Tagungsband

70. Jahrestagung der AG der Institute für Bienenforschung e.V.

LIB Länderinstitut für Bienenkunde

Kongresshotel Potsdam

28.03.-30.03.2023

ARBEITSGEMEINSCHAFT DER INSTITUTE FÜR BIENENFORSCHUNG E.V.

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SB = Studentischer Beitrag

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Hauptvortrag

Dienstag 28.03.2023, 13:15-14:15

Die Honigbiene im post-genomischen Zeitalter

The honey bee in the post-genomic era

Markus Neuditschko

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Rapid innovations in high-throughput sequencing and array technologies made it feasible to re-sequence a large fraction of any genome including honey bees. To date, mostly drone genomes build the genetic resource for diversity and genome-wide association studies (GWAS), as their haploid nature facilitates cost-efficient whole-genome sequencing. A major disadvantage of drones is that they only explain part of the genetic diversity, as multiple paternal origins are involved in the formation of honey bee colonies. Therefore, we investigated the utility of pooled worker sequences to unravel the population genetics of honey bees. Using a pool of 500 workers of 155 *Apis mellifera mellifera* colonies we identified two major quantitative trait loci (QTL) associated with recapping of *Varroa destructor*-infested brood cells. The best-associated QTL is located on chromosome 5 in a region previously found to be associated with grooming behavior, a resistance trait against *Varroa destructor*, in *Apis mellifera* and *Apis cerana*. The second best-associated QTL is located on chromosome 4 in an intron of the *Dscam* gene, which is involved in neuronal wiring. Furthermore, we analyzed reconstructed whole-genome queen genotypes from our pool-seq data experiment including 265 Western honeybee colonies form *Apis mellifera mellifera* and *Apis mellifera carnica*. Based on this data we were able to derive genomic inbreeding values (F_{ROH}) of the queens and to identify breed-specific selection signatures (e.g. the lighter striping pattern of *Apis mellifera carnica*). Here, we demonstrated the pooled worker sequences can be successfully applied to investigate the population dynamics in *Apis mellifera* including the identification of QTL and candidate genes associated with important selection criteria.

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Keywords: whole-genome sequencing, quantitative trait loci, candidate genes, conservation genetics

Session Ökologie, Wildbienen, Bestäubung, Bienenprodukte 1
Dienstag 28.03.2023, 14:15-15:00

V1. Das metabolische Profil im Darm der Bienen im Lebenszyklus einer Arbeiterin

The hindgut metabolome during the life cycle of a worker bee

Beatrice Engelmann[1], Abdulrahim Alkassab[2], Silvio Erler[1], Cassandra Uthoff[2], Ulrike Rolle-Kampczyk[1], Martin von Bergen[1,3,4], Jens Pistorius[2]

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In honey bees, a balanced microbiome-host interaction is thought to play a crucial role in bee health, but little information is available on this. As the activity and consequently the diet changes during the life cycle of a worker bee, it is crucial to assess the microbiome-host interaction at different time points.The molecules that can mediate between the microbiome and the host are thought to be metabolites produced in the hindgut.
To determine the baseline metabolic profile in the hindgut, we performed a metabolic analysis using untargeted metabolomics of hindgut samples from worker bees at different time points. Larvae were reared in vitro, marked after hatching and then placed in colonies to expose them to the typical environment. Bees were sampled after 2, 4, 7, 14, 18 and 21 days according to their markers. The hindgut was extracted, homogenised using a bead mill and the metabolites extracted by adding quenching reagents. Untargeted metabolomics analysis was performed using a qTOF mass spectrometer and more than 200 metabolites were identified and quantified in a relative manner. The metabolite profile allowed the grouping of metabolites such as ribose and 18-hydroxycortisone, which were specifically abundant in the nurse bee stage, and juvenile hormone III, which was specifically found in the wax bee stage. Targeted analysis of short-chain fatty acids showed a dominance of acetate, but also age-dependent effects on propionate production.
We conclude that hindgut metabolomics has the potential to reveal age-dependent and, in the future, microbiome-dependent effects. The incorporation of additional omics techniques may be useful to unravel subtle effects on microbiome-host interactions and thus bee' health.

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V2. Der Einfluss von Landschaftsheterogenität auf in Hohlräumen nistende Bienen und Wespen innerhalb einer stark anthropogen beeinflussten Kulturlandschaft

The effect of landscape heterogeneity within a highly anthropogenically impacted cultivated area on cavity nesting bees and wasps

Christopher Wild, Julia Zelychenko, Sebastian Hopfenmüller, Robert J. Paxton, Panagiotis Theodorou

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Habitat degradation, fragmentation and loss through agricultural intensification cause severe declines in species diversity. Cavity nesting Hymenoptera species are an important component of biodiversity by providing pollination services to many wild and crop plants, by acting as biological control agents and by being vital hosts for other animals, including species of rare parasitoid Hymenoptera. In addition, communities of cavity nesting bees, wasps and their natural enemies are potential bioindicators of habitat quality.

Here, we investigate the relative influence of habitat availability and connectivity on nest colonisation by cavity nesting bees and wasps, and their parasitoids, within the highly anthropogenically influenced Günz valley in South-West Bavaria. For the purpose of our study, we used standardised nesting sites (trap-nests) at 16 extensively managed meadows distributed across the entire Günz valley. After field placement in spring 2021, trap-nests were returned to the lab in autumn 2021, kept at ambient temperature and opened to take photos of every board and to determine their contents. We combined morpho-taxonomy and DNA barcoding for the identification of bees and wasps. Trap nests were readily occupied; overall occupancy per site varied between 14.38% and 77.78%. The average overall occupancy per site was 48.24%. Our data allow the evaluation of the importance of landscape composition and habitat connectivity for cavity nesting Hymenoptera in the agriculturally dominated region of the Günz valley. Such knowledge will inform conservation management to mitigate the negative effects of anthropogenic habitat fragmentation and loss on the diversity and abundance of cavity nesting bees, wasps and parasitoids in agricultural landscapes.

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Keywords: cavity nesting bees, species richness, trap-nests, landscape composition and configuration, *Osmia*

SB

V3. CBPV Monitoring in den Niederlanden: Im Westen nichts Neues?

CBPV Monitoring in The Netherlands: What's up in the west!

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The Chronic Bee Paralysis Virus (CBPV) can weaken honey bee colonies to the extent that they do not survive a winter. Clinical symptoms include black trembling bees, dislocated wings, bloated hairless abdomens, and dead bees in front of hives. This virus is increasingly observed in Italy, England, Southern-Germany, and the United States. We sampled 304 colonies to assess CBPV incidence in the Netherlands. A sample consisted of 30-50 living bees, per colony. Thirty deep-frozen bees were weighed and crushed per sample. Subsequently, DNA and RNA were extracted. RNA of the CBPV virus was amplified, and the number of copies was quantified by fluorescence (TaqMan RT-qPCR). Beekeepers had been asked to sample colonies of different strength and indicate whether CBPV symptoms had been observed in these colonies. The questionnaire also asked if in their experience „CBPV infections occur more frequently nowadays?". The majority of beekeepers reported not knowing whether infection was increasing (57%). The remaining beekeepers reported an increase in CBPV infection in the south (82%), east (50%), north (33%), and west (25%). The number of virus particles in samples from 304 sampled colonies indicated 93% CBPV positive and 7% negative. The numbers of virus particles did not differ significantly by region ($p=0.58$) or colony vitality ($p=0.24$), but it did differ based on symptoms ($p=0.04$). Colonies with symptoms had more virus particles than colonies without symptoms. According to the European Reference Laboratory, the threshold for symptoms is 100 million CBPV virus particles per bee. This seems consistent with our collective subjective and objective data: 5.6% of analysed samples exceeded the symptomatic threshold, while 5.2% of the colonies were reported to have shown symptoms. Although the majority of CBPV infections in the Netherlands are hidden - we note that an increase in CBPV incidence is observed among beekeepers - especially in the south of the country.

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Keywords: virus, honey bee, colony health, *Apis mellifera*

Session Ökologie, Wildbienen, Bestäubung, Bienenprodukte 2
Dienstag 28.03.2023, 16:30-17:30

V4. Einfluss der Landschaftsstruktur und Agrarumweltmaßnahmen auf die (Wild-)Bienenvielfalt in Agrarlandschaften

Interacting effects of agri-environmental measures on bee diversity and abundance in agricultural landscapes

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In recent years a dramatic decline in the biomass, abundance and biodiversity of insects has been reported affecting also wild bees. One of the most important causes of the decline in wild bee populations is the intensification of agriculture, which leads to a reduced supply of floral resources and loss of habitats. Organic farming and flower fields are two popular agri-environment schemes (AES) which have been shown to enhance bee diversity and abundance in farmland by providing floral resources. However, it remains unknown how these two AES interact at the landscape scale and how their effectiveness changes with the availability of semi-natural habitats (SNH) which might provide important nesting and foraging sites. We expected to find highest wild bee abundance and diversity in landscapes with high amounts of all three habitat types, both AES and SNH.

To examine these effects, we selected 32 landscapes with three independent landscape gradients (percentage area of organic crops, annual flower fields and SNH) and conducted transect walks, in seven locations spread across the study landscapes, over three sampling rounds to record flower visiting bees. Initial results show that a higher amount of annual flower fields in the landscape positively affects the abundance of wild bees, but only if the cover of organic crops is low. This effect could be due to the high amount of floral resources which are already provided by organic farming making an additional surplus of flowers ineffective without providing more nesting sites. SNH had no effect on wild bees, maybe because the quality of the SNH is more important than their spatial extent. Our preliminary results indicate that high amounts of AES are necessary at the landscape scale to promote wild bees. However, flower fields should be mainly implemented in landscapes with more intensive farming, i.e. low amounts of organic crops, to be most effective if no additional nesting sites are provided.

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Keywords: honeybees, wild bees, abundance, landscape composition, agri-environmental schemes

SB

V5. Unterschiede in der urbanen Wildbienengemeinschaft: Ein Muster homogener Heterogenität

Differences in the composition of the urban wild bee community: A pattern of homogeneous heterogeneity

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Land use change like increasing urbanisation is a major driver of biotic homogenisation, the reduction of species diversity due to adaptation in urban environments. Urban areas are often referred to provide habitat only for a filtered wild bee community. Therefore, it is expected that wild bee communities at different habitats in a city are comparable. In this study, the differences in wild bee community composition in a city, sampled at 49 study sites in 2019, were examined. Study sites were distributed over the entire administrative area of Braunschweig and cover a gradient from the city centre to the rural surrounding. Communities sampled on these study sites differed markedly. Dissimilarity of the community of flowering plants had a significant effect on differences of wild bee communities, whereas geographic distance between study sites and the distance to the city centre had no effect. Shannon diversity of the sampled bee community of most study sites was only slightly lower compared to the citywide bee community. This, in combination with the markedly differences in community composition, shows that cities provide suitable habitats for various wild bee communities with a mostly homogeneous diversity and these communities themselves depend mainly on the communities of flowering plants. This shows the importance of preserving and creating heterogeneous plant structures in cities to conserve or even improve the urban wild bee community.

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Keywords: wild bees, beta diversity, community composition, Shannon diversity, conservation

SB

V6. Änderung der Honigbienendichte auf artenreichen Wiesen: Beeinflusst sie das Sammelverhalten von Bestäubern?

Different honey bee densities: Do they shift the foraging patterns of wild and managed pollinators in flower rich meadows?

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Competition between honey bees and other pollinators for floral resources is of concern in light of insect losses, however, few studies to date have investigated the actual impact of increased honey bee density on foraging and fecundity of other flower visitors.

We examined how the common practice of colony migration in and out of flower-rich meadows affects flower visitation rates of pollinators on a wide range of important wild perennials.

Our experimental approach tracked floral foraging at four different locations with plant-rich meadows in Baden Württemberg, Germany in 2022. At every location, detailed pollinator counts were performed on several standardized sections of 1 m², while the honey bee density was varied every other week by migrating in five full size honey bee colonies and then removing them again two weeks later. Over a three-month period, we conducted more than 30 observation trials with 1,890 single plant observations on 67 different flowering plant species and recorded changes in flower visitation rates of honey bees, wild bees, hoverflies, butterflies and other pollinators. Important parameters, such as number of flowering units, temperature or solar irradiance, were continuously recorded during the counts, and the monitoring of honey bee foraging was supplemented by collecting pollen from the colonies and analyzing the plant source.

Our preliminary analysis (GLMM) showed a significant increase in flower visitation by honey bees on wild perennials ($p < 0.001$) when colony density was increased. When grouping all other wild pollinators together, the increased honey bee density led to a significant decrease in wild pollinator visits ($p < 0.001$). However, not all pollinator groups were affected to the same degree.

This work was funded by the Ministerium für Ernährung, Ländlichen Raum und Verbraucherschutz Baden-Württemberg.

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Keywords: honey bee density, competition, pollinator foraging, wild bees

SB

V7. Kann DNA-Metabarcoding zur Sortenbestimmung von Honigtauhonigen genutzt werden?

Can DNA-Metabarcoding be used to determine honeydew honey varieties?

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Current methods for the identification of honeydew varietal honeys rely heavily on organoleptic tastings, which are subjective classifications. We sought to establish DNA metabarcoding as an alternative method for varietal determination of honeydew honeys.

We performed 144 DNA extractions in three batches, on 134 honeys classified as fir, spruce, conifer, *Tilia*, leaf honeydew, and mixed-source honeydew using traditional methods. These extractions were sent to the bioinformatics company AIM-Advanced Identification Methods for metabarcoding to identify potential honeydew phloem-feeding insects (honeydew producers).

We found no DNA evidence from honeydew producers in 9 of the 144 DNA processed samples, 8 of which were from *Tilia*. The ninth honey was a sample analyzed in each of our batches, indicating that the third batch with very low reads had a systematic extraction issue.

The mean number of honeydew DNA reads differed significantly: fir (Mean = 513; Median = 290), spruce (177; 80), conifer (282; 178), *Tilia* (33.8; 4.5) and leaf honeydew (36.7; 33.8).

As we were attempting to establish a new method of analysis, sample preparation of honeys was imperfect with room to improve extraction methods. The DNA extracts were sometimes of low-read quality, or the DNA could not be affiliated with a specific honeydew producer species. The Physokermes species, typical on spruce trees, were difficult to detect. In future, in cooperation with AIM, we wish to improve our DNA extraction of honeys, compare results with nectar honeys, and improve DNA repositories for honeydew producers by sampling the insects directly.

To establish how reliably a specific honeydew honey is classified using traditional methods, we will conduct a blind-tasting of 30 honeys by five different analysts. These results will allow us to better understand how these honeys should be classified by the DNA metabarcoding results.

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Keywords: honeydew, DNA metabarcoding, varietal determination

SB

Session Genetik und Zucht
Dienstag 28.03.2023, 17:45-18:30

V8. Not like mother - the *Varroa* resistant traits VSH and SMR show high variability in daughter colonies.

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Breeders seek to sustainably control populations of the parasitic mite *Varroa destructor*, which threatens honey bee colonies worldwide, by developing *Varroa*-resistant honey bee through selection. Various resistance mechanisms have been investigated and three promising behavioural suites are often used in selection programmes: SMR (Suppressed Mite Reproduction), VSH (*Varroa* Sensitive Hygiene) and REC (Recapping).

Colonies displaying SMR have a high proportion of non-reproductive mites. The VSH trait results in a high removal of *Varroa* infested brood by the adult bees, while REC is the opening and recapping of brood cells by the adult bees. In our regional breeding program, the SETBie project, we focused on selecting primarily for high levels of SMR and VSH, though we also assessed REC in colonies. Over the four years of the project, we reared subsequent offspring from colonies, allowing us to assess the inheritance of the traits. SMR was measured by opening up to 450 brood cells per colony and documenting the mite's reproductive activity in singly infested cells (only one mother mite). The SMR score is the number of non-reproductive mites divided by the number of single infested cells. VSH values were determined using the direct infestation method. Both SMR and VSH values showed high variance in subsequent generations with high rates of SMR and VSH often substantially reduced in the daughter colonies, while mother colonies with low values often produced daughters with higher scores. This remarkable heterogeneity of both traits in the next generation may be partially explained by the high recombination rate of the honey bee. In some cases a complete loss of the trait was observed within three generations.

Based on this high variability, crosses of high performing colonies don't necessarily produce stellar results. Thus offspring must be evaluated for SMR and/or VSH in each subsequent generation. Assessments of SMR and VSH are time-intensive and may not be the best method for selecting for *Varroa* resistance.

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Keywords: SMR, VSH, varroa resistance, breeding

SB

V9. Genetische Verwandtschaft der Honigbienen in Deutschland mit europäischen Unterarten

Genetic relationship of honeybees in Germany to European subspecies

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While Europe is home to a great variety of regionally adapted honeybee subspecies, breeding and commercial trade shape the honeybee population in Germany. The native subspecies *Apis mellifera mellifera* was mostly replaced by *Apis mellifera carnica* and the hybrid breed Buckfast. We conducted a survey among German beekeepers, which included worker bees for genotyping on a recently developed high density SNP chip comprising 70‘814 markers. Additional workers from six European subspecies and 15 countries were collected as references to identify the subspecies of the German samples. The data set comprised 1584 genotypes of single worker bees from different hives. From Germany, 1181 samples were collected, of which 150 samples of feral and managed bees were provided by the BEEtree-monitor project (www.beetrees.org), and 166 from breeders of *A. m. carnica*. According to the questionnaire, there were 461 more samples of *A. m. carnica*, 75 of Buckfast, and 6 of *A. m. mellifera*, while the subspecies of the remaining samples was not identified. To determine the genetic diversity, genomic coefficients of relationship were calculated.

The current results suggest that the *A. m. carnica* kept by breeders are closely related to the bees of other beekeepers in Germany, and much different from the reference samples of *A. m. mellifera*. The samples of *A. m. carnica* from Germany showed clear genetic differentiation from *A. m. carnica* in other countries, probably due to regional adaptation or hybridisation. To shed more light on the internal composition of the German population cluster analysis is required.

Safeguarding the genetic diversity of honeybee populations is important not only as basis for breeding efforts, but also to face future challenges such as climate change, the transformation of landscapes, honeybee diseases and parasites, as well as changes in economic conditions for beekeeping. Monitoring the genetic diversity in Germany is the foundation to advise breeders and beekeepers.

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Keywords: Monitoring, Genetic diversity, SNP chip, European subspecies

V10. Genetische Modelle für die unterdrückte Milbenvermehrung und andere Brutmerkmale und ihre Nutzung als Selektionsmerkmale in BeeBreed.eu

Genetic Models for Suppressed Mite Reproduction and Other Brood Characteristics and their Utilisation as Selection Traits in BeeBreed.eu

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Genetic factors of the honeybee influence the reproduction of *Varroa destructor* in honey bee colonies, therefore, brood investigations for suppressed mite reproduction (SMR) and recapping of all (RECall) or infested cells (RECinf) are a promising approach for targeted selection for improved resistance to *Varroa* mites. Besides confirmation of sufficient heritability, the main challenge is to bring the strategy into breeding routine.

The results of the brood tests are represented by mixed-linear models whose genetic parameters are determined by REML / AIREML. Different model types, especially the treatment of the fixed effect, were weighed against each other using validations.

The breeding traits SMR and recapping showed high heritabilities in the studied breeding population, comparable to the already established pin test. The validations show that descendants of colonies with high breeding values reliably show improved traits. Since brood investigations can realistically only be carried out on a small fraction of the breeding population, other colonies must also be evaluated via relationships. In a breeding value estimation based on a specially tailored pedigree, many queens are evaluated even without own measurements and published on BeeBreed.eu.

The improvement of *Varroa* resistance through brood investigations now requires a continuing effort of breeders who further record the results of brood investigations and select on SMR and recapping breeding values, also taking into account the vitality of the colonies and *Varroa* infestation measurements. Meanwhile, breeding models must be permanently readjusted to form a coherent overall strategy.

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Keywords: SMR, Recapping, BeeBreed, *Varroa destructor*

Session Physiologie und Verhalten
Mittwoch 29.03.2023, 09:30-11:00

V11. Zuckerwahrnehmung in Honigbienen

Sugar Perception in Honeybees

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Sugars from floral nectars serve as a primary source of carbohydrates for honeybees (*Apis mellifera*). The molecular basis of sugar taste perception is mediated by gustatory receptors (Grs), which translate chemical stimuli into electrical signals. Although the honeybee genome comprises only three annotated and partially characterized sugar receptors (AmGr1-3), the understanding of their physiological relevance for sugar taste has been limited to the collective responses of the entire Gr-set *in vivo*. To gain a deeper understanding, we expressed AmGr1-3 heterologously in *Xenopus laevis* oocytes and investigated their sugar-induced cation transport properties using the two-electrode voltage-clamp technique (TEVC). Additionally, we generated AmGr1-3 knock-out mutants using CRISPR/Cas9 and quantified their sugar taste perception with the proboscis extension reflex (PER) to link the TEVC data to the physiological role *in vivo*. To match the wildtype (WT) and mutant sugar Gr-repertoire in the oocyte system, we performed TEVC with oocytes expressing different AmGr-ensembles. Furthermore, we employed bimolecular fluorescence complementation (BiFC) assay to verify the heteromerization of these Grs and validate our results. Our findings demonstrate that sugar specificity and desensitization-behavior of AmGr1 is modulated by heteromerization with AmGr2, but not with AmGr3. Despite AmGr2 functioning as an independent receptor, AmGr2 mutants exhibit a sugar taste like WT bees. AmGr3 may form a heteromer with AmGr2, but it remains a specific fructose receptor. Elimination of AmGr3 while retaining AmGr1 and AmGr2 in the bee abolishes fructose perception but not sucrose. Our study contributes to a better understanding of the honeybee gustatory system, highlighting the significance of AmGr1 and AmGr3 in sugar taste perception, suggesting that bees rely on these receptors to sense all relevant sugars.

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Keywords: sugar perception, gustatory receptors, *Xenopus* oocytes

SB

V12. Auswirkungen der konventionellen und der innovativen Bienenhaltung auf das Verhalten von Honigbienen

Effects of conventional and innovative beekeeping on the behavior of honeybees

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Humans have kept honeybees (*Apis mellifera*) for thousands of years. Over time, different beekeeping practices have been developed to optimise the health of the bees as well as their productivity. The varroa mite (*Varroa destructor*), is a major challenge to honeybee colony health. Under conventional methods, chemical treatments with formic acid and oxalic acid are used to reduce the mite load. In addition to these treatments, drone brood is removed in spring. This interruption of the natural structure of a colony may prevent a stable parasite-host relationship. A possible alternative to the conventional method is a more natural and innovative method of beekeeping with (1) leaving the drone brood in the hive, thereby supporting natural selection of drones. (2) During an induced brood interruption by caging the queen in late summer, which resembles the situation of natural swarming, the varroa mites cannot reproduce, because they lack the pupae as their reproduction hosts. Also, (3) one chemical treatment with oxalic acid when the hives are brood free should reduce the infestation rate.
To investigate whether our novel approach for innovative beekeeping leads to differences in mite loads, we compared varroa infestation during the year using sticky boards and the soapy water washing technique during mating season. We also compared honey harvest, where the innovative hives had a higher yield. We investigated whether there were differences in foraging behaviour. Therefore, we tagged honeybees of both methods with RFID tags. Two scanners in front of the hive then recorded when the honeybees entered or left the hive. It turned out that there were no differences in the foraging span, but the innovative kept honeybees started and stopped foraging earlier than the conventional kept honeybees.

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Keywords: *Varroa destructor,* beekeeping, brood interruption, Varroa treatment

SB

V13. *doublesex* spezifiziert die Auslösung und Aufrechterhaltung eines bestimmten Arbeiterinnenverhaltens bei der Honigbiene *Apis mellifera*

doublesex specifies initiation and sustainment of distinct worker behavior in the honeybee *Apis mellifera*

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Keywords: *doublesex,* CRISPR/Cas9, Arbeiterinnenverhalten, Trackingsystem

SB

V14. Visualisierung von *dsx*-positiven Zellen mittels Verwendung von myr-GFP im Gehirn der Honigbiene (*Apis mellifera*)

Visualisation of *dsx* positive cells using myr-GFP in the brain of the honeybee (*Apis mellifera*)

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Keywords: Kastenbestimmung, doublesex, Gehirn, GFP, CRISPR/Cas9

SB

V15. Steigende Temperaturen führen zu einer vermehrten Brutaktivität im Winter. Kann eine induzierte Brutpause die Auswirkungen auf Winterbienen verringern?

Climate change: raising temperatures increase brood activity in winter colonies. Can induced brood interruptions mitigate the impact on winter bees?

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In temperate climate zones, honeybee colonies used to stop breeding in late autumn and queens started to lay eggs again in early spring. Lately, beekeepers frequently observe brood activity throughout the winter month their colonies. Since 2016, we systematically monitored the brood activity status of 60 colonies per year in late November or beginning of December. Here, we describe a positive correlation of brood activity and average winter temperature (Pearson correlation, r = 0,741, 2-tailed).

To analyse the impact of continuous brood activity during winter month, we caged queens for a variable number of days (0, 74 or 117 days) in three consecutive years (total n = 67). In the control group (0 days), 26 of 28 queens survived, as did all 29 queens released in December (74 days) and 9 out of 10 queens released in February (117 days). All surviving queens quickly resumed to lay eggs. We found no effect of queen caging with regard to colony size: the number of workers estimated by Liebefeld method was comparable in control and caged colonies in early spring ($p > 0.05$, one-sided ANOVA, Bonferroni corrected). Interestingly, we observed a tendency for increased brood activity after the release of the queens. Although the colonies were not treated against *Varroa destructor*, we observed a significant reduction in *V. destructor* levels measured by natural mite fall on sticky bottom boards and bee infestation levels. We observed no effect on the survival of winter bees, as measured by the retrieval rate of marked bees or on food consumption. Currently, the detailed analysis of winter bee physiology, e.g. fat body quantity and gene expression levels associated to aging is ongoing.

In conclusion, we observed no negative effects of prolonged queen caging on queen survival and colony development. Caging of queens in late autumn and winter even has positive effects on *V. destructor* infestation levels.

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Keywords: climate change, brood interruption, winter bees, *Varroa destructor*

V16. Die Enträtselung des HiveMinds - Ein Multiskalenansatz zur Ermittlung von Determinanten der sozialer Hyperintelligenz

Unravelling the HiveMind - A multiscale approach to identifying determinants of social hyperintelligence

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Bees are ecologically and socio-economically essential. Their threat is among the most significant challenges of the evolving Anthropocene. The combined behaviors of individuals determine the survival and development of a colony. Eusociality allows altruistic decision making, division of labor, specialized development and morphology, additional evolutionary potential, and multilevel robustness to environmental factors. The "HiveMind" is the collective activity expressed in the complex, coordinated behavior of a colony of social insects. How the behaviors of social insects are controlled at the molecular level and affect the colony through the cells and the individual is so far unclear. We demonstrate our multiscale approach to unravel the HiveMind. Using the CRISPR/Cas9 system, we created transgenic bees carrying fluorescent proteins at the acetylcholine receptor to probe the role of the cholinergic system in bee development. We also present long-term fluorescence microscopy of embryogenesis using an mRNA injection protocol. Finally, we demonstrate an advanced video observation method to analyze the social behavior of labeled bees within cells. The study of which molecules influence social behavior is only possible with honeybees, as they fulfill all the necessary criteria of a multiscale approach: Their genome is fully sequenced, there is a high number of experimental animals through beekeeping, and they lead a eusocial lifestyle. Through a hub in the Rhine-Main metropolitan region, we want to explore development, functioning and collective intelligence to better protect bees and insects. Besides basic research, our findings may also serve as a model of social hyperintelligence to understand the functioning of our globalized, networked modern society or to decipher systems of the next level of artificial intelligence.

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Keywords: HiveMind, social behavior, acetylcholine, CRISPR/Cas9, fluorescence imaging

Session Bienenschutz und Pflanzenschutz
Mittwoch 29.03.2023, 13:00-14:15

V17. Kombinierte Auswirkungen von Fungizidexposition und begrenzten Pollenressourcen auf die Entwicklung von Honigbienenvölkern

Combined effects of fungicide exposure and limited pollen resources on honey bee colony development

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Keywords: pesticide, colony development, pollen limitation, malnutrition, brood assessment

V18. Auswirkungen kombinierter Pflanzenschutzmittel in unterschiedlichen Landschaften auf Honigbienenvölker

Effect of combined plant protection products in different landscape compositions on honey bee colonies

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Insect numbers are continuously declining, among others caused by environmental stressors such as plant protection products (PPPs). PPPs contain active ingredients, which are also available to non-target organisms, for example foraging honey bees. Therefore, mixtures of active ingredients can be found in beehives. This bears the risk of negative synergistic effects, which are not assessed in risk assessments. One relevant combination is the PPP Cantus Gold (fungicide, active ingredients boscalid and dimoxystrobin) and the PPP Mospilan (neonicotinoid, active ingredient acetamiprid), which are applied in rapeseed fields, a mass flowering resource for honey bees.

We conducted a large-scale experiment to investigate the effect of these PPPs on beehives. For this purpose, the pollen of the hives was spiked at field realistic concentrations. Each treatment (control, fungicide, insecticide, and mix) was positioned at nine different locations to investigate potential effects across a gradient of varying resource availability.

The brood development was shown to be highly affected by time with a varying influence of the underlying agricultural gradient, whereas PPP effects were compensated. Analysing the landscape composition in more detail revealed a positive correlation between increasing pollen diversity and more brood, indicating a high importance of diverse landscapes. The amount of stored nectar and honey was solely dependent on the timepoint during the observation time.

Honeybee hives seem to be quite robust to the applied PPP stressors at field realistic dosage. Potentially, the high bee number per hive helps to buffer the stressor. However, the question remains if other mixtures, concentrations, and different PPPs at different timepoints bear the same results. Thus, it is of high importance to observe wild bees and other insects, in particular solitary living ones, which need to compensate this stressor as a single individual.

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Keywords: plant protection product, synergistic effect, landscape composition, pollen diversity, honey bee hive

SB

V19. Wirkung von Pflanzenschutzmittel-Mischungen auf die Orientierung und Flugaktivität von Honigbienen

Effect of plant protection product mixtures on the orientation and flight activity of honeybees

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The decline of insects has become evident in recent years. Besides climate change, urbanization, pathogens or deforestation, the use of plant protection products (PPPs) is one of the main factors. Honeybees are exposed to mixtures of PPPs in tank mixtures or combinations of spray and seed treatments. Nevertheless, PPP mixtures are not assessed in the risk assessment, even though synergistic effects are conceivable. We studied the effects of PPP mixtures on the flight activity and orientation of honeybees. In a homing experiment, bees were marked and treated chronically with feeding solutions of the neonicotinoid Mospilan (active ingredient: acetamiprid), the non-SBI fungicide Cantus Gold (active ingredient: boscalid/dimoxystrobin), the mixture of both or a control solution. Leaving foragers were caught and released 500m from the hive. The proportion of arriving bees (Chi^2 test, $p > 0.05$) and the homing duration (Kruskal-Wallis test, $p > 0.05$) was measured but no treatment effect was found. For investigating the flight activity, newly emerged honeybees were individually labelled with RFID tags and treated for one week with the same feeding solutions as mentioned above. With the help of the RFID system, the honeybees could be monitored when entering and leaving the hive. After analyzing the initiation and ending of foraging behavior, the number of foraging days, the number of foraging trips per day and the duration per foraging trip, no negative effects of the treatments could be detected (GLMM, $p > 0.05$). However, this does not mean that the PPPs or PPP mixtures are harmless to bees. Different concentrations or combinations can lead to different results. The inclusion of wild bees in these studies and the consideration of other stressors is crucial to obtain relevant results.

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Keywords: plant protection products, neonicotinoid, non-SBI fungicide, synergistic effects, sublethal effects

SB

V20. Einsatz von RFID Technologie zur Überwachung der Flughäufigkeit und -dauer einzelner Flugbienen um die Wirkung von chemischem Pflanzenschutz auf die Aktivität von Bienenvölkern zu bewerten

Use of RFID technology to monitor the flight frequency and duration of individual forager bees with the aim of evaluating the effect of chemical crop protection on the activity of bee colonies

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V21. Wirksamkeit und Verträglichkeit von 60% Ameisensäure als *Varroa destructor* Behandlung

The efficacy and tolerability of 60% formic acid for control of *Varroa destructor*

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European medicinal veterinary product law was standardized with the EU Regulation 2019/6 from the 28, January 2022. This eliminated recognition of national authorizations, including the standard authorization currently used in Germany. As a result, formic acid (60%) and lactic acid (15%) may only be sold and applied until the end of this transitional period on January 29, 2027. Formic acid is a critical component of varroa management strategies in Germany, as it is one of the only effective varroacides that can penetrate the brood nest and treat reproducing *Varroa*. The discontinuation of this drug would have far-reaching consequences.

To assist in the registration process, we ran a large-scale experiment to collect data on the tolerability and efficacy of 60% formic acid evaporation. At a total of 6 sites, 5 g of formic acid per liter of hive volume was evaporated from single and double brood chamber colonies using the Liebig and Nassenheider Professional® dispensers. At one site, an additional group was treated using formic acid applied to a dish sponge four times at intervals of 3-4 days compared to three times at intervals of 7 days (3 ml per comb, corresponding to 0.8 g formic acid/l hive volume). FormicPro served as a positive control, negative controls remained untreated. Bayvarol or Apivar were applied as a post-treatment to determine the residual *Varroa* infestation. To evaluate impacts on bees, dead traps were installed in front of colonies and a population estimate was conducted before and after treatment, as well as post wintering. We will present data on formic acid efficacy and tolerability, as well as address current ambiguities in formic acid application.

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Keywords: formic acid, varroa, veterinary law, Nassenheider professional

Session Bienenpathologie
Donnerstag 30.03.2023, 9:30-10:45

V22. Hauptgründe für den Verlust von Honigbienenvölkern in Palästina

Main drivers of honey bee colony losses in Palestine

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Keywords: *Varroa destructor*, *Paenibacillus larvae*, *Nosema ceranae*, prevalence, honey bees

SB

V23. Parasitenbelastung wildlebender und imkerlich gehaltener Honigbienenvölker in Deutschland

Parasite burden in feral and managed honeybee colonies in Germany

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Bee parasites are the main threat to apiculture, and since many parasite taxa can spill over from honeybees (*Apis mellifera*) to other bee species, honeybee disease management is important for pollinator conservation in general. It is unknown whether honeybees that escaped from apiaries (i.e., feral colonies) benefit from natural parasite-reducing mechanisms like swarming or suffer from high parasite pressure due to the lack of medical treatment. In the latter case, they could function as parasite reservoirs and pose a risk to the health of managed honeybees (spillback) and wild bees (spillover). We compared the occurrence of 18 microparasites among managed (N=74) and feral (N=64) honeybee colony samples from four regions in Germany using qPCR. We also distinguished five colony types representing differences in colony age and management histories, two variables potentially modulating parasite prevalence. Besides strong regional variation in parasite communities, parasite burden was consistently lower in feral than in managed colonies. The overall number of detected parasite taxa per colony was lower in feral colonies (median: 5, range 1–8) than in managed colonies (median: 6, range 4–9) (generalized linear model with likelihood ratio test, $P < 0.001$). Specifically, Trypanosomatidae, Chronic bee paralysis virus, and Deformed wing viruses A and B being less prevalent and abundant in feral colonies than in managed colonies. In the comparison of five colony types, parasite burden was lowest in newly founded feral colonies, intermediate in overwintered feral colonies and managed nucleus colonies, and highest in overwintered managed colonies and hived swarms. Our study is in line with the hypothesis that the natural mode of colony reproduction and dispersal by swarming temporally reduces parasite pressure in honeybees. We conclude that feral colonies are unlikely to contribute significantly to the spread of bee diseases.

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Keywords: bee viruses, disease management, pathogen spillover, swarming, wild honeybees

SB

V24. Ein gefährlicher Biss: wie sich Lithium im Honigbienengewebe anreichert, an dem *Varroa destructor* frisst

A dangerous bite: how lithium accumulates in honey bee tissues fed on by *Varroa destructor*

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The greatest current threat to honey bee health is from *Varroa destructor*, which requires treatment. Currently there are limited treatment options. New, highly effective and easy to apply varroacides with limited unwanted side effects like pesticide residues or potential for mites to develop resistance are highly desired; lithium chloride (LiCl), which produces high mite mortality, meets many of these requirements.

To understand how LiCl functions, we screened different parts of honey bee anatomy for lithium accumulation using inductively coupled plasma mass spectrometry (ICP-MS). Our goal was to elucidate LiCl's mode of action and evaluate potential side effects such as accumulation in the hypopharyngeal glands of nurse-aged bees.

First, we dissected the digestive tract into its three main parts (crop, midgut and rectum) and detected lithium in all three compartments after only one day of feeding the compound to the colony. We found the highest concentration in the rectum, indicating a degradation and excretion of lithium from the bee's body.

Our prior research has shown that *V. destructor* feeding on previously treated adult honey bees die, and we currently hypothesize that death is due to feeding on the bee's lithium spiked hemolymph and fat body. We thus wanted to investigate if lithium accumulates in these components and how lithium builds up in the hemolymph when bees are fed for seven days. Lithium accumulated in both fat body and hemolymph, with a hemolymph concentration of 7.1 mg/kg after only 24h of feeding. After 7 days of feeding, the concentration increased slightly to 10.5 mg/kg.

Lastly, we examined the hypopharyngeal gland of 7-day-old nurse bees that were reared in colonies treated with LiCl (results pending). Should we find that lithium also accumulates in these glands, it would indicate a potential risk to queens and young larvae fed by nurse bees.

Our results provide important baseline data which can help in the approval process of this new varroacide.

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Keywords: lithium chloride, hemolymph, hypopharyngeal gland, varroa control

SB

V25. Staatliches Monitoring der Amerikanischen Faulbrut in Sachsen 2019 bis 2022

Official Monitoring of American Foulbrood in Saxony 2019 - 2022

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American foulbrood (AFB) is a notifiable disease, listed in EU directive 2018/1882. Over recent years, outbreaks of AFB have been consistently recorded in Saxony, though at a low rate. In 2019, an official monitoring system was established to detect early stages of colony infections before clinical symptoms of infection are apparent and thereby reduce the costs caused by restrictions on the movement of colonies following an AFB infection.

Every year, approximately a quarter of the registered hives of Saxony was tested for AFB, including honey from the brood nest or winter hive debris. Material from up to 12 samples (colonies) was pooled. Samples were investigated for *Paenibacillus larvae* according to the methods published by the Federal Research Institute for Animal Health, the Friedrich-Loeffler-Institut (FLI).

In total, 8146 samples from 6373 beekeepers were examined. In the first year, 3.1% of the apiaries were positive for *P. larvae.* The rate dropped during the monitoring period to 1.3% in 2022. The *P. larvae* positive samples were classified according to colony forming units (cfu). At the beginning of the monitoring period, 47.7% of the positive samples belonged to the lowest class with <10 cfu, suggesting a low intensity of infection of the colony, whereas 76.7 % of the samples in 2022 belonged to this class.

Across the monitoring period 2019-2022, positive samples were less frequently detected. Furthermore, the number of cfu per sample was reduced. Monitoring offers the opportunity to detect infections with *P. larvae* before colonies show clinical symptoms of the disease, whereupon measures can be taken to prevent the establishment of the disease.

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Keywords: American foulbrood, monitoring

V26. Validierung eines lateral flow assays für die Detektion von Amerikanischer Faulbrut und Genotypunterscheidung von ERIC I und ERIC II

Validation of a lateral flow device for American foulbrood detection and genotype differentiation of ERIC I & ERIC II

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American foulbrood (AFB) is the most devastating bacterial brood disease of honey bees (*Apis mellifera*). AFB causes colony and economic losses worldwide and is a notifiable disease in many countries. The causative agent of AFB, *Paenibacillus larvae*, can be distinguished into two main genotypes ERIC I & ERIC II that show differences in virulence and could be treated differentially. Disease diagnosis is usually conducted via visual inspection. Once a colony is suspicious, the disease has to be confirmed in the laboratory, which is often very time-consuming. The aim of the project is to develop and validate a sensitive and fast on-site diagnostic tool (lateral flow device = LFD) to diagnose AFB and distinguish between the two main genotypes (ERIC I and ERIC II) of *P. larvae*.

LFDs are based on antibody detection of antigens. Therefore, specific monoclonal antibodies (mAbs) recognizing antigens from *P. larvae* were generated in mice for the application in the LFD. The developed LFD is detecting proteins of *P. larvae* in general and of the genotype ERIC II particular. Several field strains of the genotypes ERIC I & ERIC II were successfully detected and genotyped by the LFD (n = 60). Furthermore, the lysis buffer for larvae samples was optimized for the use in the LFD. Disease detection in *in vitro* infected larvae by the LFD was compared with the PCR results of the same larvae samples. After the successful validation of the LFD in the laboratory the next validation phase will be conducted in the field in the next brood season.

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Keywords: American foulbrood, diagnosis, antibodies, *Paenibacillus larvae*

Session Ökologie, Wildbienen, Bestäubung, Bienenprodukte 3
Donnerstag 30.03.2023, 11:00-12:00

V27. Identifizierung von Sortenhonigen mittels FTIR-ATR

Identification of unifloral honeys using FTIR-ATR

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In addition to quality, the botanical origin of a honey is also decisive for marketing, since unifloral honeys can be sold more expensively. A honey can be labeled as unifloral honey "if the honey comes wholly or mainly from the indicated source (floral or vegetable origin) and possesses the organoleptic, physico-chemical and microscopic characteristics of the source" (Council Directive 2001/110/EC). The Fourier-transformed infrared spectroscopy (FTIR-ATR) in combination with discriminant analysis can be used as a low cost, fast and easy supplement to the existing analyses. The determined infrared spectrum of a honey is based on its chemical composition and consequently also differs depending on its botanical origin. Over a period of 5 years, 893 honeys of 14 different unifloral sources were classified by standard analyses. These honey samples were used to elaborate a FTIR-ATR calibration model. The mean spectrum is calculated from the reference spectra of every unifloral origin. The spectrum of a honey sample of unknown origin can now be compared to these mean spectra. The smaller the distance to the mean spectrum, the more likely the unknown sample is a honey of the corresponding botanical origin. The sample set was divided into 711 honeys used for calibration, and another 182 honeys used for validation. 92.0% of the calibration samples and 94.0% of the validation samples were identified correctly compared to the classification by standard method. In case of misclassification of the botanical origin, the honeys were often harvested during the same flowering season (*Brassica napus* vs. fruit blossom; Tilia vs. Ailanthus). This FTIR-ATR model can be used as a support of the determination of the botanical origin of unifloral honeys by classical methods.

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Keywords: FTIR-ATR, unifloral honey, honey analysis, infrared spectroscopy, botanical origin

V28. Bienen standardisiert erfassen: eine systematische Literaturübersicht zum Fang mit Farbschalen und der Erfassung des assoziierten Blühangebots

Standardizing bee sampling: a systematic review of pan trapping and associated floral surveys

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Several methodological studies and conceptual frameworks offer guidance on standardized bee sampling with pan traps (aka bee bowls, Moericke traps). Nevertheless, bee studies use a large variety of pan-trapping methodologies. The lack of standardization complicates the comparison of sampling results among studies, and so it remains in question how floral abundance around pan traps affects the number of bees sampled.

We systematically reviewed all peer-reviewed studies, which used pan traps for bee collection, were published in English until spring 2022 and were listed in the Web of Science core collection. From these studies, we extracted details of pan-trap characteristics and the methodology used to sample flower abundance and diversity around pan traps. We also obtained information on correlations between floral and bee abundance/diversity found in these studies.

Our systematic search yielded 369 references in total; detailed information was extracted from 290 studies. Some methodological aspects such as trap color (e.g. using more than one trap color) were often similar in the majority of studies; other aspects such as sampling duration, filling level or trap solution composition varied considerably. Only a small subset of studies used floral abundance and/or diversity as an explanatory variable in their analyses. In comparison to botanical surveys, these studies often simplified floral sampling methods, probably due to time constraints and the need for synchronization with bee sampling. Correlations between floral abundance/diversity and bee abundance/diversity did not indicate an unambigious relationship between pan trap results and surrounding floral context. The small pool of studies using floral context in their bee analyses indicates a great need for more research on this topic in the future, which should incorporate standardized methods.

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Keywords: bee bowls, Moericke traps, floral abundance, bee abundance, bee diversity

V29. Big Data im Honigbienen-Monitoring: Modellierung und Vorhersagen aus umfassenden Datensätzen mittels state-of-the-art Methoden der Zeitreihenanalyse

Big data in honeybee monitoring: Modeling and predictions from extensive datasets with state-of-the-art time series analysis methods

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Keywords: Trendmonitoring, MonViA, Zeitreihenanalyse, Honigbienenvitalität

Abstracts der Poster

Ökologie, Wildbienen, Bestäubung, Bienenprodukte

P1. Langzeitstudie zu standortspezifischen Veränderungen der Pollenvielfalt in Honigen

Long-term study of location-specific changes of pollen diversity in honey samples

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Our environment is subject to constant transition. Modernization of agriculture, cultivation of new crops for energy production and global warming leads to changes at all sites. This also affects the flora in our habitats and can be monitored by analyzing the pollen spectra of honey samples which provide important information about bees' pasture. Each honey sample is a reflection of the plants from which the bees have collected the nectar.

A multi-year stock analysis of the pollen diversity in honey samples all over East Germany was performed. The main focus was on the evaluation of pollen spectra at locations in semi-natural and protected areas but also pollen spectra at urban sites and areas of intensive agriculture were considered. The evaluation of the honey databases (1998-2022) resulted in 34 locations and 1.478 honey samples which provided pollen data for 9-24 years per location. The pollen types were categorized in “trees and bushes”, “economic plants” and “wild plants and others”.

One aspect of the data evaluation was the comparison of an urban location and a rural biosphere reserve site, in order to show location-related changes in the flora. The urban honeys showed a higher diversity of pollen types dominated by trees (Tilia, Ailanthus, Hippocastanea, Castanea, fruit trees). In the category “wild plants and others” myosotis is the most prominent pollen type. In the honeys from the biosphere reserve pollen from economic plants (mainly *Brassica napus*) were more present and in the category “wild plants and others” a higher dynamic of the pollen types was found. There, an increasing proportion of typical plants for flowering stripes and areas as Phacelia and Sinapis was also observed in the last 10 years.

The enormous data set will be further evaluated and can be serve as an indicator system for the biodiversity of the plants over time in different habitats.

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Keywords: honey, botanical origin, location specific, pollen diversity

P2. Honigsorte aus Bärlauchtracht (*Allium ursinum* L.). Inwiefern dient der Bärlauchpollenanteil im Honigsediment als ein Beurteilungsmerkmal?

Honey variety from wild garlic flowers (*Allium ursinum* L.). To what extent can wild garlic pollen in honey sediment be used as an evaluation criterion?

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In spring honeys harvested in 2022, many had a jelly-like consistency. Remarkably, those floral honeys had portions of wild garlic (*Allium ursinum* L.), which made them difficult to extract.
In 2022, pollen analysis of 628 honey samples from Baden-Württemberg was carried out. *Allium* pollen was found in 249 honey samples with up to a maximum of 71% of the total pollen spectrum of nectar flowers (mean: 5.2%).
A thixotropy test was carried out on 38 honey samples containing *Allium* pollen at 8.9% and above. 15 honey samples showed positive thixotropy. The proportion of *Allium* pollen in the positive samples ranged from 10 to 71%. Surprisingly, there were some thixotropy negative samples with an *Allium* pollen content up to 47%. This suggests that, factors other than the frequency of *Allium* pollen should be considered when defining the honey variety; for instance, other over-represented pollen in the honey sediment such as rapeseed or forget-me-not pollen, and physical parameters, such as the electrical conductivity of honey, color and taste. To conclude, we could not define at which percentage of *Allium* pollen, the honey could be classified as a monofloral wild garlic honey. However, we recommend performing a thixotropy test when *Allium* pollen makes up at least 10% of the total pollen spectrum in order to give the correct definition of the honey variety along wither other parameters.

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Keywords: Wild-garlic honey, *Allium*, thixotropy

P3. Intraspezifische zeitliche und räumliche Variabilität von zoophilen Pollenmerkmalen

Intraspecific temporal and spatial variability of zoophilous pollen traits

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Pollen and their analysis are becoming more and more important due to their biological and ecological importance. Microscopic pollen analysis has been established as the gold standard. But, the trend is towards automated, database-driven pollen analyses that are cheaper, less time-consuming and allow better reproducibility than the traditional microscope-based methods. Such an automated method is imaging flow cytometry linked with machine learning. It enables microscopic brightfield and fluorescence images and traits (e.g. diameter) of pollen to be recorded quickly. Based on this data, a neural network classifier can be trained, which then allows pollen identification. The accuracy of all methods is based on the reference database and its quality. Most databases of automated methods are based on only a small number of reference samples per species (e.g., two to ten samples per species from different plant individuals). Although some studies have shown that pollen traits can vary greatly within a species, most reference databases report only mean trait values. Lack of variation in reference databases can be a reason why a classifier can only be applied to diverse samples in a limited way. Our aim was to quantify spatial and temporal patterns of intraspecific pollen trait variability based on traditional traits (e.g. pollen size and shape) and additionally, new pollen traits (e.g. fluorescence, texture), taking advantage of a novel high-throughput analytic method. Specifically, we measured pollen samples from six representative European insect-pollinated herbaceous plant species using imaging flow cytometry, analysing six pollen traits. We found that most species showed significant spatial as well as temporal variability for at least one pollen trait. Our results provide evidence for the importance of considering variability in pollen traits for future pollen databases and analyses.

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Keywords: intraspecific variability, pollen traits, flow cytometry

SB

P4. Neuartige Methode zur Futtersaft Analyse von *Apis mellifera* außerhalb der Brutzeit

Novel method for brood food analysis of *Apis mellifera* during non-breeding season

Paul Siefert, Jana Bub, Bernd Grünewald

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As eusocial insects, honeybees exhibit cooperative brood care. The larvae of honeybees are fed with brood food, which is produced in the hypopharyngeal glands of nurse bees. The brood food contains millimolar concentrations of acetylcholine, crucial for proper larval development. Neonicotinoids apparently reduce acetylcholine concentration in brood food, but further research is needed. It is possible to stimulate breeding activity in winter inside of a flight room. However, to build and maintain a flight room is expensive and therefore uncommon. Here we present a low-cost method to hold bees in winter for experiments and analyse the acetylcholine concentration in brood food. We constructed a flight box with dimensions of 44 cm x 46 cm x 61 cm that accommodates up to six small bee colonies (Einwabenkästen) on the sides so that the bees have access to the box. The colonies were provided pollen and water *ad libitum* and via in-hive feeder a 1:1 solution Apiinvert and tap water. The flight box was placed inside an incubator with a UV-lamp set on a 12-hour cycle to simulate a day-night rhythm. Furthermore, the acetylcholine concentrations were analysed using a photometric acetylcholine assay kit with acetyl esterase. We developed this method to analyse how insecticides can impair acetylcholine concentrations in the brood food of the honeybee larvae. By using this method, we anticipate the possibility to a cost-effective method for testing and analysing bees throughout the year without the influence of weather or season.

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Keywords: Acetylcholine, Brood food, Incubator

SB

P5. Verfälschungs- und Rückstandsanalysen von Schweizer Bienenwachs

Analysis of adulteration and residues in Swiss beeswax

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In Switzerland, the production of the foundation sheets has been monitored for 30 years. All major commercial manufacturers in Switzerland (n=10) sampled each production batch in 2021 (n=280). Using infrared spectroscopy, all individual batches were examined for adulteration with paraffin, stearic acid, tallow, carnauba and candelilla wax. In addition, representative annual samples were prepared from the batches of each manufacturer in proportion to each batch weight. These samples (n=10) were analysed by gas or liquid chromatography and mass spectrometry for residues related to products used in beekeeping. Three of the 280 individual batches analysed for adulteration contained minor additions of paraffin (approx. 2%, 2%, 4%) below the quantification limit (LOQ) of 6.8%. No adulterants were detected in the other batches. Residue levels in the representative samples ranged from 8.5 to 38.7 mg/kg for thymol (active ingredient (a.i.) of Thymovar), from 0.07 to 1.45 mg/kg for coumaphos (a.i. of Perizin and CheckMite+), from 0.05 to 0.34 mg/kg for tau-fluvalinate (a.i. of Apistan) and from 0.03 to 1.2 mg/kg for DEET (formerly in Fabi Spray, bee repellent). Four out of ten representative samples contained traces of flumethrin (a.i. of Bayvarol) and/or DMF (degradation product of amitraz) and three samples contained bromopropylate (a.i. of Folbex VA). DCB (not authorized, protection of combs from wax moth) was detected in one sample. The analyses for adulterants and residues attest a good wax quality. No adulterants were detected in 99% of the individual batches. Thymol is not problematic for the honey quality at the detected residue levels in the wax. Products to control Varroa destructor containing coumaphos, tau-fluvalinate or bromopropylate as well as the former Fabi Spray (DEET) are no longer approved in Switzerland, so that the residue levels in beeswax will most likely further decrease in the future.

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Keywords: beeswax, residues, adulteration, honey bees, Switzerland

P6. Etablierung eines automatisierten Verfahrens zur Bestimmung der Invertaseaktivität nach DIN 10759

Establishment of an automated procedure for the determination of invertase activity according to DIN 10759

Pauline Hoffmann, Martina Janke, Hannes Beims

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The use of robots in production has become indispensable in many branches of industry. LAVES Institut für Bienenkunde Celle has the epMotion5075 liquid trading system. The determination of invertase activity in honey is to be automated in the future. A protocol for adapting the manual standard method DIN 10759 to the robot was programmed and tested. The results of the manual method were compared with the results of the developed automated method for honeys of different botanical origins and different ranges of invertase activities and evaluated by comparison with results from laboratory comparative tests and the statistical characteristics of the standard method. The verification showed no significant difference in the results between the manual and the automated method ($p=0.08791$, $n=40$). The cause of the higher standard deviation of the robot results could be attributed to edge effects. The next step is to check whether the results can be corrected by correlating factors. After subsequent validation, the automated method is to be used in routine analysis.

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Keywords: Invertase Activity, honey, automatisiert, robot, DIN 1059

P7. Städte im Spannungsfeld verschiedener Akteure – Herausforderungen und Chancen für Bestäuber

Cities caught between different actors – challenges and opportunities for pollinators

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The importance of urban habitats for humans, but also for insects and other animals, has steadily increased in recent years with a view to increasingly green urban development. The design of urban habitats requires a close integration of geography, ecology, architecture and planning, as cities increasingly suffer from heat stress and are particularly exposed to climate change. At the same time, they often provide good foraging habitat for bees and other insects throughout the year with a wide variety of flowering plants. In my work, I identify environmental and geographic factors that have an impact on honey yield of bee colonies. For this purpose, I use the long-term monitoring data of TrachtNet. This monitoring system covers bee scales all over Germany, whereas the data chosen here refer to Bavaria and Rhineland-Palatinate, covering very different landscapes. For the landscape factors, a classification based on the Sentinel-2 satellite is used.

My results show that the proportion of sealed area, which is particularly high in cities, has a significant impact on honey yield of bee colonies. On average, the honey harvest begins two days earlier at sites with a sealed area ratio of more than 21%. This effect becomes stronger the more sealed area is found directly around hive. However, tracht length is also an important factor, but did not differ at different urban sites. Average temperatures had no decisive influence on colony yield in the period 2016 - 2020.

These results prove that cities are also very good locations for honey bee colonies and can be considered in the future when planning insect-friendly cities.

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Keywords: honeybee, landscape, city transformation

SB

P8. Bienenwald Hessen - Neue Konzepte für Naturschutz, Land– und Forstwirtschaft

Bienenwald Hessen – New approaches to bridge conservation, agriculture and forestry

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The effects of climate change and diversity loss are threatening all parts of our society. This especially includes fragile forest ecosystems and pollinator communities providing crucial ecosystem services. In addition, the production of food and timber products is gaining increasing attention. These differing interests often lead to a land-use conflict between the production of food and raw materials and different conservation aims.

To elaborate new ways to solve such conflicts, we investigate different conservation-through-use approaches. Using a bottom-up attempt, we thereby link the interest of actors from agriculture, forestry and conservation to find applicable ways of reforestation, which also provide floral resources and human food supply. Therefore, three former spruce (*Picea abies*) plantations were partly sown with perennials and restocked with tree species offering good forage for pollinators. Since the initial spruce plantations died due to drought and bark beetle infestation, tree species adapted to semi-arid conditions were chosen. Besides their potential for log production and improvement of floral resources, most species enable honey production (e.g., *Robinia pseudoacacia*, *Prunus avium*) and provide other non-timber forest products like walnuts (*Juglans regia*) and sweet chestnuts (*Castanea sativa*). Thereby, forestry and agriculture can be combined to open up incomes for forest owners and belay food production. To investigate the apicultural relevance, commercial beekeeping operations gather harvest data in older stocks of the respective tree species. The economic potential will be evaluated in more detail in the next two years. In addition, the resource use of wild bee taxa will be monitored alongside with the development of honeybee colonies on the study sides. In parallel to the field investigations, a participatory study with different stakeholders is continuously conducted to ensure the practical relevance of the tested methods.

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Keywords: pollinators, non-timber forest products, reforestation, bee diversity, floral resources

P9. Vibratorische Lärmverschmutzung im Bienenstock durch Eisenbahnverkehr

Vibrational noise pollution in bee hives generated by railway traffic

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In recent years, anthropogenic noise and its effects on animal physiology and behaviour has been studied focusing mainly on airborne noise and on mammals and birds, but not on vibrational noise and arthropods. Therefore, we started to explore the effects of vibrational noise on honey bees (*Apis mellifera*), since they communicate through comb vibrations and they are often placed close to anthropogenic structures like roads and railways producing vibrations. We recorded substrate borne vibrations and airborne sound generated by trains in bee hives at different distances and sites. Vibrations were recorded on the front and back comb in three dimensions (within the comb plane and perpendicular to the comb plane) and at the base of a hive. Our results show that vibrational noise of substantial amplitude is present in the ground and in the combs. The intensity of vibrations varies depending on the ground, level, presence of obstacles and the type of train and attenuates with distance. The attenuation of the airborne sounds is found to be 6 dB per doubling of distance plus some variable linear excess attenuation. The attenuation of the substrate borne vibrations is much lower, 3 dB per doubling of distance plus a small excess attenuation. Vibrations of the combs are therefore clearly transmitted from the rails to the combs through the ground, not as airborne sound. The most intense frequencies are just the range at which bees are most sensitive and communicate. The comb vibrates in all three dimensions. Based on these data, we have developed a playback system allowing to vibrate five bee colonies artificially in a realistic way and to compare their behaviour, colony weight and colony development over a period of several months with five control colonies in order to estimate adverse effects of vibrational noise on honey bee colonies.

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Keywords: anthropogenic noise, substrate vibrations, stress, *Apis mellifera*

SB

P10. Die Größe zählt: größere Farbschalen fangen mehr Bienenindividuen und -arten als kleinere Farbschalen

Size matters: larger pan traps collect more bee individuals and species than smaller pan traps

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Although pan traps are used as an established method for sampling bees across a wide range of habitats and geographical regions, uncertainty persists as to how pan-trap characteristics influence sampling results. We investigated the effect of pan-trap diameter on sampled bee communities at agricultural sites around Braunschweig, Lower Saxony, Germany. We installed 108 pan traps at six sites, with equal proportions of color-diameter combinations per site (yellow, blue and white; 22 cm versus 12 cm in diameter). We sampled bee individuals in three rounds of 24 hours (March/April, June, August/September) in 2021. In total, we collected 1154 bee individuals, which to a large extend have been identified to species level. We observed interacting effects of pan trap color and size on the number of sampled bee individuals and species. Larger pan traps collected significantly more bee individuals and species than smaller pan traps independent of trap color (Abundance: Negative Bionomial GLMM; Species: Poisson GLMM). The estimated number of sampled species based on the same number of sampled individuals (individual-based rarefaction) was higher for large pan traps than for small pan traps at all sampling sites. Based on our findings, we advocate for the use of larger pan traps for sampling bees in order to increase trap efficacy and efficiency.

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Keywords: bee abundance, bee diversity, rarefaction, bee monitoring

P11. Konkurrenz oder Begünstigung – beeinflusst das Blütenangebot um Farbschalen die Erfassung von Bienen?

Competition or facilitation? The impact of flowers around pan traps on bee sampling results

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Although pan traps have been used for sampling bees across a wide range of habitats and geographical regions for decades, varying floral resources around pan traps may bias sampling results. This raises questions about the suitability of pan traps for bee monitoring programs that sample sites with varying flower cover.

We investigated the effect of floral context around pan traps on sampled bee communities at agricultural sites around Braunschweig in a two-year field experiment. We installed 72 pan traps at 13 sites in 2021 and 2022, respectively, with equal proportions of color-context combinations per site (yellow, blue and white; center of flower strip versus adjacent to flower strip, i.e. at 1 m distance from the edge of the flower strip). We simultaneously assessed the percent flower cover in 2.5 m radii around each trap. We sampled bees for 24 hours three times (March/April, June, August/September) each year.

In total, we collected more than 3600 bee individuals over the course of the experiment. Statistical analyses of the two-year dataset revealed effects of flower cover interacting with bee taxon on detection probability per trap (Logit GLMM) as well as on the number of sampled bee individuals per trap (Negative Binomial GLMM). Based on our findings, we encourage bee researchers to assess flower cover around traps to account for differences in trap attraction, especially when sampling bees in habitats with contrasting floral resource availability.

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Keywords: detection probability, floral abundance, bee abundance, bee diversity, bee monitoring

Genetik und Zucht

P12. Genexpressionen am geschlechtsbestimmenden Locus in Larven von *Apis mellifera*

Gene expressions at the sex-determining locus in larvae of *Apis mellifera*

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The molecular basis of sex determination is well understood in the honey bee *Apis mellifera*. In this context, the *complementary sex determiner* (*csd*) gene acts as the primary signal of sex determination based on the allelic endowment. Heterozygosity at *csd* initiates female development (2nF), while hemi- (1nM) and homozygosity (2nM) at *csd* leads to male development. The *csd* homozygous individuals (2nM) are diploid drones and have no fitness. As larvae, they are recognized and eaten by worker bees during the first 72 hours after hatching. In the present study, we will test whether the key gene *feminizer*, located downstream of csd within the sex determination cascade, and the gene *pinocchio*, located in spatial proximity to *csd*, show differences in gene expression. The dependence of the chromosomal set and the resulting sex, as well as the course of larval development, are taken into account. In this study, larvae from inbred crosses were reared *in vitro* at intervals of 24, 48, and 72 hours after hatching. Expression levels of candidate genes were analyzed by real-time qPCR. Differences in gene expression between the sexes were found during early development. These differences may contribute to the recognition of diploid drones by worker bees. Further research is needed to elucidate this complex behavioral pattern to identify all factors involved and consider them in the full context.

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Keywords: *Apis mellifera*, diploide drones, *feminizer, pinocchio*, genexpression

SB

P13. Hinweise auf einen Einfluss der Standaufstellung der Begattungskästen (Apidea und EWKs) auf die Begattungsergebnisse in der Carnica-Rassebelegstelle Jasnitz (DE-8-4) Mecklenburg-Vorpommern; LIMV e.V

Frist evidences of arrangement influences on the mating results of mating units (Apidea and single-comb-units „EWK“) in the Carnica race breeding station in Jasnitz (DE-8-4) Mecklenburg-Vorpommern LIMV e.V.

Mathias Jander, Mona Keinert, Hilger Jagau

Landesverband der Imker M-V e.V. Bienenzuchtzentrum Bantin

First evidences towards an arrangement and mating unit based effect on the successful mating rates. The arrangement of the mating units (Apidea and one-comb-units) in the breeding station Jasnitz as well as the positioning, scattered directly on the forest ground, combined on a metal stand (4 Apidea units) or on a wooden pallet on the forest ground (8 Apidea units) shows different successful mating results. Results point towards a beneficial effect, when Apidea units are used and a scattered arrangement is used.

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Keywords: breeding, carnica, arrengement, Apidea, EWK

Physiologie und Verhalten

P14. mRNA basierte Fluoreszenzmarkierung und Live-Aufnahme von Honigbienen Embryonen

mRNA based fluorescence labeling and live-imaging of honeybee embryos

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Pollinating insects play an indispensable role in both ecosystems and in agriculture. Besides the general loss in biodiversity, the ongoing decline in insect biomass during the last years therefore also poses a threat to global crop productions. One strongly affected species is the western honey bee *Apis mellifera carnica* which also has a high economic relevance. Various studies have identified key elements that are responsible for this trend such as pollution, insecticides or monocultures. Even though the mentioned factors probably influence the embryonic development, the dynamics of these processes have not yet been sufficiently addressed.

Here we present a method for non-invasive long-term fluorescence microscopy of honey bee embryos. Firstly, we developed an mRNA based protocol for fluorescence labeling of honeybee embryos.

A variety of factors were optimized to ensure viability of the embryos in the experimental setup and the imaging in general. We present an adequate imaging media that allows the necessary oxygen exchange during egg development, which is a unique requirement compared to other model organisms like *Drosophila melanogaster* or *Tribolium castaneum*. Additionally, we essayed embryo survival by comparing anterior and midventral injection and characterized spreading of fluorescence marker expression in the organism.

Taken together, these findings enabled us to record the world's first fluorescence time lapse of the honeybee's embryogenesis and hatching using light-sheet fluorescence microscopy. We show thereby that the honeybee embryo is suitable as a model organism for analyzing dynamics in embryonic development in hymenopterans. Our method serves as a first step towards enabling further research on how the changing environmental conditions already affect the first stages of the vulnerable life cycle of this important pollinator species.

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Keywords: fluorescence labeling, live-imaging, embryogenesis, light-sheet fluorescence microscopy

SB

P15. Analyse des Pflegeverhaltens von Honigbienen, die als Larven unter Pollenstress standen, mit Hilfe von multidirektionalen Videoaufnahmen innerhalb der Zellen

Nursing behavior analysis of bees which were pollen-stressed as larvae using multidirectional within-cell video recordings

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As an eusocial insect state, the honey bee (*Apis Mellifera*) engages in cooperative brood care. During the larval stage, several nurse bees feed the larvae, whose age decides on the responsible nurse bees and the type of food.

An important component for larval development is pollen, which is collected by foragers outside the colony and then stored in the colony. Depending on the availability of pollen, there may be a shortage of pollen within the colony, which results in the larvae being raised under pollen stress.

Research has shown that larvae growing under pollen stress show changes in body shape and life span. However, it is unknown whether their feeding behavior as later nurse bees also changes and if the feeding time of pollen-stressed nurse bees changes in comparison to control bees, depending on the larval age.

Here we show a novel long-term video analysis method for within-cell observation of tagged bees by recording from three sides of the colony using mirrors.

We also present preliminary results of brood care analysis of nurses raised in pollen stressed, pollen substituted, or control colonies. The accurate documentation of the feeding duration, depending on larval development day, was done by tagging nurses with colored number plates assigned to the respective group.

With the presented method we can observe exact parameters of nursing behavior of individual bees over time, as we are able to tag an entire colony. Therefore, we expect that the setup can be used as a starting point for further studies of honey bee behavior.

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Keywords: behavior, nursing, video, nutritional deficiancy, feeding

SB

P16. Unraveling The HiveMind – Eine Forschungsinitiative des Bieneninstituts Oberursel und der Goethe-Universität Frankfurt am Main

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Unter dem HiveMind versteht man die kollektive Aktivität, die im komplexen, koordinierten Verhalten einer Kolonie sozialer Insekten zum Ausdruck kommt – vergleichbar mit einem singulären Bewusstsein, der das Verhalten eines einzelnen Organismus steuert. Die kombinierten Verhaltensweisen der Individuen bestimmen das Überleben und die Entwicklung einer Kolonie. Eusozialität erlaubt altruistische Entscheidungsfindung, Arbeitsteilung, spezialisierte Entwicklung und Morphologie, zusätzliches evolutionäres Potenzial und mehrstufige Robustheit gegenüber Umweltfaktoren. Allerdings ist bisher unklar, wie die Verhaltensweisen sozialer Insekten auf molekularer Ebene gesteuert werden und sich über die Zellen und das Individuum auf die Kolonie auswirken. In einer gemeinsamen Forschungsinitiative des Bieneninstituts Oberursel und der Goethe-Universität Frankfurt am Main wollen wir am Modellorganismus Honigbiene die Entwicklung, Funktionsweise und kollektive Intelligenz eines HiveMinds erforschen. Dafür entwickeln wir einen Multiskalenansatz, dessen methodologisch-investigatives Spektrum vom Molekül bis zum Biotop recht. Folglich wurden und werden Techniken entwickelt, die in dieser Kombination nur in dieser Initiative angetroffen werden können, wie unter Anderem das Herstellen stabiler transgener Honigbienen-Linien, die Fluoreszenz-Lebendbeobachtung sich entwickelnder Embryonen oder die Langzeit-Beobachtung von Verhaltensmustern im Bienenstock. Neben Fragen aus der angewandten und Grundlagenforschung können unsere Erkenntnisse auch als Modell der sozialen Hyperintelligenz dienen, um zum Beispiel Funktionsweise unserer globalisierten, vernetzten modernen Gesellschaft zu verstehen.

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P17. 'Vitalbiene' – Auswirkungen innovativer Bienenhaltung auf die Leistung und Vitalität von *Apis mellifera* L.

'Vitalbiene' – Effects of innovative beekeeping on performance and vitality of *Apis mellifera* L.

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The 'Vitalbiene' project compares *Apis mellifera* L. colonies under different treatment concepts in terms of health, performance, population development and mating success. In an innovative approach (IN) an artificial brood interruption in summer was induced followed by an oxalic acid treatment in the brood-free state. Neither drone brood removal nor winter treatment were applied. The control group (CO) was managed according to a conventional treatment concept (drone brood removal, summer treatment with formic acid, winter treatment with oxalic acid).
Starting in July 2021, two apiaries were set up with 8+8 colonies each. Colonies were evenly distributed to the groups based on colony strength and mite infestation. Population dynamics and parasitization were recorded over the course of the years.
The results show a significant effect of treatment groups on mean infestation levels during drone rearing (mite infestation of bee samples [% ± s.e.m] in calendar week 19: IN = 2.3% ± 0.5; CO = 0.2 ± 0.1; $p \leq 0.001$, one-sided ANOVA). Yield in 2022 (harvest [kg ± s.e.m]: IN: spring = 26.1 ± 2.7, summer = 13.8 ± 4.3; CO: spring = 23.8 ± 1.9; summer = 25.7 ± 2.2) was significantly higher in CO in summer ($p = 0.002$) but not in spring and total yield (n.s.). The innovative approach led to a higher colony strength before winter (number of bees before winter ± s.e.m: IN = 8845 ± 719; CO = 7243 ± 465; $p = 0.06$) as well as to a higher overwintering index (2022-23; IN: 0.99 ± 0.04; CO: 0.87 ± 0.04; $p = 0.04$).
These encouraging results will be followed up by further investigations in the laboratory and in the field, focusing on the mating success of drones. The potential of the innovative approach in terms of supporting natural selection will thus be further investigated.

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Keywords: *Varroa destructor*, summer brood interruption, honey bee health

SB

P18. Welche Möglichkeiten gibt es für den Einsatz von Bienenzählern aus Sicht der angewandten Forschung?

From an applied science perspective, what is a possible use of bee counters?

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For more than 100 years, scientists have been trying to count honey bees automatically, and various counter models have been developed for this purpose. In theory, benefits from automated counters can be manifold, i.e. a continuous recordings of bee traffic could provide data on flight behavior, foraging performance, negative environmental influences, and swarming. In practice, very few of these parameters have been scientifically and thoroughly evaluated. We therefore adopted two protocols that allowed us to assess the impact of i) acute forager loss and ii) sudden brood loss in adult honey bee colonies. At three different sites in Germany, two colonies were manipulated with i) and ii), respectively. Flight activity was recorded for 28 days with a bee counter (one BeeCheck under each colony) and population development was estimated three times with the Liebefeld method. Both parameters were compared with an untreated control group. Our results showed that the manipulated colonies clearly flew less in i), as we expected. However, this effect diminished after 21 days, when the subsequent brood cycle was completed and new foragers could be recruited. In contrast, brood manipulation (ii) did not have such a clear effect on the number of flights, suggesting that foragers may have endured longer than usual. Hence, our protocols were appropriate for challenging and providing display to the counters with the parameters assessed. In our opinion, there is great potential in the use of automated counters in applied apidology but only high quality instruments are capable of making correct counts. Therefore, it is necessary to advance the field with thorough validation methods to ultimately improve the counters for scientific use, which is one of the goals of our current project, VIBee (www.vibee-project.net).

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Keywords: bee counter, flight behavior, swarming, precision beekeeping, validation protocol

P19. Überwachung der Flugaktivität einzelner gechipter Bienen

Monitoring the flight activity of individual chipped bees

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P20. Untersuchungen zu Metabolomveränderungen von Honigbienenlarven (*Apis mellifera* L.) nach Exposition durch Pflanzenschutzmittel-Tankmischungen

Metabolomic-based analysis on the response of honey bee larvae (*Apis mellifera* L.) after exposure to a pesticide tank mixture

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Pesticides can cause lethal and sublethal effects on honey bees. However, little is known about sublethal effects on bee larvae, altering the development of the brood. This study aimed to identify suitable biomarkers, which could be related to biologically relevant changes in the metabolomics of larvae caused by organismal responses after exposure to pesticides.

To identify such biomarkers a semi-field study was conducted, compromising a forced exposure of whole colonies after spray application in flowering *Phacelia tanacetifolia* during bee flight. Bees were exposed either to an insecticide containing chlorantraniliprole or to a mixture of the insecticide with fungicides assumed to enhance the toxicity of the insecticide.

After application, individual larvae (L3) were sampled from the brood nest and subsequently analysed for changes in their metabolism by GC-MS/MS. Additional larvae were used to quantify the actual exposure to the active substances via residue analysis and to link the exposure level to changes in their metabolic profile.

The results indicate that the treatment with the test substances is associated with an exposure of honey bee larvae shortly after spray application. The measured residues and changes in metabolite abundance point to a correlation between the exposure of honey bee colonies and metabolic response of honey bee brood. Thus, the study provides a first step towards the identification of biomarkers as indicators of sublethal effects on bee larvae.

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Keywords: metabolomics, honey bee larvae, pesticides, sublethal effects

SB

Bienenschutz und Pflanzenschutz

P21. Die florale Pilzgemeinschaft nach Behandlung mit einem häufig angewandten Fungizid.

Nectar fungal community after application of a commonly used fungicide.

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Agricultural practice can include the application of plant protection products as well as other substances to guarantee plant survival and productivity. These products may not only affect invertebrate biodiversity or associated plant communities but also the microbial community of the crop plant. In particular, fungicides are of major interest; being active against fungal plant pathogens, they may harm native fungal communities of the flower. Because nectar microbial community mostly consists of yeast fungi, application of fungicides is one of major concerns for pollination. An impaired nectar microbial community may distort attractiveness of the treated plants to bees and consequently affect their pollination service.

Here, floral samples were collected from control and treatment fields at three different locations in Germany and at three different time points before and after application of the fungicide Pictor® Active (boscalid and pyraclostrobin). Yeast community in nectar samples was investigated using a cultivation-based approach and sequence-based identification. Fungal total abundance (CFUs), species richness, and Shannon diversity differed strongly among locations and sampling time. None of the analysed factors showed to differ between control and treatment fields, though the effects of the fungicide were more pronounced for basidiomycetous species. The observed changes in species richness and total abundance values do not necessarily reflect plant treatment with fungicides, but may be driven by abiotic factors or changes in pollinator diversity and visitation patterns, all not tested here.

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Keywords: microbiota, nectar, fungicide, pollinator, yeast

P22. Semi-field study investigating the effect of tank mixtures containing chlorantraniliprole and EBI-fungicides on honey bees

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Risk assessment of plant protection products (PPPs) for their possible effects on non-target organisms, including honey bees is conducted before authorization. Tank mixtures are often common farmer practice, and mostly their impact on honey bees is not routinely assessed. Previous laboratory studies reported possible synergistic effects of the combination between chlorantraniliprole and EBI-fungicide. To enable a realistic assessment, a semi-field study with spray application in flowering Phacelia was conducted. The experiment included four treatments (control, chlorantraniliprole, chlorantraniliprole + prochloraz, chlorantraniliprole + tebuconazole). The tested PPPs were applied at the maximum recommended application rate of 60 g chlorantraniliprole/ha, 675 g prochloraz/ha, and 375 g tebuconazole during bee flight. Several parameters were investigated, including mortality, flight activity and behavior, and colony development. No effects were observed after the application of chlorantraniliprole alone. On the other hand, several intoxication symptoms were observed two hours after application of the tank mixtures. Higher number of moribund bees and dead bees were also observed on the first and second days after application compared to the control. Adverse effects of the tank mixtures were found on the colony development compared to control over the experimental period. In conclusion, exposure to a combination containing chlorantraniliprole and EBI-fungicides poses a high risk to honey bees under semi-field conditions. Further studies with such tank mixture under realistic field conditions are necessary to evaluate the potential risk to honeybees.

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P23. Rapsanbausysteme mit Begleitpflanzen zur Schadinsektenabwehr und Insektizid-Reduktion (Raps-OP)

Oilseed rape cropping systems with companion plants for insect pest control and insecticide reduction (oilseed rape OP)

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Among other factors, insect pests are a significant influence in canola production. Increasing resistance and the limited choice of insecticides exacerbate the problem. At the same time, pollinator species are disappearing, especially in poorly structured agricultural landscapes. An innovative solution would be to cultivate plants in mixture with or next to rapeseed that are more attractive to rapeseed pests than the rapeseed itself, so-called trap plants ("sacrificial plants") for "diversionary feeding". These can either be tolerated there or targeted ("attract and kill"). At the same time, these plants, such as turnip rape, early rape varieties, marrow stem cabbage, etc., extend the flowering period, which, together with a significant reduction in insecticides, should have a positive effect on pollinators and antagonists of the rape pests.

In order to investigate the described measures qualitatively and quantitatively with regard to their attractiveness for pollinating insects, the respective trial plots or the insect flight are filmed with a camera once a week during the individual flowering phases and evaluated. The advantage of this is the reproducible possibility of pollinator counting while at the same time avoiding trapping or killing.

After the first year of the experiment, it became apparent, especially in the later course of the flowering period, that the variants with the early rape variety, the marrow cabbage and the turnips were visited 1.5 - 7 times more frequently by wild pollinators than the main crop rape. Honeybees also particularly preferred turnip and medullary cabbage (1.5 and 2.8x more often than the main crop, respectively).

The preliminary results seem to support the hypothesis mentioned at the beginning that a targeted sowing of diversionary plants or forage plants for pollinators significantly increases the attractiveness of a conventional rapeseed area for honey bees and wild pollinators and can thus be another building block for increasing biodiversity.

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Keywords: Biodiversität, Pflanzenschutz, Bestäuberschutz, Kamerabeobachtung

SB

P24. Pflanzenschutz im Hopfenbau: Erhöhte Wirkstoffbelastung für Honigbienen?

Crop protection in hop growing: increased exposure to active substances for honey bees?

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Hops are one of most frequently with pesticides treated crops in Germany. Since the cultivated female hop provides neither nectar nor pollen, hop yards are considered unattractive to pollinating insects. In order to examine, if intensive plant protection measures in hop cultivation led nevertheless to an increased exposure of honey bees to such active substances, bee bread samples were taken from colonies located near hop yards ("hop group", n=36) and analyzed by residue analysis (QuEChERS, GC-MS/MS, LC-MS/MS). Bee colonies at other locations with intensive agriculture, but without hop cultivation, were used as a reference group ("arable land group" n=34).

The bee bread samples from the vicinity of hop yards showed a significantly higher number of detectable active substances per sample than those from the reference group (median 12.5 vs. 8.5 active substances/sample, Mann-Whitney-U-Test, p=0.002). The total amount of active substances in the samples from the hop group also exceeded that in the samples from the arable land group (median 0.16 vs. 0.06 mg/kg, Mann-Whitney-U-Test, p=0.046). Substances classified as hazardous to bees (B1/B2) were detectable in 47.2% of the samples from the hop group and in 15 % from the arable land group. The higher levels of contamination in the bee bread of the hop group were mainly due to insecticides, acaricides and fungicides used to control hop-typical pathogens.

The results of this study show that honey bee colonies in the vicinity of hop yards are exposed to higher levels of pesticides than colonies at other agricultural sites. However, the detectable amounts of active substance in all samples were far below the acutely toxic concentrations for honey bees. Since the hop plants themselves are not attractive to pollinators, the hop-typical contaminants in the bee bread are likely to be due to flowering weeds or cover crops within the sprayed hop yards or the spray mixture drifting to adjacent areas.

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Keywords: hop growing, crop protection, residues, bee bread

P25. DeBiMo: ein Vergleich der Pflanzenschutzmittelrückstände in Pollen in Deutschland und den Vereinigten Staaten

Long-term Monitoring: A comparison of pesticide residues in pollen in the USA and Germany

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The German Bee Monitoring Project (DeBiMo) was launched in autumn 2004/2005 to document winter losses and continues to monitor honey bee health year-round, with 10 colonies in each apiary monitored three times per year. Each year approximately 120 apiaries are monitored throughout Germany for mortality, colony strength, disease load (*Nosema* spp., *Varroa destructor*, viruses), and the pesticide exposure through stored pollen (bee bread). Pollen samples (N = 2,091) were analyzed for up to 474 different pesticide products and their metabolites. Here we present the results of the DeBiMo pesticide analysis since 2009 and compare them to a similar long-term monitoring scheme conducted in the United States from 2011-2017, where they analyzed pollen samples (N = 1,055) for 218 different pesticide products and their metabolites.

In Germany we documented an increase in the amounts of fungicides (X^2 = 32.80, df = 12, p = 0.0010) detected over time and a significant reduction of insecticides (X^2 = 70.29, df = 12, p < 0.0001), in line with changes in agricultural use. The mean number of different pesticide products detected per sample was 5.60 ± 0.1 products, with up to 34 different residues detected in a single pollen sample. Network analysis was used to determine which pesticides frequently co-occur and three common clusters of 2-4 products identified. Although we find more residues per sample in Germany than in the United States, these occur at lower concentrations. Thus, the risk in Germany from pesticide contaminated pollen to honey bee health is substantially lower than in the USA, reflecting the precautionary principle used in pesticide regulations within Germany.

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Keywords: pesticides, pollen, monitoring, hazard quotients, risk assessment

P26. Neonicotinoide Notfallzulassung bei Zuckerrüben: Rückstände in einer bienenattraktiven Folgekultur?

Emergency use of neonicotinoids in sugar beets: are their pesticide residues in the subsequent planting of a bee-attractive flowering crop?

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Although previously banned in the EU, thiamethoxam seed-treated sugar beet was planted in restricted areas in Baden-Württemberg in 2021 using an emergency authorization of neonicotinoids for this important cash crop. Eight hectares of this treated seed were planted on one of the agricultural trial fields belonging to the University of Hohenheim. The use of neonicotinoids for plant protection is controversial, as they are highly toxic to bees in very low concentrations when bees come into direct contact with them. In 2021 we mimicked a worst-case scenario of weeds growing in amongst the sugar beet crop by planting phacelia in the same field as the sugar beets under a tented part of the field. Traces of the active ingredient thiamethoxam were found in the pollen and nectar of the plants, indicating that low residues could be collected by foraging bees if farmers tolerated flowering weeds in their treated beet fields. In 2022, we sowed an entire field of phacelia where the treated sugar beets had grown the year before. These flowers were highly attractive to both honey bees and bumble bees. We maintained four honey bee colonies at the field edge. Samples were taken from returning foragers from all experimental colonies. We analyzed their corbicular pollen loads and the nectar expelled from their honey crop. We also analyzed phacelia influorescences and stored honey. None of these samples contained any thiamethoxam residues, despite the low limit of quantification (LoQ) used 0,1 µg/kg. Emergency authorization of thiamethoxam for sugar beets was an exception in 2021 and remains prohibited in Germany until further notice. This project was financially supported by the Ministry of Food, Rural Areas and Consumer Protection in Baden-Württemberg.

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Keywords: pesticides, residues, sugar beets, phacelia, neonicotinoid

SB

P27. Unterschätzte schädliche Wirkung von entomopathogenen Nematoden auf Bienen

Underestimated harmful effect of entomopathogenic nematodes on bees

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Entomopathogenic nematodes (EPNs) have received much attention as alternative biological-control agents to conventional synthetic agrochemicals. Since, EPNs are considered natural enemies, registration is often based on limited or no data. For the first time, we show that exposure to a commercial EPN product can significantly reduce honeybee survival and nematodes can successfully replicate in infected adult bees. Newly emerged honeybees (*Apis mellifera*) and larvae of the greater wax moth (*Galleria mellonella*) were exposed to dry and wet foliage residues at a low (0.25 Mio/m2) and medium (0.5 Mio/m2) field-realistic concentration of *Steinernema carpocapsae*. Mortality was assessed over 96 h and nematode reproduction was evaluated in all dead individuals. EPN exposure significantly reduced wax moth larvae survival across both treatments (p's < 0.001), resulting in an increased mortality rate of 80%. Honeybee survival was also significantly reduced (p's < 0.001). However, the data revealed a dose-dependent effect wherein the medium concentration led to a significantly higher mortality rate (55%) than the lower dose (43%) when compared to the non-exposed control. Nematode reproduction was significantly higher in wax moths than in honeybees (p < 0.001), yet no significant difference was observed between the low and medium treatments for either species (p>0.56). Mean nematode reproduction per individual wax moth and bee was 126'695 and 4'370, respectively; representing a 29-fold increase in wax moths. The data show that EPN exposure can adversely affect honeybees. Due to the vast lack of data regarding potential adverse effects of EPNs on non-target pollinating insects, our results underline the urgent need to act cautiously when considering foliar application of EPNs on crops. Further research is required to adequately address the potential risk of EPNs to bees and other non-target species regarding foliar and soil application.

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Keywords: Entomopathogenic nematodes (EPN), honeybee, registration, NTA, pollinating insects

P28. Ameisensäure verbessert die Lernleistung von Honigbienen

Formic acid makes bees learn better

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Honey bees increasingly suffer from the parasitic mite *Varroa destructor*. Formic acid is an effective treatment to protect honey bee colonies and keep them healthy. But little is known about side effects of this treatment on honeybees. In the present work the influence of a formic acid treatment on sucrose responsiveness and cognition of the honey bees has been investigated. Classical olfactory conditioning using the proboscis extension response (PER) was employed to quantify learning and memory abilities. Sucrose responsiveness was tested prior to training using the PER to increasing concentrations to sucrose. In our study, bees of different developmental stages were treated with 460 g of the 60% formic acid for two weeks. All bees were trained at adult age.

Our results show that formic acid did not affect sucrose responsiveness. However, formic acid significantly improved appetitive learning performance both in bees treated as larvae and in bees treated as foragers. One possible explanation for this could be that formic acid treatment induces stress in honey bees, which could lead to enhanced levels of stress hormones such as octopamine and dopamine. Both amines are involved in learning and memory formation in insects. Enhanced octopamine and/or dopamine levels might lead to an improved learning performance in honeybees.

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Keywords: parasite control, organic acid, classical conditioning, responsiveness to sucrose

SB

P29. 10-Tage chronisch orales Testdesign für Solitärbienen, *Osmia* spp.

10 day chronic oral test design for solitary bees, *Osmia* spp.

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The risk assessment for non-Apis bees has not been extensively validated due to limited availability of standardized methods. So far, new methodologies have been designed and ring-tested to assess the acute oral and contact toxicity of plant protection products to adult solitary mason bees (*Osmia* spp). First attempts of a chronic oral test have been conducted following the OECD test guideline 245 for the honey bee. Elements as duration, replication and validity criteria for control survival have been used from the chronic oral toxicity bee test. Here we present results for the horned mason bee *Osmia cornuta*. Biological observations or other adverse effects and consumption measurements were carried out. In order to account for evaporation, evaporation controls were included to correct consumption estimates. The average *Osmia cornuta* body weight was 129 ± 16 mg. Consumption in the control group was 157 ± 35 mg sucrose solution/bee/d. Control survival after 10 days was ≥ 90% for *Osmia cornuta*. Dimethoate, usually used as a positive control in bee toxicity tests, was tested in a dose-response design. The LC_{50} endpoint derived from this test was 0.75 mg a.s./kg diet. Similarly, the LDD_{50} endpoint was 0.069 µg a.s./bee/day. Further testing is necessary to derive a standardized and reproducible test protocol.

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Keywords: risk assessment, toxicity test, solitary bees, non-*Apis* bees

P30. Testdesigns für Mauerbienen (*Osmia* sp.) Halbfreiland- und Freilandstudien nach der neuen revidierten EFSA Bienen Guidance

Semi-field and field studies on Mason Bees (*Osmia* sp.) according to the new revised EFSA Bee Guidance

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In October 2022 EFSA published the draft of the new revised guidance on the risk assessment of plant protection products (PPP) on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), which should be the basis of the risk mitigation of solitary bees by PPPs in the near future. In Annex C, EFSA provides detailed guidance on how semi-field and field studies should be designed and how reliable results for the risk assessment for honeybees, bumblebees and solitary living mason bees (*Osmia* sp.) should be obtained. For mason bees, the guidance is largely based on a ring test protocol developed and published by the non-Apis working group of the International Commission for Plant-Pollinator Relationships (ICPPR).

As member of this working group, tier3 solutions participated in the development of a test protocol and ring-testing for semi-field testing of this solitary bee species. The experience gained from this ring test, as well as the experience gained from solitary bee field effect studies with the mason bee *Osmia bicornis* conducted since 2014, was used to develop test designs that meet the requirements of the new EFSA revised bee guidance.

A test design for a semi-field study in tunnels as well as a test design for field studies will be presented and possible study endpoints as well as the necessary time schedule will be discussed.

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Keywords: risk assessment, solitary bees, methods, plant protection products, mason bee

P31. Ovarien Entwicklung in Mikrokolonien von *Bombus terrestris*

Ovarian Development in microcolonies of *Bombus terrestris*

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One of the remaining challenges is to set-up a standardized study design to assess effects on bumblebee colony health and reproduction under laboratory conditions to contribute valuable data required for risk assessments. Therefore, the use of microcolonies is increasing. Here it is assumed that in a group of isolated bumblebee workers, one becomes dominant and starts egg laying. Since recent experiments differ in the number of produced offspring, our aim was to figure out how many bumblebees in a microcolony have developed ovaries at various times and contribute to the number of offspring produced.

Five worker bumblebees of unknown age were taken from commercially available standard hives (*B. terrestris*) to form a microcolony. During the test the microcolonies were provided with ad libitum access to a pollen-Api-Invert mixture and Api Invert. The test included five test groups, which were deep frozen after 7 (n=5), 14 (n=5), 21 (n=5), 28 (n=5) days after initiation of the microcolony and just before hatch of the second generation (n=15). To evaluate the development of the ovaries the deep-frozen bumblebees were dissected.

The evaluation of this test involves the mean number of bumblebees with developed ovaries per test group and its standard deviation. The test group which was deep frozen just before hatch of the second generation had a mean value of 5 (±0). Whereas all other groups showed variations. On average 4.2 (±0.7) bumblebees of the test groups which were deep frozen on day 7 after initiation had developed ovaries. The microcolonies which were deep frozen on day 14 had on average 4.8 (±0.4) bumblebees whose ovaries were developed. The test groups which were deep frozen on day 21 and 28 show both a mean value of 4.6 (±0.5).

The conclusion is, that more than one worker of the initial microcolony might contribute to the number of offspring produced. For the interpretation of microcolony data, this should be considered.

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Keywords: Ovarian Development, *Bombus terrestris*, Microcolony, hazard assessment

Bienenpathologie

P32. Quantifizierung der Rekombination innerhalb und zwischen zwei Genotypen des Krüppelflügelvirus in der Honigbiene

Quantifying recombination within and between two genotypes of deformed wing virus in the honey bee

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When transmitted by the ectoparasitic mite *Varroa destructor*, deformed wing virus (DWV) is the major threat for honey bee (*Apis mellifera*) populations across the temperate world. Even though its two widespread genotypes, A and B, are virulent, recombinants between these genotypes have been hypothesised to be even more virulent for their hosts. Here we investigate the extent to which viral recombination occurs by injecting (mimicking *V. destructor* transmission) two genotypes into the same honey bee pupa, thereby promoting the opportunity for recombination. After 3 days incubation at 35°C, we then extracted RNA from pupae and sequenced it on an Illumina next generation (NGS) machine (150 bp PE reads). Yields were between 2×10^7 and 7×10^7 paired-end reads per NGS library (per individual pupa), of which, on average, >50% were derived from DWV. We then employed a variety of bioinformatics tools to identify recombinants: fastp (for trimming the raw short NGS reads and merging the overlapping ones), rnaviralspades (for reference assembly of the original inocula used to inject pupae), hisat2 (to map the reads to a reference sequence), varscan (for variant calling), cliqueSNV (for haplotype calling) and ViReMa (for recombination calling). We found signals of recombination events not only between viral genotypes but also within variants of a viral genotype. However, it is clear that the NGS short read technology may introduce false positives and artifacts which do not allow one to confirm the existence and the rate of recombination events, and for which third generation sequencing technologies promise a solution.

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Keywords: DWV, NGS, viral bioinformatics, virulence

P33. Entwicklung und Charakterisierung eines Antikörpers gegen DWV

Development and characterization of an antibody against DWV

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One of the major threats for honey bees are infections with deformed wing virus (DWV) which in combination with *Varroa destructor* parasitization strongly correlate with colony losses. Early detection of DWV infections in colonies could help to reduce colony losses and prevent spreading of the DWV syndrome. However, virus diagnostics for honey bees is currently only possible by *reverse transcription* polymerase chain reaction (RT-PCR), an expensive and time-consuming lab procedure. The availability of specific antibodies against DWV would enable the development of cheap and fast immunoassays for easy monitoring of this virus in honey bee colonies. In addition, if used in research projects such antibodies could further our understanding of DWV infection pathogenesis.

For the development of anti-DWV monoclonal antibodies, we decided to use a recombinantly expressed capsid protein of DWV (DWV-VP2) as antigen for immunisation of mice. To obtain the recombinant antigen, the gene coding for DWV-VP2 was cloned into a GST- tag vector and expressed in *E. coli*. The recombinant protein recDWV-VP2 was successfully purified from *E. coli* culture supernatants via column chromatography and used for commercial (ASKA Biotech) production of monoclonal antibodies. We validated the specificity of the obtained monoclonal antibodies for the antigen recDWV-VP2 used for immunisation by dot blots and Western blots. Using Western blots, we also analysed the ability of the antibodies to detect DWV particles and DWV-infected bees. One antibody was not only able to detect recDWV-VP2 and purified DWV-particles in Western blots, but also to distinguish between bees that had tested DWV- positive and DWV- negative in RT-PCR assays. We will now check whether this antibody also performs well in immunohistochemistry and immunoprecipitation assays and whether it can be used for the development of an enzyme-linked immunosorbent assay (ELISA) for the detection of DWV.

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Keywords: virus, pathogen, protein expression, diagnostics

SB

P34. Optimierung der Virusdiagnostik mithilfe einer multiplex-RT-PCR

Optimization of molecular virus-diagnostic by multiplex-RT-PCR

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International studies and monitoring programs in Europe and other countries repeatedly show that the biggest problem in beekeeping worldwide is varroosis caused by the ectoparasitic mite *Varroa destructor*. The term varroosis describes a symptom complex that is primarily determined by the viral infections transmitted or activated by the *Varroa* mite. Both monitoring programs and laboratory experiments show that viruses are decisive for the severity of bee damage in the course of varroosis, which can then lead to the death of the entire colony and is responsible for sometimes catastrophic winter losses. The damage characteristic of varroosis is not fully understood, either at colony level or at the level of the individual bee. However, in order to be able to assign damage or symptoms to a specific virus infection, it is essential to have a reliable and efficient method for detecting bee viruses. Although the virus diagnostics established at the LIB have proven to be very reliable, they are based on the individual detection of the respective viruses. But this approach is time-consuming and costly. Therefore, we have established and optimized a multi-method for the detection of up to seven bee viruses in one assays and present here our results on the establishment of this method and the evaluation of its suitability in comparison to the individual virus detection protocol used so far.

The simultaneous diagnosis of as many bee pathogens as possible will significantly improve the diagnostic situation. A so-called multi-method is an indispensable prerequisite to methodically update the practical diagnostics and the rapid recording of the infection status of bee colonies and to be able to better counter colony losses due to bee diseases in the future through improved diagnostics.

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Keywords: honey bee viruses, molecular diagnostic, multiplex RT-PCR

P35. Erfassung der Flugleistung von Honigbienen (*Apis mellifera*)

Determination of the flight performance of honey bees (*Apis mellifera*)

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The health and survival of honey bees (*Apis mellifera*) is threatened by a variety of parasites and pathogens. Amongst them, the viral pathogen deformed wing virus (DWV) and the microsporidium *Nosema ceranae* are discussed as main causes of colony mortality, especially in winter. However, infections with these pathogens are not necessarily fatal for the colony or individual bee. Both DWV and *N. ceranae* also cause covert infections, i.e. infections that do not cause obvious visible symptoms at the individual level and do not lead to colony loss, but can still affect bee health and performance. The symptoms of covert infections are usually difficult to detect and sophisticated bioassays such as classical learning experiments (PER) are required to measure such effects.

PER experiments have been widely used to detect cognitive impairment as a sublethal effect in infected adult bees. While the influence of *Nosema* infection on learning and memory formation has so far yielded contradictory results in several studies, we were able to demonstrate with PER experiments that DWV-B infection of the brain leads to a significant reduction in the cognitive abilities of otherwise healthy-looking animals.

In order to expand our spectrum of bioassays and also be able to analyse the physical fitness of bees, we constructed a carousel-like mechanical device for determining the flight performance of individual honey bees. With the help of our flight carousel, we were able to obtain objective data on the flight distance and flight duration of individual forager bees, which provided valuable information about the fitness of the individual bees tested. We here present our first data on the flight performances of healthy bees compared to bees infected with DWV or *N. ceranae*. Having established two bioassays that can measure cognitive and physical fitness of individual bees, we will now be able to characterize the impact of sublethal infections with pathogens in honey bees at an unprecedented level.

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Keywords: bee pathogens, infection, flight performance

SB

P36. Vergleich der Wirksamkeit der Behandlung gegen *V. destructor* mit dem „Herba Strip“ und dem „Bayvarol“

Comparison of the effectiveness of treatment against *V. destructor* with “Herba Strip” and “Bayvarol”

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“Herba Strip”, is reported to be successfully used as a one-time summer treatment against *V. destructor.* According to the manufacturer, each strip contains 0.1 mg of flumethrin with extracts of pyrethrum, thyme and camphor. Beekeepers reported “Herba Strip” to be effective in suppressing varroosis, without resistance in *V. destructor* mite even after six consecutive years of use.

In 2021 and 2022, at the Agricultural Department of the Polytechnic in Rijeka, was tested its effectiveness on apiaries in the vicinity of Rijeka. The control treatment was carried out with Bayvarol (flumethrin). Two strips of “Herba Strip” or “Bayvarol” were placed in the brood of each treated bee colony.

In 2021 experimental treatment with „Herba Strip“ after a 10-day test period, “Herba Strip” reduced the initial *V. destructor* infestation from 3.26% to 0.00%., while in the same period, Bayvarol lowered the infestation from 0.80% to 0.22%.

After a 42-day test period, “Herba Strip” reduced the initial *V. destructor* infestation from 6.62 % to 0.34% in 2021, and from 1.43% to 0.48% in 2022. On different apiary in 2022 season “Bayvarol” lowered the infestation from 5.32% to 1.17%.

It was noticed that during the treatment with “Bayvarol”, the brood was mostly removed from under the strips on both sides, while under the strips with “Herba Strip” the brood was not removed. Since the treatment with “Herba Strip” had slightly better results than with “Bayvarol”, it was concluded that “Herba Strip” is effective in the treatment against *V. destructor* infestation. However, before possible further use of “Herb Strip” in EU, it should be checked whether there are any substances in its composition, which are not harmless for the beekeepers during its use, and if there are eventual residues left in the bee products after the treatment.

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Keywords: efficacy, varroa treatment, “Herba Strip”

SB

P37. Anwendung verschiedener Genotypisierungsmethoden zur Analyse der genetischen Diversität von *Paenibacillus larvae*-Isolaten in ausgewählten österreichischen Proben

Using different genotyping methods to assess the genetic diversity of *Paenibacillus larvae* isolates in selected Austrian field samples

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The gram-positive endospore forming bacterium *Paenibacillus larvae* is the causative agent of American foulbrood, a severe bee disease of honeybees (*Apis mellifera*). The genotypes of *P. larvae* differ in their virulence and consequently cause colony loss at a different pace. Several genotyping methods exist for strain identification. The most frequently applied method is the repetitive element sequence based PCR (rep-PCR) using enterobacterial repetitive intergenic consensus (ERIC) primers. This method has led to the discovery of five different genotypes, ERIC I-V of which only ERIC I and ERIC II are commonly detected in field samples. More recent genotyping methods, such as Multi-Locus-Variable-Number-of-Tandem-Repeat-Analysis (MLVA) and Multi-Locus-Sequence-Typing (MLST) provide better reproducibility and higher discriminatory power.

We have used these genotyping methods to assess the genetic diversity of *P. larvae* infections throughout Austria (2011-2021). Selected isolates of *P. larvae* were analyzed using ERIC (n=136), MLVA (n=142) and MLST (n=39) genotyping. The results showed that 74% of samples were assigned as ERIC I, 24% as ERIC II and 2% were not assignable. MLVA identified 26 different profile types. Based on this, 39 isolates showing highest genetic diversity were selected for MLST, which led to the identification of 11 sequence types, including four new sequence types. In conclusion, ERIC genotyping showed ERIC I was the predominant genotype in Austria. Recent genotyping methods facilitate in-depth analysis of *P. larvae* infections due to high discriminatory power.

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Keywords: *Paenibacillus larvae*, genotyping, ERIC, MLVA, MLST

P38. Vorstellung des koordinierten AFB-Monitorings in Berlin

Presentation of the coordinated AFB monitoring in Berlin

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The highly infectious, notifiable bee disease American foulbrood (AFB) is caused by the spore-forming bacterium *Paenibacillus larvae*. Sources of infection include undetected diseased bee colonies or neglected apiaries. The spread usually occurs through robbing or improper beekeeping practices. In Berlin, the risk of spread is exacerbated by the high density of bees, the high level of migratory activity and the increasing proportion of poorly trained beekeepers.
In 2020, Freie Universität Berlin began setting up the "Coordinated AFB Monitoring" as part of the Berlin Bee Strategy. The aim is to gain more insight into the prevalence of AFB in the long term and to reduce the number of foulbrood outbreaks and thus mitigate the economic and intangible damage. Through systematic and prophylactic sampling, the spores of *Paenibacillus larvae* can be detected very early, often even before a clinical outbreak.
Brood comb honey samples are taken free of charge on a voluntary basis and analysed at the Institute for Bee Research Hohen Neuendorf (LIB). The brood comb honey samples are taken by the beekeepers themselves or by trained samplers. In order to ensure the validity of the results, the samples are taken during the period without nectar flow and before or after winter feeding. Depending on the weather conditions and the course of the year, the samples are taken from the beginning of March to the end of September. The suitable bee sites for sampling are selected using an AFB heat map. Using the web platform, the probands can select the locations and enter the necessary data. Based on the area-wide honey samples, risk-based heat maps are generated in order to minimise AFB outbreaks in the long term through suitable prophylactic measures.

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Keywords: Amerikanische Faulbrut, Monitoring

P39. Die Rolle der Flagellen beim Schwärmen, der Biofilmbildung und der Virulenz von *P. larvae*

The role of flagella in swarming motility, biofilm formation and virulence of *P. larvae*

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The Western honey bee *Apis mellifera* is an indispensable pollinator in natural and agricultural ecosystems worldwide. It is therefore of great concern to human society that *Apis mellifera* is threatened worldwide by various parasites and pathogens that affect the health of individual bees and colonies. The most devastating bacterial honey bee brood disease is American Foulbrood caused by the Gram-positive, spore-forming, peritrichously flagellated bacterium *Paenibacillus larvae*. Infection with *P. larvae* begins when the ingested spores of *P. larvae* germinate in the midgut lumen of the young honey bee larvae. After massive proliferation of the vegetative bacteria in the midgut, *P. larvae* attacks the midgut epithelium with the help of various virulence factors and thus manages to invade the larval hemocoel, which leads to the death of the diseased larva. *P. larvae* then decomposes the larval carcass into a ropy mass that eventually dries into a scale tightly adhering to the lower rim of the brood cell, consisting of billions of spores. During the pathogenesis of *P. larvae* infection, not only molecular mechanisms of the single bacterial cells play an important role, but also cooperative multicellular behaviours. For the *P. larvae* genotype ERIC II, we have recently demonstrated the cooperative behaviours of swarming motility and biofilm formation. In particular, swarming motility is generally driven by the filamentous appendages of bacteria, the flagella. However, flagella have also been shown to play different roles in biofilm formation. To investigate the role of flagella in swarming motility, biofilm formation and virulence of *P. larvae* ERIC II, we constructed a gene knockout mutant that lacks production of the filament forming protein flagellin. We here present our data showing that the *P. larvae* mutant was no longer able to form flagella, and consequently lost the ability to swarm, exhibited altered biofilm formation and had a significantly reduced virulence potential.

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Keywords: *Paenibacillus larvae*, flagella, swarming, biofilm

SB

P40. Untersuchungen zur Charakterisierung von *Paenibacillus larvae* Immune Inhibitor A (InhA) - einer möglichen Metalloprotease

Investigation and characterisation *of Paenibacillus larvae* Immune Inhibitor A (InhA) – a putative metalloprotease

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One of the most devastating bee diseases responsible for significant colony losses is the epizootic American Foulbrood, which caused by the gram-positive, spore forming bacterium *Paenibacillus larvae* and only affects the larval stages of honeybees. For the development of sustainable measures against this fatal brood disease, it is essential to understand the molecular basis of host-pathogen interactions and bacterial virulence factors. The two genotypes *P. larvae* ERIC I and ERIC II that cause AFB outbreaks worldwide differ in their virulence due to differences in their virulence factors.

Proteases have been suspected for decades to play a role in the virulence of *P. larvae*. We therefore analysed the profiles of secreted proteases at different stages of the *P. larvae* life cycle, such as the exponential and stationary phase of growth in liquid culture or the proliferation and sporulation phase of *P. larvae* grown on agar plates.

For the genotype ERIC II, the M6 metalloprotease Immune Inhibitor A (InhA), a proven virulence factors of some pathogenic *Bacilli*, has been discussed as a possible virulence factor. To further our understanding of InhA and its role in the pathogenesis of *P. larvae* ERIC II infections, we have recombinantly expressed and purified both InhA and the active site of InhA as GST-fusion proteins in *E. coli*. We will present our initial results from testing these recombinant proteins for proteolytic activity against a variety of possible substrates.

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Keywords: *Paenibacillus larvae*, American Foulbrood, virulence factors, proteolytic enzymes

SB

P41. Ein Vergleich verschiedener Probenmaterialien für die Diagnose der Amerikanischen Faulbrut bei Honigbienen

A comparison of different matrices for the diagnosis of American Foulbrood of honey bees

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American Foulbrood of honey bees (AFB) is a notifiable epizootic disease in most countries, caused by the Gram-positive, spore forming bacterium *Paenibacillus larvae*. Infection with *P. larvae* is fatal to the larvae of the Western honey bee (*Apis mellifera*) and usually leads to the death of the entire bee colony. In controlling AFB, it is particularly important to identify infected colonies as early as possible to reduce the spread and potential economic losses due to colony loss. This is because the early identification of an infected but not yet clinically diseased colony gives beekeepers the opportunity to get rid of the infection by taking sanitary measures such as the shook swarm method. In contrast, in the case of a clinically diseased colony in which the pathogen *P. larvae* has been detected in addition to characteristic clinical symptoms, most authorities consider eradication of the affected colonies the only sustainable control measure. Identification of infected colonies can be achieved by detection of *P. larvae* spores in sample material from the hive like brood comb honey, adult bees and hive debris. In order to assess the suitability of these three matrices for the detection of *P. larvae* spores, samples of all three matrices were spiked with different but defined amounts of spores. These samples were then used to compare which matrix is best suited for the detection of *P. larvae* spores. Our results show that concomitant bacteria are the biggest problem in reliably detecting *P. larvae* spores and that they are predominantly present in debris samples but less so in adult bee and brood comb honey samples. We conclude that due to the sensitivity and the detection limit for *P. larvae spores*, as well as the low amount of concomitant bacteria, brood comb honey and adult bees were confirmed to be the most suitable matrices for the detection of *P. larvae* spores. In contrast, hive debris should only be used when neither of the other two matrices are available.

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Keywords: *Paenibacillus larvae*, laboratory diagnostics

SB

P42. Wann trifft *Paenibacillus larvae* auf mikrobielle Konkurrenten in der Honigbienenlarve?

When does *Paenibacillus larvae* encounter microbial competitors in honey bee larvae?

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Paenibacillus larvae is the causative agent of American Foulbrood, a worldwide occurring disease of honey bee larvae. *P. larvae* is a gram-positive and spore forming bacterium, of which only the spores are infectious. The larvae are infected by food contaminated with *P. larvae* spores. The spores germinate in the gut of the larvae and after massive proliferation, the vegetative bacteria kill the host by invading the hemocoel. The larval carcass is decomposed into a ropy mass by the pathogen, resulting in a pure culture of *P. larvae* in the ropy mass. The ropy mass dries to the characteristic foulbrood scale and the vegetative bacteria sporulate to the infective spores. After cleaning the brood cell, the nursing bees transmit the spores to the next larva or contaminate the brood comb honey. Honey bee larvae are considered sterile when they hatch, but they are described to acquire the gut microbiota through feeding, i.e via brood comb honey as part of their diet. To identify putative candidates for the larval microbiota, bacteria cultivated from brood comb honey from several colonies were identified via 16S-rRNA-gene sequencing. Most of the sequenced species were gram-positive and spore-formers. Exposure bioassays were performed with honey bee larvae infected with *P. larvae* in the absence or presence of spores of the microbiota species we identified. Survival and mortality were monitored and no significant difference was measured. Live and dead larvae fed with the microbiota species were analyzed by fluorescence *in situ* hybridization (FISH). No vegetative bacteria were detectable in live larvae. However, in dead larvae, strong signals were detected via the FISH-probes about 24 hours after death. Thus, competition between *P. larvae* and the microbiota plays a role during the saprophytic phase of the *P. larvae* life cycle in larvae and not during the commensal and invasive phases of pathogenesis.

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Keywords: *Paenibacillus larvae,* fluorescence *in situ*-hybridization, honey bee larvae, microbiota

SB

P43. Signifikant, aber nicht biologisch relevant: *Nosema ceranae* Infektionen und Winterverluste

Significant, but not biologically relevant: *Nosema ceranae* infections and colony winter losses

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Managed and wild insect pollinators play a key role in ensuring that mankind is adequately supplied with food. Among the pollinating insects, the managed Western honey bee (*Apis mellifera*), which provides about 90% of commercial pollination, is of special importance. Hence, diseases as well as disease causing pathogens and parasites, that threaten honey bees, have become the focus of many research studies. The ectoparasitic mite *Varroa destructor* together with deformed wing virus (DWV) vectored by the mite have been identified as the main contributors to colony losses, while the role of the microsporidium *Nosema ceranae* in colony losses is still controversially discussed. In an attempt to solve this controversy, we statistically analyzed a unique data set on honey bee colony health comprising data on mite infestation levels, *Nosema* spp. infection status and winter losses continuously collected over 15 years. We used various statistical methods to investigate the relationship between colony mortality and the two pathogens, *V. destructor* and *N. ceranae*. Our multivariate statistical analysis confirmed that *V. destructor* is the major cause of colony winter losses. When using cumulative data sets, we also found a significant relationship between *N. ceranae* infections and colony losses. However, determining the effect size revealed that this statistical significance had no biological relevance, because the deleterious effects of *N. ceranae* infection are normally masked by the more severe effects of *V. destructor* on colony health and therefore only detectable in the few colonies that are not infested with mites or are infested at low levels.

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Keywords: honey bee viruses, molecular diagnostic, multiplex RT-PCR

P44. Effekte erhöhter Honigbienendichten auf den Reproduktionserfolg und das Nahrungssuchverhalten von *Osmia bicornis*

Effects of increased honey bee densities on the reproductive success and foraging behavior of *Osmia bicornis*

Michael Glück[1], Raghdan Alkattea[1], Ulrich Ernst[1], Peter Rosenkranz[1], Kirsten Traynor[1], Manuel Treder[1]

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The reasons for the current decline in wild bee species are heavily debated, and it is still unclear if they compete with *Apis mellifera* for floral resources as there are few studies investigating actual effects on wild bee fitness under field conditions. We therefore used *Osmia bicornis* as a model organism to investigate the impact of different honey bee densities on the reproductive success and the foraging behavior of wild bee species under field conditions.

Over an eight-week period we conducted two full replications, varying the honey bee density in four study sites of flower-rich meadows in Baden-Württemberg by migrating five full-size honey bee colonies into two of the study sites. Every two weeks we relocated the honey bee colonies to the other sites, which allowed us to compare high and low density at the same locations and expose every development stage of *O. bicornis* to different honey bee densities. In total, we released 50 *O. bicornis* cocoons at each of 16 nesting sites (N = 800) and observed 800+ created cells in 2,400 nesting holes. Additionally, we collected pollen from each nesting site and honey bee colony for a detailed pollen analysis and valuated the weight of pollen provisioning and larvae inside each nest cell.

Our preliminary results (GLMM) showed significant effects of the study site ($p < 0.005$) and season ($p = 0.003$), but we found no effect of increased honey bee density on the reproductive success of *O. bicornis* ($p = 0.61$). The ongoing pollen analysis and nest evaluations will provide a deeper insight into possible effects on the foraging behavior and nest-building of *O. bicornis*.

Based on our current data, site-specific factors had a greater impact on the reproductive success of *O. bicornis* than increased honey bee densities. Moreover, our results establish a promising experimental set-up for further studies on the fitness of solitary wild bee species.

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Keywords: competition, wild bees, honey bee density, reproductive success, fitness

Organisatorisches

Allgemeines

- garantierter Check-in im Hotel am 28.03.2023 ab 15 Uhr
  Es lohnt sich aber auch vorher schon mal an der Rezeption nachzufragen, ob das Zimmer schon fertig ist.
- Check-out am 30.03.2023 bis 11 Uhr
  Das Gepäck kann dann bis Tagungsende im Gepäckraum verbleiben.
- Frühstück findet im Hotelrestaurant Zeppelin statt
- Es besteht die Möglichkeit wichtige Informationen wie z. B. Stellenausschreibungen an einer Pinnwand zu veröffentlichen.

Exkursionen am 29.03.2023

Biosphäre Potsdam

Treffpunkt: 14:50 Uhr vor dem Hotel (siehe Karte)

Mit dem Bus fahren wir dann gemeinsam zur Biosphäre Potsdam. Dort gibt es eine Führung (Beginn: 15:30 Uhr).

Stadtführung Potsdam

Treffpunkt: 15:50 Uhr auf dem Luisenplatz (direkt am Bassin, siehe Karte)

Vom Hotel aus kann der Luisenplatz direkt mit der Straßenbahn erreicht werden (Linie 91, Haltestelle: Bhf Pirschheide). Nach einer etwa 10-minütigen Fahrt steigen Sie an der Haltestelle Luisenplatz-Süd/ Park Sanssouci aus.
Die Fahrt findet im Tarifbereich Potsdam AB bzw. Berlin C statt. Fahrkarten können in der Tram gekauft werden. Zu beachten ist, dass beim Kauf einer 4-Fahrten-Karte (Potsdam) das erste Ticket dann bereits entwertet ist.

Führung durch den Park Sanssouci

Treffpunkt: 15:50 Uhr am Grünen Gitter in der Allee nach Sanssouci (siehe Karte)

Vom Hotel aus kann der Luisenplatz direkt mit der Straßenbahn erreicht werden (Linie 91, Haltestelle: Bhf Pirschheide). Nach einer etwa 10-minütigen Fahrt steigen Sie an der Haltestelle Luisenplatz-Süd/ Park Sanssouci aus.
Die Fahrt findet im Tarifbereich Potsdam AB bzw. Berlin C statt. Fahrkarten können in der Tram gekauft werden. Zu beachten ist, dass beim Kauf einer 4-Fahrten-Karte (Potsdam) das erste Ticket dann bereits entwertet ist.

Abendveranstaltung am 29.03.2023

Abendveranstaltung im Kongresshotel Potsdam

Beginn: 19 Uhr

Die Abendveranstaltung mit Live-Band, DJ und Buffet findet im Hotel-Restaurant Zeppelin statt.

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Impressum

Die 70. Jahrestagung der AG der Institute für Bienenforschung e.V. wird organisiert vom:

Länderinstitut für Bienenkunde Hohen Neuendorf e.V.
Friedrich-Engels-Str. 32
16540 Hohen Neuendorf

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www.honigbiene.de