



Action FA0803



COLOSS Work Shop

Standardized protocols for honey bee vitality and diversity

26.-27.02.2010

Lab of Agricultural Zoology & Entomology

Agricultural University of Athens

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**NATIONAL AGRICULTURAL
RESEARCH FOUNDATION**



**AGRICULTURAL UNIVERSITY
OF ATHENS**



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Agenda

TIME	PROVISIONAL PROGRAM
25.02.2010 (Thursday) Astor Hotel Arrival and informal social gathering in the evening	
20:00	Welcome dinner in roof garden of Astor Hotel
26.02.2010 (Friday) Lab of Agricultural Zoology & Entomology, Agricultural University of Athens	
09:00 – 09:30	Registration
09:30 – 10:30	Welcome and organizational matters
10.30 – 11.30	Subgroups' meeting
11.30 – 12.00	Coffee break
12.00 - 13.30	Subgroups' meeting
13.30 – 14.30	Lunch.
14.30 – 16.00	Joint meeting of both Subgroups
16.00 – 20.00	Visit to the New Museum of Acropolis
20:30 – open	Social dinner
27.02.2010 (Saturday) Lab of Agricultural Zoology & Entomology, Agricultural University of Athens	
09:30 – 11:00	Subgroups' meeting
11:00 - 11.30	Coffee break
11:30 – 14:00	Joint meeting of both Subgroups
14:00– 15.00	Lunch, end of workshop meeting

Local Organizers: Maria Bouga, Fani Hatjina

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Interactions between the genetic origin of the bees, the environment and pathogens – a part of Coloss experiment going in Poland

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In the beekeeping season of 2009 within an international project "Prevention of honeybee Colony Losses" three experimental apiaries were created in Poland. The apiaries were located in various parts of the country with different climatic and foraging conditions. Altogether 124 colonies with queens belonging to 8 lines of bees were prepared. In each apiary queens belonging to 4 different lines were introduced: south-east part of the country, Kunki - 37 colonies: CarG GR1 from Pulawy, Poland (10 colonies), CarP Kortowka from Olsztyn, Poland (10 colonies), MacB Macedonica from Bulgaria (10 colonies), Mel P Augustowska from Poland (7 colonies); central Poland, Bronowice near Pulawy - 44 colonies: CarC from Croatia (7 colonies), CarG GR1 from Pulawy, Poland (13 colonies), CarP Kortowka from Olsztyn, Poland (12 colonies), Car V Veitshöchheim from Germany (12 colonies); and in northern Poland, Gasiory - 43 colonies: CarC from Croatia (12 colonies), CarK from Kirchhain, Germany (11 colonies), CarL from Lunz, Austria (9 colonies), CarP Kortowka from Olsztyn, Poland (11 colonies). All colonies were similar strength before wintering. In October, in the colonies were observed different amounts of brood, depending on the localization of the apiary. All experimental colonies were treated in autumn against *Varroa* mite with Apiwarol AS with amitraz in the form of fumigation and with oxalic acid. In some of experimental colonies *N.apis* or *N.ceranae* was found, in very few colonies both, *N.apis* and *N.ceranae*.

Experimental set up and first evaluation of the genotype/environmental interactions experiment in Greece

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A number of 40 honey bee colonies were prepared in the summer of 2009 to receive 40 newly mated queens originated from *A.m. macedonica* (from Greece), *A.m. ligustica* (from Italy), *A. m. carnica* (from Germany) and *A.m. carnica* (from Bulgaria).

The colonies were uniformly prepared by using frames with no brood from original colonies situated in the apiary of Hellenic Institute of Apiculture in N. Moudania-Greece. The first evaluation of the colonies' status was performed on 22nd of October 2009. Using the same methodology with all the other partners of the experiment, we evaluated the strength of the colonies in terms of number of bees, the brood area of the colonies, the infestation level of Varroa on adult bees, and we took the total weight of the colonies (hives+ bees+ food). Samples of bees were also taken for future analysis of viruses.

The analysis of the genetic origin of the colonies will be determined next spring.

In average, all colonies were relatively small, with little honey storages and small brood areas. Average Varroa levels were about 2 mites per 10g of bees and average weight of the colonies was 18 Kg. No treatment for Varroa was performed. Two months later, on 22nd of December 2009, a 2kg sugar cake was given to all colonies.

As all groups were relatively uniform in strength, we expect to see the first evidence of a possible better adaptation even from this over-wintering time.

Breeding projects in Italy

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The Italian bee research unit CRA-API is responsible for coordination of breeding activities inside the National queen breeders registry, which was set up by Ministerial Decree in 1997. The aim of the registry is to protect and improve the native Italian races *Apis mellifera ligustica* and *A. m. sicula*. The research unit organizes performance testing, anonymous distribution of queens, courses for testers, data collection, biometric and genetic analysis. The traits currently screened in routine performance testing are honey production, docility, swarming tendency and, to a lesser extent hygienic behaviour. However a project is underway to introduce vitality traits in routine testing of the registry queens, and to include these in the breeding values, which are calculated according to the modified BLUP method by the Hohen Neuendorf Institute (Germany).

In the EU Rural development policies framework, several beekeepers' Associations, in collaboration with CRA-API, are planning to set up specific *A. m. ligustica* conservation programmes, in which vitality traits will be given priority. Furthermore, besides ongoing efforts for *A. m. sicula* conservation in Sicily, a new reintroduction project is underway. It is therefore important to have reliable protocols to plan and coordinate vitality testing methods and conservation techniques. Discussion among international breeding experts and comparisons of similar breeding and re-introduction schemes established in different countries will contribute to this aim.

Influence of selected queens on colonies losses

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Colony losses survey in Republic of Croatia has been organized in collaboration with Croatian Agricultural Agency. From the totally surveyed 89813 colonies from 910 beekeepers average colony losses were 10.26% during 2008 year. Incidence of colonies losses at apiaries of 8 surveyed queen breeders was 2.81% in the same period.

Total colony losses during 2008 year on surveyed apiaries

	Beekeepers	Queen breeders
No. of wintered colonies 2007/08	87962	1851
Lost colonies	9026	52
Total colony losses during 2008 (%)	10.26	2.81

Main reasons of losses according to beekeepers opinion:

Reason of loss	Beekeepers	Queen breeders
1. Varroa (%)	42.39	48.08
2. Problems with queens (%)	20.34	17.30
3. Nosematosis (%)	16.02	3.85
4. Food shortage (%)	12.97	9.62
5. Unknown	8.28	21.15

In 2008 year beekeepers did not reported presence of typical CCD symptoms.

Allozymes as possible markers for the discrimination of honey bee populations

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Honeybee is a well-adapted insect with great economic importance and exists in different ecological conditions in the world. Traditionally, taxonomy of the honey bee *Apis mellifera* has been based on morphology and at present, 29 subspecies are recognized on the basis of classical morphometry. The natural populations of local honeybees are subject to strong genetic contamination which resulted from the intensive transfer of different subspecies through whole Europe. Different biochemical-genetic analyses have been used extensively in studying the genetic diversity of organisms and are appropriate for studying of *Apis mellifera* variability also. As allozymes are different forms of the same enzyme protein coded by the different alleles of the same gene, data about their polymorphism could be useful for understanding the subspecies discrimination and revealing the existence of hybrid zones between them. Allozymes could also be used as genetic markers in characterization of genetic differentiation among the honey bee races and populations, in analyzing the phylogeny of *A. mellifera* and to detect significant genetic differences between commercial and feral honey bee populations.

By usage of electrophoresis in starch and PAA gels, polymorphism of different allozyme systems (MDH, ME, EST, PGM, HK, ALP, etc.) have been investigated. Two to seven alleles on polymorphic loci studied have been reported by different authors.

For characterization of populations from different honey bee races, allele frequencies, mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium, Nei's genetic distance (D), and Wright's fixation index (F_{ST}) could be calculated using BIOSYS-1. Phylogenetic trees could be constructed by UPGMA and Neighbour-Joining methods using the PHYLIP software package.

Varroa infestation as colony health assessment and vitality indicator in Genotype-Environment experiment

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The ectoparasite mite, *Varroa destructor* is the most destructive parasite of honey bees, *Apis mellifera*. *Varroa* infestations cause weight loss, reduced longevity in adult bees which result in low productivity or colony mortality. *Varroa destructor* also shows that it is a vector of various viruses. Thus it is the key component of the vitality evaluation of the GxE experiment to be followed. The parameters related to *Varroa* infestation measures are the infestation level as percent infested brood or adults and the intensity of infestation as number of mites per pupal cell. The threshold infestation level is usually considered as 10%, and the intensity of infestation is considered as moderate in case of 1-4 mites per pupal cell, high infestation if 5-6 mites per pupal cell is encountered. The *Varroa* infestation and intensity levels are related to colony sizes and production.

On the other hand, other factors such as genetic differences between the honey bee races being tested will be affecting the *Varroa* infestation levels since they are found to differ in hygienic behavior, biting, and grooming behaviors. Remaining differences will be attributed to the environmental conditions the colonies are maintained and comparisons will be made between the races and the locations.

Standardization of the method, timing, and intervals of assessment of *Varroa* infestations as well as other parameters will be finalized during the workshop. Those methods and protocols will be applied to the vitality tests of the three races at three different climatic regions that are started as part of the GxE experiment of COLOSS Working Group 4.

Comparing genetic diversity for European population

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Fitness and vitality are not synonyms. Fitness is define in the sense of Darwin and relates to the reproductive output of individuals or colonies. In contrast WG4 has chosen the term vitality to describes bees health in general and in particular the capacity to withstand environmental challenges, like climatic changes, parasite and pest pressure, with minimal assistance from the beekeeper.

The importance of genetic diversity has been noted at the individual, the colony, the population, subspecies and species level in honey bees. There are examples of reduced fitness at the individual and colony level, due to reduced genetic diversity. At the higher levels, the capacity to adapt to changes in the environment demands for genetic diversity. Therefore, genetic diversity at those levels is important too, and of course in closely connected to that of the lower levels. Breeding often leads to a reduction of genetic diversity and as a result could be as detrimental to honey bee population vitality.

Comparison of genetic diversity for various populations of bred honey bees from Europe and wild bees of Africa seem to support this view, however there are additional factors to consider.

I present data to examine the claim that reduction in genetic diversity is the result of breeding. Bees were collected from a range of population of several subspecies, and indeed the level of diversity is considerable higher in African bees. However, closer examination of the results from Europe is needed to infer to what level breeding impacts on genetic diversity. Another claim is that the introduction of foreign subspecies has a major impact on established local populations. I will discuss the available methodologies to examine this. Do we need to consider to protect the diversity of African bees?

Update of the Genotype – Environment Interaction Test in France

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Two different sites are use in France for the Genotype – Environment Interaction Test in the framework of COLOSS. One is located near Toulouse and managed by Olivier Celle, including 30 colonies. The other near Avignon includes 30 colonies managed by Yves Le Conte. The queens were introduced in the colonies lately, just after the Apimondia meeting in Montpellier. Most of the queen were accepted and were ready for wintering. We will present the update of this experiment, including differences in survival rate, development and behavior of the different stocks.

Sustainable breeding strategies and the conservation of honey bee biodiversity in Europe

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The existing subspecies and ecotypes of honey bees in Europe represent an important resource for breeding of disease resistant strains. Several methods are being used to characterize European honey bee populations and much information has been collected over the years, however, it is only partly accessible in reference data bases. One of our aims is to create a published and accessible reference data base that will be of use to scientists and apiculturists working in the field of European honey bee biodiversity and conservation.

To include criteria relevant to vitality into honey bee breeding and enable sustainable breeding strategies based on regional populations, standard methods to assess the status of colonies, including their health condition will be developed. This includes parameters of colony strength, brood area and food status, and especially the level of infestation with *Varroa destructor* and other relevant diseases. Methods will be adapted and validated according to regional conditions of climate and environment in Europe. International breeding recommendations including characters related to colony vitality will be designed, consisting of both the theoretical framework and technical and methodological aspects.

Morphometric studies of certain lines of *Apis mellifera carnica* Pollman from Serbia

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During the previous decades the natural populations of honeybee *Apis mellifera carnica* Pollman at the territory of Serbia has been exposed to migratory beekeeping and uncontrolled imports and crossbreeding with the other strains of honeybee.

For the purpose of their further breeding, researches upon the metric morphology of certain individual populations of choice at the territory of Republic of Serbia. During research period three selected lines of honey bee from three different localities in Serbia (East-line 1, West – line 2 and South Serbia-line 3) were studied. The metric morphology examinations have been conducted upon the sample of 50 bees taken from three geographically rather distant sites of the Republic of Serbia. Each bee has been fixated at the 70% ethanol, and then the permanent microscopic preparations have been made of the right forewing.

The preparations have been viewed through the Leica XTL-3400D binocular microscope, and the measurements of lengths and ten angles (A4, B4, D7, E9, G18, J10, J16, K19, L13, N23) of the forewing and the cubital index have been conducted by the IL 1009 software package in compliance with the Ruttner (1978) methods.

Morphometric studies have shown that the bees from South Serbia had the biggest value for angles B4 (110,3°), D7 (100,8°), E9 (22,6°), G18 (94,5°), J16 (92,5°), L13 (15,1°), N23 (93,9°). The three Carnica groups are very similar u pogledu veličine uglova. However, value of cubital index was varied from 2,6 in line 2 to 2,3 in lines 1 and 3. It is important for further preserve authenticity of the species and improvement of the selection of domestic honey bee.

Relationship between genetic origin of honeybee queens and their performance

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Four honey bee lines originated from 4 different regions of Serbia and Montenegro and other 4 honey bee lines originated from 4 different regions of Greece have been set up for experimentation during summer 2009. The aim of the experiment is to evaluate the performance of the specific queens for 3 years and to find the existing relationship between their genetic origin and their performance.

Classical morphometrics, geometrical morphometrics and mitochondrial DNA analysis are the tools to be used in order to define the genetic differences between the populations from each country. At the same time, performance characteristics such as strength of colony (population), brood area, aggressiveness, swarming, hygienic behaviour, honey storage, honey yield, pollen storage will be evaluated 4 times per year and for the duration of 3 years.

Working plan for genotype and environment test in China

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To reveal interactions between genotype and environment, *Apis mellifera* subspecies /strains in China will be studied. A comparative test on vitality has established in various locations Of China, such as: Liaoning province (temperature zoon), Beijing (temperature zoon), zhenjiang province (sub-tropic zoon) and Hainan province (tropic zoon). The local *Apis mellifera* (*A. m. ligustica* & *A. m. carnica*), which has been kept in China since 1930s, newly imported subspecies/strains, the honey bee queens were imported to China in the last 10 years, and honeybees in their export areas of Europe, will be studied. Both Breeding protocol and testing design that has been discussed in WG4 will be transferred into the testing areas of China, the *Tropiladaps clareae* will be an extra parameter were test in China. In the coming workshop, I would like to discuss with WG4 members on more detailed conducting technique and working partners.

Relationship between Varroa Mite Infestation levels and honey bee colonies survival rate

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In autumn/winter 2009 in 5 different bee yards in Republic of Macedonia total of 70 colonies were controlled for estimation of Varroa mite infestation by usage of method of washing adult bees. In average more than 30 grams of bees were sampled from side frames on the top super in the hive, weight and washed by soapy water in laboratory. The mites were counted on fine mesh, data were analyzed on colony and bee yard level and indexed as number of mites in 10 grams of bees.

The average mite infestation in 10 grams of bees was: bee yard 1 - 0,45, bee yard 2 - 0,54, bee yard 3 - 0,60, bee yard 4 - 16,37 and bee yard 5 - 2,73.

All colonies were controlled once per month for evaluation of the influence of mite level infestation on honey bee colony survival. During the autumn/winter seasons in 2009/2010 4 controls were performed (October, November, December 2009 and January 2010).

In control period there were not evident negative effects on colonies winter cluster size or survival rate. On contrary, in January 2010 we observed 50% colony losses (5 colonies out of 10) in bee yard 4. This bee yard was with highest infestation level of 16,37 mites on 10 grams of bees.

Additional analysis, regarding the number of bees and brood cells in the colonies, is going to be made after completion of whole winter period. This investigation is a part of GEI common experiment.

List of participants

Bouga	Maria	Greece
Büchler	Ralph	Germany
Charistos	Leonidas	Greece
Costa	Cecilia	Italy
Hatjina	Fani	Greece
Ivanova	Evgeniya	Bulgaria
Kence	Meral	Turkey
Kezic	Nikola	Croatia
Kokkinis	Michalis	Greece
Kryger	Per	Denmark
Le Conte	Yves	France
Meixner	Marina	Germany
Nedic,	Nebojsa	Serbia
Panasiuk	Beata	Poland
Rasic	Sladjan	Serbia
Shi	Wei	China
Uzunov,	Aleksandar	former Yugoslav Republic of Macedonia ¹
		¹ UN temporary reference
Wilde,	Jerzy	Poland