Action FA 0803

COLOSS WORK SHOP

WG4

"HONEY BEE VITALITY AND DIVERSITY - STATUS OF THE GENOTYPE - ENVIRONMENT INTERACTIONS EXPERIMENT AND THE SUBSPECIES DISCRIMINATION METHODS COMPARISON "

01 - 03 MARCH 2011

PLOVDIV

BULGARIA

DEPARTMENT OF DEVELOPMENTAL BIOLOGY
BIOLOGICAL FACULTY

PLOVDIV UNIVERSITY "PAISII HILENDARSKI"

LOCAL ORGANIZER:

PLOVDIV UNIVERSITY“PAISII HILENDARSKI”

BIOLOGICAL FACULTY

Department of Developmental Biology

CO-ORGANIZERS:

NATIONAL BEE BREEDING ASSOCIATION

AGRICULTURAL UNIVERSITY – PLOVDIV

UNION OF SCIENTISTS IN BULGARIA – PLOVDIV

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AGENDA

TIME	PROGRAM
01.03. 2011 (Tuesday) **Place: Union of Scientists in Bulgaria – Plovdiv: 6, Mitropolit Paisij Str.**	
10:00 – 12:00	WG4 registration at the House of Bulgarian Scientists - Plovdiv.
12:00 – 12:45	**Welcome and organizational matters** Greetings from the Rector of the Plovdiv University and the Head of the Department of Developmental Biology
12:45 – 13:30	**GEI experiment** Ralph Büchler, Stefan Berg; Malgorzata Bienkowska, Beata Panasiuk, Yves Le Conte, Cecilia Costa, Winfried Dyrba, Fani Hatjina’ Leonidas Charistos, Evgeniya Ivanova, Plamen Petrov, Nikola Kezic, Seppo Korpela, Per Kryger, Hermann Pechhacker, Aleksandar Uzunov, Jerzy Wilde **First results of the international genotype-environment interaction experiment** **Discussions**
13:30 – 14:30	Lunch
14:30 – 16:00	**GEI experiment** Malgorzata Bienkowska, Beata Panasiuk, Jerzy Wilde **Progress or problems with the Polish part of GEI experiment?** Cecilia Costa, Marco Lodesani, Kaspar Bienefeld **Presence of genotype – environment interactions in *Apis mellifera ligustica* in Italy** Seppo Korpela, Lauri Ruottinen, Ari Eskola **Performance of four bee strains of the GEI experiment in Finland in 2009-2010**

	Varoa, Nosema, hygienic behaviour: Aleksandar Uzunov, Hrisula Kiprijanovska, Ilija Petrovski, Ivan Dodevski, Sreten Andonov **Analysis of *Varroa* mite population development in relationship with brood area, bee population and initial infestation level in experimental honey bee colonies** Kalinka Gurgulova, Plamen Petrov, Rumen Valchovski ***Nosema* and *Varroa* Infestation in Local Bulgarian Honeybee** Slađan Rašić, Mića Mladenović, Ljubiša Stanisavljević **Hygienic behaviour of four different bee lines from Serbia and Montenegro** **Discussions**
16:00 – 16:30	Coffee break
16:30 – 18:30	Visit the Old Town Plovdiv
19:00	Welcome dinner in Restorant Philippopolis: 29, Saborna Str.
02.03. 2011 (Wednesday) **Place: Agricultural University – Plovdiv: 12, Mendeleev Str.**	
09:00 – 10:30	Greetings from the Rector of the Agricultural University – Plovdiv **Genetic variability** Marina Meixner, Per Kryger, Roy Francis, Maria Bouga **Genetic variability of honey bee origins used in the GEI experiment: microsatellite analysis** Evgeniya Neshova Ivanova **Genetic variability of honey bee origins used in the GEI experiment: preliminary data of alloenzyme analysis** Stanimila R. Nikolova, Evgeniya Ivanova **Investigation on genetic variability in honey bee population from Bulgaria**

	based on microsatellite analysis **Discussions**
10:30 – 11:00	Coffee break
11:00 – 13:00	**Morphometry:** Nebojša Nedić, Mića Mladenović, Ljubiša Stanisavljević, Goran Jevtić **Analysis of some morphometric characters of honeybees from Serbia** Rahsan Ivgin Tunka, Evgeniya Ivanova, Meral Kence **A test of the discrimination power of traditional and geometric morphometric methods on honey bees (*Apis mellifera*) from Bulgaria and European part of Turkey** Leonidas Charistos, Mića Mladenović, Fani Hatjina **Performance comparison between four different Greek honey bee lines** **Discusions** Round table: “Honey bee Diversity and Vitality” with students and scientists (optional) or Discussion about future activities and possible joint publication of WG4
13:30 – 14:30	Lunch
14:30 – 18:30	Visit to apiary of National Bee Breeding Association: Base Vinitca
20:00	Social dinner in Restorant Megdana: 11, Odrin Str.
03.03. 2011 (Thursday) **Place: Union of Scientists in Bulgaria – Plovdiv/Biological Faculty of Plovdiv University**	
09:30 – 10:30	Discussion on standardized methodologies for the BEE BOOK and other matters.
10:30 – 11:00	Coffee break
11:00 – 12:30	Discussion on future plan activities of WG4.
12:30– 13:30	Lunch
13:30 – 14:00	Concluding the meeting

FIRST RESULTS OF THE INTERNATIONAL GENOTYPE-ENVIRONMENT INTERACTION EXPERIMENT

Ralph Büchler*1), Stefan Berg2); Malgorzata Bienkowska3), Beata Panasiuk3), Yves Le Conte4), Cecilia Costa5), Winfried Dyrba6), Fani Hatjina7), Leonidas Charistos7), Plamen Petrov 8), Evgeniya Ivanova 9), Nikola Kezic10), Seppo Korpela11), Per Kryger12), Hermann Pechhacker13), Aleksandar Uzunov14), Jerzy Wilde15)

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The international experiment to estimate the importance of genotype-environment interactions on honeybee vitality and colony losses was started in July 2009 with 621 colonies, involving 18 strains of European honeybees in 16 test locations spread all over Europe.

The common test protocol considers colony survival, bee population in autumn, spring and summer, productivity, swarming, gentleness, hygienic behavior and the infestation with Varroa, Nosema and viruses. No chemical treatments against Varroa and diseases were applied since 2010. In most test apiaries, all brood combs were withdrawn once during season in order to reduce the level of Varroa infestation.

23,8 % of the colonies were lost until the end of May 2010 and another 16,2 % were lost until the end of January 2011. Besides problems with the queens (23,1%), most losses were linked with symptoms of Varroa disease (25,6%), Nosema or defecation (7,9%) or weakness and robbery (5,8%). No clear symptoms were observed in 32,6 % of the cases.

The data analysis shows a strong influence of the test location on the strength of the colonies at all control intervals. However, we can also observe highly significant differences between the different strains and highly significant interactions between genotype and environment.

Regarding the Varroa infestation of bee samples, significant effects of the location, but not of the genetic origin have been observed. However, in the case of Nosema infection, the test environment, the genotype, and interactions between them all show highly significant effects.

The tested genotypes clearly differ in their honey productivity, gentleness and swarming tendency which can at least partially be explained as a consequence of different breeding intensity for these classical selection characters. However, it is important to note that even regarding these characters highly significant genotype – environment interactions can be observed.

To sum up our primary results, we can state a high relevance of interactions between honeybee genotypes and different environmental conditions within Europe. Obviously, the genetic adaption of honeybees to a specific environment influences its population dynamics, health status, and productivity. Consequently, the conservation of European honey bee diversity and the support of local breeding activities should be pushed forward.

GENETIC VARIABILITY OF HONEY BEE ORIGINS USED IN THE GEI EXPERIMENT: MICROSATELLITE ANALYSIS

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One of the main goals of COLOSS WG 4 is to establish a common protocol for the discrimination of honey bee populations. In Europe, a host of methods are used to determine the subspecies origin of honey bees. In WG4 different methods are currently applied to analyze samples of the colonies that are part of the common GEI experiment. DNA microsatellite data will be combined with classical and geometrical morphometrics as well as mitochondrial DNA analysis to compare the various methods.
The data from 371 colonies of *Apis mellifera mellifera*, *A. m. ligustica*, *A. m. carnica*, *A. m. anatoliaca*, *A. m. macedonica*, *A. m. siciliana*, and *A. m. caucasica* will contribute to the documentation of the genetic origin of each colony involved in the common experiment and to the establishment of a published and accessible reference database that will be of value to scientists and apiculturists working in the field of European honey bee biodiversity and conservation. Preliminary results from the microsatellite analysis will be presented, and samples for mtDNA will be distributed in Plovdiv, Bulgaria.

GENETIC VARIABILITY OF HONEY BEE ORIGINS USED IN THE GEI EXPERIMENT: PRELIMINARY DATA OF ALLOENZYME ANALYSIS

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Different methods are used to determine the subspecies origin of honey bees. COLOSS WG4 "Diversity and vitality" has the purpose to find common approaches for discrimination of honey bee populations in Europe. Genetic enzyme analysis is a classical method for studying the gene pool and genotype structure of populations and gives reliable information about genetic heterogeneity and gene flow among populations. In this aspect, in common GEI (genotype – environment – interactions) experiment of COLOSS WG4, data of enzyme analysis will be combined with microsatellite and mitochondrial DNA data, and classical and geometrical morphometrics in order various methods to be compared.

In this preliminary investigation, genetic variation in 14 honey bee populations of *Apis mellifera mellifera*, *A. m. carnica*, *A. m. ligustica*, *A. m. macedonica* and *A. m. siciliana* located in different regions in Europe was studied on 2 enzymic systems (MDH and EST) corresponding to 2 genetic loci. Allozyme analysis revealed that both loci were polymorphic in all populations studied. Three alleles were detected at MDH-1 locus (MDH 65, MDH 80 and MDH 100) and six – at EST-3 locus (EST 80, EST 88, EST 94, EST 100, EST 105 and EST 118. It was found that the mean number of alleles per locus varied from 2.5 to 3.5 and the observed and expected heterozygosities (H_o and H_e) ranged from 0.136 and 0.240 (in *A. m. macedonica* from Greece) to 0.483 and 0.446 (in *A. m. mellifera* from France), respectively. The estimated mean F_{ST} value was 0.2065 which shows that 20.65% of the overall genetic diversity observed was among populations. The values of genetic distance ranged from 0.00 to 0.472.

Data from this study will contribute to the documentation of the subspecies genetic origin involved in the common experiment and to the establishment of a published and accessible reference data-base that will be valuable for scientists and apiculturists working in the field of European honey bee biodiversity and conservation.

PROGRESS OR PROBLEMS WITH THE POLISH PART OF GEI EXPERIMENT?

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In the beekeeping season of 2009 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 bee queens belonging to 8 lines were prepared. Afterwards, groups of colonies with bee queens of 4 different lines were situated in each apiary: south-east part of the country, Kunki - 37 colonies: CarG GR1 from Pulawy, Poland, CarP Kortowka from Olsztyn, Poland, MacB Macedonica from Bulgaria, Mel P Augustowska from Poland; central Poland, Bronowice near Pulawy - 44 colonies: CarC from Croatia, CarG GR1 from Pulawy, Poland, CarP Kortowka from Olsztyn, Poland, Car V Veitshöchheim from Germany; and in northern Poland, Gasiory - 43 colonies: CarC from Croatia, CarK from Kirchhain, Germany, CarL from Lunz, Austria, CarP Kortowka from Olsztyn, Poland.

All the colonies were of similar strength before wintering with 13.309 of bees on average (from 11.854 to 15.456). In October, different amounts of brood were observed in the colonies. The highest number of cells with brood was noted in colonies in northern Poland (20.302) compared to central and south-east (respectively 1654 and 1589). In autumn, all experimental colonies were treated against *Varroa* mite with Apiwarol AS with amitraz in the form of fumigation and with oxalic acid. The highest number of mites fallen on hive board after treatments was observed in apiaries in Kunki and Gasiory (respectively 4.5 and 3.5 mite per 100 bees) and the lowest in Bronowice (1.1 mite per 100 bees). In some of the colonies *N.apis* or *N.ceranae* was found, while in very few colonies - both, *N.apis* and *N.ceranae*. Number of colonies lost during winter 2009/2010, recorded in mid-April was: in Kunki-15, in Bronowice-9 and in Gasiory-2. Losses of colonies reached on average 24% , furthermore the most of lost colonies were *Apis mellifera mellifera* (71%).

In the spring of 2010 different number of bee workers and brood cells were observed in particular apiaries. Analysis of dead bees collected from bottom boards showed significantly higher infestation with *Nosema* spores in colonies that were lost compared to surviving ones. In all the colonies that did not survive the winter, autumn infection with *Varroa destructor* mite was higher than in the surviving colonies. In Polish climatic conditions the bees cleared an average of 24% of damaged cells with the maggot (from 0% to 76%).

In 2010, a total of 90 colonies were prepared for winter (34 colonies less than in 2009).

PRESENCE OF GENOTYPE – ENVIRONMENT INTERACTIONS IN *APIS MELLIFERA LIGUSTICA* IN ITALY

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Genotype–environment interactions (GxE) are defined as genetic variation in phenotypic plasticity. When GxE occur, one genotype may be superior to another in a certain environment, but inferior to the same genotype in a different environment. The recent interest in locally adapted genotypes together with awareness of the negative consequences of biodiversity loss, has made breeders and scientists more aware of the importance of GxE.
The aim of this study was to assess whether in Italy, a country with many different environments but with large scale commercial breeding and a long tradition of selection towards general productive superiority, there are still locally adapted honey bee populations. To verify the existence and magnitude of GxE interaction three *A. m. ligustica* subpopulations were compared for honey production and spring development, in three habitats differing in flora and climate.

The results from a total of 165 colonies showed significant interactions for both considered traits. Interestingly, for two of the considered origins, it was clear that colonies produced most when kept in their region. This study will provide useful information for national coordinated breeding programs, in terms of choice of testing stations but also for biodiversity conservation policies.

PERFORMANCE OF FOUR BEE STRAINS OF THE GEI EXPERIMENT IN FINLAND IN 2009-2010

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Test sites in the common GEI experiment extend from Sicily in south to Jokioinen, Finland in north. Survival and performance of the strains could be expected to be significantly different among strains if some strains at a testing site originate from very different environments. In Finland we test strains LigF, CarL and CarB and MacGR. Of these, MacGR could be seen as a representant of a strain from a different environment.

Low food consumption is one property of bees in bees adapted to northern long winters. In winter 2009-2010 we could not find any statistical differences in winter food consumption between groups LigF, CarL and CarB, but MacGR consumed significantly (P=0,03) more than others. The disadaptation of MacGR to harsh conditions was also seen as statistically lower overwintering index (** difference in comparison with CarL). This lead also to more time needed to get colonies in good foraging strength and thus, honey yield was also lower in MacGR than in other groups (** difference in comparison with CarB). Autumn populations of the test groups did not differ significantly in autumns 2009 and 2010, so we get another set of wintering indices next spring.

Mite growth did not differ significantly between groups, but there are wide differences in varroa infestations within groups in autumn 2010. At least partly this can be caused by infestation pressure from foreign apiaries.

ANALYSIS OF *VARROA* MITE POPULATION DEVELOPMENT IN RELATIONSHIP WITH BROOD AREA, BEE POPULATION AND INITIAL INFESTATION LEVEL IN EXPERIMENTAL HONEY BEE COLONIES

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In late summer 2009, three testing bee yards were established country wide for evaluation of honey bee genotype and environment conditions interaction. The testing colonies were monitored in accordance with previously defined methodology for performance testing and sampling. The data were compared with results from other experimental colonies in 13 locations across Europe, which have applied the same methodology. The *Varroa* management program was based only on implementation of brood removal methodology in the mid summer period.

Varroa mite infestation level was evaluated by the method of washing bees. Levels of infestations were indexed as a number of mites in 10 grams of bees. The monitoring was performed on quarterly basis. First annual results (autumn 2009 / autumn 2010) indicated variance of the data. Still, the preliminary analyses of the data shows continuous development of *Varroa* mite population in the experimental colonies. According to the reached infestation level in autumn 2010 there are indications that the introduced brood removal control method has limited effects. In addition to the research, the differences were analyzed in relation with brood area, bee population and initial infestation on individual colony and group level.

During the testing period the mite infestation level in the experimental colonies in average increased from 1.7 (autumn 2009) to 12.1 mites /10 gr. of bees (autumn 2010).

NOSEMA AND *VARROA* INFESTATION IN LOCAL BULGARIAN HONEYBEE

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The aim of this study was to establish the *Nosema* and *Varroa* infestation in the local type honeybee colonies from different regions of Bulgaria. To determine *Nosema* invasion and *Varroa* infestation, a screening of 412 honeybee samples (colonies) from 98 apiaries in different regions of the country was made. Samples were collected during the period March – July 2009. The samples were investigated for microsporidian spores presence using light microscope analysis and results were confirmed by molecular diagnosis (PCR) to differentiate two types of *Nosema* – *N. apis* and *N. ceranae*. The infestation level of *Varroa* was determined by counting of mites per 100 bees. The microscopic analysis results showed that total 102 samples collected from 44 apiaries were positive for *Nosema* spores. The molecular investigation (PCR) showed presence of 146 positive colonies among 45 of all apiaries studied. *Nosema apis* was identified as present only in 2 samples from one apiary. At the same time, *Nosema ceranae* was observed in 144 samples among 44 of all apiaries studied. *N. ceranae* was found to be more prevalence than *N. apis*. Being more sensitive, PCR method gave good possibility to identify both types of *Nosema*. *Varroa* was found in the 97 samples collected from 50 apiaries. The spring samples were more infected by *Nosema* and had less infestation level of *Varroa*. Vice versa – the summer samples were more infested by *Varroa* and less invaded by spores of *Nosema*.

HYGIENIC BEHAVIOUR OF FOUR DIFFERENT BEE LINES FROM SERBIA AND MONTENEGRO

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In four honey bee lines, originated from 4 different regions of Serbia and Montenegro, classical morphometrics, geometrical morphometrics, mitochondrial DNA analysis and performance characteristics such as strength of colony (population), brood area, aggressiveness, swarming, honey storage, honey yield, pollen storage and hygienic behavior were investigated.

Hygienic behavior of honey bee is a very important trait, closely associated with most diseases of adult stage, as well as bee brood diseases.

Since hygienic colonies demonstrate resistance to brood diseases such as American foulbrood and chalkbrood, it may be worthwhile to increase the expression of hygienic behaviour through selective breeding, and thus strengthen these potential characteristics associated with resistance to V. destructor.

All tested lines differed in obtained results when implementing pin test even from the first year (2009).Differences between lines were in number of uncapping, and completely cleansed cells after larvae killing.

COMPARISON IN GENETIC DIVERSITY OF *A. M. MACEDONICA* AND *A. M. CARNICA* POPULATIONS FROM BULGARIA, GREECE AND SERBIA BASED ON ENZYME ANALYSIS

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Genetic variation of honey bee populations from six different locations corresponding to tree geographic regions in Bulgaria (North-western, North-central and North-eastern) and populations of *A. m. macedonica* from Greece, and *A. m. carnica* from Serbia was studied on 6 enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 genetic loci. Allozyme analysis revealed that all loci studied were polymorphic in almost all populations studied. Four alleles were detected at MDH-1 locus, three – at ME, five – at EST-3, three – at ALP, two – at PGM and three – at HK. For the Bulgarian populations, it was found that: 1) the mean number of alleles per locus varied from 1.8 to 2.5; 2) the estimated percentage of polymorphic loci was between 50% and 100%; 3) the observed and expected heterozygosities (H_o and H_e) ranged from 0.17 to 0.221 and 0.250 to 0.315, respectively; 4) the estimated mean F_{ST} value from allozyme data was 0.0443 which shows that 4.43% of the overall genetic diversity observed was among populations, as opposed to 95.57% within populations; 5) the values of genetic distance range from 0.002 to 0.036.

Genetic variation of honey bee populations from Bulgaria (local type *A. m. rodopica*), Greece (*A. m. macedonica*) and Serbia (*A. m. carnica*) was characterized based on the enzymic analysis also. There was found that: 1) all of the sixth loci studied were polymorphic in most of the populations; 2) the observed and expected heterozygosities ranged from 0.163 (Serbia) to 0.236 (Bulgaria) and from 0.248 (Greece) to 0.263 (Serbia), respectively; 3) Nei’s genetic distances range from 0.012 (between Greece and Bulgaria) to 0.157 (between Serbia and Bulgaria); 4) in UPGMA dendrogram two clusters were formed - Bulgarian and Greek populations are clustered together in first branch and Serbian population is grouped in the second cluster. Hypothesis that honey bees from Bulgaria and Greece might be two different ecotypes of *A. m. macedonica* has been discussed.

INVESTIGATION ON GENETIC VARIABILITY IN HONEY BEE POPULATION FROM BULGARIA BASED ON MICROSATELLITE ANALYSIS

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There are many different studies concerning race status and the degree of genetic diversity of local Bulgarian honey bee which are mainly based on classical morphometry and partially on biochemical-genetic analysis. In the last years, different DNA analyses have been used in order to clarify the race status of the local honey bee as a part of European genetic resource of *Apis mellifera*. The aim of the present study was to characterize genetic variability in honey bee populations from different regions in Bulgaria using microsatellite DNA analysis. A total of 1460 honey bees were analyzed. Individual DNA extractions were performed using Salting-out extraction method from tissue procedure (Qiagen Inc, Valencia, CA). DNA quality and quantity was checked by OD260/280 (Molecular Devices Inc, CA, USA) and Nanodrop 8000 (Fisher Scientific, USA). All honeybee DNA samples were analyzed for nine microsatellite loci; Ac011, A024, A043, A088, Ap226, Ap238, Ap243, Ap249 and Ap256. DNA amplification was done by Qiagen Multiplex PCR kit (Qiagen Inc. CA, USA) following two separate PCR amplification protocols. Aliquots of fluorescently labeled amplified DNA were mixed with formamide and GENESCAN-450 (ROX) Size Standard (PE Applied Biosystems, CA, USA) and genotyped on the ABI PRISM® 3130xl DNA Sequencer using the GeneScanTM Analysis v2.5.2. Population-genetic statistics were computed using GENEPOP software v 4.0 (Raymond M. & Rousset F, 1995.). Genetic diversity within populations was evaluated by computing allele frequencies and observed and unbiased expected heterozygosity. F-statistics were estimated using the estimators of Weir and Cockerham (1984). Deviation from Hardy-Weinberg equilibrium was tested and the Fis estimator calculated for each data set.

All nine loci were found to be polymorphic in all populations studied. The Bulgarian population of honey bees was characterized by possessing 188 alleles at 9 microsatellite loci, where 60 alleles could be described as rare with frequency distributions of less than 5% in all sub-populations. The heterozygosity in the Bulgarian bee populations was relatively high, ranging from 0.261 for locus Ap243 to 0.896 for locus Ap256. Number of alleles per locus was found to range between 10 at A043 locus to 38 at Ap256 locus.

Pairwise Fst were quite low, but still sufficient to indicate significant differences between some of the populations. Pairwise Fst values ranged from 0.019 to 0.116.

The results of this study will give additional information about genetic variability of Bulgarian honey bees. It will be useful for characterization of their race status and also for their comparison with other subspecies spread in Europe.

PERFORMANCE COMPARISON BETWEEN FOUR DIFFERENT GREEK HONEY BEE LINES

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The performance of four different lines of Greek honey bees was evaluated in consecutive years, in respect of population, brood production, pollen and honey stores, as well as hygienic behaviour, Nosema and Varroa prevalence during the seasons. The methodology used was the same as the one described for the GEI experiments running by WG4.

At the same time, molecular and morphometric analysis was performed in order to define possible subspecies differences among the populations. The scope of the study is to determine a) the specific characteristics of these lines, b) their genotypic differences and the way these could be used for future subspecies discrimination and c) to use the best lines for further breeding. Selective crosses between the lines could also be evaluated for their performance later on.

All honey bee queens were naturally mated and originated from different regions of the country, but the evaluated colonies were situated in one area. Here the first year's (2009) results on the queens' performance are presented.

ANALYSIS OF SOME MORPHOMETRIC CHARACTERS OF HONEYBEES FROM SERBIA

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Given that Serbia has a heterogenous relief and climatic conditions in which honeybees are reared, it is considered that thanks to adaptable abilities of honeybee various local forms adapted to specific environmental conditions have been created. Also, migratory beekeeping and trade in queens have aditionally exposed native population of bees to differentiation. For comparative morphometric investigations 50 worker bees were collected from each of the six groups originated from different localities in Serbia. The measurement of eight lenght on the hind wing (L1:L8) was done with stereo microscope Leica XTL-3400D, and software package IL 1009 in accordance with the standard method. From a total of eight lenght distances measured on the hind wing, seven linear character (length of L2, L3, L4, L5, L6, L7 and L8) had the highest value in bees from group 3 (southern Serbia). For character L1 maximum value (0.947 mm) was found in the group 5 (Eastern Serbia). Based on the results of the length of characters L5, L7 and L8, bees from group 3 (Southern Serbia) were highly significantly different ($P \leq 0.01$) than the all other five groups of bees. Minimum value of the characters L3, L6 and L8 were found in group 6 (eastern Serbia) and this group of bees were highly significantly different ($P \leq 0.01$) from all other groups of bees. Greatest variation was for the character of L5 (coefficient of variation: from 11.639% to 19.734%), whereas the lowest variability was for the character L8 (coefficient of variation: from 1.436% to 2.090%). The results showed significant differences between the groups of bees and investigation of eight section on the hind wing could be included in the next wider morphological analysis to contribute to a clearer separation of groups of bees that meet the standards for Carniolan strain.

A TEST OF THE DISCRIMINATION POWER OF TRADITIONAL AND GEOMETRIC MORPHOMETRIC METHODS ON HONEY BEES (*APİS MELLİFERA*) FROM BULGARIA AND EUROPEAN PART OF TURKEY

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Morphometric variation of honeybee populations from Bulgaria and Turkey is analyzed with both traditional and geometric morphometric methods.

Honey bee workers collected from 11 locations in Bulgaria and one from Thrace region of Turkey. A total of 346 samples were used in morphometric measurements.

Eight morphometric characters, six metric measurements on the wings, and length of wing vein c and d on a total of 346 honey bee workers were used in classical morphometry, nineteen landmarks on the same samples were used for geometric morphometric analyses. The results of geometric approach was compared with the traditional multivariate analysis.

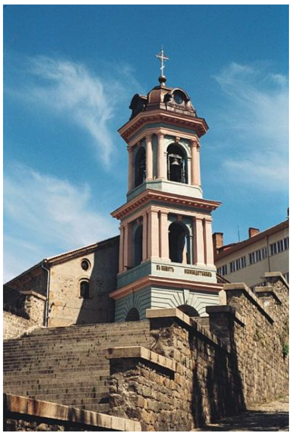
Both methods were successfully separated Bulgarian and Turkish honeybee populations. Wing shape analysis with classical morphometrics had a better separation of Bulgarian and Turkish bees along the first axis of CVA which has size component in it. In geometric morphometric analysis the scatter of Turkish and Bulgarian bees overlapped to some extent. The results indicated that the lengths of wing veins c & d (indicated 16, 17, and 18th landmarks) and also the upper wing veins (demonstrated with 7, 8, 10, 11, 14, and 15th landmarks) were observed to be narrower to inwards as the altitude decreases to sea level. However, there is no such differences between the different habitats such as forest, shrubs, and cultivated areas.

Geometric morphometrics discriminate the taxa in terms of shape differences independent of the environmental effects. Thus it can be used to unveil the evolutionary relationship between the subspecies compared.

Geometric morphometric analysis reflects differentiation between shapes of honeybees in different populations in comparison to classical morphometrics where size differences contribute to separation.

The Antique Theatre Plovdiv

Saint Nikola Church



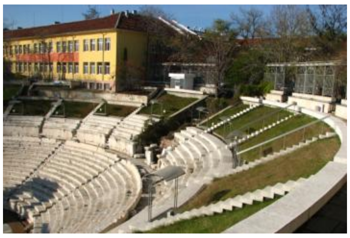
The Virgin Mari Church

Saint Dimitar Church

Saint Marina Church

House of Bulgarian Scientists Plovdiv

The Old Town Plovdiv



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