Resistance to varroa and VSH behaviour in the honey bee

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PROCEEDINGS

of the COLOSS/Research Network for Sustainable Bee Breeding Spring Workshop

9-10 February 2015

Kirchhain (Germany)

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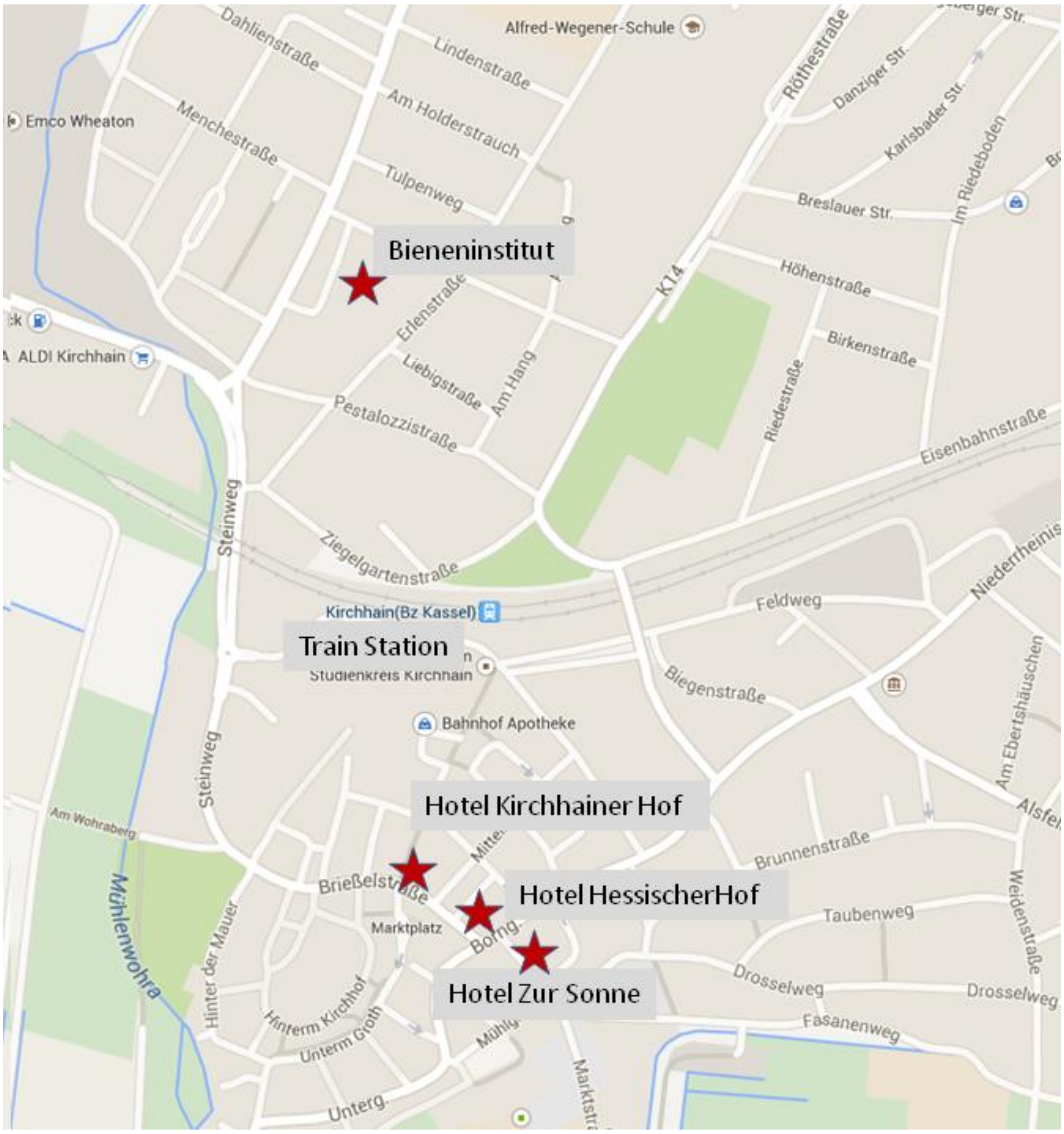
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Marktstr

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Agenda	
8 February 2015	
19:00 -	Arrival Informal gathering at the **Restaurant «UNIKAT»**, Kirchhain (directly at the train station)
9 February 2015	
08:30 - 09:00	Registration on site; Registration fee 20 €
09:00 – 10 :30	**Session 1: General - Chair : Marina Meixner** **Welcome and workshop organization** Introduction and welcome to new members administrative issues **Presentations (10 min)** Jerzy Wilde: Total antioxidant capacity of honey bee hemolymph in relation to age, exposure to pesticides and comparison to antioxidant capacity of seminal plasma Adam Tofilski: Circular movement of spermatozoa inside spermatheca Fani Hatjina: The use of ‘train of virgin queens’, a control system for the natural mating of honeybee queens Alex Uzunov: New perspectives for breeding vital European honey bees - The SMARTBEES project Discussion
10:30 – 11:00	**Coffee break**
11:00 – 13 :00	**Session 2: Sustainable Bee Breeding: why and how - Chair: Cecilia Costa** Norman Carreck: Sustainable Bee Breeding: Why And How? Presentation of synopsis of chapters by the assigned authors Discussion
13:00 - 14:00	**Lunch**
14:00-15:30	**Session 3: VSH protocols - Chair: Ralph Büchler** **Presentations (10 min)** Cecilia Costa: Testing for the trait “Varroa Sensitive Hygiene” Bjørn Dahle: Varroa tolerant honeybees in Norway Maja Drazic: Varroa infestation of the worker brood Marin Kovacic: Monitoring of Varroa destructor population and hygienic behaviour on apiary in Baranja region (Croatia) Fanny Mondet: Resistance to Varroa and VSH behaviour in the honey bee Sladjan Rasic: Advances in honeybee breeding of *Apis mellifera carnica* in tolerance to *Varroa destructor* mite Vicky Soroker: Attempt to breed for more hygienic bees Ralph Büchler: Strategy and preliminary results of VSH selection in a local Carnica population
15:30 – 16:00	**Coffee break**
16:00 – 18:00	**Lab session:** Varroa Sensitive Hygiene - Identification of Varroa reproductive stages Discussion

From 19:00 -	**Dinner at the Gasthaus zur Sonne, Kirchhain** (at your own expense)

10 February 2015	
9:00 – 10:15	**Session 4: Population differentiation and conservation** **Chair: Marina Meixner** **Presentations (10 min)** Benj. Basso: Genetic and genomic characterization of French bee populations Irfan Kandemir: Rediscovery of Central Anatolian honeybee (*Apis mellifera anatoliaca*): Description, Conservation and Dissemination J. Kretavicius: Inheritance of some morphological traits in honeybee without yellowish Per Kryger: Pipeline for data, in preparation for the analysis of honey bee DNA microsatellites Nebojsa Nedic: Morphometrical characteristics of honey bee front wing in the region of south-west and central Bosnia and Herzegovina Melanie Parejo: Genetic diversity of Swiss honey bee populations – an update
10:15 – 10:45	**Coffee break**
10:45 – 12:30	Discussion on common projects: Update on Wing reference database Sequencing project/SMARTBEES: status report and coordination of further acticvities
12:30 – 13:30	**Lunch**
13:30 – 15:30 15:30- 17:00	**Session 6: Discussion in separate working groups** - Popular book - VSH protocols - Common projects - RNSBB website issues Report of working groups Concluding session
17:00 -	**Train ride to Marburg** **Visit of the city with guided tour** **Dinner at the Gasthaus Sonne in Marburg**

List of participants

Name	Institution	Country
Marina Meixner	**Local organizer**	
Ralph Büchler	**Local organizer**	
Aleksandar Uzunov	**Local organizer**	
Annely Brandt	Bieneninstitut Kirchhain	
Reinhold Siede	Bieneninstitut Kirchhain	
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Benjamin Basso	ITSAP-Institut de l'abeille, Avignon,	France
Malgorzata Bienkowska	Research Institute of Pomology and Floriculture, Division of Apiculture, Pulawy	Poland
Maria Bouga	Agricultural University of Athens	Greece
Norman Carreck	International Bee Research Association / University of Sussex	United Kingdom
Eliza Cauia	Institute for Beekeeping Research and Development - Romanian Beekeepers Association, Bucharest	Romania
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Bjørn Dahle	Norwegian Beekeepers Association (NBA) & Norwegian University of Life Sciences (UMB)	Norway
Maja Drazic	Croatian Agricultural Agency, Zagreb	Croatia
Andone Estonba	University of the Basque Country, Bilbao	Spain
Roy Francis	Uppsala University	Sweden
Stefan Fuchs	Institut für Bienenkunde, Oberursel	Germany
Pierre Giovenazzo	Département de biologie, Université Laval, Québec	Canada
Fani Hatjina	Hellenic Institute of Apiculture, Nea Moudania	Greece
Irfan Kandemir	Ankara University, Ankara	Turkey
Nikola Kezic	Faculty of Agriculture, University of Zagreb	Croatia
Marin Kovacic	Agricultural Faculty, Osijek	Croatia
Justinas Kretavicius	Institute of Biochemistry, Vilnius University	Lithuania
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Adam Tofilski	Agricultural University of Krakow	Poland
Jerzy Wilde	Apiculture Div., Warmia and Mazury University, Olsztyn	Poland

Abstracts

The Research Network for Sustainable Bee Breeding/COLOSS Spring Workshop
Kirchhain (Germany), 9 – 10 February, 2015

GENETIC AND GENOMIC CHARACTERIZATION OF FRENCH BEE POPULATIONS

Benjamin BASSO*, Alain Vignal, Maxime Béguin, Dave Wragg, Jean-Pierre Bidanel, Yves Le Conte

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INRA and ITSAP started in 2014 a research project to study the genetic diversity of bees used by French beekeepers. At least 20 French bee populations (including the main bee populations used by beekeepers and major black bees conservatories) as well as several European populations used as references are included in the analysis. The analysis is performed by complete sequencing of one male per colony and around 30 colonies per population. Sequencing of haploids individuals rather than diploids limits the depth of coverage required and allows the sequencing of many individuals at a relatively low cost. The first results shows a large diversity of origin and a variable purity levels of *Apis mellifera mellifera*.
In parallel, ITSAP-Bee institute started in 2014 a testing apiary with the aim of phenotypically comparing major bee populations selected in France and testing / developing new selection criteria to be used by professional beekeepers. The testing apiary is filled by queens provided by several French beebreeders partners. The experimental station is putting a strong emphasis on the evaluation of the resistance of the colonies tested to varroa, as finding appropriate criteria to select for resistance to this parasite is considered as a major priority.
In this context, research projects on VSH selection will be essentials in the following years.

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PROTECTION OF MELLIFERA BEES IN POLAND

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The Convention on Biological Diversity, was adopted by the United Nations States in 1992 in Rio de Janeiro and then ratified by 194 countries. It initiated increased international activity to protect the biological diversity in agriculture, including livestock genetic resources.It includes protection of biodiversity which requires conservation and sustainable use of biological diversity of organisms throughout the country. Central European bee, Apis mellifera mellifera, originally appeared in Poland, with the exception of the southern territories of Podkarpacie. Import of other bee subspecies, which has been observed in recent decades, significantly reduced both, the number of colonies with mellifera bees and the area of their occurrence. Despite many favorable characteristics, especially higher resistance to diseases, better wintering and the ability to use weak nectar flows, the bees were eliminated due to their lower productivity and aggressiveness. Recently, conservation program for four lines spread over the north-eastern and central parts of Poland has been carried. Augustowska bee line occurs in its natural habitat in the Augustow Forest (north-eastern Poland), and similarly Kampinoska line, which population is located in Kampinos Forest (near Warsaw). Asta line was created during the interwar period of the local bees that were originally kept in the southern part of the central Poland. Północna line was formed using the genetic material of the Pomeranian bees. The line occurs now in the western part of the Warmia-Mazury region. The activities related to the protection of Mellifera bees started already in the 70s of last century in especially created isolated breeding areas. The aim of the program is to preserve the population by increasing number of colonies; preserve the phenotypic traits in accordance to the reference model line; preserve the typical biological and useful characteristics (as early development, disease resistance, good wintering ability, ability to store large amounts of pollen, increased nest defense, high wax production) and the reintroduction of queens to the environment and the only moderate improvement of bee stock. Each of the four bee lines has one leading conservation stock. Its main objective is rearing and insemination of bee queens, evaluation of breeding and production values, selection, and promotion of the material and distribution among beekeepers. In addition to the leading conservation stocks, there are also cooperating conservation apiaries. Their main objective is keeping the required number of colonies, transfer best queens to the leading conservation stock, making the phenotypic evaluation of colonies and rearing larvae and drones for reproduction.

These conservation programs are intended to preserve the genes typical for the Mellifera bees, which are characterized by better adaptation to environmental conditions.

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SUSTAINABLE BEE BREEDING. WHY AND HOW?

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The COLOSS Working Group 4 / Research Network for Sustainable Bee Breeding, has produced a number of important publications. These have included the reviews on “methods for discrimination of honey bee populations as applied to European beekeeping” and “methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies”; the two *BEEBOOK* Chapters on “Standard methods for rearing and selection of *Apis mellifera* queens” and “Standard methods for characterising subspecies and ecotypes of *Apis mellifera*”; and the papers that made up the *JAR* Special Issue on “Honey bee genotypes and the environment”. These papers were, however, all aimed at the bee scientist, but members of the group are very aware that there are no suitable books for the practical beekeeper that cover these topics. After discussion at various COLOSS meetings, an original idea to turn these two *BEEBOOK* chapters, together with that on “Standard methods for instrumental insemination of *Apis mellifera* queens” into a simple manual for beekeepers, has grown into a much more ambitious project. The aim is to produce a popular book which will draw on all aspects of the work of the group, especially the results of the genotype environment interactions experiment. This book will reassess the traditional view of bee breeding, with a new emphasis on local breeding efforts to conserve and develop local strains of honey bee best suited to local conditions.

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Testing for the trait "Varroa Sensitive Hygiene"

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Soon after the arrival of *Varroa destructor* on European honey bees, honey bee scientists noticed that the reproduction rate of Varroa in colonies could differ quite greatly. Two scientists working in the USDA bee laboratory in Baton Rouge, Harbo and Harris, focused on this trait to breed varroa-resistant stock. Initially it was thought that some colonies were able to reduce reproduction of varroa mites inside the cell, and the trait was named "Suppression of Mite Reproduction", and selection was performed by testing colonies for rate of non-reproductive mites in portions of capped brood. Studies on colonies thus selected showed that they performed well in hygienic behavior tests and specific experiments suggested that the low proportion of reproducing mites was due to their preferential removal by worker bees, so the trait was renamed "Varroa sensitive hygiene". In the United States much attention has been dedicated to this trait by scientists and breeders, and VSH stock is used in commercial operations. Some studies have tried to investigate the genetic control of this trait, however the mechanism by which varroa population growth in these colonies is reduced is not yet well understood. The specific hygiene behavior has not been confirmed by subsequent experiments, and a certain role of the brood in suppressing mite reproduction seems to be present.
In recent years, breeding programs in several European countries have included hygienic behavior and / or varroa infestation levels or growth indexes, but testing for VSH / SMR has not yet been applied large scale. Some scientists and breeders have started assessing the presence of this trait and many questions have arisen as to the best way to proceed. Within the Research Network for Sustainable Bee Breeding we have developed a protocol to evaluate this trait with a view to including it as a basic trait in breeding programs.
During the workshop we will share our experiences so far and discuss the critical points of application of the protocol.

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VARROA TOLERANT HONEYBEES IN NORWAY

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Varroa destructor was first detected in Norway in 1993 and its distribution now covers most of the country. Most beekeepers use a single treatment with oxalic acid to control Varroa, but some beekeepers do not control Varroa by chemical treatment or other radical biotechnical methods. Bee samples obtained from some of these beekeepers reveals Varroa infestation rates well below the damage threshold and they report winter losses not different from beekeepers following the recommended Varroa treatment strategy. To explain the observed Varroa tolerance, we initiated a study of mite reproduction in tolerant and susceptible (control) colonies in 2014. The study will continue in 2015-2017 and include several suggested resistance mechanisms such as VSH, shortened pupae development time and interactions with beekeeping management methods.

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Varroa infestation of the worker brood

Maja Dražić[1], Janja Filipi[2], Saša Prđun[3], Nevenka Jerbić[3], Jerko Jukić[3], Joso Brajković[4], Sreten Andonov[5], Dragan Bubalo[3] i Nikola Kezić[3*] (nkezic@agr.hr University of Zagreb Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia)

Varroa infestation of worker brood was monitored in 20 honeybee colonies from 11 May to 7 July 2014. Colonies were randomly selected from commercial apiary close to city of Vrbovec, Croatia. Honeybee colonies were not treated against varroa before and during experiment. Measurements were performed in 14 days interval. Colony weight, number of bees and brood cells, adult bees' infestation using sugar dusting, worker brood infestation in the center and on the side of brood area (400 cells) were examined. In average infestation of bees was 3.16% and infestation of worker brood cells 5.03%. The number of infested cells in the center (5.02) and on the side (5.05) was not significantly different (p>0.05). The average proportion of varroa without offspring was higher in the center in comparison to the side of brood area.

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THE USE OF 'TRAIN OF VIRGIN QUEENS', A CONTROL SYSTEM FOR THE NATURAL MATING OF HONEYBEE QUEENS

Fani Hatjina

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As part of the project '*CHARTA MELISSA- Saving, Improving and Maintaining germplasm of Greek bee populations - The characterization and identification of A.m.macedonica, A.m.cecropia and A.m.adami (macedonian, cecropian and cretan bee) through natural mating and artificial insemination in order to create the basis for the national program to maintain and improve the Greek bee populations"*, we are planning to use a novel system for the control of natural mating of A. mellifera queens that has been developed by Mr Jo Horner, an Australian queen breeder. Whether it is called the 'Koehler Method' or 'Horner's System' or 'Moonlight mating station' this method has significant advantages over Instrumental Insemination.
We call this system the 'Train of Virgin Queens'. The system does not require geographical isolation. By manipulating the ambient light and the temperature, we can delay the flight time of selected queens and drones so that their time of flight to congregation areas is later than that of feral drones. To delay the mating flight, mating nuclei are confined within a darkened cool room at 13–15 C^{o} for 2 days prior to mating. The mating nucs are on several trolleys that slide on rails, so they can easily be pushed by one operator from the cold room out into the open. The key advantages of this system over instrumental insemination are that it is technically easier and the quality of queens may be better because of the natural mating.
https://www.youtube.com/watch?v=YPW1tmBjoQ0 (uploaded video by Gilles Ratia)

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Rediscovery of Central Anatolian honeybee (*Apis mellifera anatoliaca*): Description, Conservation and Dissemination

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Central Anatolian honeybees (*Apis mellifera anatoliaca*) were adapted to Anatolian steppe climate and floral diversity in thousands of years of evolutionary period and survived from diseases and parasites. Morphological, biochemical and genetical properties of this honeybee subspecies were partially studied by F. S. Bodenheimer, F. Ruttner, Br. Adam and several Turkish bee scientists. Central Anatolian honeybees were used to obtain Buckfast honeybee by Br. Adam who visited Turkey three times. On the other hand with the migratory beekeeping continuing for 50 years, as in the case of other races Central Anatolian honeybees were also hybridized, genetically polluted, lost its properties and became unproductive. ANG foundation combating for the conservation of natural resources has just initiated the similar project as in Caucasus honeybees ''Description, and conservation of Central Anatolian honeybees and proliferation in its region'' was started with the partial support from TAGEM, Ministry of Food, Agriculture and Animals. In the frame work of this project apiaries consisting of the original skept honeybee colonies were evaluated. Purchased from suitable apiaries, skept hives were brought to the isolated conservation apiaries. Isolated conservation apiaries were established in a valley located about 75 km North of Ankara. After surveying the region, the valley and its environs were found to be free of other bee colonies. In conservation apiary, colonies were maintained using artificial insemination and this methodology will be continued. Also during conservation period colonies were studies with their physiology and morphological properties. This study was planned to continue for three generations. In the last generation molecular methods such as microsatellites (the most variable four loci) and mtDNA (RFLP and sequencing) markers were surveyed. Based on the data obtained during the project, application will be made to the Ministry of Food, Agriculture and Animal for the registration of the Anatolian honeybees. In the later years breeding Anatolian queen bees were marketed to the use of the beekeepers. Until now, from 50 colonies obtained from five apiaries were evaluated morphologically and physiologically, according to the initial observations: physiological properties such as overwintering, spring build up, and swarming were determined in Central Anatolian honeybees. The number of colonies reached to 300 and the morphological analyses were completed. Characterization of microsatellites were in progress. The production of hybrids between Anatolian queen and Caucasus drones are planned and will be distributed to beekeepers to test the performances in the next phase of the project.

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Monitoring of Varroa destructor population and hygienic behaviour on apiary in Baranja region (Croatia)

Marin Kovačić, Nikola Kezić*, Janja Filipi
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On the apiary in Baranja (Pčelarstvo Kovačić) in the 2014. year was selected 36 from 400 colonies for performance test. On the selected colonies average daily natural mite mortality was 0, monitored from 03. to 17. of April on the screened bottom boards. Average varroa infestation on adult bees monitored by powder sugar dusting was 2,3 on the 20. May and 3,6 on the 15. July. On the 36 colonies in the 20. June was performed pin test. Average 24 worker brood cells remained after 24 h.

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INHERITANCE OF SOME MORPHOLOGICAL TRAITS IN HONEYBEE WITHOUT YELLOWISH

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In the northern countries of EU bee strains without yellowish widely propagate and is considered to be the most popular. It is due to hard and long lasting winters. In Baltic countries selection and breeding practically is not done. So, many beekeepers import much Queens into these countries and later from this stock rear Queens themselves and propagate them for selling. In Lithuania still is not present any serious bee breeding work or program.

In future will be created breeding program focusing on the scientific knowledge. In Lithuania, and neighbours states such as Latvia and Poland bees without yellowish are reared in general (Carniolan, Caucasian and dark bee). Therefore, a problem arises, how to keep pure bees in the mixing region and distinguish them. The simplest and the cheapest way to find the purity is morphological analysis of separate parts of worker bee. But it might be that some measurements and traits will not change after crosses of pure race queens with another strains drone. Then the incorrect conclusion about the bee colonies purity could be done.

Our purpose was to determinate, how separate bee parts and secondary measurements will inherited from their parents. To achieve this purpose, I bring pure sister queens of separate strains and instrumentally crosses them with another strain drones. Six groups containing six colonies in each were created. In the Fall, 30 worker bees from each colony were taken, decapitated with ether and conserved until morphological measurements. Then it was done morphological measurements and data calculated. Cubital index was calculated such: venation B:A x100 and expressed as relative percent value. Also was introduced inheritance coefficient (IC), which show how strong traits is inherited and impacted from one or other of parents, or maybe from both. It could vary from 0 to 1. It was calculated from morphological values of traits, for example, if IC is 0.5, this means, that this trait was inherited from both parents equally. Also than gene impact was also equally from both parents, when is forming trait. If IC is nearly 1.0, than trait is impacted just from one of the parent. It seems, that is similar as dominant and recessive dependence, or intermediate gene impact in genetic science.

All our data is presented in table. Here is shown, that in crossed bees many traits changed. Tongue length is more impacted by Queen genes (Carnica and Caucasica), but in case with native dark bee, value was slightly affected from drone (see table 1).

Cubital index are more impacted by father, especially in crosses Carniolan with Caucasian, and vice versa. In the case, when one of parent was native dark bee, value was determined only from these strain independently who were parents. In the other words, cubital index in worker wing arise mostly from native dark bee, did not much impact other strains of this value.

Conclusions. If is doing bee breeding with Caucasian stock, best way is to measure length of the proboscis, cubital index, discoidal chief. In Carniolan bee selection, to distinguish it from other enough to measure cubital index, discoidal chief. For the native dark bee mostly important to keep short proboscis.

Table 1. Mean values of morphological traits and inherintance coefficients (IC) of bee races

♀ × ♂	Length of proboscis, mm	IC		Cubital index, %	IC		Discoidal chief, %			IC	
		♀	♂		♀	♂	-	0	+	♀	♂
Apis mellifera mellifera (*A. m. m.*) n=150	$6,15 \pm 0,028^{a*}$	–	–	$59,05 \pm 1,39^{a}$	–	–	75,1	22,8	2,1	–	–
Apis mellifera carnica (*A. m. car.*) n=150	$6,51 \pm 0,023^{b}$	–	–	$42,87 \pm 1,11^{b}$	–	–	3,2	2,1	94,7	–	–
Apis mellifera caucasica (*A. m. cau.*) n=150	$7,09 \pm 0,024^{c}$	–	–	$53,68 \pm 1,22^{c}$	–	–	80,2	12	7,8	–	–
A. m. cau. × *A. m. car.* n=180	$7,00 \pm 0,021^{c}$	0,84	–	$43,38 \pm 1,18^{b}$	–	0,95	5,8	15,7	78,5	–	0,71
A. m. car. × *A. m. cau.* n=180	$6,76 \pm 0,018^{d}$	0,57	–	$53,81 \pm 1,44^{c}$	–	1,0	64,3	14,6	21,1	–	0,68
A. m. cau. × *A. m. m.* n=180	$6,65 \pm 0,028^{dbe}$	0,53	–	$58,18 \pm 1,14^{a}$	–	0,85	63,4	23,8	12,8	–	–
A. m. m. × *A. m. cau.* n=180	$6,54 \pm 0,042^{be}$	0,58	–	$58,45 \pm 1,18^{a}$	0,84	–	70,2	16,0	13,8	–	–
A. m. car. × *A. m. m.* n=180	$6,34 \pm 0,031^{f}$	–	0,52	$52,41 \pm 1,38^{c}$	–	0,57	57,3	14,0	28,7	–	0,57
A. m. m. × *A. m. car.* n=180	$6,35 \pm 0,029^{f}$	–	0,55	$49,58 \pm 1,45^{cd}$	0,61	–	20,6	14,0	65,4	–	0,62

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Pipeline for data, in preparation for the analysis of honey bee DNAmicrosatellites

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In the processing of genetic data we have developed a strategy for systematic processing, which we describe in the following. Information about each individual bee is stored in a database: Sampling date and location, worker/drone/queen, larvae/pupae/imago, colony no, sampler, suspected or confirmed subspecies status. In addition in some cases it may be relevant to store information about health status, behaviour, colour.

For each set of 380 bees we receive four batch files, representing the four individual sequencer runs needed to amplify all 38 loci. Each batch file containing the output from the sequencer, which has been checked and double checked by eye for errors, containing the alleles status for each individual bee. Each original batch file, is a long list ordered first according individuals and the each loci allele size. The batch files are sorted and combined to contain first by loci and then by bees, and the loci are then placed into a new table containing a column for the individual ID, and the two columns for each loci containing in order first th smallest and the largest allel. For drones only a single column is used for each loci. As an additional check-up two microsatellites AC087 and AC088 are included in separate batches, which in fact contain information as to the same loci. It can be seen from the sequence information, that the difference between the allele size is 11 basepairs and it allows first to check that no mix up has occurred between these two batch and if indeed the control sequencing has worked proper. Once the data files are brought in order, the data are copied into a template for GenAlEx. Inside GenAlEx a large number of basic population genetics indexes can be calculated, but the real strength is the option to export the data into a wealth of other data formats thus reducing the risk of introducing errors in when making numerous additional data files, for instance for Structure, GenePop, GeneClass, Arlequin, Flock and Trace, Spagedi.

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RESISTANCE TO VARROA AND VSH BEHAVIOUR IN THE HONEY BEE

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For several years, the INRA has been interested in unravelling the basis of chemical communication between honey bees and Varroa, with the aim of understanding the origin of the resistance of some honey bee colonies to the parasite. In the last three years, priority was given to the study of chemical communication around VSH behaviour.
In partnership with ITSAP, the INRA is currently launching a research program focused on Varroa resistance and the VSH trait, with the aim of identifying genetic markers for the VSH trait that can be used in breeding programs to select for Varroa resistant colonies. The selection program will start in 2015, by phenotyping colonies originating from a large variety of French honey bee populations for the VSH trait. In parallel, the basis of VSH behaviour at the individual level will be investigated.

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Morphometrical characteristics of honey bee front wing in the region of south-west and central Bosnia and Herzegovina

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The region of Bosnia and Herzegovina has an excellent preconditions for development of beekeeping. Owing to different relief and climatic characteristics there exists a wide range of honey bee pastures. In such different ecological conditions, due to adapting to natural conditions, it is presumed that in Bosnia and Herzegovina (BIH) exist locally adapted geographical ecotypes of honey bees. In previous research, morphometrical characteristics of honey bees from BIH were only partially studied. Therefore, the aim of this investigation was to analyse a part of morphological characters in order to help distinguish indigenous honey bee varieties. Samples were collected from four different locations covering the territory of south-west and central Bosnia and Herzegovina and compared with the honey bee sample taken from the border region of Serbia and Bosnia and the sample of Carniolan bees from Slovenia.
Fifteen worker bees of each sample were dissected and 14 characters on the front wing were measured according to standard procedure. A descriptive statistical analysis was carried out and comparisons between locations were determined by Duncan's test. All measurements of characteristics for the bees were analysed by multivariate discriminant analyses as well.
The angles A4 (, B4, L13, K19, G18 and cubital index (CI), as well as the length and width of front wing were found to show significant differences among locations ($P<0.01$).
The results of multivariate discriminant analyses showed significant differences existed between six groups of bees. Owing to this analysis the samples of bees from Gornji Vakuf (BIH) and Bajina Bašta (Serbia) were found to be different from the other bee groups from BIH and Slovenia. This difference was mostly contributed by the A4, L13, K19, CI characters and the length of the front wing.
The results of the research show the existence of significant variability of honey bee in the region of Bosnia and Herzegovina and implicate the existence of different subpopulations of honey bees which could be a result of adaptation to local geographical living conditions or conditioned by anthropogenic factor. It is necessary to spread the research to a whole territory of BIH which will contribute to a clearer separation of groups of bees that meet the standards for Carniolan strain.

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ADVANCES IN HONEYBEE BREEDING OF *APIS MELLIFERA CARNICA* IN TOLERANCE TO *VARROA DESTRUCTOR* MITE

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From the first infestation of honey bee parasite *Varroa destructor*, on apiaries in eastern Serbia during the seventies of last century, almost forty years have passed. During that time, varroa mite has inflicted severe damage to beekeeping, in our country as well as in other countries worldwide.
To prevent the decline of bee colonies on a large-scale, beekeepers have mainly depended on the use of various chemical preparations, which led to a larger or smaller negative impact on the quality of bee products. Additionally, varroa mite has, in time, become resistant to different applied antivarroa preparations.
From the beginning of parasite invasion, it was noticed that a certain number of bee colonies has been surviving. Based on experiences with other agricultural (animal and plant) species, it could be assumed that natural selection would in time bring to balance the interest of the host – honey bee and varroa mite, whose nature is not to disappear together with the host. Regarding that, directed artificial selection should have produced bees more tolerant to varroa mite.
Research within an American-Yugoslav science project in “Apicenter” in the second half of the eighties proved this was possible. Then, after three selectioned generations, colonies of a more tolerant line survived, and less tolerant went to decay. Since the process was one-sided, such bees were less productive. After completing the project, such an approach to resolving the problem with varroa mite, was modified.
Since midnineties, program of selection to honey productiveness was continued, considering the intensity of varroa mite infestation - of individual bee colonies, as well on lines, and since 1998 in total per year for the whole selective apiary of about 80 – 90 honey bee colonies.
A long-term control of varroa mite infestation and taking into regard of this factor during selecting queens within bee selection to honey productivity and reproducing of queens, brought to stabilize relations between the honey bee as a host and varroa mite as a parasite. This enables beekeepers that use “Apicenter” queens to keep bee and prevent varroa mite with minimum intervention in autumn with acceptable preparations.
We believe that this achievement during the last six years (2009-2014) was mainly the result of selection for tolerance to varroa mite under our natural conditions or the influence of some other factors. Similar long-term infestations monitoring of other honey bee genetic populations did not happen in Serbia.

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Genetic diversity of Swiss honey bee populations – an update

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Genetic diversity at the colony level however is important for colony fitness. And the genetic diversity at the population level determines the fate of the population for the future. It is the potential to adapt to changing environments including climate change and new emerging diseases. This adaptive capacity is valuable and thus a priority for conservation biology.
In Switzerland, the endemic ecotypes of *A. m. mellifera* are adapted to the alpine environment with long winters and short foraging seasons. Previous studies using microsatellites have already shown that hybridization between subspecies in sympatric areas does occur regularly. This process can lead to loss of genetic diversity through introgression of foreign genes into the native gene pool. Therefore, we started a project on the genetic diversity of Swiss honey bee populations, in order to investigate in more detail the extent of admixture as well as the current population structure of Swiss honey bees, and the effectiveness and success of mating apiaries and conservation areas. In late spring and summer of 2014, honey bee drone brood samples were collected from entire Switzerland, including amongst others *A. m. carnica* and *A. m. mellifera* from conservation and non-conservation areas. Drones were collected because of their haploidy, which simplifies subsequent bioinformatic analyses. After DNA extraction and purification, we sent the haploid DNA samples to the GeT-sequencing platform (Toulouse, France) for whole-genome next-generation sequencing. The next step will be to apply a bioinformatics pipeline for mapping and SNP (single nucleotide polymorphism) calling. Further downstream population genetics data analyses will be performed with various software, amongst others with ADMIXTURE.
Whole-genome sequencing can unravel in much more detail the extent of admixture and genetic diversity of different Swiss *A. mellifera* populations. Moreover, comparing neutral and non-neutral molecular markers of the subspecies of different lineages allows an insight into the adaptive genetic variation of the endemic ecotypes.

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TOTAL ANTIOXIDANT CAPACITY OF HONEY BEE HEMOLYMPH IN RELATION TO AGE, EXPOSURE TO PESTICIDES AND COMPARISON TO ANTIOXIDANT CAPACITY OF SEMINAL PLASMA

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Total antioxidant capacity (TAC) was measured for the first time in honey bee hemolymph and seminal plasma using a commercial antioxidant assay kit (Sigma-Aldrich). Hemolymph was characterized by higher TAC activity compared to seminal plasma (1.98 vs. 1.38 mM of Trolox for hemolymph and seminal plasma, respectively, P≤0.05). Neither storage nor freezing affected TAC activity. TAC activity increased with age of bees (1.18 vs 1.97 mM of Trolox for 1 and 30 day old bees, respectively, P≤0.05). Hemolymph of 30 day old bees were characterized by four times higher protein concentration. Exposure to imidacloprid (IMD) affected TAC activity of hemolymph of one day but not 30 day old honey bees. TAC activity in hemolymph of one day old bees decreased after addition of 5 and 200 bmp IMD from 2.37 to 1.46 mM of Trolox (P≤0.05). Our results suggest that antioxidant protection of honey bees is related to age and may be disturbed by exposure to IMD.
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Circular movement of spermatozoa inside spermatheca

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It is generally believed that spermatozoa stored inside spermatheca remain motionless, however, some studies have reported the contrary. We have instrumentally inseminated queens with spermatozoa stained with fluorescent stain Hoechst 33342. This made it possible to observe their behaviour inside spermatheca. The observations were made 1, 4, 8, 16, 24, 26 hours after insemination and 72 days after insemination. During first 8 hours after insemination density of spermatozoa inside spermatheca was increasing, however, their movement was stationary. Only small fraction of the spermatozoa displayed relatively fast forward movement. Completely different behaviour of the spermatozoa was observed 16 hours after insemination and later. At that time we have observed circular movement of the spermatozoa inside spermatheca. Numerous circles were visible at one time. The circles were located close to the spermathecal wall. Movement of the spermatozoa was also observed in spermathecae of naturally inseminated queens. The marble like pattern of the spermathecae was changing. The changes were relatively slow and well visible only when they were video recorded and played at high speed.
The movement of the spermatozoa inside spermatheca can be related to competition between spermatozoa. Entrance to the spermathecal duct is located at the spermathecal wall and spermatozoa close to the wall are more likely to enter the duct and fertilize an egg. Therefore, spermatozoa can compete for place closer to the spermathecal wall. Moreover, the circular movement of the spermatozoa can lead to segregation of alive spermatozoa next to the wall and dead once closer to the centre. In this situation release of dead spermatozoa from spermatheca would be less likely.

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NEW PERSPECTIVES FOR BREEDING VITAL EUROPEAN HONEY BEES - SMARTBEES PROJECT

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In order to preserve the natural honey bee diversity, improve the performance and vitality, reduce colony losses and diminish the dependence on therapeutic treatments the European Commission supported a research project entitled „Sustainable Management of Resilient Bee Populations„ or SMARTBEES. The project is going to support local breeding activities in all European honey bee subspecies with a special focus on so far neglected strains.
The SMARTBEES (Sustainable Management of Resilient Bee Populations) consortium is a multidisciplinary team of 16 European institutions and experts, coordinated by Länderinstitut für Bienenkunde Hohen Neuendorf e.V from Germany, which aims to provide a solid framework for the long-term improvement of honey bee health and genetic diversity.
Our strategy focuses on identification, breeding and propagation of locally adapted honey bees with high performance and resistance traits to *Varroa destructor.* This is implemented as the main task of the Working Package No 6, coordinated by the Landesbetrieb Landwirtschaft Hessen Bieneninstitut from Kirchhain in Germany. To standardize methods for management and testing of test colonies around Europe, we developed a manual "Performance testing protocol". In addition numerous training workshops will be organized around Europe for participating honey bee breeders and beekeepers.
We are encouraging all interested parties (breeders, beekeepers, institutions, associations etc.) to join the program and to enhance the possibilities for breeding vital honey bee populations. More details can be found on www.smartbees-fp7.eu.

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Attempt to breed for more hygienic bees

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Recent increase in colony losses and Varroa mite resistance to coumaphos and lower efficacy of the Amitraz treatment chemical treatments has raised awareness' for sustainable Varroa control.
In attempt to establish Varroa IPM program, we evaluate the possibility to initiate a breeding program for honeybees more tolerant to Varroa based on "local" honey bee population. The local" honey bees are believed to be a mix between *Apis mellifera ligustica*, *caucasica* and Buckfast. The original local bees *Apis mellifera syriaca* no longer exist. The trait we have chosen for selection is hygienic behavior. The study is conducted in Zrifin Research Apiary at Volcani center. The honey bees in the apiary are locally bread lines based mainly on *Apis mellifera ligustica*. We started the project two years ago by comparing hygienic behavior for two generations on about 100 colonies representing 11 matrilines. The evaluation was conducted three times a year.
We have found that, in the summer months, the hygienic behavior is stronger than in the spring and the differences between the lines is greater. Some lines showed constancy over the two generations, suggesting trait heritability. We plan to continue the breeding effort by using artificial insemination and evaluating both the intensity of hygenic behavior and its impact on the Varoa infestation as well as on general colony performance.

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