# Research Network of Sustainable Bee Breeding
# 2021 Online Workshop
# Tentative Agenda

### Tuesday March 16, 15:00 CET

15:00 Introduction and Overview of Recent Task Force Achievements - Marina & Cecilia

15:30 Discussion
1) COLOSS funding of 2500 CHF for the Task Force. Suggestion to split into five portions for open access support for common publications.

Feel free to break off a piece of chocolate and have a sip of coffee.

**Presentations**

16:00 Fani Hatjina et al - "SafeAgroBee" Safeguarding agroecosystem resilience under climate change through efficient pollination and sustainable beekeeping

16:30 Ralph Büchler et al - EurBeST study on resistant honey bees in Europe

17:00 Marin Kovacic et al - BeeConSel (Joint Effort for Honey Bee Conservation and Selection)

Please leave time for discussion after each talk.

### Wednesday March 17, 15:00 CET

15:00 Brief welcome back

15:10 Dario de Nart, Cecilia Costa et al - Subspecies classification by image recognition

15:40 Dora Henriques et al - A SNP assay for assessing diversity in immune genes in the honey bee (Apis mellifera L.)

Short break

16:20 Melanie Parejo et al - Developing a SNP-based paternity assignment test in honey bees for evaluating effectiveness of mating stations

Please leave time for discussion after each talk
16:50 New common Projects -GEI 2.0, SNP genotyping, REC project of TF Survivors

# "SafeAgroBee" Safeguarding agroecosystem resilience under climate change through efficient pollination and sustainable beekeeping

Fani Hatjina [1] and SafeAgroBee Consortium [2]

(1) Department of Apiculture HAO-DEMETER/ HAO-API, Greece
(2) SafeAgroBee Consortium members

SafeAgroBee is a new 3-year PRIMA project for collaboration in the Mediterranean basin. The consortium consists of 13 partners, originated from 8 Mediterranean countries.

Its aim is to contribute to adaptation and mitigation of the effects of climate change and other drivers negatively influencing the sustainability and the resilience of the agricultural system in the Mediterranean basin, ensuring the income of farmers and food security; in short ensuring resilience and sustainability.

In SafeAgroBee we focus on beekeeping and pollination provided by both wild and managed bees as important drivers in ruling food security and human existence. To this aim, SafeAgroBee will specifically address the following: 1) examine the resilience of bee pollinators (Apis and non Apis bees) on a changing environment towards pollination services and productivity by a) documenting wild and domesticated bee contribution to the pollination of key crops; b) determining the carrying capacity of several crops as a novel approach for bee productivity and c) by projecting historical climatic data and bee related data in today's conditions; 2) investigate the adaptability of local bee populations and the application of optimal practices under climate change in order to ensure sustainable beekeeping by monitoring the development and the performance of local populations and their resistance to diseases; 3) support the development of mitigation strategies ensuring the health of the bees and provide advice for the beekeepers by comparing the health and productivity of the honey bee colonies between different agricultural ecosystems and by performing alternative and new strategies to control bee diseases; 4) develop innovative monitoring tools and precision apiculture systems for advanced data acquisition by building on sounds, bee movements and heat detection, also enhancing business potential; 5) test and validate novel models for predicting the health of the bees, as for example the Health Status Index, as well as their productivity in terms of honey and pollination services based on two strong conditions, i) on previous experience, developments and knowledge and ii) on the large data sets with very accurate data and from diverse climatological conditions; 6) adopt a Citizen Science approach to interact and collaborate with all stakeholders and the citizens thus performing a multi-actor approach at all levels; furthermore by forming an active Advisory Board will ensure the usefulness and credibility of the new farming strategies to be recommended; 7) contribute to SDGs.

# EurBeST study on resistant honey bees in Europe

Ralph Buechler [1], Cecilia Costa [2], Fanny Mondet [3], Aleksandar Uzunov [4], Marin Kovacic [5], Marina Meixner [1], Yves le Conte [3], Sreten Andonov [4]

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(4) Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje 1000, Macedonia
(5) J.J. Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, V. Preloga 1, 31000, Osijek, Croatia

The EurBeST study explores possibilities for increasing the varroa resistance of commercially available honey bees by selective breeding and analyses ways to improve beekeepers’ access to resistant material.

Analysis of the EU market for reproductive material, including queen production and trade, shows high diversity of organisation, but also a weak development of breeding structures in some countries. EU beekeepers are mostly satisfied with the quality of breeding material, except for varroa resistance traits. Despite a growing demand, no established market for varroa-resistant stock in Europe exists, and supply of queens is limited.

To provide reliable data regarding the performance of resistant stocks, specific lines were compared under commercial conditions in five case studies, including traditional traits and varroa resistance (VSH, SMR, REC, hygienic behaviour). Strong genotype-environment interactions affected many traits, highlighting the importance of local adaptation. Beekeepers’ own lines produced more honey, but the test lines had fewer mites at the end of the study. Some lines from long-term selection programs combined good productivity and improved varroa resistance.

A cost-benefit analysis provides insights into the economic aspects of queen production, colony evaluation, and selection for varroa resistance.

Based on the results, recommendations are being developed for beekeepers involved in breeding and for policy makers. The findings will be presented and discussed in detail during an online conference in April 2021 (https://www.eurbest.eu/Contact/eb-conference/).

**BeeConSel**

Marin Kovačić [1], Janez Presern [2], Bjorn Dahle[3], Sreten Andonov[4], Aleksandar Uzunov[4], Borce Pavlov[5], Goran Aleksovski[5], Zlatko Puskadija[6].

(1) Faculty of Agrobiotechnical Sciences Osijek, Croatia

(2) Agricultural Institute of Slovenia, Slovenia

(3) Norwegian Beekeepers Association, Norway
(4) Saints Cyril and Methodius University of Skopje, North Macedonia

(5) Macedonian association for conservation of Macedonian native honey bee (MacBee) North Macedonia
(6) University of Osijek, Croatia

The project "Joint Effort for Honey Bee Conservation and Selection" (BeeConSel) aims to research the queen mating through (1) the observations of the honey bee queen nuptial flights and (2) the measurement of success of mating control strategies (island, valley, forest, highland, non-isolated flatland and "horner" method) by the usage of DNA microsatellites that enable the characterization of the patrilines. In the frame of the first work package, our main activity is to overview the status of current mating control strategies in project partner countries (Slovenia, North Macedonia and Croatia) and we have developed a survey on this manner. Survey also includes part with queen production. Now we are thinking to go beyond our project obligations and to expand the survey on more countries and through this workshop we are seeking for interested partners who are willing to conduct a survey among breeders.

We will continue the meeting on Wednesday the 17th of March 2020, hope to see you all.

## **A SNP assay for assessing diversity in immune genes in the honey bee** (*Apis mellifera* L.)

Dora Henriques [1], Ana R. Lopes [1], Nor Chejanovsky [2], Anne Dalmon [3], Mariano Higes [4], Clara Jabal-Uriel [4], Yves Le Conte [3], Maritza Reyes-Carreno [3], Victoria Soroker [2], Raquel Martin-Hernandez [4,5], M. Alice Pinto [1]

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(5) Instituto de Recursos Humanos para la Ciencia y la TecnologIa (INCRECYT-FEDER), Fundacion Parque Cientifico y Tecnologico de Castilla-La Mancha, 02006 Albacete, Spain.

With a growing number of parasites and pathogens experiencing large-scale range expansions, monitoring diversity in immune genes of host populations has never been so important because it can inform on the adaptive potential to resist the invaders. Population surveys of immune genes are becoming common in many organisms, yet they are missing in the honey bee (Apis mellifera L.), a key-stone species that has been severely affected by biological invasions. To fill the gap, here we identified single nucleotide polymorphisms (SNPs) in honey bee immune genes and then developed an assay to be genotyped using the high-sample-throughput iPLEX MassARRAY system that can be readily used for population surveys. The assay was constructed using SNPs detected in whole-genome scans of 123 individuals originating from a wide geographical area, representing seven A. mellifera subspecies and three evolutionary lineages (M-Western European, C- Eastern European, A- African). In this dataset, a total of 35,782 SNPs distributed through 180 genes were found. Only polymorphic SNPs (MAF>0.05) SNPs located in putatively functional regions were retained. Other filtering criteria inked to the MassARRAY® MALDI-TOF genotyping system were used and an assay with 107 SNPs was obtained. A total of 16 SNPs were discarded either due to inconsistent calls and/or misidentification of heterozygous positions. The final assay contains 91 quality-proved functional SNPs covering 89 innate immune genes. The 89 genes belong to several families and pathways, such as IMD, JAK-STAT, Toll and RNAi. This gene set also includes genes that have been found to be expressed in honey bees infected with the viruses SINV, IAPV, DWV and Nosema spp. This medium-density-SNP assay was applied to 156 samples from four countries (Portugal, Spain, France and Israel) and the admixture analysis clustered the samples according to their lineage and subspecies, suggesting that the immune SNPs can be also used to reconstruct population structure. This newly-developed SNP assay can be applied to monitoring diversity in immune genes, identifying the genetic basis of disease susceptibility, and even inferring genetic structure.

# Developing a SNP-based paternity assignment test in honey bees for evaluating effectiveness of mating stations

Melanie Parejo [1], Egoitz Galartza [2], Andone Estonba [1]

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(2) ERBEL, Iberian Bee (Erle Beltz) Breeding Association, Zaldibi-Basque Country, Spain

Honey bees have a special mating biology, where young queens mate multiple times in flight far away from their colonies at so-called drone congregation areas (DCAs). To advance towards set breeding goals, mating control is crucial. Geographically isolated mating stations with selected strong drone-producing colonies are widely used throughout Europe in an effort to guide mating of the virgin queens that are placed at the stations for several weeks during spring and early summer. To test the reliability of such mating stations to control mating, we are developing a paternity assignment test for honeybees based on the recently published genotyping tool for subspecies assignment that includes 4165 SNPs (single nucleotide polymorphisms). We genotyped for these SNPs 25 drone-producing colonies of the mating station in Ataun, Basque Country, as well as 150 worker offspring of 16 queens mated at the station. To determine the degree of relationship between possible “fathers” (i.e., drone producing colony) and worker offspring, we calculated the kinship coefficient defined as the number of minor alleles shared between two individuals, divided by the average number of minor alleles between the two individuals, also taking into account the probability of identity by state equaling zero P(IBS=0) quantified as two individuals presenting opposite homozygous opposite allele calls. Using our approach, we could identify first-degree relations (parent-offspring) and assign worker bees to their putative “father”. We encountered some difficulties, amongst other low genotyping rate of some samples, and give recommendations for future studies.

## Subspecies classification by image recognition

Dario De Nart [1], Cecilia Costa [1], Gennaro Di Prisco [2], Emanuele Carpana [1]

(1) CREA Research Centre for Agriculture and Environment
(2) IPSP-CNR Institute for Sustainable Plant Protection

We investigated whether image recognition techniques applied to honey bee wings could be used to discriminate among honey bee subspecies. Samples of honey bees from a subspecies reference collection kept at CREA, augmented by specifically collected samples, were used to obtain a dataset consisting of eight classes (7 European subspecies and Buckfast) with 9887 wing image samples.

Four well-known convolutional neural network models were considered in the experiment: ResNet 50, MobileNet V2, InceptionNet V3, InceptionResNet V2. The latter two gave the highest recognition accuracy (0.96 and 0.97 respectively). The Inception ResNet model achieved slightly higher scores of Precisions (the fraction of positive values that are true positives) and Recall (the fraction of true positive samples correctly labelled by the system) for most classes. All tested models significantly outperformed traditional morphometric evaluation. We conclude that automatic image recognition and machine learning, applied to the honey bee wings, could represent a useful tool for fast classification of honey bee subspecies for breeding and conservation aims.