharmacologically characterise these GPCRs of the mite. We will test the same substances (in particular formamidines and essential oil components) for their agonistic or antagonistic effectiveness on orthologous receptors of the mite and the honeybee. From this in-vitro approach, we expect to identify candidate compounds that are non-toxic (or only slightly toxic) for bees but highly toxic for mites with justifiable costs.

for your remarks:

10.7

**Gene-knockdown in *Varroa destructor*.**

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*Varroa* are recognised as a major cause of honeybee death worldwide largely by their involvement in pathogen transmission and virulence. The emergence and spread of resistance in *Varroa* populations to the few effective and registered pesticides available has resulted in the need for alternative control strategies. Unfortunately, the relatively close relatedness between the honeybee and *Varroa* makes the discovery of specific pesticides capable of effective killing of the mites and yet benign to the bees very difficult. We have demonstrated that double-stranded RNA-interference is effective in suppressing genes within *Varroa*. This approach harnesses the natural anti-viral immune reaction present throughout the plant and animal Kingdoms and offers a potential way of both studying the roles of certain genes and pathways in the mite-bee relationship and, importantly, has potential in controlling mite populations without harming the bee.

10.8

**The epizootic investigations of apiaries with massive colonies depopulation syndrome in the East provinces of Poland.**

Chmielewski MW

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The flumethrin or fluvalinate applications conducted in 2010 resulted in an average mite mortality at 50 apiaries; respectively 20.21% ( $\pm 14.62$ ) and a 30.28% ( $\pm 10.47$ ) reduction in the number of mites in lightly infested colonies as well as 91.30% ( $\pm 4.26$ ) and 94.24% ( $\pm 3.14$ ) in highly infested colonies. The investigations of the samples revealed a “*Varroa* problem” [severe *V. destructor* infestation, bees with deformed wings (DWV) or ABPV infection detected] in 78% of the apiaries. In 68% of the apiaries a severe *Nosema* spp. infection was detected. The winter bee colony mortality in apiaries showed losses ranging from 30% to 70%. The presence of *V. destructor*, *Nosema* spp, and the following viruses were confirmed: ABPV, CBPV, IAPV and DWV. Co-infection with three or four pathogens was noted in over 70% of bee colonies. Infestation of *V. destructor* was found in 91.7% of the colonies, while the infection of deformed wing virus (DWV) was noted in 76%. ABPV was detected in 35% of the examined colonies and CBPV was found in only 7.8% of them. Severe colony losses in the examined apiaries could be attributed to the wide prevalence of *V. destructor* with DWV and ABPV infections, and/or *Nosema* spp. infection. (Pohorecka K., 2011).

for your remarks:

10.9

**Development of new techniques to control *Varroa destructor*.****Pflugfelder J**

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Screening procedures are based on experimentally simplified test conditions that represent the complex natural context to identify those with biological activity. Screening for a lead compound or a biological antagonist requires the appropriate bioassays to assess if the test item has biological activity that increases the mortality of the target organism. The criteria for good screening tests are: high throughput, cheap and reproducible. Here we present methods for the screening of lethal effects on *Varroa destructor* and *in vitro* tests for the screening of compounds that disturbs reproduction. The first results from the screening for lethal effects on *V. destructor* using entomopathogenic fungi and a compound that blocks the reproduction in the *in vitro* tests are promising.

for your remarks:

10.10

**Evaluation of honeybee stocks for *Varroa* tolerance in Argentina.****Palacio MA\*, Lanzavecchia S, Merke J, Agra M, Martinez A, Camacho B, Fourquet G, Figini E, Scanapiecco A**

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*Varroa* tolerance of several honeybee populations from different eco-climatic regions in Argentina is being evaluated. Apiaries were established in subtropical and temperate climates. Local (LS), commercial (CS) and hygienic behaviour-selected (HBS) stocks were considered. As first approach for this evaluation, three major aspects of the beehive were periodically monitored for all stocks: population dynamics of *Varroa* and honeybees, viruses' prevalence and hygienic behaviour. Bees from each stock were genetically characterised using mitochondrial markers and microsatellites. In subtropical climates, LS showed lower values for phoretic *Varroa* and *Varroa* in brood cells when compared to HBS but higher values for hygienic behaviour were registered in HBS. Africanised and european haplotypes were detected in LS and HBS, respectively. In one of the apiaries from temperate region, HBS colonies showed lower values of *Varroa* reproduction than CS colonies and have been maintained without the use of acaricides for five generations. The first results obtained in each eco-climatic are discussed in the frame of the use of improved procedures of management and production.

**P10.1 Varroa control: comparison of three queen-trapping methods.**

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*Apis mellifera* has suffered from the synergistic effects of *Varroa destructor* and pesticides such as neonicotinoids. Reduced or avoided acaricide use has caused many beekeepers to search for alternative ways to contain the mite without harming the bees. Queen caging is one method that can be employed in summer after the main honey production, and in which the resultant block of brood seems useful against *V. destructor* and allows colony resumption for the winter. Sadly, caging methods differ widely and generally are not scientifically validated. Thus, trials that discontinued the colony brood by queen caging were designed and conducted to evaluate a commercially-available caging device with small uniform spaces against both single- and double-caged frames in Piedmont, Italy. Four groups, five hives each, were treated as follows:

1) Treatment with Apilife Var® only (control), 2) two-frame cage, 3) single-frame cage, and 4) “Scalvini” queen excluder cage. Each treatment was maintained for 22 days; the hives were then treated drop-wise with a sugar solution containing an oxalic acid (OA) based formulation, API-Bioxal®. Tests periodically considered colony development (adult bees, brood), and the number of mites dropped during the trial as well as after the winter OA treatment. Treatments 2 and 3 showed quicker queen egg-laying recoveries than did the others. No significant differences were found relative to the considered parameters.

**P10.2 Bidirectional transfer of RNAi between honeybee and *Varroa destructor*: gene silencing and control.**

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The mite *Varroa destructor* is an obligatory ectoparasite of the honeybee (*Apis mellifera*) and is one of the major threats to apiculture worldwide. Recently, *Varroa* resistance to acaricides has become a global concern and alternative means of control are needed. In recent years, plant-to-parasite RNAi transfer has been documented, suggesting a potential strategy for next-generation pest control. We previously reported that honeybees fed on double-stranded RNA (dsRNA) with a sequence homologous to that of the Israeli acute paralysis virus are protected from the viral disease. Here we show that dsRNA ingested by bees is transferred to the *Varroa* mite and from mite on to parasitised bee. This cross-species, reciprocal exchange of dsRNA between bee and *Varroa* engendered targeted gene silencing in the latter, and resulted in an over 60% decrease in the mite population. Thus, transfer of gene-silencing-triggering molecules between this invertebrate host and its ectoparasite could lead to a conceptually novel approach to *Varroa* control.

**P10.3 Organic and chemical *Varroa* mites control in honeybee (*Apis mellifera carnica*) colonies.**

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Organic and chemical treatments were assessed in order to establish the efficacy against *Varroa* mites. Oxalic acid solution (OA), consists of 2.9% oxalic acid and 31.9% sugar in water and thymol based products Timovar (Andermatt BioVet AG) and Apiguard (Vita Europe Ltd., UK) were applied in a controlling the mite. Flumethrin, fluvalinate or amitraz were also comparatively applied in colonies. The flumethrin or fluvalinate applications, triggered an average mite mortality at the two apiaries: 19.11% ( $\pm 14.62$ ) and a 39.28% ( $\pm 10.47$ ) reduction in the number of mites in slightly infested colonies and 94.30% ( $\pm 4.26$ ) and 96.24% ( $\pm 3.14$ ) in highly infested colonies. The relative mite mortality after four OA applications, two Timovar or two Apiguard applications, in colonies with capped brood was 41,80% ( $\pm 14,31$ ), 14,35% ( $\pm 10,71$ ), and 18,93% ( $\pm 13,56$ ) respectively. On the second side two Apiguard applications and single amitraz treatment resulted in reducing the mite populations by 19,71% ( $\pm 12,61$ ) and 23,89% ( $\pm 14,25$ ) respectively. In the Mediterranean located apiary, Timovar and Apiguard treatments triggered 59,02% ( $\pm 17,28$ ) and 46,50% ( $\pm 13,33$ ) of the total mite reduction. The results indicate that OA trickling, two thymol formulations or Amitraz fumigation are of limited use during periods with brood. The possible use of OA and thymol based products against the *Varroa* mite in honeybee colonies and complementary apicultural methods performed in the apiaries is demonstrated.

**P10.4 Survey on winter losses in Sweden.**

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The estimated number of beekeepers in Sweden is about 12.000 with approximately 120.000 colonies. Each year since 1920 beekeepers have sent in reports about their beekeeping to the Swedish Beekeepers Association. These reports include information about e.g. the number of hives, the honey yield and the losses. According to these reports the average yearly losses 1920-2011 was around 12,9%, varying between 6 and 22%.

Since 2009 we have carried out web based surveys on winter losses. The questionnaires we have used have been based on questionnaires developed by WG1 within the COLOSS network. The winter losses according to these surveys have been the following: 2008/2009, 17,5% (n=7.354), 2009/2010, 24,7% (n=13.598), 2010/2011, 14,5% (n= ). Preliminary data by the end of May 2012 show that the losses 2011/2012 were around 10-11% (of around 21.000 colonies). The losses have generally been higher in the areas of Sweden where *Varroa* is present compared to areas without *Varroa*. Inadequate *Varroa* control appears to be one of the main reasons for colony losses in Sweden. But our data show that even other factors play a role. In addition to the web based survey we have conducted a survey on overwintering among 1.200 randomly selected beekeepers. Data from the web based surveys, from the survey among the randomly selected beekeepers as well as results from surveys on pathogens will be presented.

**P10.5** Microbial contamination of a *Beauveria*-based biopesticide against *Varroa*.

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Biological control of *Varroa destructor* would resolve many problems, such as pesticide resistance and contamination, experienced with chemical control. A biopesticide, formulated with commercially-prepared conidia of a strain of *Beauveria bassiana*, strain 05002, isolated from *Varroa*, was tested against *Varroa* in southern France. In contrast to previous field experiments with laboratory-prepared conidia, treated hives had lower survivorship and higher mite infestation levels. The biopesticide was subsequently found contaminated with two strains of *Pseudomonas fluorescens* bacteria, PSP1 and PSP2, identified via biotyping and sequencing data of the 16s rRNA and rpoB regions. On nutrient agar the growth of fungus strain 05002 was suppressed in the presence of the bacteria when cfu densities were about equal. Experiments using caged adult bees showed that newly emerged bees ingesting diet and sugar solution treated with PSP1 and kept at 33°C died sooner than those treated with strain 05002, so PSP1 may have impacted bee health. In further lab experiments, *Galleria mellonella* larvae treated with another *B. bassiana* strain, GHA, and the bacterial strain PSP2 lived significantly longer than those treated with either GHA alone or GHA and PSP1, indicating that PSP2 interfered with *Beauveria* virulence. These experiments indicate the importance of monitoring for contamination as well as the potential for microbial interactions in situations even without contamination.

**P10.6** Comparative field study of oxalic acid in glycerol and sucrose aqueous solutions for *Varroa* control.

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The aim of this study was to evaluate under field conditions the acaricide efficacy of trickling treatment with oxalic acid (OA) dissolved in 33% (v/v) glycerol (OA-G) compared to the currently used 50% (w/v) sucrose solution (OA-S) in different seasonal time periods. This was encouraged by previous laboratory studies showing that efficacy of OA-S solution, but not OA-G solution, is influenced by relative humidity (Milani et al, 2001). Five different field trials were performed in winter 2010-2011 (two apiaries), in summer 2011 (two apiaries) and in winter 2011-2012 (one apiary) treating a total of 58 broodless colonies. The treatment was performed using 5ml/frame of OA-G or OA-S solution respectively. Approximately 7-21 days after OA treatment, all colonies underwent acaricide control treatment using amitraz and/or fluralinate. During the trials, the mite fall of each colony was recorded weekly, and adverse effects, temperature and relative humidity were monitored. The cumulative mite fall after OA and control treatment was considered to be 100%. Overall, the tolerability and effectiveness of the two solutions were similar, with the OA-G solution being slightly less effective than the OA-S in four out of five treated apiaries. Further field trials are underway to definitely assess if any progress in *Varroa* control may arise from the use of OA-G solution in field conditions. This study was supported by Regione Lombardia, STRANOVA Project.

**P10.7** Comparative evaluation of Acrinathrin and Bayvarol at control of varroasis.

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*Varroasis* is most important parasitic disease of honeybees. In this study, the effects of Gabon PA92 and Bayvarol was evaluated. 30 bee hives in Nazarabad region of Alborz province in three experimental groups were examined as Control, Gabon and Bayvarol. In each hive we took 50 adult and 50 brood and their mites were quantitated as primary infestation. Following using drugs infestation rate was determined again. In treatment groups infestation rate were reduced significantly ( $p < 0.01$ ). Efficacy rate was 95.8 and 98.5 in adults and 96.4 and 98.1% in brood for Bayvarol and Gabon respectively. Although efficacy of Gabon was a little more than Bayvarol but this difference was not significant ( $p > 0.05$ ).

**P10.8** Coordinated treatment of *Varroa destructor* – the Swiss approach.

Pflugfelder J

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Beekeepers suffer each year from high winter losses that are caused by *Varroa destructor*. Low treatment efficacy and reinvasion of *V. destructor* mites from infested hive are the main reasons for the frequent high colony losses. Both are not realised by the beekeepers and affect even the most responsible beekeeper. Reinvasion is an important mechanism that ensures the survival of the mite when the parasitised colony collapses. It has been shown that up to 300 mites per day can enter a colony late in the season over distances up to 2km. Here we present preliminary results of a field trial where we investigated the efficacy of an area wide *V. destructor* treatment on 1600 colonies in the canton of Bern. In an area of 154km<sup>2</sup> the colonies from 167 bee yards have been treated at the same time. The efficacy of this coordinated treatment, the mite reinvasion and winter survival of the colonies will be compared to a control area where the treatment against *Varroa* has not been coordinated.

**P10.9 Spread and strain determination of *Varroa destructor* in Madagascar since its first description in 2010.**



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In 2010, *Varroa destructor* has been detected for the first time in Madagascar on the endemic bee, *Apis mellifera unicolor*. The local honeybee contributes to pollination of both endemic and important economic species. In early 2010, *Varroa* spread was restricted to three districts around Antananarivo.

The aim of this study was first to survey infested areas, then to analyse *Varroa* mitochondrial genetic diversity and identify its lineage(s). Female *Varroa* mites were sampled from honeybee workers and drone brood cells. mtDNA genetic diversity and mtDNA typing via partial sequencing of the cytochrome oxidase one gene (cox-F and cox-R primers) were analysed.

In 2012, *Varroa* spread was investigated at a large spatial scale. A total of 641 honeybee colonies from 116 apiaries distributed in 26 districts all around the country were examined for the presence of *Varroa* mites. Only six out of the 26 districts investigated were infested. *Varroa* mites were found in two districts, adjacent to those already infested near Antananarivo, in the center part of the country (123 colonies infested in 20 apiaries). Our survey revealed the presence of mites in a sixth district, located on the northeastern coast of Madagascar in the Analanjirofo region (30 colonies infested in six apiaries). Mitochondrial DNA lineage, and haplotype diversity are presented.

**P10.10 Acrinathrin residue in honey and wax and evaluation on varroa-cidal effects of Gabon PA92 & Apistan at Iran.**

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Varroosis is one of the most important diseases of honeybees which caused by *Varroa jacobsoni*. In this study we evaluated varroa-cidal effects of Acrinathrin(Gabon PA92) and Apistan at three province of Iran, also the probable residue of Acrinathrin in honey and wax was quantitated. We selected 60 honeybee hives in each provinces of Tehran, Mazandaran and Eastern Azarbayjan that were divided to four experimental groups randomly including Control, Gabon-1 (one strip was used), Gabon-2 (two strips were used) and Apistan. After quantitation of primary infestation of varroosis at all hives of total groups and using Gabon and Apistan in their groups we quantitated infestation rate of all groups again by sampling of 100 adult and 100 brood from each hive of all groups at all provinces. In time of honey extraction, we took five sample of honey and wax in groups of Control , Gabon-1 and Gabon-2 at all provinces and Acrinathrin residue were quantitated by GC method. (ECD,Ni 63, Detector: CP Sil BP1). The efficacy of Gabon-1 (with one strip dose), Gabon-2 (two strip) and Apistan at provinces of Tehran, Mazandaran and Azarbayjan was 97.48, 99.43, 93.85%, 97.84, 99.3, 94.21% and 96.81, 98.97, 93.27% respectively. There were not significant difference between efficacy rate of each same treatment group at all provinces ( $p < 0.01$ ). Although the efficacy rate of Gabon-1 is more than Apistan but there was not significant difference ( $p < 0.01$ ). According to the drug residue results, Acrinathrin residue was not detectable (ND) in all samples of honey and wax.

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### Abbreviations:

 Student contribution

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