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Association of Institutes for Bee Research
Report of the 55th Seminar in Hohen Neuendorf
11–13 March 2008

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55. Jahrestagung in Hohen Neuendorf
vom 11.–13. März 2008

Association des Instituts de Recherche sur les abeilles
Comptes rendus du 55e Congrès à Hohen Neuendorf
11–13 mars 2008

List of reports (* after the title indicates that no abstract of this report is published).

Verzeichnis der Referate (* bedeutet, dass zu diesem Titel keine Zusammenfassung aufgeführt ist).

Liste des communications (* après le titre indique que le résumé de la communication n'est pas publié dans ce numéro).

Invited talk
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Conférence inaugurale

1. Parasites and humoral defense in the model of *Bombus* (Bumblebees). *P. Schmid-Hempel**

Parasiten und Immunabwehr im Modellfall von *Bombus* (Hummeln).

Parasites et défense humorale chez le modèle *Bombus* (bourdons).

Biology, physiology, behavior

Biologie, Physiologie, Verhalten

Biologie, physiologie, comportement

2. Why do pollen foragers perform better in associative learning than nectar foragers? *R. Scheiner*

Warum lernen Pollensammlerinnen besser als Nektarsammlerinnen?

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3. "Sniffer Bees": Can honeybees learn the odor of queens with different kin relation? *R. Alkattea, H. Steidle, P. Rosenkranz*

„Schnüfflerbienen": Können Bienen den Duft von Königinnen mit unterschiedlichen Verwandtschaftsbeziehungen lernen?

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Localisation des processus d'apprentissage et de mémoire dans le cerveau de l'Abeille : anesthésie locale des corps pédonculés.

6. Experience contributes to an improvement of hygienic behaviour in honeybee workers, *Apis mellifera*. *S. Härtel, H.M.G. Lattorff, M.O. Schäfer, J.S. Pettis**

Beitrag von Erfahrung zur Verbesserung des hygienischen Verhaltens von Honigbienenarbeiterinnen.

L'expérience contribue à améliorer le comportement hygiénique des ouvrières d'abeilles.

7. Heatseeker – heat as a trigger for trophallactic activities in the honeybee. *R. Basile, J. Tautz**

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Les vieilles nourrices favorisent la fertilité – du nouveau sur la détermination des castes chez l'Abeille.

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Pathologie / Varroose / Abwehr

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Abstracts

2. Why do pollen foragers perform better in associative learning than nectar foragers ? *R. Scheiner* (Technische Universität Berlin, Institut für Ökologie, Franklinstr. 28/29, 10587 Berlin, Germany)

We studied the associative tactile learning performance of pollen and nectar foragers under laboratory conditions. In this paradigm, bees learn to associate a tactile stimulus with a sucrose reward. Pollen foragers reached a significantly higher level of acquisition than nectar foragers ($P < 0.05$, pollen foragers = 46, nectar foragers = 42, Fisher exact Probability Test). To test whether these learning differences were related to differences in the evaluation of the rewarding sucrose stimulus, we analysed

sucrose responsiveness in returning foragers using the proboscis extension response. Pollen foragers displayed a significantly higher sucrose responsiveness than nectar foragers ($Z = 8.01$, $P < 0.001$, pollen foragers = 504, nectar foragers = 507, Mann Whitney U Test). We hypothesised that the learning differences of pollen and nectar foragers were a result of their differences in sucrose responsiveness and next compared the learning performance of pollen and nectar foragers with the same sucrose responsiveness. They did not differ in their tactile acquisition. In a series of experiments we found that sucrose responsiveness correlates positively with tactile acquisition. Bees with high responsiveness performed well, while those with low responsiveness performed poorly. Differences in the learning performance of bees with different responsiveness could be compensated by giving bees equal subjective rewards, depending on their responsiveness. Further, learning differences between genetic strains of bees and between bees tested at different times of the year could also be explained by differences in their sucrose responsiveness. We have now first indications that the relationships between sucrose responsiveness and associative learning found under laboratory conditions also apply under free-flying conditions.

3. "Sniffer Bees": Can honeybees learn the odor of queens with different kin relation? *R. Alkattea*[1]*, H. Steidle*[2]*, P. Rosenkranz*[1] ([1]Universität Hohenheim, Landesanstalt für Bienenkunde, 70593 Stuttgart, Germany; [2]Universität Hohenheim, Institut für Zoologie, 70593, Stuttgart, Germany)

The Proboscis Extension Reflex (PER) can be used to show the learning ability of honeybees. Recently, it was also used for practical application such as detection of smuggled goods, explosives and drugs at airports. In our work, the PER was applied to investigate the ability of honeybees to learn the odor of a queen and to differentiate it from the odor of another queen, which had a well-defined kin relation to the queen used for the learning process. The aim of our study was to use the "sniffer bees" as biosensors and analyze the olfactory cues which are responsible for the individual recognition of the resident queen within a honeybee colony. Hungry workers were trained to learn a queen's odor by touching the antennae with sugar syrup and offering the queen odor shortly after the syrup was being licked by the worker. The queen ordor was offerred by presenting a queen within a perforated plastic tube helpd closely to the test bees but without direct contact. After 5 repetitions for the learning phase another queen was offered to the trained workers without sugar rewards. We conducted the following tests: (i) conditioning with a virgin queen (odour + reward) and testing (odour only) once with its sister (n = 100) and once with an unrelated queen (n = 100), (ii) conditioning with a mated queen and testing with its sister (n = 92) and with an unrelated mated queen (n = 92), respectively. In virgin queens, there was no significant difference in worker responses to the learned and test odors in all test series. This means that workers could not differentiate between the odours of different virgin queens at all. In mated queens, however, workers could highly significantly differentiate between individual queens independent of the kin relation between learned and tested queen ($P < 0.005$, χ^2-test). These results are in contradiction to our bioassays where foreign queens related to the resident queen were better accepted by the workers than unrelated ones. This could mean that kin related recognition cues do not depend on volatile bouquets (PER) but rather on stimuli elicited through non-volatile compounds of the cuticle of the queen. Worker bees perceive these compounds in the bioassay by direct contact and licking of the queen.

5. Localization and dynamics of the information transfer between the brain hemispheres after side-specific conditioning of the honeybee, *Apis mellifera*. *B. Grünewald*[1]*, C. Bartsch*[2]*, M. Giurfa*[3]*, J.-M. Devaud*[3] ([1]Institut für Bienenkunde, Polytechnische Gesellschaft, FB Biowissenschaften, Goethe-Universität Frankfurt am Main, Germany; [2]Institut für Biologie – Neurobiologie, Freie Universität Berlin, Germany; [3]Centre de Recherches sur la Cognition Animale, Université Paul Sabatier Toulouse II, France)

The honeybee mushroom bodies play important roles during complex forms of learning and memory retrieval. In addition, output neurons from the mushroom bodies may mediate information transfer between the two hemispheres after side-specific learning. Bees are able to learn an odour with only one antenna and the response to the CS is retrievable firstly on the ipsilateral side directly after training and later also on the contralateral side. We analysed the dynamics of the information transfer between the brain hemispheres. For this, we used a method to locally and reversibly block the neural activity within the mushroom body α-lobes using injections of the local anesthetic, procaine. In a first experiment bees were conditioned bilaterally and procaine was unilaterally injected into the α-lobe. Thus, we blocked the retrieval on the injected side

whereas the conditioned response on the untreated side was unaffected. This shows that the ipsilateral mushroom body is essentially involved during memory retrieval; that procaine is a valuable local narcotic in bees and that it stays locally within the injected side. In a second experiment, we tested the dynamics of the memory transfer. For this, bees were injected unilaterally (ipsi- or contralateral) at different times during the learning process (unilateral conditioning), e.g. before or directly after the side-specific training or before the retention tests to investigate the dynamics of the information transfer between the brain hemispheres. Injections immediately before or after conditioning did not influence the contralateral response during later tests, whereas ipsilateral injections directly before retrieval reduced the contralateral performance. We therefore conclude that information transfer does not take place during acquisition. Rather, our results show that the memory trace after side-specific odour learning is limited to the trained side and that the information is transferred to the contralateral side via the α-lobes only during retrieval.

13. Social dynamics of brood warming: simultaneous measurements of honeybee temperatures and brood cell temperatures of a brood area. *J. Lein*[1], *M. Becher*[2], *R.F.A. Moritz*[2], *S. Fuchs*[1] ([1]Institut für Bienenkunde (Polytechnische Gesellschaft) FB Biowissenschaften der Goethe-Universität Frankfurt am Main, Germany; [2]Institut für Biologie, Martin Luther Universität Halle-Wittenberg, Germany)

Social activities in honey bees are thought to be based on a division of labor, depending on the readiness for work of the individual workers. Though many highly developed theoretical models exist, there are very few empirical analyses. We studied the extent to which a small group of honeybee workers engage in brood warming, how they react to a warming stimulus, and how the performance of the group is composed of the individial contributions. The bees were studied within an experimental set-up integrating for the first time infrared thermography with temperature measurements of a sealed brood area. The infrared thermography allows us to quantify all thorax temperatures of individual brood warming honeybee workers, leading to a detailed picture of the group's activities. A temperature-multiplexer simultaneously measured the temperature of the brood area through 248 sensors inserted from the back of the brood comb into the opposing brood cells. The graphical illustration of the data shows a detailed picture of the temperature distribution of the brood comb and allowed us to investigate the heating success of the whole group. This experimental set-up enabled us to analyse in detail the brood warming behaviour of composite groups of geneticly diverse honeybees, e.g. races or patrilines. Using this method we can study numbers and intensities of individual brood warming efforts, and their effect on the temperaure of the comb, thus providing a better understanding of the regulatory processes in social activities.

14. Improvement in the pupal development of artificially reared honeybee larvae. *U. Riessberger-Gallé*[1], *J. Vollmann*[1], *R. Brodschneider*[1], *P. Aupinel*[2], *K. Crailsheim*[1] ([1]Institut für Zoologie, Karl-Franzens-Universität Graz, 8010 Graz, Austria; [2]Unité expérimentale d'entomologie, INRA, Le Magneraud, Surgères, France)

The feeding protocols to rear honeybee larvae artificially in the laboratory have been improved during past years (Aupinel et al. (2005), Bull. Insectol. 58, 107–111), but rearing of morphologically intact adults has been ignored. Horizontal microtiter plates with plastic queen cups lead to wing deformations and humpbacks in adult bees, because larvae and pupae hold vertical positions. Since these morphological deformations may affect the behaviour of adult bees, we developed a method to rear honeybees horizontally in the pupal stage. The (white eyed) pupae are rotated carefully inside their plastic cups facing towards the opening on the 11th day after grafting to ensure later emergence. Capping of brood cells is simulated by sealing the rearing plates with a thin, almost transparent and perforated wax layer to prevent pupae from falling out when plates are positioned vertically. The wax layer is prepared by melting 2.5 g of wax foundation between two sheets of baking paper and shaping it with a rolling pin. Plates held in a vertical position (like combs in a colony) allow pupae to develop horizontally as in natural brood cells. When metamorphosis is finished the honeybees can eclose from the cups autonomously by biting through the thin wax layer, a parameter for their viability and fitness. None of the individuals reared this way displayed humpbacks or other morphological deformations. This simple and cost-efficient improvement guarantees the optimal morphological development of artificially reared adult honeybees.

15. Dynamics of body weight in honeybee larvae: artificially versus naturally raised. *U. Riessberger-Gallé, J. Vollmann, K. Crailsheim*

(Institut für Zoologie, Karl-Franzens-Universität Graz, 8010 Graz, Austria)

Various methods to raise honeybee larvae artificially are described in the literature. We used a method described by Aupinel et al. (2005) (Bull. Insectol. 58, 107–111). The goal of this study was to compare the increase in weight of artificially and naturally raised larvae. Larvae of age 5–10 h were transferred to plastic queen cups in the lab, while their sisters were immediately put back in their mother colonies for further development. The investigations were carried out in June–July 2007 using three colonies. After 1–8 days of development the individuals were taken out of their plastic cups or brood cells in the comb and weighed. We also compared the weight of artificially and naturally raised adult bees 0–0.5 h after their emergence. In all three colonies, the naturally raised larvae were significant heavier (*t*-test; $P < 0.05$) than their artificially raised sisters of the same age, at least after the third day of development. Naturally raised larvae were capped inside the colony at the age of 5–5.5 days, and they started to elongate and reach their maximum weight of 154.2 mg (± 10.4) at this time. During the following days, a significant decrease in weight was measured. Artificially raised larvae reached their maximum weight (127.9 mg, ± 30.6) after 6–6.5 days of development, and started to elongate at the age of 7.5–8.5 days. A significant decrease of weight was measured in only one colony from day 7 to 8. Comparing the weight of the two groups we found a significantly reduced increase of weight and also a delay in larval development in the artificially raised larvae. Artificially raised individuals appear to be able to compensate for this lag, because we found no significant differences when comparing the weights of the adult bees of the two groups after emergence (artificially: 108.7 ± 13.2 mg versus naturally: 110.5 ± 9.1 mg).

20. South-Eastern limit of distribution of *Apis mellifera meda* in Iran. *M. Pour Elmi*[1,2], *S. Fuchs*[2] ([1]Islamic Azad University Tschalus, Iran; [2]Institut für Bienenkunde (Polytechnische Gesellschaft) FB Biowissenschaften der Goethe-Universität Frankfurt am Main, Oberursel, Germany)

Apis mellifera meda, first described in 1929 by Skorikov, was shown by Ruttner et al. (1985) to consist of six subpopulations. Of these, 4 are from the main distribution area, Iran; the other two are from Iraq and Eastern Turkey. Only a few further studies added to this status. Here, we investigate 7 bee samples from 3 locations in Sistan Belutschistan, close to the border of Pakistan. Until now it was assumed that *Apis mellifera* is absent in this region because of the dry-hot climate. Three samples from Iranshar, one from Khash and 3 from Zahedan were measured in 38 morphometric characters taken from each of 10 workers of each colony. Data were analysed by discriminant analysis and cluster analysis, and were related to data from 67 samples from *A. m. meda* and 128 samples of adjacent subspecies taken from the Oberursel data bank. The morphometric analysis showed a clear separation between the samples from Belutschistan and the other samples from *A. m. meda*. In particular, they were characterized by smaller body size, shorter and broader forewings and shorter legs in relation to *A. m. meda* from Iran. In many measurements they were intermediate to the *A. m. meda* bees of Iraq, but do not share their narrower wings and high wax mirror index. Inclusion of further subspecies into the analysis showed that the bees of Belutschistan, notwithstanding their particularities, unequivocally belong to *A. m. meda.* Morphometric distances to the next related subspecies, *A. m. anatoliaca* and *A. m. syriaca*, were about double to that to *A. m. meda* from southeastern Iran or from Iraq. The presence of *A. m. meda* bees in Sistan Belutschistan thus further extends the eastern limit of distribution of *A. mellifera* by about 300 km from the until now documented easternmost occurrence in Kerman, South-East Iran. At the same time, it enriches the spectrum of *A. m. meda* by a clearly deviating subpopulation, whose peculiarities and similarities to *A. m. meda* from Iraq may be interpreted as adaptations to the extremely dry-hot climate. This subpopulation is separated by still another 600 km from the known range of *A. cerana*, to which it showed no morphometric relation, but possibly this distance might be even less.

21. The first *Apis florea* discovery in Jordan. *N. Haddad*[1], *J.R. de Miranda*[2] ([1]National Center for Agricultural Research and Technology Transfer, Baqa' 19381, Jordan, drnizarh@yahoo.com; [2] School of Biological Sciences, Queen's University Belfast, 97 Lisburn road, Belfast, BT9 7BL, Northern Ireland)

The dwarf honeybee *Apis florea* is originally native to South East Asia, but in recent years its geographic range has been steadily expanding westwards, both naturally and accidentally via global transportation (Mogga and Ruttner, 1988). It has been reported from in Iraq (1990), Oman (1990), Yemen (1990), Saudi Arabia (1990) and Sudan (1983). Throughout its range in the Arabian

Peninsula and Africa, *A. florae* has proved to be a highly successful colonizer; well adapted to the hot arid conditions of both the urban and rural landscapes and seemingly unaffected by competition from any local *Apis mellifera* colonies (El-Shafie et al., 2002). A survey found dozens of colonies, mostly in the trees lining the parks and roads of the city, and a few were found inside old buildings and unused rooms. When nesting in tree cavities, the bee exhibited a preference for introduced Eucalyptus (*Eucalyptus camaldolensis*), native desert Acacia (ö*Acacia arabica*) and Ziziphus (*Ziziphus spina-christi)* trees. The size of nest comb ranged from 10×10 cm to 40×35 cm. It is currently found only at less than 45 above sea level, although this is more likely an artefact of human activity rather than a physiological or biological barrier: there is a far greater abundance of forage and nesting trees near the shore line, in the wealthier parts of towns, than in the poorer areas higher up the hill slopes.

25. Multiplication of acute bee paralysis virus in primary bee cells. *R. Siede, M. König, R. Büchler, H.-J. Thiel* (LLH, Bieneninstitut Kirchhain, Institut für Virologie (FB10), JLU Giessen, Germany)

Significant progress in the field of bee pathology is expected from the use of cell culture systems. However, neither a honey bee cell line nor an alternative insect cell line supporting the growth of bee viruses is available. This project addresses this gap: We aimed to obtain primary bee cells which are permissive for acute bee paralysis virus (ABPV) replication.

Abdomina of white eyed pupae were opened by a short incision. Body fluid leaking out was aspirated, suspended in ringer solution, washed, resuspended in L15-medium and split into three parts. One part was infected with a crude ABPV suspension from experimentally infected bees. The second was mock infected with an extract from healthy bees. The third served as non-infected control. After washing, cells were seeded in plates filled with supplemented L15 medium. The cells were harvested at 0, 2, 2.6, 4, 6, 8, 25, 48, 66, 120 and 144 hpi and the amount of ABPV quantified by real time PCR for both the supernatant and the cells. Approximately 3 hpi, the amount of cell-associated virus increased. At 4 hpi the viral load was significantly higher than at the starting point ($P < 0.001$, randomization test, REST© 2005). The cell bound fraction reached its maximum at 25 hpi showing 2.5×10^4 times more viral nucleic acid compared to 0 hpi. Later on the amount of viral RNA decreased slightly to a relative amount of 3.8×10^3 at 144 hpi. Within supernatants the amount of detectable viral RNA decreased during the first hours after infection. The minimum was found at 2.6 hpi. The maximum yield of ABPV in the supernatant was detected 25 hpi, when the supernatant contained 4.3×10^3 fold more copies of ABPV. In conclusion, primary cells obtained from abdominal body fluids of bee pupae supported the replication of ABPV.

26. Development of a real time RT-PCR assay for the absolute quantification of Deformed wing virus. *S. Gisder*[1], *P. Aumeier*[2], *C. Yue*[1], *E. Genersch*[1] ([1]Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany; [2]Department for Behavioural Biology, Faculty for Biology, Ruhr-University Bochum, Germany)

Deformed wing virus (DWV) is a plus-stranded RNA virus pathogenic for both honeybees and bumble bees. Outbreaks of clinical DWV infections in honeybees have been reported to be associated with *Varroa destructor* infestation, while in the absence of *V. destructor*, DWV causes true covert infections in honeybees. To further analyze the intricate interactions between honeybees, their ectoparasitic mite *V. destructor* and DWV, as well as the transmission of DWV between the mite and the developing bee, we established a quantitative real time RT-PCR assay. For this purpose we produced an external DNA standard by amplifying a viral genomic fragment of 1520 bp in length via RT-PCR generating an amplicon containing a specific 354 bp-region of the viral genome to be amplified for quantification. The DNA standard was shown to be stable for at least two months and had a reaction efficiency of 99.2% in the range between 10^1 and 10^7 genome equivalents. This external standard enabled us to determine the absolute DWV genome equivalents in mite and bee samples instead of the relative viral titres by comparing ct-values as reported in the literature so far.

27. Efficacy of varroa treatment with rotenone in strips in the region of Rijeka – Croatia. *D. Šekulja* (Polytechnic of Rijeka, Croatia)

Rotenone ($C_{23}H_{22}O_6$) is a slow-acting poison which interferes with the electron-transport system in the mitochondria, acting as a general inhibitor of cellular respiration, and therefore target species have a poor chance to develop resistence against it. It is rapidly broken down in soil and water and is ultimately converted to carbon dioxide and water. Over the last six years rotenone in strips was used

as a routine varroa treatment in Slovenia and Croatia, where it has proven to be very efficient. The efficiency of rotenone in strips was evaluated according to the method for determining the number of varroa in colonies, (Büchler et al., ADIZ, 7/2006). For this purpose, two sets of bee samples were collected, one shortly before the treatment, the other 4 weeks later. Control colonies were treated with Checkmite+, containing Coumaphos as the active ingredient. All together 185 bee colonies located on 5 different apiaries in the surroundings of Rijeka were involved in the experiment. Within the group of 162 colonies covering one Langstroth super, the average initial infestation level of 17.09% (i.e. 17 mites on 100 bees) dropped after the treatment to a level of 0.55%. In the control group, consisting of 21 colonies, infestation dropped to 0.13%. In a parallel experiment in which 23 colonies covering two Langstroth supers placed in one apiary were treated with double doses of medicaments, the initial invasion of 25.01% dropped to a still high infestation of 7.35% after the Rotenone treatment. In the control group treated with a double dose of Check-Mite+, infestation dropped to 0.32%. However, all colonies survived the winter with just one additional treatment with oxalic acid in December. According to practical experience, as well as to the results of this work, rotenone in strips might be considered as a good alternative drug for varroa treatment.

29. Selection for Varroa tolerance: concept and results of a long-term selection project. *R. Büchler*[1], *C. Garrido*[1], *K. Bienefeld*[2], *K. Ehrhardt*[2] ([1]Landesbetrieb Landwirtschaft Hessen, Bee Institute, 35274 Kirchhain, Germany; [2]Länderinstitut für Bienenkunde, 16540 Hohen Neuendorf, Germany)

In cooperation with the bee breeder association "Arbeitsgemeinschaft Toleranzzucht", an increasing number of *A. m. carnica* test colonies were evaluated for performance under field conditions from 2004 (490) to 2007 (1645). The characters assessed were the development of Varroa infestation (measured by the relation of natural daily mite mortality during *Salix* blossom and mite infestation of bee samples in the beginning of July) and the hygienic behavior (measured by repeated pin-tests). All data were combined and processed to estimate breeding values. The heritability of mite infestation development was calculated as $h^2 = 0.24$ and that of hygienic behavior as $h^2 = 0.29$, with a highly significant genetic correlation between both characters of $r = -0.57$. In each year, after finishing the field test at the end of July, some colonies were chosen and kept without any treatment in an isolated test yard close to Kirchhain. Of this total of 86 colonies, 55.8% survived until the following spring, while the rest died, showing clear symptoms of varroosis. Compared to the colonies that died, surviving colonies had higher breeding values for mite infestation (104.7 versus 98.4; $P = 0.037$) and hygienic behavior (105.0 versus 102.5; $P = 0.141$), thus confirming the suitability of the field test characters used to select for higher mite tolerance. By comparing the adult bee population before and after wintering, survivors with good overwintering performance were selected as breeder colonies, especially for the production of drones on isolated mating stations. Several tolerance mating stations have been established, where drones are reared under high infestation pressure in colonies that remained untreated for a long time. Thus, natural differences in drone fitness will be integrated into the selection process.

32. Sex pheromones trigger the mating behaviour of *Varroa destructor*. *B. Ziegelmann*[1], *J. Steidle*[2], *P. Rosenkranz*[1] ([1]Universität Hohenheim, Landesanstalt für Bienenkunde, 70593 Stuttgart, Germany; [2]Universität Hohenheim, Institut für Zoologie, 70593, Stuttgart, Germany)

The copulation of the ectoparasitic mite *Varroa destructor* takes place within the sealed brood cell, mostly between the adult male offspring and his sisters. Using the bioassay developed at our lab we could confirm our previous studies showing that varroa males favour young freshly moulted females compared to the old mother mites; non-adult deutochrysalis were even not attractive. However, it was not clear whether this preference behaviour of the males is elicited by volatile compounds of the attractive stage of the female mites. Therefore, solvent extracts of young attractive females were tested in the bioassay. Two deutochrysalis were used as unattractive dummies, one treated with extract and one with the solvent as control. The behaviour of a male was recorded for 5 min. 30 repetitions were performed and different solvents and concentrations, respectively, were tested. By the use of non-polar solvents (pentane, dichlormethane) we did not receive positive reactions of the tested males. Only ether extracts of young female mites elicited the typical behavioural cascade in the males during the copulation behaviour and males tried to copulate only with the treated but not with the control dummy ($n = 30$, $P = 0.03$ ANOVA). However, there is evidence that; depending on the concentration, the extract could have attractive or

repellent effect on tested males. Surprisingly, we could also find positive but weaker reactions of the males toward extracts of other developmental stages of female varroa mites which are not attractive at all as living stage. Obviously, the attractive compound is present in several developmental stages of varroa mites but is only on the surface of freshly moulted females available in sufficient volatile concentrations. Through this study we could prove that a volatile female sex pheromone is involved in the mating of *V. destructor*. Using our active extract it should be possible to analyze the compounds of the sex pheromone in detail. For the future, this may offer a further approach for biological control of varroosis.

33. Do rats leave sinking ships ? Preference of *Varroa destructor* mites for adult bees of different infestation or familiarity level. *I. Joachimsmeier, P. Aumeier, W.H. Kirchner* (AG Verhaltensbiologie und Didaktik der Biologie, Ruhr-Universität Bochum, Germany)

Phoretic *Varroa destructor* mites clearly distinguish between adult honeybees of different age or task. We evaluated whether this preference behaviour might in addition be affected by the infestation level or familiarity of potential hosts. Three highly infested colonies were repeatedly exposed to robbery events. Outgoing forager bees on average carried twice as many mites when leaving the colony (infestation level of entering vs. leaving bees: 15.8 ± 20 vs. 32.7 ± 15; chi^2 $P < 0.001$), whereas infestation degree of robbing bees quadruplicated during their stay inside this hives (2.1 ± 1 vs. 9.6 ± 5; chi^2 $P < 0.001$). Due to their generally higher infestation level, the "normal" foragers largely contributed to mite export from their colony. However, foreign bees engaged in robbing, seem to be more attractive to phoretic mites. The preference behaviour of single mites was tested in a laboratory bioassay where groups of 20 bees were offered to them in small boxes. The mites or bees differed in respect of their home-colonies' infestation level or their origin. Mites slightly preferred foreign hive bees versus corresponding bees originating from the same colony (10 ± 2 vs. 9 ± 3; Wilcoxon-test; $P = 0.014$). However, forager bees offered alive or dead (bioassay modified after Rosenkranz (1993), Apidologie 24, 486–487) did not differ in attractiveness. Neither infestation level of the original colony of the tested mites nor of potential hosts had an impact on this preference behaviour. *V. destructor* mites to a certain extent prefer alien bees to bees of their original host colony. Whether this behaviour might contribute to the spreading of mites into hitherto weakly infested new "hunting grounds", remains to be clarified.

35. Identification of entomocidal toxins in *Paenibacillus larvae*. *A. Ashiralieva*[1], *A. Fünfhaus*[1], *R. Borriss*[2] *E. Genersch*[1] ([1]Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany; [2] Institute for Biology, Humboldt University Berlin, Chausseestr. 117, 10115 Berlin, Germany)

American foulbrood, a worldwide bacterial disease of honeybee brood caused by the grampositive bacterium *Paenibacillus larvae*, is one of the most serious bee diseases. Typing of all *P. larvae* strains with ERIC PCR revealed four *P. larvae* ERIC-genotypes. The time course of disease progression has genotype-specific differences in the proportion of larvae dieing after cell capping. Since only ERIC I and ERIC II genotypes are of clinical importance, these two genotypes were compared by Subtractive Suppression Hybridization (SSH), to identify virulence factors of *P. larvae*. We here describe the results of the subtraction of the ERIC II-genome from that of ERIC I, identifying genes unique for ERIC I. In addition to numerous hypothetical proteins, transposase genes and some genes related to metabolism we also identified two toxin genes. A fragment of 573 bp showed homology to an ADP-ribosyltransferase of a binary toxin from *Bacillus thuringiensis* and another fragment of 1365 bp showed homology to the mosquitocidal larval toxin from *Bacillus sphaericus*. We named these new toxins binary larval toxin (Blt) and *Paenibacillus* larvacidal toxin (Plt), respectively. Further analysis revealed additional paralogues of these toxins in the genome of *P. larvae* ERIC I. This is the first report of entomocidal toxins in *P. larvae*. We suggest that these toxins are putative virulence factors. Functional studies will now prove the role of Plt and Blt in the pathogenesis of AFB.

36. Developmental stage-specific expression of antimicrobial proteins in the haemolymph of the honey bee after artificial infection. *K. Randolt, O. Gimple, H. Gätschenberger, H. Beier, J. Tautz* (BEEgroup, University of Würzburg, Am Hubland, 97074 Würzburg, Germany)

Like all insects, honey bees have developed an effective innate immune response to combat invading pathogens. As social insects with a very high population density, honey bees offer a unique possibility to study the immune status with respect to their developmental stage and their social role. The

components of the humoral and cellular immune system are best studied in *Drosophila*. Although many features are conserved in most insects, our study of the honey bee's immune system has revealed some unique properties. We have employed a proteomic analysis in combination with mass spectrometry of immune proteins transiently synthesized in the haemolymph of worker larvae, pupae and adult bees after aseptic wounding and challenge with bacteria. Our results reveal that in vitro reared fourth instar larvae react with a strong humoral response to wounding and septic injury as evidenced by the transient synthesis of hymenoptaecin, defensin1 and abaecin. The same three antimicrobial peptides (AMPs) are induced in white-eye and red-eye pupae after bacterial challenge, albeit at reduced rates. In contrast, young adult worker bees respond with a broader spectrum of immune reactions that include the activation of prophenoloxidase, expression of the three AMPs detected already in larvae and pupae and the synthesis of two novel polypeptides with calculated masses of about 30 and 65 kDa. The latter protein belongs to a family of type B esterases and lipases that are participating in various metabolic reactions in many organisms. The non-classified 30 kDa protein appears to be unique for the genus *Apis*. Future investigations will elucidate the role of these two newly discovered proteins in the immune response of adult bees.

37. First results of genotyping of *Paenibacillus larvae* from the Austrian Federal Province of Upper Austria. *I. Loncaric, I. Derakhshifar, H. Köglberger, R. Moosbeckhofer, J. Oberlerchner, M. Riedel* (Institute for Apiculture, Austrian Agency for Health and Food Safety (AGES), 1226 Vienna, Austria)

Paenibacillus larvae, the etiological agent of American foulbrood (AFB), is reported to cause this disease in honeybee larvae worldwide. In Austria, AFB is a well-known disease, which causes severe economic losses in affected apiaries. The range of notified outbreaks varied from 49 to 383 cases during the years 1998–2006. It was the aim of this study to genotype *P. larvae* isolates from the federal province of Upper Austria to gain some data from Austria for the first time. Sixty three *P. larvae* strains from Upper Austrian were tested. Samples consisted of brood which showed clinical symptoms and previously had tested positive for AFB, and extracted or brood comb honey, which previously had tested positive for spores of AFB. For rep-PCR experiments, DNA was extracted as described by Loncaric et al. (2008, in press). DNA was amplified by using ERIC 1R-ERIC2, BOX A1R and MBO REP1. Differences in banding pattern between the strains were named according to the nomenclature of Genersch and Otten (2003) (Apidologie 34, 195–206). MBO-REP type B always correlated with Eric II and MBO-REP type b with ERIC I. Using ERIC-PCR technique, two genotypes could be differentiated: ERIC I (37% of isolates) and ERIC II (63%). Using combined typing by BOX- and REP-PCR, four different genotypes were detected: ab (29%), aB (55%), Ab (8%) and AB (8%). The genotype aB (55%) has, up to now, not been reported applying the same technique.

38. How does *Paenibacillus larvae* breach the midgut epithelium? *D. Yue*[1], *M. Nordhoff*[2], *L.H. Wieler*[2], *E. Genersch*[1] ([1]Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany; [2]Institute for Microbiology and Epizootics, Faculty for Veterinary Medicine, Free University Berlin, Germany)

American foulbrood (AFB) is a bacterial disease of honeybee larvae caused by the spore-forming bacterium *Paenibacillus larvae*. Although AFB and its etiological agent are described now for more than a century, the general and molecular pathogenesis of this notifiable disease is poorly understood. Using fluorescence *in situ*-hybridization (FISH) performed with a *P. larvae*-specific, 16S rRNA-targeted oligonucleotide probe, we recently demonstrated that *P. larvae* spores ingested by young larvae germinate in the midgut, the vegetative bacteria then colonize the larval midgut and massively proliferate in the midgut lumen prior to eventually breaching the epithelium. Penetration of the epithelium was thought to occur via phagocytosis. To analyze this important step of the infection process we analyzed hundreds of infected larvae via FISH. However, no *P. larvae* bacteria were ever detected intracellularly, as we expected for a presumably phagocytosed bacterium. Instead we could show that *P. larvae* invaded the paracellular space obviously destroying cell-cell junctions, indicating that *P. larvae* penetrated the epithelium via the paracellular route rather than via phagocytosis. Outside the gut lumen *P. larvae* could be detected between the epithelial and the underlying smooth muscle cell layer, suggesting that the bacteria were able to degrade the basement membrane and/or to interfere with cell matrix adhesion. In the haemocoel *P. larvae* was distributed intercellularly, between the cells of the fat body and other larval tissues, but

could never be detected intracellularly. Therefore, our data obtained with specific detection of *P. larvae* bacteria via FISH analysis provided strong evidence that the paracellular route is the main route for escaping the midgut lumen and entering the haemocoel.

39. Molecular differences between *Paenibacillus larvae* ERIC III/IV and ERIC I. *A. Fünfhaus, A. Ashiralieva, E. Genersch* (Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

Paenibacillus larvae, the causative agent of the American Foulbrood (AFB), is a gram-positive, spore forming bacterium. AFB is a fatal disease of the honeybee brood and distributed worldwide. Four different genotypes (ERIC I- ERIC IV) of this pathogen are described. They differ from each other in colony and spore morphology as well as in virulence and metabolism. A PCR-based method, the Suppression Subtractive Hybridization (SSH), was used to identify genetic differences between genotype ERIC I and the genotypes ERIC II/IV. The subtraction has been carried out in both directions: ERIC I-ERIC III/IV and opposite ERIC III/IV-ERIC I to obtain sequences unique for ERIC I and ERIC III/IV. The obtained sequences from ERIC I genotype missing in genotypes ERIC III/IV exhibited homology to synthetases for non-ribosomally produced antibiotics and to ABC transporters. Sequences specific for genotypes ERIC III/IV and missing in genotype ERIC I showed homology to tetracycline resistance determinant tetV, antigen-like proteins, and flagellar proteins. Our results show that SSH is a powerful tool for the identification of genetic differences between *P. larvae* genotypes. At the moment the functional importance of the identified genes for the pathogenesis of AFB and the observed differences in virulence between the genotypes is being analyzed.

45. Acute toxicity of organic acids on *Apis mellifera* following oral application. *M. Harz, E. Rademacher* (Freie Universität Berlin, Institute of Biology/Neurobiology, Königin-Luise-Str. 28–30, 14195 Berlin, Germany)

We evaluated the acute oral toxicity of citric acid (CA), ascorbic acid (AA) and acetylsalicylic acid (ASA) on *Apis mellifera* in laboratory tests. These three acids are of comparable acidity like other acids (e.g. oxalic acid) used as drugs for honey bees. Groups of ten bees per cage and three cages per concentration were used for every acid. The concentrations used (0.0125M, 0.025M, 0.05M, 0.075M, 0.1M) correspond to a dosage of 22 µg up to 210 µg per bee fed in sugar-water. All trials were replicated once. The temperature and relative humidity averaged 22 °C and 61%. After 24 h of starvation bees were fed individually with 10 µL solution. Controls received sugar-water only. The bee mortality was determined 24, 48 and 72 h after application. The total bee mortality 72 h after application of CA and AA averaged 33.1 and 17% respectively, which was not significantly different from controls, even in high dosages. Regarding ASA, 22 µg were well-tolerated, but in higher dosages significantly higher bee mortality was observed (U-Test, $P < 0.05$). A correlation between bee mortality and dosage was found ($r^2 = 0.93$). CA and AA showed similar bee mortality (8.0, 12.3, 10.7% and 7.5, 3.5, 5.2%, respectively, test intervals of 0–24, 24–48, 48–72 h), which was not significantly different from controls nor in comparison to both acids. ASA, however, had a significantly higher bee mortality (37.8%, U-Test, $P < 0.05$) in the first 24 h. Surprisingly these acids, although of comparable acidity and applied in the same dosages show highly different bee mortality. This might be caused by other factors than the acidity, possibly other molecular properties of the acids. It was previously described that the efficacy of oxalic acid against *Varroa destructor* does not only depend on the pH-value. To understand more about the pharmacological process involved we will now analyse haemolymph samples from treated bees using HPLC.

46. Combined *Varroa destructor* treatment with formic acid and thymol. *S. Berg, F. Schürzinger* (LWG, Fachzentrum Bienen, 97209 Veitshöchheim, Germany)

We compared thymol and formic acid treatment, both individually and in combination for their efficacy as summer treatments for control of *Varroa destructor*. A number of 50 colonies, divided in five groups (1–5), 10 colonies each, were treated after last honey harvest in 2007 according to the following scheme: (1) Thymovar® (2) ApiLife Var® (3) formic acid (60% formic acid, 2 mL per comb from above) on foam tissue followed by Thymovar® (4) formic acid (60% formic acid, 2 mL per comb from above) on foam tissue followed by ApiLife Var® (5) four times formic acid (60% formic acid, 2 mL per comb from above) on foam tissue in 4 days intervals followed by only one treatment with Thymovar®-wafers. In December oxalic acid (trickle method) was used to determine the efficacy of treatments. Percent mite mortality in the thymol-treatments group 1 and 2 averaged 97.6 ± 2.2%, resp. 98.5 ± 1.1%. The efficacy of the combined

treatments was in 97.6 ± 1.3% in group 3 and 96.9 ± 2.6% in group®4. The treatment in group 5 resulted in an efficacy of 94.9 ± 1.9%, whereby the four formic acid treatments alone had an efficacy of 70.8 ± 5.9% due to a cold snap with daily maximum temperature of 16 to 18 degree during time of treatment. There were no significant differences in the efficacy between the different treatments (Duncan, $P > 0.1$). The efficacy of all treatment methods was independent of the number of *V. destructor* (ranging from 160 to 7860/ colony) and the strength of the colonies (Kruskal-Wallis, $P > 0.25$, each variable). Also the colony strength after over wintering showed no differences (Kruskal-Wallis, $P > 0.06$). All different treatments showed a high efficacy of more than 95% in average and there were no negative effects on the colony strength of the combined treatments. However, the insufficiency of the formic acid treatment in group 5 was striking. Since the formic acid treatment of group 5 is a solely used treatment in Germany, the survival of colonies would have been critical.

47. Methods to evaluate the dependence of hygienic behaviour in honey bees on the parasitation with *Varroa destructor*. *I. Illies, S. Berg* (LWG, Fachzentrum Bienen, 97209 Veitshöchheim, Germany)

Hygienic behavior of worker honey bees (uncapping and removing dead or injured brood) is a behavioral mechanism of defense against *Varroa destructor*. Hygienic behaviour can be evaluated by measuring the removal of experimentally or naturally infested brood cells. In this study, we used two different methods to measure the removal of infested brood and mites. Brood removal in "high" and "low" infested brood combs was compared by observing 100 cells between capping and just before hatching of the brood by marking cells on foil (n = 14). There was no significant difference in the percentage of brood removal ($P = 0.128$, U-test) between the two comb types, although the percentage of infested cells inthe "high" infested combs significantly decreased over time from more than 30% to 12% ($P = 0.005$, Wilcoxon). The percentage of infested cells was determined by opening 100 cells 1 and 9 days after sealing. In a second experiment, hinged combs equipped with a foundation of Plexiglas® (glass combs) were used, which allowed cell inspection after sealing. Combs from 11 colonies were observed, and cells were identified as infested or non-infested control cells (104–200 cells per comb). 21% of all cells identified as infested after capping were not infested at the time of emergence. The mean percentage of removed cells was significantly higher in infested brood cells (15.3%) compared to the control cells (7.5%, $P < 0.05$ Wilcoxon). Worker bees thus were able to open the cappings of mite-infested brood without removing the brood (as described for *Apis cerana*). The mites then left the opened cells or were removed by the bees, and the brood cells were subsequently recapped. Because it is difficult to quantify hygienic behavior at the time of emergence, the described method using glass combs enables the determination of the number of both the removal of infested brood and the removal of mites without removing the brood.

48. On the quantitative influence of drone brood excision on *Varroa destructor*. *J. Radtke, P. Neuberger* (Länderinstitut für Bienenkunde, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

Studies on the effect of excising capped drone brood have so far led to highly varying results. Studies by Rosenkranz and Engels (1985) (ADIZ 19, 265–271) reveal that these depend on the positioning of the drone brood within the bee colony and on how the drone brood is removed. Twenty three bee colonies were separated into two groups at the beginning of rape bloom. Each colony received one building frame per brood chamber placed in proximity to the brood nest. In 12 test colonies, the capped drone brood in the building frames was excised in intervals of 7 to 14 days from the beginning of May to mid-August. The 11 control colonies were not treated. On 05/02/2003, the average infestation of bee samples containing an average of 701 ± 12 bees did not differ between the test colonies and the control colonies (1.44% ± 0.37 and 1.27% ± 0.28, respectively). However, on 08/04/2003, average infestation level was 85% lower in the test colonies compared to the control colonies (3.01% ± 0.70 and 20.51% ± 6.55; $P < 0.05$; *t*-Test). The degree of bee infestation thus doubled in test colonies over the period between 05/02 until 08/04/2003 while it increased 16-fold in the control colonies ($P < 0.01$; analysis of variance for repeated measurements). After treatment with formic acid, starting on 08/06/2003, 78% fewer mites were found in the test colonies compared to the control colonies (average number of *Varroa* mites 1.263 ± 260 and 5.802 ± 1.105, respectively; $P < 0.01$; *t*-Test). Although both test and control colonies were initially equally strong, the test colonies had developed significantly better than the control colonies until late summer. By extrapolating to equal colony strength,

the resulting mite number would be 8.841 mites per colony in the control group and mite numbers would be significantly lower in the test colonies (86%) compared to the extrapolated control colonies. The net weight increase of the test colonies was 70.6 kg, which is 14% more than that of the control colonies. However, this was not statistically significant.

49. The first detection of honey bee viruses in Jordan using RT-PCR methods. *N. Haddad*[1], *J.R. de Miranda*[2] ([1]National Center for Agricultural Research and Technology Transfer, Baqa' 19381, Jordan; [2]Department of Biological Sciences, Queen's University Belfast, BT9 7BL, Belfast, Northern Ireland)

Mortality of honeybees is a serious problem that beekeepers have to face periodically in Jordan and worldwide. The Ajlon province is the second most important region in Jordan for beekeeping, in terms of the number of beekeepers and managed colonies. Adult worker bees were collected from thirteen colonies in seven widely dispersed apiaries from the Ajlon province and were assayed for the presence of six honeybee viruses (ABPV, BQCV, CBPV, DWV, KBV, and SBV) using RT-PCR. Out of the 13 colonies examined, 92% were infected with DWV, 8% with SBV, and 16% with ABPV. None were infected with CBPV, BQCV, or KBV. Nearly all the samples from colonies that showed high bee mortality were infected with at least one virus or were co-infected with more than one virus. These preliminary results show the presence of several bee viruses, in particular DWV, in dying Jordanian bee colonies and are similar to surveys conducted in other countries (Tentcheva et al., 2004; Todd et al., 2007; Cox-Foster et al., 2007). Further research is needed to determine which other factors are also differentially associated with colony mortality, such as infestation with parasitic mites (*Varroa destructor*, *Acarapis woodi*), *Nosema apis* and *N. cerana*, bacterial diseases, and any possible effects of chemical treatment of colonies or foraging resources.

50. Transmission of Deformed Wing Virus between pupae and mites. *C. Yue, E. Genersch* (Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

Deformed wing virus (DWV) is a bee-pathogenic, plus-stranded RNA virus which can be detected in all life stages of the honey bee in the absence of visible disease symptoms. Clinical symptoms in adult bees are dependent on the transmission of DWV by *Varroa destructor* during honeybee pupal development. So far, the intricate relationship between DWV as the viral pathogen, *V. destructo*r as viral vector, and the honeybee pupa as host is poorly understood. To elucidate the transmission routes of DWV between mites and pupae, we molecularly analyzed the viral populations in mite infested brood cells. We extracted DWV RNA from freshly hatching bees and their accompanying mites and sequenced the L-gene. The L-gene is known to be quite variable in other RNA viruses and, therefore, is considered a good candidate for the analysis of viral-sequence heterogeneity in organisms or populations. The obtained sequences were compared using ClustalW alignment. Supporting the theory of the quasi-species nature of RNA viruses, we could not confirm any consensus sequence for the L-gene. Instead, the sequence of the L-gene of DWV was shown to be highly variable. Only 11% of the analyzed bees had identical sequences, the remaining bees showed many sequence variations. In contrast, when analyzing the L-gene sequence of the mites corresponding to a certain bee, the sequences found in the mites were 100% identical with each other and, more importantly, with the sequence found in the bee, suggesting that each "brood cell community" (i.e. the developing bee and the mites present in one brood cell) is harbouring a certain DWV mutant from the mutant cloud RNA viruses tend to form. This mutant is characterized by a DWV master sequence which develops due to the constant exchange of DWV between the pupa and the mites during the mites' feeding acts.

51. Overwinter colony losses in Austria and Southern Tyrol in 2007/2008. *K. Crailsheim, R. Brodschneider, H. Kovac, U. Riessberger-Gallé* (Institut für Zoologie, Karl-Franzens-Universität Graz, 8010 Graz, Austria)

Extremely high colony losses were reported in early 2007 in the U.S.A. and Turkey. This phenomenon was termed Colony Collapse Disorder, or CCD. In late February through April 2008, we conducted a survey at 7 beekeeping conventions. 447 beekeepers were questioned concerning their over-winter colony losses representing 18 371 colonies in autumn 2007. "Small" (< 50) and "larger" (≥ 50 colonies) beekeepers were compared across 3 different regions. Of 2937 colonies present in autumn 2007, "small" Austrian beekeepers (n = 140) from the south and east lost 18.0%, with 65.7% of beekeepers having losses of fewer than 20% of their colonies. "Larger" beekeepers in south and east Austria (n = 45) had 6856 colonies and lost 13.3%, with 68.9% of beekeepers having losses of fewer than

20% of their colonies. Of 2960 colonies present in autumn 2007, "small" beekeepers from Salzburg and Tyrol (n = 124) lost 10.0%, with 86.3% of beekeepers having losses of fewer than 20% of their colonies. "Larger" beekeepers in Salzburg and Tyrol (n = 22) had 1 614 colonies and lost 12.2%, with 86.4% of beekeepers having losses of fewer than 20% of their colonies. Of 2145 colonies present in autumn 2007, "small" beekeepers from Southern Tyrol (n = 97) lost 15.6%, with 77.3% of beekeepers having losses of fewer than 20% of their colonies. "Larger" beekeepers in southern Tyrol (n = 19) had 1859 colonies and lost 8.4%, with 84.2% of beekeepers having losses of fewer than 20% of their colonies. The most frequently stated reasons for overwinter losses were infestations with *Varroa sp.* and queen loss. No extraordinary losses or symptoms as reported for CCD occurred during the winter 2007/08 in our investigation area. We therefore conclude that the losses presented here are in the normal range for the climate in the investigation area.

56. Characterisation of honey species by SPME-GC/MS and multivariate statistical data analysis. *C. Bartzsch, J. Trompelt, K.-H. Feller, W. Schmidt* (Fachbereich Medizintechnik und Biotechnologie, Fachhochschule Jena, Carl-Zeiss-Promenade 2, 0745 Jena, Germany)

Up to now, the floral origin of honey is diagnosed by pollen analysis – a time consuming method needing expert knowledge. With the advent of ultrafiltration in honey processing, this procedure is not longer suitable. In the present work, headspace solid phase micro extraction in combination with gas chromatography – mass spectrometry was employed to analyse the aroma profiles of different unifloral honeys. For each floral origin, between 6 and 12 honey samples were investigated and more than 70 substances were identified from their chromatograms. Rather than focussing attention to special marker substances of the honey origin, differences in the overall flavour profile were analysed. Statistical methods, especially discriminant analyses, were used to detect characteristic group properties of single honey origins. It was possible to distinguish between 7 unifloral honeys (robinia, chestnut, heather, rape, lime, sea buckthorn, sunflower); as well as honeydew and summer flower honeys with a reliability of 97%. Differentiation involved 15 variables representing the peak areas of substances in the chromatogram relevant for separation. The *P*-value was lower than 0.000005 in all cases and a cross validation yielded a 92.5% recognition. Hence unknown honey samples could be recognized and allocated to their floral source, although the data set is still in development. More floral sources and more honey samples per origin should be included in the model in order to uncover fluctuations within a group and better to detect differences between the groups. **64. Use of honey bees (*Apis mellifera*) for pollination in wild flowers – the example of *Cornus sanguinea.*** *J. Radtke, E. Etzold* (Länderinstitut für Bienenkunde, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

The usefulness of the honey bee (*Apis mellifera*) pollination performance in cultivated plants has been documented in numerous studies. In the present study, a wild plant, *Cornus sanguinea*, was observed. Immediately before the onset of blossoming on 05/14/2007, honey bee visits were prevented from 108 flower clusters containing an average number of 51 ± 2 blossoms on three bushes. For this, 5 branches per bush were supplied with a cage (mesh inner width 2.5 mm). At the same time, 137 freely blossoming flower clusters with an average of 50 ± 2 blossoms were examined. The bushes were located approximately 50 m from an apiary in an area with varied plant growth. *Robinia pseudoacacia*, which densely populates the area and is frequently visited by bees, was blooming at the same time. Despite a rich array of *R. pseudoacacia*, *C. sanguinea* was well visited. Among all of the insects (n = 255) that were observed during mornings and evenings over the course of several days, 91% were honey bees. In contrast, solitary bees, syrphid flies, beetles, wasps, and butterflies taken together amounted to 9%. Average fruit setting for the blossoms on freely blossoming branches was 16% on 07/11/2007. However, this differed among the bushes within a range of 9 to 24%. On the caged branches, fruits were only found occasionally and scattered (0.2%) but without retarding the growth of the others. It is assumed that their blossoms touched the cages and that they were pollinated from the outside. Because of the large share of honey bees among the insects observed on *C. sanguinea* during the same period in which *R. pseudoacacia* was in bloom, it can be deduced that they were highly attracted to *Cornus sanguinea*. At the same time, the results indicate that the honey bee plays an important role in its pollination.