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**UNIVERSITÄT  
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Division of Apiculture- Institute of Animal Science  
HELLENIC AGRICULTURAL ORGANIZATION 'DEMETER'

# The COLOSS / Research Network of Sustainable Bee Breeding Spring 2016 Workshop

## Proceedings

**Thessaloniki, Greece,  
22-23 April 2016**

# The COLOSS / Research Network of Sustainable Bee Breeding Spring 2016 Workshop

## Topics

The main topics of the meeting include:

- The results of the VSH Common experiment
- The presentation of results / discussion and planning of publications
- The Book project: "Sustainable bee breeding: why and how"

## When & Where

The meeting will be held for two days, 22nd and 23th of April (see tentative agenda below), In GRAND HOTEL PALACE, in Thessaloniki, Greece (<http://www.grandhotelpalace.gr/>)

## Registration- fees

There will be a 10 € registration fee (payable on site) which will help to cover all coffee breaks, lunches and 1 social dinner but not dinner on 23rd April. Max number of participants =25

At the moment **no** reimbursement for travelling or accommodation is likely.

Registration deadline: 20th of March 2016.

For registration please use the link provided in the website

## Excursion

An optional excursion will be organized (on morning of 24th April) for visiting prehistoric Caves at Petralona and sea side in Chalkidiki peninsula if at least 15 people will register for this directly to [fani.hatjina@yahoo.com](mailto:fani.hatjina@yahoo.com).

However, those wishing to take part in the excursion will have to pay an additional 30 € fee. A reminder note will be sent to those registered for the workshop

## Abstract

The submission of an abstract is recommended but not required, unless you wish to have it published in the proceedings

## Instructions to get to Thessaloniki and the hotel

- By air

Thessaloniki Macedonia Airport (SKG) is an international airport with direct flights from several European capitals or through Athens International Airport. It is situated about 15 Km from our venue location.

There is a Bus service from Macedonia Airport of the city Thessaloniki: In details, from Macedonia Airport you take Bus No 78 route towards KTEL Macedonia Terminal and get off at the stop "ORIZOMILOI". It is at the opposite side of the road of GRAND HOTEL PALACE. (305 - 307 Monastiriou str. Thessaloniki, Tel. 2310 549000 Fax. 2310 549149) <http://www.grandhotelpalace.gr>)

In the following link you can find the time table and the route for the bus service from INTERCITY bus Terminal to AIRPORT and back (Bus NO 78)  
<http://oasth.gr/#en/routeinfo/list/61/12/73/>

For night hours there is Bus No 78N ,  
<http://oasth.gr/#en/routeinfo/list/146/74/73/>

Additional information:

(Bus No 08 takes you from the same bus stop just outside of the Hotel to the city centre and Bus No 35 takes you from the city centre to the castle area).

- By train from neighbouring countries.

There is also the possibility of travelling by train or bus.

Information for train services can be found in the following links.

<https://forum.rail.cc/istanbul-athens-train-t2369.html>

<http://www.thetrainline-europe.com/train/thessaloniki-to-skopje~7300008~6500001>

- By bus from neighbouring countries.

There are bus connections between Thessaloniki and Istanbul, Sofia, Bucharest and Tirana (please contact [fani.hatjina@yahoo.com](mailto:fani.hatjina@yahoo.com) for more details)

### **Accommodation**

Special prizes have been achieved for the hotels in the list if you book through

**Halkidiki Tours: For Reservation and payment**

Tel: +30 23730 21062

email: [halkidikitours@live.com](mailto:halkidikitours@live.com)

### **Touristic information**

For more information about Thessaloniki, please visit:

<http://www.thessaloniki.travel/index.php/en/> or

<http://www.greeka.com/macedonia/thessaloniki/>

## Tentative Schedule

21st APRIL, 2016

Time	ARRIVAL 2nd Group RNSBB
20:00	Informal gathering at hotel (GRAND HOTEL PALACE)

22nd APRIL, 2016

Time	Session 1 – RNSBB
08:30 - 09:00	<b>Registration</b>
09:00 - 11:00	Welcome and workshop organization Introduction and welcome to new members Administrative issues
11:00 - 11:30	<b>Coffee break</b>
<b>Session 2 – RNSBB</b>	
11:30 - 13:00	VSH Common experiment Presentation of results Discussion
13:00 - 14:00	<b>Lunch</b>
<b>Session 3 – RNSBB</b>	
14:00 - 16:00	VSH Common experiment – discussion and planning of publications and conference presentations
16:00 - 16:30	<b>Coffee break</b>
16:30 - 18:00	VSH Common experiment – continued
20:00-	<b>Walk at the sea side of the city- Social dinner</b>

23rd APRIL, 2016

Time	Session 4 – RNSBB
08:30 - 10:30	Update on suspecies discrimination methods / inventory / database
10:30 - 11:00	<b>Coffee break</b>
<b>Session 5 – RNSBB</b>	
11:00 - 13:00	Book project: “Sustainable bee breeding: why and how” - presentation of drafts for individual chapters Book project –next steps
13:00 - 14:00	<b>Lunch</b>
<b>Session 3 – RNSBB</b>	
14:00 - 16:00	<b>Visit to White Tower</b>
16:00 - 16:15	<b>Coffee break</b>

16:15 - 18:00	Collection of Ideas for next common experiment Miscellaneous topics Synopsis of the meeting Next meeting – where and when?
20:00-	<b>- Dinner</b>

24th APRIL, 2016

<b>Time</b>	<b>Excursion day</b>
10:00	Visit Peralona Caves and sea side of Chalkidiki <b>(optional)</b>

<b>LOCAL ORGANIZER</b>
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## **SUMMARY**

During the workshop there were several interesting presentations on ongoing breeding and conservation research which led to lively discussions on various topics. Organization of the group was also discussed, mainly focusing on the web page development/improvement/ maintenance, introduction of new members and the membership fee.

Regarding the scientific part, there were several presentations on the common investigation on SMR/VSH behavior, including preliminary results and critical methodological points. Several hours were dedicated to discussing what has been achieved till now and how to proceed in the coming season and following years. More specifically a great variability across European colonies seems to exist, and the critical point is now the repeatability of the test.

The subspecies differentiation project is continued, and a workshop devoted to this subject might be possible next year.

The 'Sustainable bee breeding' book was also discussed and plans for its realization were made. An editorial board was formed to support Norman in this task and the next deadline (Eurbee in Romania) was set for chapter drafts.

Several proposals were also made for the location of the next Spring meeting: proposed locations were: Tel Aviv (Israel), Ohrid (Rep. Macedonia), Bologna (Italy), Pulawy/Kracow (Poland).

The decision will be taken in September.

## **SUBMITTED ABSTRACTS**

### ***Common investigation on SMR/VSH behavior in European honeybees***

*Ralph Büchler*

Data from brood collected in 2015 (total 334 colonies, 284 colonies with at least 10 single infested cells): decision to use for a publication which will show the variability existing in European honey bee colonies, and will include indications on minimum numbers of cells / colonies to be considered (after statistic simulation). Contributing members should send additional information by 25th May. *Plan for 2016 has to be drafted.*

### ***Some critical points in the evaluation of VSH and the results of a first study in Romania.***

*Eliza Cauia,*

*Adrian Siceanu,*

*Gabriela Oana Visan*

The study was carried out in the frame of SMARTBEES project, in the November 2015-January 2016 period. In this study were used high infested bee brood samples collected from 23 honeybee colonies from a single apiary. The VSH trait was evaluated following the improved protocol of evaluation elaborated in the frame of RNSBB network. The results show a relative high infestation level with varroa between 9,52% and 60% with an average of 36,34% and a non-reproductive rate between 18% and 38%, with an average of 26,73%, expressed as delay of reproduction rate, infertile rate and no male rate. Because this non-production rate strongly depends on the accuracy of evaluation in terms of appreciation of right stages of bee brood and varroa brood it is very important to pay attention to some critical points that we noticed during the evaluation procedure especially the stage of varroa young females and the stage of bee

brood depending on their pigmentation degree. The presentation will illustrate by images and cases situations some critical points in the evaluation process.

***Monitoring of honey bee diversity in the Republic of Macedonia***

*Miroljub Golubovski,*

*Borche Pavlov,*

*Hrisula Kiprijanovska,*

*Aleksandar Uzunov,*

*Marina D Meixner*

In the last decade the honey bees in the Republic of Macedonia have been subject to morphological and molecular studies (Uzunov *et al.*, 2009, 2014) which reported that this local population belongs to the subspecies of *Apis mellifera macedonica*, initially described by Ruttner (1988). However, in the context of the risk from globalization and transhumance activities, such as queen marketing and migratory beekeeping, there is a continuous need for monitoring the diversity status of this population.

Recently, Europe-wide actions were taken within the SMARTBEES project for surveillance of European honey bee diversity, analyzing numerous morphological and molecular markers. Concerning the honey bee population in the Republic of Macedonia 20 locations were identified from where 30 to 40 worker bees and 10 drones per colony will be sampled. The criteria for location identification and sampling procedure are in accordance with the SMARTBEES ([www.smartbees.eu](http://www.smartbees.eu)) recommendations.

The samples will be subject to morphometrical and molecular characterization in the laboratories of the Bee Institute in Kirchhain, Germany, the University of Aarhus and GenoScan A/S in Denmark.

**Keywords:** *honey bee, diversity, markers, Apis mellifera macedonica, Republic of Macedonia*



## ***Preliminary screening for Varroa Sensitive Hygiene trait in Italian bees***

*Cecilia Costa,*

*Gabriele Marzi,*

*Raffaele Dall'Olio*

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. 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.

At CREA-API, in July 2014 we collected brood combs from 17 colonies belonging to one of the institute's breeding programs and using the RNSBB protocol (first version) analysed them for presence of the VSH trait (considering the delay of reproduction, and not the presence of the male mite). We found a great variability, with values ranging from 0% to 67%. Brood infestation ranged from 1% to 19%.

At the end of September 2015 we collected brood combs from 13 colonies belonging to the National Queen Breeders Registry, present in our performance testing apiary, and analysed them using the RNSBB protocol (2015 version) for presence of the VSH trait (also considering presence of the male mite). In this case analyses was easier due to higher infestation of the brood (from 21% to 67%), although in some cases the amount was insufficient. Rate of delayed reproduction was higher compared to the previous year (23% to 85%). We found a high rate of absence of males (35%- 92%).

During the workshop we will share our experiences so far and discuss the critical points of application of the protocol.

## ***Hygienic behavior in locally bred honeybees and its impact on Varroa infestation***

*Rya Zelzer,*

*Paz Kahanov,*

*Yosef Kamer,*

*Ilia Zaidman,*

*Malgorzata Bienkowska,*

*Abraham Hefetz,*

*Victoria Soroker*

As in most of the world the Varroa mite *Varroa destructor* is the major problem for Israeli apiculture. It was reported in Israel in mid-eighties of the last century and since then treatments by synthetic chemical insecticides were widely implemented. However the efficacy of those proved to be temporal, making it clear that the approach needs to change towards integrated Varroa management with Varroa resistance as one of the major component in this strategy.

The aim of the present research is to evaluate locally bred bees in Israel for frequency of hygienic behavior, its impact on the *Varroa* mite infestation and the heritability of this trait. The bees bred in our apiary are basically *Apis mellifera ligustica*, but other honey bee strains were sporadically introduced in the past to improve honey productivity.

We have started this research about three years ago by screening colonies with freely mated queens for hygienic behavior using the “pin test”. In parallel we assessed the tested colonies for colony strength, health and honey production. After the first year we selected colonies that represented two extremes: most hygienic and least hygienic, and screened the second generation using for the above traits. In this generation we also tested seasonal changes in Varroa infestation. The results show that the daughter colonies are similar in their hygienic behavior to their mother, while no effect on honey production was found. Furthermore, significant negative correlation was found between Varroa infestation and the level of hygienic behavior. These results are promising and suggest heritability of hygienic behavior in local populations. We pursue our selection studies by implementing artificial insemination.

## ***Apis mellifera anatoliaca: characterisation, conservation and dissemination***

*Irfan Kandemir,*

*Ahmet Inci*

*Apis mellifera anatoliaca* is the main honeybee subspecies distributed across the Anatolian peninsula and form several different ecotypes in isolated areas. During the course of evolution this subspecies adapted to Anatolian step climate and floral diversity, and survived from diseases and parasites for thousands of years. Since 1990's morphological, biochemical and genetical properties of this honeybee subspecies were partially studied many scientists worldwide. And also this subspecies were used to form hybrids, the one well known is the Buckfast honeybee by Br. Adam. However since last half a century, migratory beekeeping practices hybridized, genetically polluted the Anatolian honeybees and lost its favorable properties and became unproductive. To save this biological diversity a project is proposed "Description, and conservation of Central Anatolian honeybees and proliferation in its region" by ANG foundation and partially supported by TAGEM, Ministry of Food, Agriculture and Animals. In the framework of this project, apiaries consisting of the original honeybee colonies were visited and where it is possible colonies purchased and placed in the isolated conservation apiaries. After survey of the valley and its environs, the conservation apiary is established and the colonies were maintained using artificial insemination. The project continued for three years and ended on March, 2016. During three years morphological characterization done on each year and for the last three generations were compared. In the last generation, all the original colonies were samples and subjected to molecular analysis of mtDNA and nuclear DNA. Two mtDNA gene regions were amplified and sequenced. No variation found in COI-COII intergenic region and in CytB partial sequence. 10 microsatellites markers were also surveyed and compared among four original locations. The molecular analysis resulted in that four locations were not different from each other and formed a big population. Colony build up, wintering, honey yield, swarming tendency, and aggressiveness were tested for all the colonies. Last year several queen bees were reared and distributed to beekeepers for their performances. Also from the scientific data obtained from morphometrical

measurements, gene sequences and microsatellite allele frequencies, registration application will be made on May 2016 to register the Anatolian honeybee and the conservation apiary to the Ministry of Food, Agriculture and Animals. Currently the number of colonies reached to 300 and hybridization experiments will be followed in the new season. The production of reciprocal hybrids between Anatolian and Caucasus honeybees are anticipated and will be distributed to beekeepers to test their performances.

### ***Computer software for identification of honey bee subspecies***

*Adam Tofilski,*

*Anna Nawrocka,*

*Irfan Kandemir,*

*Stefan Fuchs*

Within honey bee (*Apis mellifera*) there are recognized more than 20 subspecies. It is well known that the subspecies differ in wing venation which can be used for their identification. However, there is lack of tools which can be used for the identification. The aim of this study was development of fast and easy identification method of honey bee subspecies. The method was based on wing measurements.

The study was based on 1800 wing images representing 180 honey bee colonies, 24 subspecies and four evolutionary lines. The wing images were obtained from Morphometric Bee Data Bank in Oberursel, Germany. On the wing images 19 points were marked manually using computer mouse. Methods of geometric morphometrics was used to analyze coordinated of the points. Canonical variate analysis allowed to correctly classify all the colonies. Identification of subspecies and evolutionary lines of honey bees based on wing measurements proved to be very efficient, which confirms earlier studies. The obtained results were used to develop computer software for identification of the subspecies. The software is freely available for both beekeepers and scientists.

In order to use the software at least ten workers from one colony should be collected and their forewings dissected. Images of the wings should be obtained using camera or scanner. The wing images should be opened in the software and 19 characteristic points need to be marked on each wing. The software calculates probabilities that the measured colony belongs to each of the subspecies. The colony should be classified as subspecies with the highest probability.

***Mugla Honey Bee (Apis mellifera anatoliaca) Breeding Project Against Varroa***

*Rahsan Ivgin Tunca,*

*Devrim Oskay,*

*Servet Arslan,*

*Kemal Karabag*

Honeybee (*A.mellifera* L, Insecta: *Hymenoptera*) is an important organism due to its contribution to pollination and it has some important production besides being an important model organism for behaviour genetic studies. These substantial activities of honeybee has been ceasing because of high death rates related with various reasons. *Varroa destructor* is the most commonly known of them which has a negative influence on the honey bee health. In the current project was carrying on Muğla Honey Bee populations in which ecotype of *A.m. anatoliaca*. The subject of of the project was to select of the resistance honeybees against varroa and reare hygienic honeybee populations.

Closed population breeding program was applied in the selection program and the selection works will take next three years. Selection program was started with a hundred colonies and the colony numbers will be increased to two hundreds at end of the project. Three different tests (sugar powder for adult bees, examine brood cells and bottom board) are applied in order to detect varroa infection levels. According to counting results, we determined selection indexes and scores for breeding program. This breeding project is supported by Mugla Beekeeping

### ***The Greek Train of Virgin queens***

*Fani Hatjina*

<https://youtu.be/3PHUmeiRvC8>

Under the three-year program 'Improvement of conditions of production and marketing of apiculture products 2014-2016' Regulation 1234-1207 of the EC. In the Division of Apiculture - Institute of Animal Science, we are carrying a research project entitled 'Preservation, improvement and conservation of genetic material of Greek bee populations - CHARTA MELISSA - the characterization and identification of the Macedonica, Cecropia and Cretan bee through natural and artificial mating methods- to create the basis for the National conservation program and improvement of Greek bee populations'. As part of this project the implementation of an innovative method would be for the controlled, yet natural mating of queen bees. Until recently the only way to ensure the genetic origin of the drones and controlled mating was the artificial insemination, method demanding, expensive and not at all easy to use by each producer. The 'Train of virgin queens' (TVQ) is an innovative method which has been applied only in New Zealand. The method requires a cage, in which the virgin queens kept at a temperature of 14-15 C and in the darkroom, the nuclei are rolling on rails. At the afternoon and when all available free drones have returned to their colony a) we release the selected drones and b) we pull over the rails with the nucleous with the virgin queens, place them in specific positions and release the queens them for natural insemination The 'Train Virgin queens 'ensures the placement of hives always the same positions for the correct orientation of queens and couplings only with selected drones.

The first time it was used in Greece, the efficiency of the matings was above 50%. This year, it will be tested for both macedonica and cecropia queens, and at the same time observations will be made on the behavior of the queens.

## LIST of PARTICIPANTS

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