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BEE SHOP
"Bees in Europe and Sustainable HOney Production"
Specific Targeted Research project – STREP
Tracking the food "from the farm to the fork"

DELIVERABLE

D 10.7 Final Consolidated Report

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1. Project execution

1.1 Project objectives

Honey is among the oldest food products of mankind and beekeeping is deeply rooted in every European culture. Numerous European and national regulations control honey quality, which reflects both the high nutritional and societal value of the product. Yet in an environment with increasing chemical pollution and the wide use of agrochemicals, honey runs high risks of becoming chemically polluted. In addition a broad spectrum of chemicals is used to treat honeybee diseases, further contaminating honey with sometimes highly toxic compounds.

The BEE SHOP was a network of nine leading European honeybee research groups in honey quality, pathology, genetics and behaviour as well as selected beekeeping industries, which all share a common interest in promoting Europe's high honey quality standards. The prime goal was to reduce potential sources of honey contamination due to both foraging contaminated nectar and chemotherapy of honeybee diseases. The BEE SHOP therefore dealt with the development of biological resistance to pests and pathogens to avoid chemotherapy. Selected European honeybee races and populations have been screened for their disease resistance potential to the main pressing pathogens. Differences in foraging patterns among European honeybees and their underlying mechanisms have been studied to identify behavioural traits reducing contamination. The impact of honey quality on disease prevention in honeybee colonies has been studied by analysing antimicrobial properties of plant and bee derived compounds in bee products. Newly developed tools for testing honey quality and authenticity now allow for inspections of honey according to the current EC directives on honey quality and organic beekeeping. Differences in disease susceptibility in honeybees has been genetically analysed by QTL mapping and major candidate loci in the genome have been identified with the aid of the published honeybee genome to allow for selection of specific target genes in both drones and queens before insemination. These genes may greatly accelerate the selection progress in honeybee breeding allowing for the swift establishment of resistant but efficient stock.

1.2 Network partners

P1: Martin-Luther-Universität Halle-Wittenberg, Germany
P2: Swedish University of Agricultural Science, Uppsala, Sweden
P3: Queen's University Belfast, Belfast, UK
P4: Centre National de la Recherche Scientifique, Gif sur Yvette, France
Université Paul Sabatier CNRS, Toulouse, France
P5: CEBAS, Murcia, Spain
P6: Universität Hohenheim, Stuttgart, Germany
P7: Slovak Academy of Science, Bratislava, Slovakia
P8: CRA - Unità di Ricerca di Apicoltura e Bachicoltura, Bologna, Italy
P9: Bee Research Institute, Dol, Czech Republic

1.3 Work performed

This section is structured according to the departmental structure of the BEE SHOP network.

1.3.1 Honey Department

The aim of the Honey Department in the BEE SHOP project was the evaluation of honey quality and authenticity, through the development of new instrument for the verification of the botanical origin and the presence of impurities.
Honey quality is not only important for the consumer but also for the colony itself, because of its antimicrobial properties, the presence of specific proteins added by the honeybee and of plant compounds and their metabolites, which can have a positive impact on honeybee health. The Honey Department therefore also had a strong focus on the physiological properties of honey which can be beneficial for honeybees and for the prevention of bee diseases.

WP 1: Honey quality for consumers and pathogen defence

The first aim was to develop improved standards for honey authenticity based on chemical analyses on secondary plant metabolites in honey. Unifloral honeys from 18 different botanical origins (Acacia, Canola, Cherry blossom, Chestnut, Corbezzolo, Eucalyptus, Heather, Lavender, Lucerne, Orange blossom, Rododendron, Rosemary, Sunflower, Tilia, and Taraxacum) were collected from beekeepers across Europe. Three honeys from non-EU countries were imported: Litchi and Baobab honey from Madagascar, Manuka honey from New Zealand. In addition Acacia, Tilia, Citrus and Eucalyptus nectar was collected directly form the flowers by glass capillary, while chestnut nectar was collected by extracting the honey stomach from honeybees foraging on chestnut threes. The samples were analysed for verification of the botanical origin by the traditional chemico-physical, palynological and sensorial methods, as indicated in the Council Directive 2001/110/EC concerning honey requisites. They were then submitted to the following analyses:

1. presence of phytochemical markers specific for the botanical origin;
2. presence of protein components which can be important for the determination of honey quality and authenticity;
3. evaluation of honey quality in respect of description and production;
4. evaluation of antibacterial properties and anti quorum-sensing activities.

Detection of floral origin markers for unifloral honeys

The BEE SHOP developed HPLC analyses of floral nectar phytochemicals as the gold standard method for the determination of plant metabolites in honey, often exceeding classical pollen analyses for the verification of the floral origin of honeys. In particular, flavonoids and other phenolics, volatile compounds, aromatic and degraded carotenoid-like substances, aminoacids, aromatic aldehydes and heterocycles proved to be reliable tools for verifying the floral origin of honey.
In addition to the honey samples also nectars directly collected from the flowers were analysed in selected species by HPLC-MS-MS methods. The phenolic constituents of nectar proved to be reliable markers. For example myricetin, tricetin and luteolin were characteristic Eucalyptus honey, kynurenic acid related compounds for chestnut

honey, terpenoids for Linden honey, hesperetin for Citrus honey, and kaempferol rhamnosides for Acacia honey.

Royal jelly proteins and honey authenticity

The presence of royal jelly (RJ) proteins in honey is an important maker for honey authenticity. A novel, rapid, reproducible and sensitive enzyme-linked immunoassay (ELISA) of quantitative determination of predominant of RJ protein apalbumin1 in honey has been developed by the BEE SHOP. The sensitivity of the essay was tested by adding of various amounts of apalbumin1 to sugar syrups. Surprisingly, apalbumin1 concentration was also dependent on the botanical origin of the honey. The highest content of apalbumin1 was determined in chestnut honey, in comparison with acacia honey and rape honey. The lowest amount of apalbumin1 was detected in honey obtained after feeding of bee colony by saccharose syrup. This ELISA test has a potential of wide application to prove the authenticity of honeys, to reveal their adulteration with low-cost syrups and for the quantitative determination of the apalbumin1 protein in honey, pollen and RJ. The method is simple, only small amounts of honey are needed for testing, and there is no need for preliminary treatments of the honey sample. Similarly, the DNA of the producing honeybees could be identified in honey. This tool allows to verify the local honeybee race which has produced the honey, as requested for organic beekeeping

Evaluation of honey quality in respect of description and production

Two new techniques were introduced for the evaluation of botanical origin and for the detection of adulteration of honey by sugar syrups: Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) and High Resolution Nuclear Magnetic Resonance (HR-NMR).

For the determination of botanical origin, 82 honey samples from 4 different botanical origins (robinia, chestnut, citrus, polyfloral) were scanned by DRIFTS. The procedure was able to achieve a classification accuracy near 100%. 71 honey samples from 5 different botanical origins (robinia, citrus, chestnut, eucalyptus and polyfloral) were analysed by HR-NMR using multiple bond correlation spectroscopy (HMBC). After cross validation the two procedures yielded a prediction accuracy of 92% and 97% respectively.

For the detection of adulteration, a sample of robinia honey was fractionated and artificially adulterated with commercial sugar syrups: Adulterated samples were compared to 100 samples of certified authentic honey of different botanical origin. The combination of DRIFTS and HR-NMR allowed for a classification capability beyond 95% showing that these methods are most suitable not only for a qualitative but even for a quantitative determination of adulteration.

Evaluation of antibacterial properties and anti quorum-sensing activities of honey

Honey inhibits bacterial growth by a high sugar concentration andhydrogen peroxide. In addition antimicrobial activity is also due to protein compounds added by the honeybee and by foraged compounds from plants and their metabolites. Antibacterial activity of honey was evaluated from three different aspects.

a) Antibacterial properties of honey relating to prevention of bacterial bee diseases

The antibacterial activity of selected Italian honeys was tested against pathogenic and non-pathogenic bacteria isolated from the bee hive. Agar well diffusion was

performed on honey samples in order to determine both total and non-peroxide antibacterial activity.
Most honey samples showed an inhibiting activity against the tested bacteria, mainly due to the hydrogen peroxide. The highest level of activity was observed for Manuka honey against *Bacillus subtilis. Melissococcus plutonius* was the most sensitive pathogen to the antimicrobial power of honey. The antibiotic activity was significantly lower for the strain of *Paenibacillus larvae*.

b) Antimicrobial potential of proteins added by the honeybee to honey
The antimicrobial potential of honeybee proteins was tested in vitro. The protein fractions of cherry honey, rape honeys and honeydew showed an inhibition of *P. larvae* growth. This was the first report that honey contains antibiotic peptides against honeybee pathogens. This discovery changed the traditional view on honey as source of energy for honeybee and opens a new horizon for molecular studies on individual proteins and peptides of honey and for their physiological function in honeybee colony.

c) Detection of antimicrobial and anti-quorum sensing compounds of honey
Since the direct antimicrobial activity of unifloral honeys were rather weak against various bacterial strains, the anti quorum-sensing (anti-QS) activities of various honeys were tested in bacterial strains in which quorum sensing activated the pigment violacein. The BEE SHOP showed that 29 honey samples inhibited the AHL production, even at the lowest concentration (0.1 g/mL). Honeys from the same floral origin but obtained at different European regions showed similar anti-QS activity.
Since phenole content had little or no effect on the antipathogenic activity of honeys. Non-phenolic compounds associated with floral origin were detected in the HPLC chromatograms of chestnut, linden and tilia honeys. but were lacking in orange and rosemary honeys. The anti-quorum sensing activity of chestnut honey and the water soluble and water insoluble fractions were analyzed by HPLC-MS-MS detection of acyl-homoserine lactones produced by different foodborne pathogens (*Yersinia enterocolitica* and *Aeromonas hydrophyla*) and spoilage bacteria (*Erwinia carotovora*). The addition of honey reduced the biofilm formation of these bacteria, thus reducing the quorum sensing.

1.3.2 Pathology Department

The past decades saw a constant decline of beekeeping throughout the European Union. One main reason for colony losses is the accumulating number of pathogens, some of which have developed resistance to chemical treatments. Diseases affecting the brood (e.g. *Varroa destructor*, *Paenibacillus larvae*, viruses) have been identified as the most dangerous pathogens. The use of chemical pesticides, though potentially in the short-term successful at the individual colony level, will not eradicate diseases at the population level if the pathogen has a high transmission rate and a high infectivity.
In order to efficiently control diseases using novel and sustainable strategies, it is essential to understand the infection processes both at the level of the individual bee and at the level of the colony. Obviously it is fundamental to understand the spread of disease not only inside the colony but also in a population of colonies. Therefore, the Pathology Department of the BEE SHOP has studied infection processes at all organizational levels exhibited by honeybees and produced some groundbreaking new

discoveries, as outlined below. Based on the obtained results we present strategies that increase bee tolerance to disease, diminish pathogen virulence and break infection pathways and compiled this information into a manual aimed at practice.

The parasitic mite *Varroa destructor* is a main obstacle to profitable beekeeping in Europe and elsewhere. Control of this mite is often based on chemicals that end up as residues in honey and other bee products. Nevertheless, populations of European honeybees exist that apparently coexist with *Varroa* mites, and where chemical control is not needed for colony survival. The BEE SHOP has examined the nature of such relationships to determine traits in the host and the parasite that have allowed for increased tolerance and/or reduced virulence, respectively, making identification of genes for host resistance or management for less virulence in the parasite population a realistic option. Complicating factors in the *Varroa*-honeybee relationship are virus infections associated with *Varroa* mite infestations. The BEE SHOP has taken advantage of recent progress in the molecular characterisation of these viruses and developed tools to characterise and quantify virus transmission and replication in relation to mite infestation levels and type of bee.

American foulbrood (AFB) is caused by the spore-forming bacterium *Paenibacillus larvae*. Although this disease has been identified >100 years ago, and can be treated by antibiotics, it still plagues beekeeping in the EU. The BEE SHOP Pathology Department has demonstrated a wide variation in virulence between different isolates of *P. larvae*. We also demonstrate for the first time in full sized colonies that the most virulent strain at the individual larval level actually is the least virulent strain at the colony level. Furthermore, we have combined *in vitro* larval infections, full sized colony infections and inter- and intracolonial transmission rate studies to determine the significance of virulence and transmission for the epidemiology of AFB. Genetic variance in both pathogen virulence and host tolerance has been further analysed within the BEE SHOP Genetics Department.

Nosema apis is a microsporidian parasite of honeybees with less obvious impact than the aforementioned diseases. It was included in the BEE SHOP for contingency reasons for AFB in WP 4. However, the need for a contingency plan never materialised. Nevertheless, the work done on *N. apis* is reported here under WP 4.

WP 2: Mechanisms of pathogen transmission and disease tolerance of colonies

The objectives of WP 2 were to investigate pathogen transmission and disease tolerance at the colony level for AFB, *Varroa* mites and virus infections associated with *Varroa* mites.

Most importantly, BEE SHOP has contributed new insights into inter-colony disease transmission by documenting actual rates of transmission in the field (AFB) and discovered venereal infection pathways. Based on results obtained recommendations for practice on breaking or altering transmission routes, and reducing pathogen virulence are included in the manual for breeding of disease resistant bees reported under this WP.

American foulbrood (AFB)

The BEE SHOP studied both between and within colony transmission of AFB and demonstrated the impact from distance to diseased colonies for the probability of

contracting AFB and show that close range (<500meters) inevitably produce new disease outbreaks, whereas distances >2km have little impact on disease transmission. Regarding within colony transmission, we demonstrate that honey serves as a reservoir for infective spores that may lead to disease outbreak. Furthermore, we document the accumulation of infective spores in the feces of wintering bees, possibly providing a new route for within colony spore transmission. Such contaminated feces may be deposited within the hive. As cleaning bees become contaminated, they may also transmit infective spores to susceptible larvae as they are being fed.
To study colony level resistance to brood disease in general and AFB in particular, we have evaluated different techniques for measuring the hygienic behaviour of honey bee colonies. From these results, it is clear that pin-killed brood is removed faster than brood killed by liquid nitrogen that in turn is removed faster than brood killed in the deep freezer. Recommendations for hygienic behaviour evaluations are included in the manual for disease resistance breeding.

***Varroa* mites**

BEE SHOP has documented that at least in one population of honey bees that have survived mite infestations, the reduced mite population growth is not related to source of mites but linked to the genetic background of the bees. This is most encouraging for future selection work towards mite tolerance. Unfortunately, reduced production of both drone and worker brood appears to be linked to survival, which is not a desired trait.

Virus infections

To study colony level mite resistance, BEE SHOP has quantified the relationship between mite and virus infections and tried to establish threshold levels for both *Varroa* infestations (Task 2) and virus infections (Task 3) when damage to colonies can be expected. Although, there is a significant positive correlation between mite loads and some virus titers, the definition of a joint threshold level for mites and virus infections remains to be a problem. It is also difficult at best to develop an individual based epidemiological model to probe the potential effects of mite or virus resistance that might cause increased colony level tolerance. Nevertheless, the BEE SHOP showed a close relationship between mite loads and deformed wing virus (DWV) titers suggesting that mite control will also control virus infections most of the time.
BEE SHOP has documented within colony transmission of DWV through vertical transmission, when DWV infected queens lay infected eggs that develop into infected larvae and pupae. Between colony transmission of DWV can be accomplished through venereal transmission where drones carrying infected semen contaminate queens and spread the infection both directly via stored semen in the spermatheca or indirectly via infecting the ovaries of the queen, subsequently causing within colony vertical DWV transmission. Furthermore, we have investigated the effect from removal of the mite vector on virus infection persistence in adult bees and pupae. Detectable DWV infections diminish more rapidly in the brood and persist much longer with higher titers in the adult bee population.

WP 3: Variance among pathogens

The objectives of WP 3 were to genetically characterise variants of the major honeybee pathogens in the EU, to develop quantitative methods for detection and to correlate different variants with individual larva and colony level virulence.

American Foulbrood (AFB)

Based on data collected in BEE SHOP on AFB transmission and virulence, a theoretical model on the evolution of virulence of AFB strains has been developed. It is the first model of its kind for a honey bee pathogen and suggests that there may be reverse relationship between colony level virulence and individual level virulence in AFB strains.

BEE SHOP has tested the hypothesis that the least virulent strains of AFB at the larval level are the most virulent at colony level, and vice versa in the field. In strain competition field experiments, we demonstrate that spore build up over time in infected colonies is higher for the least virulent strain at the larval level. This greatly increases our understanding of how apiculture may influence virulence evolution in AFB and suggests strategies for virulence management.

Varroa mites and their viruses

BEE SHOP has sampled adult bees and brood to study virus titres in honey bee colonies with no, low and high mite infestation rates and how infections vary over the season. Interestingly, we have found DWV also in honey bee colonies without mites, although at very low titers. In a future project, these isolates will be compared to isolates where the mites have persisted for decades, to study virus evolution when the mite vector is introduced. Although the intention was to include several bee viruses in the temporal sampling, only DWV, Sackbrood (SBV) and Black Queen Cell Vírus (BQCV) could be found.

Varroa-transmitted viruses

BEE SHOP has developed quantitative RT-qPCR protocols for the quantification of all honeybee viruses that have so far been sequenced, including the most critical viruses linked to colony collapse. For DWV, the most important virus associated with *Varroa* mites, BEE SHOP has developed genetic markers by which variants can be characterized and quantified, again using real time RT-qPCR, based on nucleotide and amino acid sequence comparison. By detailed characterisation of these known virus infections, and by discovering a new *Varroa* mite associated virus infection (*Varroa destructor* Macula-like virus – VdMlV), BEE SHOP has greatly improved the emerging field of honey bee virology both by providing analytical tools now available to scientists world-wide and by new discoveries.

WP 4: Variance in disease tolerance among honeybees at the individual level

The objectives for WP 4 were to determine mechanisms that cause disease tolerance in honey bee larvae and adults with the intention of providing material for genetic mapping by the Genetics department.

American Foulbrood (AFB)

The *in vitro* rearing system of honeybee worker larvae, had been adapted for rearing of individual drone larvae and used to analyse variation in susceptibility of honeybee strains to AFB (LD_{50}). Furthermore, the susceptibility of individual worker larvae of

different genetic background (races) to AFB infections were explored. The data demonstrate that substantial differences do exist but also that there is a substantial variation within races. This means that tolerance for AFB should be monitored in the bee population as a whole and not within specific subgroups.

***Varroa* mites**

The BEE SHOP could show that mite reproduction success is lower in the Gotland population honeybees (not treated against Varroa) than in populations of bees with mite control. Z-8-heptadecene from the larval cuticle has been shown to either stimulate or reduce *Varroa* reproduction. BEE SHOP showed that although Z-8-heptadecene may influence mite reproduction, it is not a compound by which larvae through synthesis can impact on mite reproductive success. Thus, the chemical ecology of the mite-bee relationship remains elusive.

***Varroa*-transmitted viruses**

In spite of substantial efforts, reliable laboratory bioassay in individual larvae (feeding assays, injection assays) for measuring DWV virulence proved impossible to establish. We therefore adapted natural DWV infection routes (oral, venereal-vertical and *Varroa*-mediated) to screen individual bees susceptibility to DWV infection, for subsequent genetic DWV-resistance analysis and for cataloguing differences in DWV susceptibility between bee races. For the genetic screening we used incorporated venereal-vertical DWV infection into the crossing scheme, to produce recombinant F1 drones and back-crossed worker progeny, and these were delivered to the Genetics Department for preliminary testing. For the bee races we used oral and *Varroa*-mediated transmission to establish infections. The conclusions of these experiments were that the variation between replicates was too large to establish the significance of any differences between the four bee races.

1.3.3 Behaviour and Genetics Department

One way to avoid chemotherapy altogether is selection for disease resistant stock. However, various selection programmes for disease resistance in honeybees in the past had only mixed success, primarily because the testing and selection of the phenotype of honeybee colonies is extremely time consuming (usually 2 yrs per generation). Since queens and drones mate in flight (20 m above ground) mating control is difficult unless highly skilled and laborious instrumental insemination of queens is used. At the same time Europe harbours a huge wealth of various indigenous honeybee subspecies, with a vast potential to reveal mechanisms for disease resistance. A tool for swift selection of disease resistance and mating control is lacking.

The Genetics and Behaviour Department in the BEE SHOP aimed to develop both swift molecular tools for confirming mating control and selection of resistant colonies, based on target genes, which control specific disease resistance in honeybees. Another way to avoid honey contamination is to control the behaviour of bees while foraging for nectar and therefore control of the sources from which honey is produced. Instead of using classical methods for evaluating pesticide impact through assessing lethal doses, the BE SHOP profited from its integrative expertise on honeybee behaviour and neurobiology, with a special emphasis on learning and memory.

WP 5: Mapping QTL genes for disease resistance

Resistance against American Foulbrood (AFB)

For QTL mapping of individual larval resistance against AFB haploid drone larvae from a single queen were infected with AFB under controlled *in-vitro* conditions and mortality was recorded. Then resistant (R) and susceptible (S) phenotypes were identified and tested in a bulk segregant analysis with 672 microsatellite markers spanning the entire genome. This allowed to identifiy geneomic regions linked to the traits with a resolution of 20 cM or 1 Mb. We detected a only a single candidate region on chromosome 3 comprising three genes which so far have no known function in immunity or disease resistance. Gene expression studies yielded a Rab GTPase activator (Gene ID: GB10089) as the most likely candidate.

Resistance against the *Varroa* mite

Theresistant honeybeepopulation on the island of Gotland provided the bees tested for Varroa resistance at the individual drone level. We tested whether a *V. destructor* female reproduced in the cell with a drone larva. When the *V. destructor* female reproduced the drone was identified as sensitive while when the *V. destructor* female did not reproduce, the drone was identified as resistant.

Simlar to the mapping approach for AFB we used bulk segregant analyses to identified regions of interest in th egenme that were linked to the phenotypic classification. Drones were then individually genotyped for one to three markers in these regions to confirm or infirm their interest (56 and 82 markers respectively). All resistant (R) individuals and a similar number of sensitive (S) ones were genotyped. Significant results were obtained for only four regions for each colony (8 candidate regions).

New markers were defined in the candidate regions and used to genotype the drones. Significant results were observed in only two candidate regions; one located on chromosome 6 and the other on chromosome 7.

Despite a large genotyping effort (2111 markers genotyped on DNA pools in BSA and 201 markers genotyped on individual drones DNA sample), only two candidate regions could be confirmed in only one colony and these regions explain only a small part of the resistance. Moreover, the size of the candidate regions are large and do not allow to define a small number of potential genes to analyse further.

Nevertheless, a very interesting candidate (Dscam) has been found which confirms that the method was relevant.

Refined mapping tools: recombination hot spots and linkage disequilibrium

The detection of QTLs relies on the resolution of the genome scan. This procedure needs to be rationalised by the description of density and strength of possible recombination hot spots. In that way, a pilot study has been conducted on the two smallest chromosomes 15 and 16. Besides family studies, association studies allow the mapping of QTL from population data using linkage disequilibrium indices. This approach is well developed on the human genome. It supposes that a link is established between genetic distances on the map and linkage disequilibrium in the population for the same markers. Pilot studies on the same chromosomes (15 and 16) have also been performed on population data. We could show that for physical distances below 10 kb, about 80% of linkage disequilibrium are significant. This proportion decreases to 50% above 150 kb. This implies that linkage disequilibrium

could be useful for QTL detection only at close distance in honey bee. It could be used in a second step to confirm candidate genes in population studies.

WP 6: Developing SNP markers for disease resistance

The aim of this part of the BEE SHOP was to develop SNPs for available alleles of the QTL genes which have been identified to control resistance phenotypes against one of the diseases studied in WP 5. Since in WP 5 for both, individual larval resistance against AFB and Varoosis, only candidate regions have been identified containing three up to 50 genes, this part of the project has to remain incomplete until the candidate regions are further narrowed down or alternative ways have been found to confirm candidate genes. This might include additional mapping studies on different populations and/or whole genome expression studies using microarrays. First experiments into this direction have been undertaken by the BEE SHOP network but the necessary verification of these preliminary results will exceed the time span of the reporting period.

WP 7: Sustaining European honeybee races

The conservation of endemic European honeybee subspecies had high priority in the BEE SHOP since native subspecies are important reservoirs of local adaptations, ultimately determining the survival and pollination success of honeybees in the wild. We quantified the current status of European honeybee populations by employing a novel genetic marker tool kit to estimate the density of honeybee colonies in different regions of Europe, detect the existence of wild colonies and test for the reliability of different mating apiaries. Our results showed that wild honeybee populations have all but disappeared in many European countries. Because the remaining honeybee populations in Europe are mainly composed of managed bees, we decided to review the past and present honeybee genetic diversity and relate it to the different beekeeping practices observed across the continent. By surveying the apicultural practices of 33 European countries, we provided a comprehensive review of the magnitude and nature of the beekeeping industry in the continent. The density of managed hives showed a clear political, rather than geographical distribution across the continent, showing that sociocultural differences can profoundly influence the density of honeybees across Europe. In the light of these results we provide guidelines for future conservation efforts, highlighting the importance of promoting responsible beekeeping to preserve native European honeybee subspecies

WP 8: Foraging behaviour and contamination of honey with agrochemicals

Studies on honeybee foraging behaviour are crucial in the frame of BEE SHOP as they allow potential control of the behaviour of bees while foraging for nectar and therefore control of the sources from which honey is produced. The goal of this work package was to study whether it is possible to induce avoidance of specific substances in honeybees as a consequence of aversive learning. In this way, we aimed at training bees to reject cultures treated with undesirable molecules, which would be paired with neutral molecules associated with aversive experiences. Using a combination of behavioral and neurobiological approaches we provided the first demonstration of aversive learning in bees in the laboratory. We showed that odor-electric shock associations can be built in this context so that bees learn to extend the sting to the

odorant previously punished. We identified dopamine as the substitute of aversive reinforcements in the honeybee brain. We demonstrated that such aversive learning leads to long-term memories in honeybees and that these memories depend on protein synthesis and can last, eventually, the whole life of a honeybee. Aversive learning is indeed aversive as freely-moving bees explicitly avoid the odor punished in the laboratory. Moreover, within a hive, individual bees differ in their preparedness to respond to aversive stimulation thus providing a basis for division of labor. Forager bees are more sensitive to punishment and learn better about aversive experiences. This guarantees that aversive learning in a foraging context is a relevant goal. Before initiating an olfactory imprinting in the aversive modality we determined whether such imprinting was possible in the appetitive modality. We showed that precocious rewarded odor experiences lead to biased odour choices at the adult stage and that such biases are accompanied by dramatic reformatting of the adult honeybee brain supporting this long-term memory for odors imprinted at the young age. An equivalent study on precocious aversive learning should complete this study. Finally we used electrophysiological and behavioral experiences to determine whether honeybees are able to sense bitter substances. Our findings indicate that such a capacity does not exist in bees which would need to be able to sense the presence of bitter compounds per se.

1.3.4 Extension Department

The extension department was essential for providing honeybee colonies, bee samples and honey samples. It provided the main link to the industry. In addition it was instrumental for conducting WP9.

WP 9: Novel techniques for reducing residues in honey

The extension department has developed a suite of novel ways of disinfecting hives and apicultural equipment. These have been published in a manual on hygiene at the apiary. Disinfection techniques include treatments with a wide variety of compounds which must be used with the appropriate expertise. These treatments of the apicultural equipment will prevent undesired spread of bacterial diseases at the apiary before any clinical symptoms occur in the hives and hence reduce treatment of colonies and prevent contamination of honey with antibiotics, fungicides and other undesired chemicals. The activities were realized in direct co-operation with eight partner beekeepers that provided over 1100 honeybee colonies. We gained likewise from our partners more than 60 completed questionnaires for the assessment of beekeeping level and pertinently for the evaluation of bee colony losses..All partners received procedure guidelines and we obtained results of experiments on the treatment of bee colonies by selected compounds for apiary hygiene including organic acids and thymol.

2. Dissemination and use

Section 1 - Exploitable knowledge and its use

Exploitable results, defined as knowledge having a potential for industrial or commercial application in research activities or for developing, creating or marketing a product or process or for creating or providing a service were not foreseen and have not been developed within the BEE SHOP network. All other results are free to use by the apicultural industry and open to the public.

Section 2 – Dissemination of knowledge

The BEE SHOP used a multilayerd dissemination strategy incluging scientific publicaiotns, publications for the general public, oral presentation on conferences and seminars, schooling of extension specialists and interviews in the public media. Table 2.1 gives an overview on the most important activities. A final but most sustainable dissemination effort will be the publication of a monograph of the BEE SHOP results. NOVA publishers have agreed to produce a hardcover book comprising all results produced by the BEE SHOP network. Deadline for the manuscript is April 2010 nad production is foreseen by the end on 2010.

Overview table (2.1)

Date	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Feb 2006	Web-site	General public	International	open	*all*
March 2007	AG-Bienentagung Veitshöchheim, Germany	Research	International	150	P1, P6
June 2007	International Conference on Innate and Adaptive Immunity. Crete, Greece	Research	International	500	P7
Aug. 2007	XI. Congress of the European Society for Evolutionary Biology (ESEB) (Uppsala, Sweden)	Research	International	1.500	*all*
March 2008	AG-Bienentagung (Liebenwalde, Germany)	Research	International	150	P6
Sept. 2008	Eurbee3, EU Congress of Apidology. Belfast UK	Research	International	500	*all*
March 2009	AG-Bienentagung (Schwerin, Germany)	Research	International	150	P1, P2, P6
May 2009	Molecular Tools Workshop Bern, Switzerland	Research	International	25	P1, P2, P3
Sept. 2009	41. Apimondia (Montpellier, France)	Industry, Research	International	10.000	*all*

Section 3 - Publishable results

Publication list of the BEE SHOP network

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