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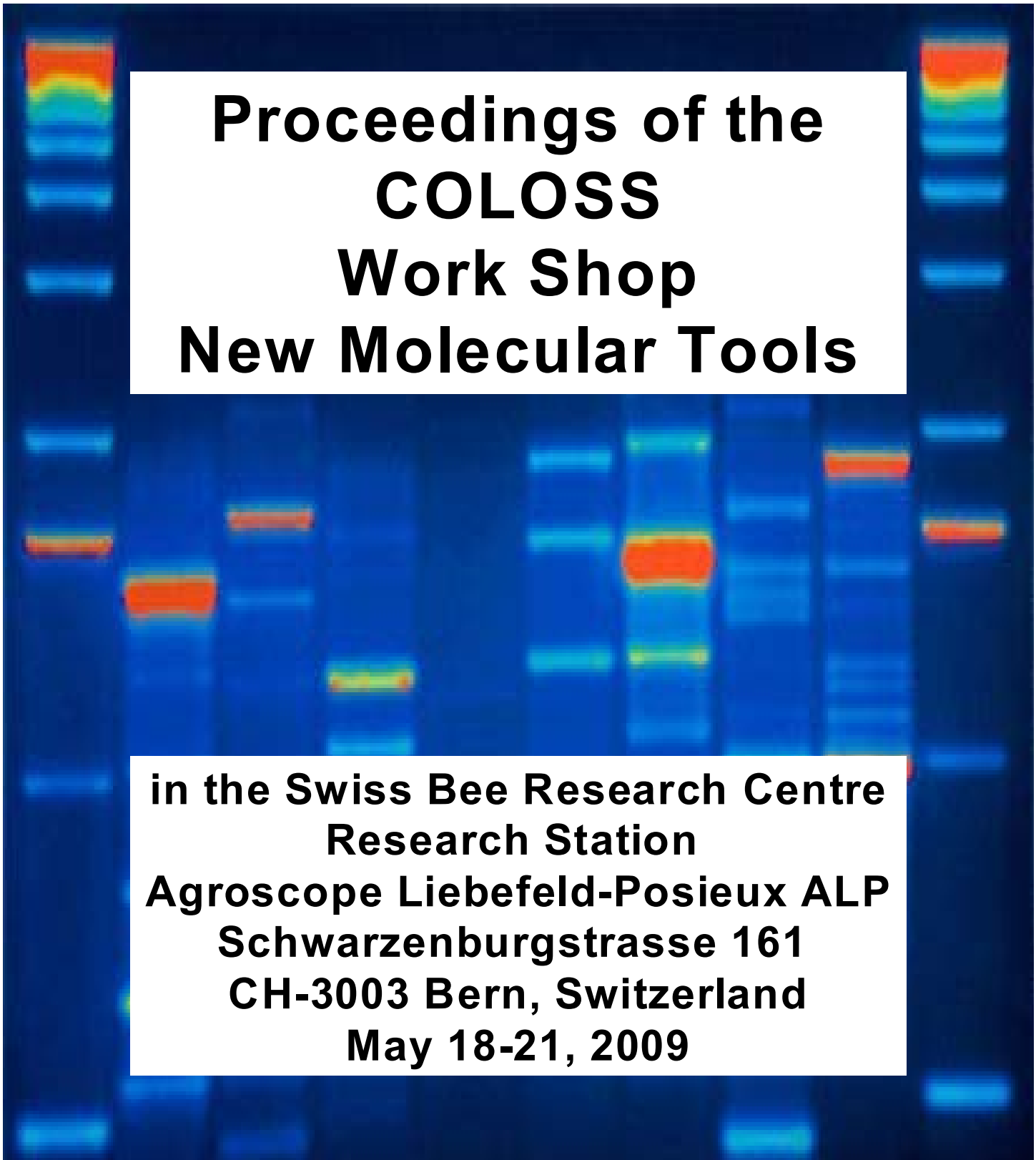
Action FA0803

Proceedings of the COLOSS Work Shop New Molecular Tools



in the Swiss Bee Research Centre Research Station Agroscope Liebefeld-Posieux ALP Schwarzenburgstrasse 161 CH-3003 Bern, Switzerland May 18-21, 2009

Dear colleagues

On behalf of the local organising team, it is my personal pleasure to welcome you to the Work Shop New Molecular Tools at our Swiss Bee Research Centre, Research Station Agroscope Liebefeld-Posieux ALP.

I would like to thank all the people who have helped to organise and conduct this meeting.

Appreciation is also addressed to all contributors for submitting their abstracts, which I hope will stimulate rewarding discussions on diagnosis and hypothesis-driven research on the causes of honeybee colony losses.

Financial support is granted by COST via the Action FA0803 COLOSS.

I am looking forward meeting all of you, and hope you will enjoy this work shop.

Peter Neumann, Action Chair

Bern, Switzerland, Wednesday, 08 April 2009

The Local Organising Committee for the Work Shop

Vincent Dietemann, Peter Neumann, Marc Schäfer

Technical support

Brigitta Rosta, Michael Eyer

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Agenda

TIME	PROGRAM
18.05.2009 (Monday) – Hotel Glocke	
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09:30 – 10:00	Registration and Coffee
10:00 – 10:10	Welcome
10:10 – 11:00	Laurent Keller “Gene by social environment interactions on gene expression and behavior in ants”
11:00 – 11:30	Coffee break
11:30 – 12:30	Jay Evans “Molecular diagnostics and the causes of honey bee declines”
12:30 – 14:00	Lunch
14:00 – 15:00	Axel Brockmann “Quantitative peptidomics and the role of neuropeptides in honey bees foraging behavior”
15:00 – 15:20	Cédric Alaux “Finding molecular markers of infection and resistance to environmental stressors in honey bees: the use of genome-wide transcriptomic”
15:20 – 15:40	Ales Gregorc “The study of lethal and sublethal effects on honeybees”
15:40 – 16:00	Dieter Behrens “Lethal infection thresholds of *Paenibacillus larvae* for honeybee drone and worker larvae (*Apis mellifera*)”
16:00 – 16:30	Coffee break
16:30 – 17:30	Open discussion: molecular tools and colony losses (incl. 5 min talks) chaired by Peter Neumann
19:00 – open	Social event in the old town of Bern
20.05.2009 (Wednesday) - Swiss Bee Research Centre	
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9:50 : 10:10	Rudolfo Jaffé “Can mating flights hinder the vertical transmission of a honeybee (*Apis mellifera*) virus?”
10:10 – 10:30	Tamas Revay “Genetic diversity of Hungarian honeybee colonies based on morphological and RAPD markers”
10:30 - 11.00	Coffee break
11:00 – 12:30	Poster Session (19 contributions)
12:30 – 14:00	Lunch
14:00 – 15:30	Open discussion: methodological pitfalls or what to learn from negative results (incl. 5 min talks) chaired by Maria Navajas
15:30 – 16:00	Coffee break
16:00 – 17:30	Open discussion: new frontiers (incl. 5 min talks) chaired by Jay Evans
18:30 – 20:30	Guided evening tour through the old town of Bern from Bern Central Train Station to Bärengraben
21.05.2009 (Thursday) – Field Excursion: beekeeping at the Top of Europe	
8:00 – 16:00	1 day excursion to the Berner Oberland (see details below)

Registration on site is required. Registration fees: 50.- €

Excursion – beekeeping at the Top of Europe

Beekeeping in Switzerland is very distinctive and practiced under Alpine conditions. We will visit a typical Swiss apiary just below the truly magnificent Top of Europe. The departure from Bern to Kleine Scheidegg is scheduled on 21.05.2009 at 8:00 am. Participants will return to Bern on 21st May before the evening (~16:00).

Accommodation

Please register asap online by yourself with Hotel Glocke to ensure that all of us can stay in one place. The Hotel Glocke (http://www.bernbackpackers.ch/english.htm) is right in the centre of the beautiful old town of Bern (UNESCO world heritage site) and is reasonably cheap, thereby within the COST reimbursement limits. Those among you preferring other accommodation please don't hesitate to contact me.

CONFERENCE LOCATIONS	
Hotel Glocke Backpackers Bern	**Swiss Bee Research Centre**
Rathausgasse 75 CH-3011 Bern Tel: +41 31 311 37 71 Fax: +41 31 311 10 08 info@bernbackpackers.ch http://www.bernbackpackers.ch	Agroscope Liebefeld-Posieux Research Station ALP Schwarzenburgstrasse 161 CH-3003 Bern Switzerland
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Lethal infection thresholds of *Paenibacillus larvae* for honeybee drone and worker larvae (*Apis mellifera*)

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We compared the mortality of in-vitro reared worker and drone larvae of a single queen after infecting with two dosages of Paenibacillus larvae, the causative agent of American Foulbrood (AFB), finding a delayed onset of mortality and a lower mortality rate in drones compared to workers. We furthermore determined the absolute number of P. larvae cells in all deceased individuals by rt-PCR, finding a higher cell number in drones compared to workers and a higher cell number in the higher dosage groups. Gender differences were shown to be body-size dependent, suggesting a body-size dependent lethal threshold for the number of P. larvae cells per amount of larval tissue. The difference between dosage groups suggests a considerable variance of lethal thresholds within the offspring of a single queen. We propose bacterial growth rate and individual lethal infection thresholds to be key parameters in larval AFB resistance.

Quantitative peptidomics and the role of neuropeptides in honey bee foraging behavior

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Honey bees are one of the model organisms to study the mechanisms and evolution of social behavior. To a large extent, social behavior is based on behaviors already present in solitary species, which during social evolution are then placed under the control of social interaction and regulation. In honey bees, for example, foragers collect food (nectar and pollen) not for their own immediate consumption but for their colony's nutritional needs. The forager's decisions about whether to search for food, and if so, whether to search for carbohydrate (nectar) or fat and protein (pollen) are dependent on the colony's and not its own physiological condition. A central issue is to understand how social information affects the molecular pathways and neural circuits in the brain to affect behavior.
Accumulating evidence suggests that neuropeptide signaling plays a prominent role in regulating social behavior. However, research on the behavioral function of peptides is still in its infancy. Our lab focuses on developing experimental assays to study behavioral functions of neuropeptides. We have collaborated with the Sweedler lab (UIUC), who have established a quantitative peptidomic procedure, which allows detecting changes in multiple brain peptides in animals behaving under natural conditions. We have used this approach to study changes in brain peptide dynamics as a function of foraging behavior. This talk will review some of the latest findings in these areas.

Long-term analyses of patriline composition of a honeybee colony

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A honeybee queen mates with a number of drones a few days after she emerges as an adult. Spermatozoa of different drones are stored in her spermatheca and used for the rest of the queen's life to fertilise eggs. Sperm usage has been thought to be random, so that the patriline distribution within a honeybee colony would remain constant over time.
In this study we assigned the progeny of a naturally mated honeybee queen to patrilines using microsatellite markers at the queen's age of two, three and four years. No significant changes in patriline distribution occurred within each of two foraging seasons, with samples taken one and five months apart, respectively. Overall and pair-wise comparisons between the three analysed years reached significant levels ($p<0.05$, Fisher's exact test). Over the three-year period we found a trend for patrilines to become more equally represented with time.
We discuss changes in patriline composition due to mixing processes in the queen's spermatheca, following incomplete mixing of different drones' sperm after mating. This could result in diverse resistance or susceptibility against parasites of the colony in various years.

How to detect and quantify an honeybee virus: The CBPV (Chronic bee paralysis virus).

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The Chronic bee paralysis virus (CBPV) is the aetiological agent of an infectious and contagious disease of adult honey bees known as chronic paralysis. Over the past few years, the outbreak in France of trembling symptoms caused by CBPV has led our laboratory to conduct studies in order to improve the knowledge on this agent and on the disease. Full-length nucleotide sequences for the two major RNAs of CBPV have been characterized, leading to the development of, firstly, molecular diagnostic tools that can be used to detect genetically variable viral isolates and, secondly, a Real-Time PCR viral quantification technique. This two step real-time RT-PCR assay based on TaqMan technology using a fluorescent probe (FAM-TAMRA) was developed to quantify chronic bee paralysis virus (CBPV) genome in bee samples. According to an absolute standard curve obtained with a plasmid containing a partial sequence of CBPV, this assay provided linear detection over a 7-log range (R^2>0.99) with a sensitivity of 100 copies, confirmed by reliable inter-assay and intra-assay reproducibility. In order to evaluate the CBPV TaqMan methodology, the CBPV genomic load was quantified in bee samples coming from experimental infections but also from naturally infected colonies and various samples of insect. These results validate this method for chronic bee paralysis virus quantification.

Finding molecular markers of infection and resistance to environmental stressors in honey bees: the use of genome-wide transcriptomic

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Microarray technology is widely used for the routine prognostic screening of numerous human pathologies. The development of this high-throughput system monitoring the expression of thousand of genes enables one to characterize the molecular events occurring in pathologies. Once an organism has been exposed to an environmental stress, it responds with a series of physiological changes, many of which are the result of changes in gene expression. Since the publication of the honey bee genome and the availability of the new honey bee oligonucleotide microarray containing all the genes of the bee genome, microarrays have been successfully used to identify molecular markers of behavioural and physiological states. Now, genome-wide transcriptome analysis promises, as in human, to screen genes that characterize the nature of honey bee stressors including diseases or exposure to pesticides. Another perspective of this genomic approach includes the identification of genes that are potential markers of honey bee resistance to pathogens. Ultimately, these molecular markers would potentially allow for a clinical screening and the identification of resistant honey bee population to pathogens for breeding selection.

Investigation of honey bee losses in Austria 2006 – 2008

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In the years 2006 to 2008 honey bee losses in Austrian apiaries were investigated in order to find their causes. 532 bee samples from colonies (with different and more or less distinct disease symptoms) from 109 apiaries were investigated for their pathogen and parasite status by light microscopy (*Nosema* sp., *Malpighamoeba mellificae*, *Acarapis woodi*) and PCR-methods (ABPV, BQCV, DWV, SBV, CBPV and KBV) or visual inspection for varroa infestation (50 bees and 50 brood cells/sample, respectively). 50 samples from 32 apiaries were suspected for pesticide poisoning. Residue analysis was carried out by GC-MS and LC-MS-MS-methods. The most prevalent virus was DWV (63 %), followed by ABPV (56 %), BQCV (53 %,) SBV (33 %) and CBPV (4 %). KBV (n = 62) was not detected. Some differences in virus prevalence were observed according to type of virus, season of sampling and federal provinces of Austria. *Varroa* infestation level was significantly higher in samples from dead bee colonies (15 %) compared to weak or queenless colonies (1 %) or normally developing colonies (3 %). *Nosema* sp. was more often detected in bees from dead colonies compared to normally developing colonies. Cysts of *Malpighamoeba mellificae* were only found in two (0.4 %) out of the 494 examined bee samples. There was no incidence of tracheal mite in 387 investigated samples. Poisoning by pesticides was confirmed only in 4 cases, two of intentional and two of accidental poisoning by the use of plant protection products.

Molecular diagnostics and the causes of honey bee declines

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Honey bee populations often shown puzzling declines for which no immediate cause can be identified. In the United States, an episode of decline was most noticeable in the winter of 2006-2007, leading to diverse efforts to identify possible biotic and abiotic causes. Obstacles for genetic diagnoses of bee pathogens or genes related to bee health occur at the level of sampling, preservation, and diagnosis. Especially among the viruses, diagnostic tests are prone to differences across strains in nucleotide sequence and are generally unsuccessful in finding novel pathogens or parasites. We have used known and newly developed genetic markers to survey declining and healthy colonies collected across the United States. Colonies identified as being under collapse showed nearly twice the number of pathogens found in both historic and current healthy colonies and pathogen loads (pathogen transcript copies) were also higher in these colonies. Pathogen identities differed across geographical scales ranging from subpopulations (apiaries) to states, indicating that collapsing colonies are generally more vulnerable to attack. We found high levels of the microsporidian pathogen Nosema apis in collapsing colonies from western states, widespread abundance of Nosema ceranae in both collapsing and healthy colonies, and an association between levels of a common bee trypanosomal parasite and colony health. Among the viruses, Kashmir Bee Virus was most strongly associated with declining bee colonies in our survey.

Quantification of *Nosema apis* and *Nosema ceranae* using real-time PCR

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Nosema apis and Nosema ceranae are intracellular microsporidian parasites infecting the midgut epithelial cells of adult honey bees. N. ceranae was considered to be restricted to Apis cerana, but is nowadays a parasite found also in A. mellifera colonies across most of the world. Recent surveys and experimental work suggest that N. ceranae is a serious threat to the global beekeeping industry. It has been suggested that N. ceranae induces significantly higher mortality in honey bees than N. apis, but little is known about their comparative virulence. By developing and applying molecular genetic techniques, i.e. quantitative real-time PCR (qPCR), this work aimed to elucidate within host competition between the two parasites, using mixed infections. The outcome of the experiments, the relative virulence of the two parasites at the level of individual bees and the development and validation of the qPCR will be discussed.

PCR DETECTION OF *NOSEMA APIS*/*NOSEMA CERANAE* SPORES IN HONEY SAMPLES

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The present study describes the development of a PCR assay for the detection of Nosema apis/Nosema ceranae spores in honey samples. Briefly, deoxyrubonucleic acid isolation and PCR assay were tested on 1 mL honey samples (in triplicate) experimentally infected with different Nosema ceranae spore dilutions (from 1x106 to 1x10 spores). The QIAamp® DNA Mini Kit was used to extract DNA with a pre-incubation step with chitinase or lysozime to lyse the rigid cell wall. Therefore, DNA was amplified by PCR according to Higes et al. (2006). To investigate the practical value of this assay, 30 commercial honey samples, of different colour and consistency, were tested.
The assay showed high sensitivity and reproducibility, since it amplified Nosema ceranae DNA extracted from honey samples experimentally infected with 0,5x102 spores/ml. Comparable results were obtained by using either chitinase or lysozime to lyse the spores wall. Moreover, no amplification products were ever observed after the examination of aforementioned commercial honey samples. The method was very sensitive and reproducible, despite time consuming and labour intensive. Its application to bee hive products, such as wax and pollen other than honey, might be helpful for a screening of beehive health status, based on the presence of Nosema spores in different hive matrices, also without clinical signs.

The study of lethal and sublethal effects on honeybees

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A number of cell biology techniques were employed to assess cellular changes induced in bees after inoculation of bacterium Paenibacillus larvae and separately with different pesticides and acaricides. The immunohistochemical localisation of the heat shock proteins (Hsp70 and Hsp90) and histone proteins in healthy and pesticide treated bees has been studied to evaluate sublethal effects on bees.
Collapsed honeybee colonies and varroa mites from these colonies were analysed for acute bee paralysis virus (ABPV) and deformed wing virus (DWV) using RT-PCR assay. The majority of sampled worker bees were infected with both viruses (64%), while only DWV was determined in 22% and ABPV in 5% of the examined colonies. Both, worker bees and varroa mites, were infected with ABPV in 65% and with DWV in 70% of colonies. We have also analysed newly reared and mated queens in the seasons 2006 and 2008 on the presence of four viruses (ABPV, DWV, SBV and BQCV) in order to establish the incidence of viral infections in queens. The highest percentage of infected queens was detected for DWV appeared in 65 %, BQCV in 25 %, SBV in 8 % and ABPV in 4 % of queens. Honeybee colonies in queen rearing apiaries were only Nosema cerane infested, using PCR method.
Our data show that cell biology and molecular methods are useful for studying honeybee pathology and indicate possibilities for monitoring the sublethal and lethal effects of infective and chemical environmental stressors on honeybee.

Molecular classification of bacterial associates of honeybees

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In honeybees, several species of bacteria can proliferate in the haemolymph. However, previous reports are contradicting, creating demand for re-evaluation of bacterial associates. Here we use molecular methods to identify bacteria from the haemolymph of workers. Haemolymph samples were taken from 295 live workers (N=35 colonies throughout Switzerland). Samples were plated on low-selective media and cultured. To identify bacteria, individual pure cultures were PCR-RFLP analyzed (16S rRNA gene and Cfo I) and partially sequenced (16S rRNA). In 80 out of 295 samples, bacterial growth was found. The PCR-RFLP analyses revealed a total of 11 different bacterial species. Most common was *Lactobacillus kunkeei* (76 %). Infections with one species only (N=58) were significantly more often found than those with two (N=2; χ^2=60, p<0.001). Our data confirm that bacteria can be found in the haemolymph, but most of which are usually considered as being non-pathogenic (e.g. *L. kunkeei* in the gut). The key question appears to be, how bacteria get into the haemolymph of bees, which is the underlying reason for diseases such as septicaemia. This transmission should be addressed in further experiments.

Can mating flights hinder the vertical transmission of a honeybee (*Apis mellifera*) virus?

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Males of the honeybee (*Apis mellifera*) fly to specific drone congregation areas (DCAs), which virgin queens visit in order to mate. From the thousands of drones that are produced in a colony, however, only very few succeed in copulating with a queen. Hence, a strong selection on males acting during their mating flights, is likely to hinder the vertical transmission of pathogens affecting flight performance. Deformed wing virus (DWV) is a worldwide spread honeybee virus, associated with malformed wings and severely reduced life span in highly infected bees. Although vertical transmission was recently reported in DWV, it is not yet clear if it represents a regular transmission mode of this virus. By collecting drones at a DCA and comparing the DWV loads found in their sperm with those of a control sample of drones collected at their maternal hives, we tested if drone mating flights can hinder the vertical transmission of DWV. In addition, relying on wing fluctuating asymmetry as a measure of phenotypic quality, we measured selection taking place during drone mating flights. Although we found evidence for a strong selection against wing asymmetry during drone mating flights, no differences were found between the DWV loads of drones collected at their maternal hives or at the DCA. Our results suggest that, by remaining asymptomatic, DWV is adapted to being vertically transmitted by honeybee drones.

Gene by social environment interactions on gene expression and behavior in ants

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In this talk I will discuss how interactions between genes and social environment influence behavior and social organization. In particular, I will show that, in ants, worker behavior and gene expression profiles are more strongly influenced by indirect effects associated with the genotypic composition of workers within their colony than by the direct effect of their own genotype. This constitutes an unusual example of an “extended phenotype,” and suggests a complex genetic architecture directly and indirectly influencing the individual behaviors that, in aggregate, produce an emergent colony-level phenotype. I will finally discuss of these gene by environment interactions underlie the presence of two distinct modes of social organization.

Effects on virus infection dynamics following removal of mites (*Varroa destructor*) from honey bee colonies

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Apis mellifera colony mortality caused by honey bee viruses has been associated with severe infestation of the ectoparasitic mite *Varroa destructor*. The mite has been shown to vector virus infections between adult bees and between adult bees and brood stages. Using quantitative RT – PCR we studied the virus dynamics over time in adult bees, pupae, and mites in infested bee colonies treated with the acaricide Apistan® compared to non-treated infested control colonies. A preliminary screening of the test colonies for all viruses associated with V. destructor infestation showed deformed wing virus (DWV), sac brood virus (SBV), and black queen cell virus (BQCV) to be present. Detailed results will be presented in poster format.

Integration of viral sequences into the bee genome: how to distinguish between viral infection and expression of integrated sequences

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Diagnosis of viral infection in honeybees is usually performed by RT-PCR with virus-specific primers. Recently it was shown that segments of IAPV sequences (and possibly of DWV?) have been reversed transcribed and integrated into the bee genome. Some of them were shown to be transcribed from a native bee gene as chimeric RNAs. Once such integration is stabilized in the host genome, it becomes a heritable trait and is passed on to the host progeny. Therefore, if primers of integrated viral sequences are employed they might amplify the transcripts of the integrated sequence rather than the viral sequence and lead to errors. It might even happen that a bee that has never been exposed to a virus will be diagnosed as infected, while in fact, it inherited a viral segment from progenitor. Methods for discerning viral sequences from transcripts will be demonstrated.

Cross-species amplification of microsatellite loci in stingless bees

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Polymorphic microsatellites are very usefull molecular markers which have wide-ranging applications in the field of genetics, including kinship and population studies. The development of microsatellite markers through transfer of primers from related species (cross-species amplification) remains a little-explored alternative to the de novo method of developing microsatellite primers. Here are listed loci that showed a high cross-species amplification success in Apidae, mainly in stingless bees.

Molecular methods to study honeybee responses to environmental stressors

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We are interested in how honeybees interact with their environment. In particular what are the genetic basis of the bee responses to both biotic (Varroa and viruses) and abiotic (chemicals) environmental stressors. For example, we know that not every bee is equally susceptible to Varroa. How do some bees survive to the parasite aggressions while others perish? What are the traits involved in the observed adapted bee phenotypes? These are some of the questions we attempt to answer by measuring gene expression in bees experimentally confronted to different challenges. We are also interested on methods derived from bioinformatics that are used to identify the regulated genes involved in targeted biological functions

The analysis of mtDNA variation in some isolated honeybee lines in Serbia

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Since Serbia has a heterogenous relief and climate conditions in which the honeybee is being raised, it is thought that thanks to adaptable capacities of honeybee a different geographical ecotypes of honeybee have been created. Also migration of beekeepers was rather intensive so honey bees were moved all over the country. For this reason the objective of this research was to determine the differences of selected lines in the subspecies *Apis mellifera carnica* Pollman on the territory of Serbia and to conduct the analysis of mtDNA in honeybee in order to determine the variability of studied lines. Total DNA was extracted from the legs of worker bees. DNA was extracted by means of JETQUICH Tissue DNA Spin kit. All the PCR amplifications were conducted in the programmed thermocycler GeneAmp® PCR System 9700. The sequences of 5'-end mtDNA were aligned with published sequences of 49 different mtDNA haplotypes of honeybee. In all our samples the differences which correspond to C2E haplotype described in the samples of honeybees from Serbia were found as three polymorphic sites such as follows: two A-T transversions and one insertion of nucleotide A. However, in the group 4, for the first time in honeybee from phylogenetic line C, one transition G-A was observed 12 places before polymorphic place 4 in relation to haplotype C2E and one deletion of T nucleotides of 20 base places besides the aforementioned polymorphic place 3. On the basis of this research it can be concluded that in Serbia there exists one level of hybridization in honeybees. However, some of the honeybee populations have retained the original characteristics of the breed, what is of importance for the conservation of local population of honeybees in a future period.

Genetic diversity of Hungarian honeybee colonies based on morphological and RAPD markers

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The only native and legally licensed honeybee race in Hungary is the Carniolan bee (*Apis mellifera carnica*). All the queen breeding colonies are under control by „Hungarian bee breeding code", that fix wanted productivity, trait elements and morphological characteristics. Four out of these morphological characteristics are considered important as length of the mouthpart, cubital index, deformity of the cubital cell and the colour of the tergit plate. The markers give the possibility to compare different lines on morphological basis as well. According to a more year breeding program participating stations apply regular freshening. Tested apiaries included honeybee colonies originated from different parts of the country. Analyses based on random DNA polymorphism (RAPD) was carried out on 19 colonies at the apiary of 6 different breeders. Extracted DNA samples were tested with 20 different primers generating 15 variable markers. Distance matrices were calcuated from both dataset (morphological traits and DNA polimorphism). It has been clearly evident that no one of the breeders's stock demonstrate higher similarity between their families as compared with samples from different breeders. Mantel test showed low correlation between genetic and physical parameters. Results of principle component analysis also proves that the tested portion of the honeybee stocks of Hungarian breeders presents high diversity. This might be an important factor in the good productivity, the resistance against parasites and the so called Colony Collapse Disorder which has not been demonstrated yet in Hungary.

Activation of Immune-Related Genes by Pathogen Derived Elicitors

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Bees are permanently exposed to attacks of parasites and pathogens. Survival of colonies depends on behavioral defense traits, such as polyandrous mating, swarming or brood hygiene and on the functionality of the immune system of each individual bee. Pesticides and nutritional shortages are believed to destabilize the immune system, eventually resulting in colony fatalities. As a prerequisite for testing this hypothesis, meaningful immune indicators have to be identified. Here, we focus on the expression of immune related genes after stimulation with elicitors. Brood combs were removed from colonies and transferred to an incubator (34°C, ca. 70% rH). After hatching and a transient stay in cages, the bees were injected with solutions containing lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PG), Paenibacillus larvae, and a crude preparation of acute bee paralysis virus (ABPV). Bees were sacrificed by freezing ten hours after injection, and the total RNA was extracted. Based on published protocols we established SYBR-green real time PCR assays for abaecin, apidaecin, defensin 1, hymenoptaecin, prophenoloxidase (ProPO) and for two genes from the JAK-STAT and the TOLL signal pathways. As expected from previously published results, LPS, LTA, P larvae, and PG proved to be efficient elicitors. While the genes for abaecin, apideacin and hymenoptaecin were upregulated, the expression of defensin1 and ProPO was hardly influenced at all. The ABPV preparation did not show prominent effects on gene regulation with the exception of hymenoptaecin, which appeared to be the most indicative gene with the highest up-regulation of more than 400-fold.

Prospects of Croatian beekeeping with regard to current situation and achievements

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According to the data of Croatian agricultural agency (CAA) there are 3 402 beekeepers with 314 943 honeybee colonies registered in the 2007 on Croatian professional and sideline apiaries. Data for 2008 are still processing. Systematic surveys of colony losses are not established yet in Croatia. In order to collect and present preliminary data of colony losses in 2009, a survey was conducted among beekeepers from different locations in Croatian Continental region during February and March 2009. A total of 135 beekeepers with 10 618 colonies filled in the anonymous questionnaire. A survey results showed that beekeepers lost total 1 797 colonies (16.92 %) during winter period 2008 / 2009. 507 colonies (4.77%) were lost during January and February 2009. According to beekeepers reports, losses were caused by diseases (38.58%) and technological problems (queens and food storage – 30.16%). 13 surveyed beekeepers did not register losses and 30 of them did not specify loss causes, which covers 31,38% of total colony losses. Carniolan bee (*Apis mellifera carnica*) is autochthon in Croatia. There are three known distinguishing ecotypes of Carniolan bee in Croatia - pannonian, mountain and mediterranean ecotype. They are developed in distinctive regions, where climate, relief and vegetation have caused their biodiversity. Differences in morphometric and behavioral characteristics are confirmed between them. Croatia has achieved significant selection progress so far, but there is a need for further breeding improvements. Croatia should make an effort to alleviate a conflict between selection and biodiversity of autochthon Carniolan bee. New molecular tools could certainly contribute to achieving this goal.

Key words: colony losses survey, Carniolan bee, ecotypes, selection

Presence of *Nosema ceranae* in honey bee colonies in Poland

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Nosema infection is very common in Polish apiaries. It was found in 92% of apparently healthy colonies when dead bee samples collected in 1995 - 1996 in nine apiaries, situated in different parts of Poland, were investigated. In 2007 dead bee samples with *Nosema* spp. spores, originating from ten colonies, of four Polish apiaries, were sent to Centro Regional Apicola in Marchamalo (Guadalajara, Spain). There the team of Professor Aranzazu Meana and Dr. Mariano Higes, using PCR analysis, diagnosed N. ceranae infection in all received samples. Though it is advisable to use live bee samples to investigate Nosema spp. presence by PCR our lab usually receives dead bee samples from beekeepers. We introduced the preliminary treatment of such samples with sodium hypochlorite before performing PCR test. The investigation of samples collected in 1994-1996 revealed that *N. ceranae* was present in Polish apiaries already in 1995. Analysis of current bee samples revealed the presence of *N. ceranae* in 82 % of *Nosema*-positive bee colonies in which increased mortality of bees or disappearing of bees was observed.

Passage of natural deformed wing virus sequence polymorphisms during different transmission routes between honeybees

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This study concerns different aspects of the transmission of viral pathogens implicated in *Varroa* associated colony losses. We recently discovered that natural populations of deformed wing virus (DWV) can be quite variable, and that this variation mostly consists of a polymorphism of two or more distinct sequence variants, each surrounded by largely non-overlapping mutational spectra. Among the possible causes of such polymorphisms are differences in transmission efficiency between the variants for alternative transmission routes, which may furthermore be linked to the known tissue specificity of deformed wing virus. Deformed wing virus accumulates in the salivary and hypopharyngeal glands (trophallactic transmission), the hindgut lumen (fecal-oral transmission) and in the sex organs (venereal – vertical transmission). It is also transmitted epidemically by *Varroa destructor* when feeding on bee haemolymph during the pupal and adult stages. In these experiments we investigated whether different naturally occurring variants were favoured during different transmission routes, either qualitatively (establishing a new infection) or quantitatively (viral titres during transmission). The results suggest that independent of the route of transmission, the different variants of DWV are transferred proportionally from the infected honeybees or *Varroa* parasites to the new honeybee hosts. If there is no selective pressure during the transmission of these DWV variants, the question of the evolutionary origin of the natural DWV polymorphisms remains unanswered.

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