IMPORTANCE OF ADAPTATION OF BEE POPULATIONS ON THEIR LOCAL ENVIRONMENT – THE EUROPEAN INITIATIVE AND ITS RESULTS IN THE GREEK TERRITORY – THE GREEK INITIATIVE FOR THE CONSERVATION AND BREEDING OF THE LOCAL POPULATIONS

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Beekeeping in Europe and in the world today

For millions of years honey bees have survived close to humans, in a way that not only the beekeeping practice but also the different populations surviving in each environment constitute a kind of an 'inheritance'. To successfully survive in the wide range of habitats where they naturally occur, as a result of the natural evolutionary process, the honey bees developed specific adaptations to different environmental conditions; they also developed into many different geographical subspecies and into a wide variation of ecotypes[1,2]. Form Büchler et al[3] we quote:

> "The honey bee sub-species are also described as 'geographic sub-species' since their distributions correspond to distinct geographic areas. Even within Europe there is a wide range of climatic and vegetation zones which favoured differentiation, and at present about 10 subspecies of *A. mellifera* are recognized on the basis of morphometric and genetic markers[4]. Some of these subspecies were found to be more attractive than others for beekeeping, which as an economic and social activity plays a crucial role in the sustainable development of rural areas by providing important ecosystem services via pollination, thus contributing to the improvement of biodiversity of plants and farming crops[5]. An understanding of the genetic variability of bee populations and their adaptation to regional environmental factors such as climate and vegetation, prevailing diseases and agricultural practices is an important prerequisite for understanding problems in the health of honey bee colonies.

Hatjina et al[6] also noted:

> Thus, long-term adaptations express suitable population dynamics of the bee colony, which enable the colony to make the most of the available resources and to successfully resist threats like unfavourable seasonal living conditions[7], disease and parasite pressure[8,9]. Adaptations can be recognised by genotype –

1 Ruttner, 1988.
2 Meixner et al., 2010.
3 Büchler et al, 2014.
4 De la Rúa et al., 2009.
5 EU Parliament Report, 2011.
6 Hatjina et al, 2014.
7 Parker et al., 2010.
8 Fries et al., 2006.
9 Le Conte et al., 2007.

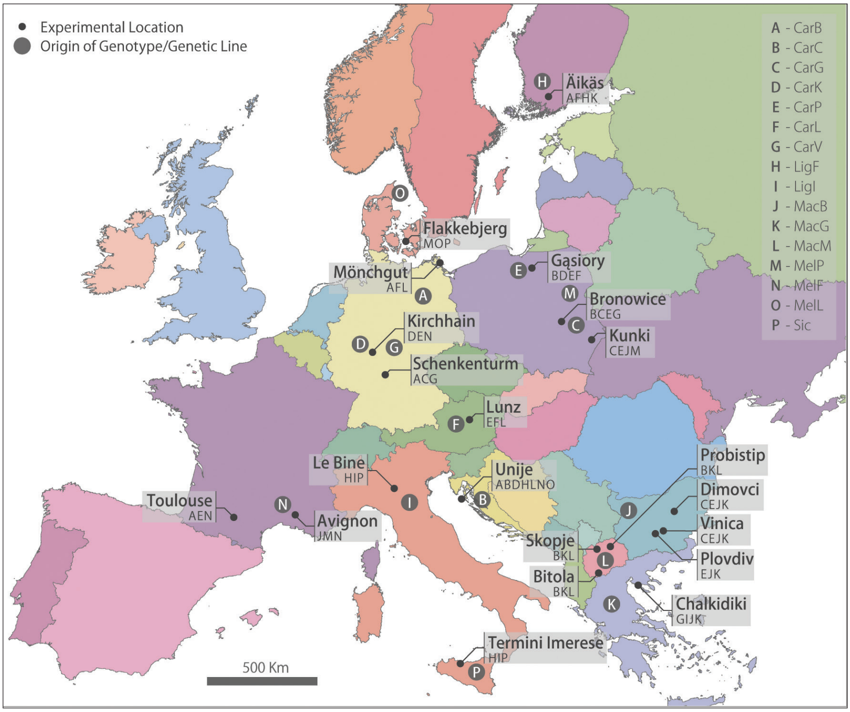

Fig. 1 Map of Europe showing the 21 locations covering the 11 countries participating in the European Genotype X Environment Interactions and the local populations used indicated by capital letters. Copyright International Bee Research Association. Reprinted from Francis et al[11].

environment interactions (GEI), in which distinct genotypes vary in the degree to which their phenotypes are affected by environmental conditions.

In light of the above, European -wide experimentation was conducted by several members of the COLOSS (Prevention of COlony LOSSes, Association, www.coloss.org) in order to study the complex interactions between honey bee colonies and their environment. We do know that distinct genotypes may vary in the degree to which their phenotypes are affected by specific environmental conditions - this phenomenon is known as "genotype-by-environment interactions" (GEI). Presence of the GEI indicates that the phenotypic expression of one genotype may be superior to another genotype in one environment but inferior in another environment. The different environmental conditions combine microclimate, vegetation, competition, enemies and the beekeeping practice. Different genotypes differ in how they react to the different environments and interaction explains the diversity in adaptability and superiority of some genotypes to specific environmental conditions. The same logic explains why no single genotype is the most suitable for all environments. The adaptability of a genotype may also explain the possible resistance in some of the pathogens. Therefore we conducted a very large experiment involving 11 countries and comparing 16 different strains of honey bees (**Table I**) in 21 different environments for two and a half years, with respect to characters such as colony development, honey yield, overwintering, survivability, swarming and susceptibility to diseases.

The experimental apiaries were distributed across Europe, reaching from Finland in the North to Sicily and Greece in the South and from France in the west to Poland in the East (**Fig. 1**). Individual work with the results published in a special issue of the Journal of Apicultural Research 2014[3,10,11,12,13,14,6]. A comprehensive report of the main findings of the above experiment can be found in American Bee Journal, issue of June 2015[15]. A significant difference it was observed in survival time between the local and foreign populations without therapeutic intervention. While in any given area, the foreign colonies survived an average of 470 days, the average survival time of the local bee colonies was 553 days.

10 Costa et al, 2012.
11 Francis et al, 2014a.
12 Meixner et al, 2014.
13 Francis et al, 2014b.
14 Usunov et al, 2014.
15 Meixner et al, 2015.

Table I. The 16 genotypes used in the GEI experiment and their origin

Genotype	Subspecies	Origin
CarB	Carnica	Bantin/ Germany
CarC	Carnica	Croatia
CarG	Carnica	GR1/Pulawy/ Poland
CarK	Carnica	Kirchhain/ Germany
CarP	Carnica	Kortowka/ Poland
CarL	Carnica	Lunz/ Αυστρία
CarV	Carnica	Veitshöchheim/ Germany
LigF	Ligustica	Finaland
LigI	Ligustica	Italy
MacB	Macedonica	Bulgaria
MacG	Macedonica	Chalkidiki/ Greece
MacM	Macedonica	Skopje/ FYROM
MelP	Mellifera	Augustowska/ Poland
MelF	Mellifera	Avignon/ France
MelL	Mellifera	Laeso/ Denmark
Sic	Sicula	Sicily/Italy

The main conclusions of this great experiment were the following:

- no single strain showed superior performance at all locations, therefore there is no genetic superiority but good adaptability
- each genotype may respond differently to different environments
- local bee populations have developed mechanisms which render the 'upper' of the 'foreign' populations in the survival, growth and sometimes productivity in the particular environment
- we need to improve and develop local populations in desired directions such as productivity and disease resistance, but we should not depend on imported genetic material

Adaptability result in Greece

In Greece the genotypes tested were CarV- *A. m. carnica* from Germany, LigI- *A. m. ligustica* from Italy, MacB- *A. m. macedonica* from Bulgaria and MacG- *A. m. macedonica* from Halkidiki-Greece (**Fig. 2**). We used 40 beehives, 10 for each genotype and kept them in an area away from other apiaries. No treatment for varroa mites was administered, other than the original treatment with oxalic acid before the introduction of the new queens. After 2 and half years, from the 40 beehives used in Greece, only 7 were alive in March 2012 (**Table II**). Of these only one was headed by Italian queens and the rest by Greek *macedonica* queens. Furthermore, it was also shown that the local colonies also produced more honey (**Table II**).

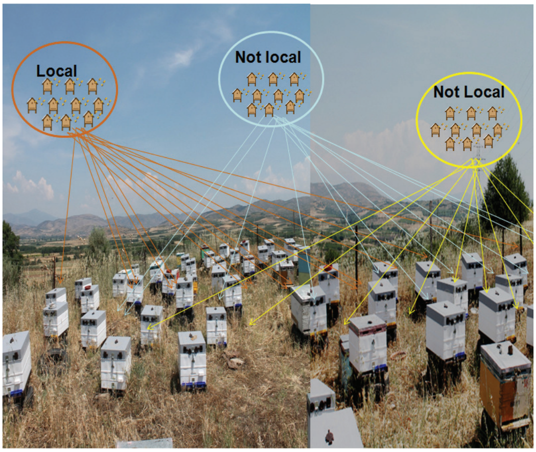

Fig. 2 Schematic presentation of the population origin in CO-LOSS- GEI experiment.

Table II. Average days of survival of different genotypes in Greece

Genotype	Average Days of survival	Kg of honey produced
CarV	384	15
LigI	428	27
MacB	503	25
MacG	580	29

Figure 3 shows the dynamics and the wintering ability of the survived colonies in terms of colony population, with the Greek colonies starting with higher population in spring. Interestingly, the population of colonies in spring was negatively correlated with varroa infestation in previous autumn, an expected result with what is known so far from the biology and development of the varroa mite.

It is believed that the adaptation of populations to abiotic environment may maintain the genetic diversity needed for the resilience to diseases and better exploitation of food sources. With the above

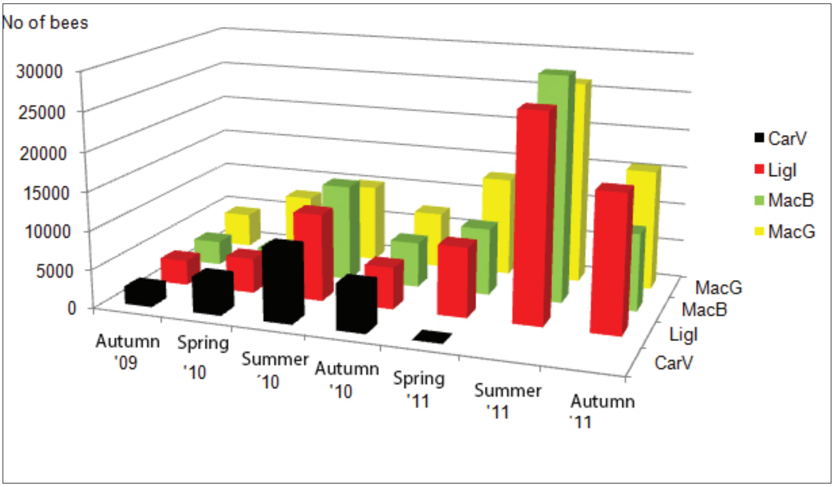

Fig. 3 Colony population (in honey bee numbers) of the survived colonies during the experiment.

experiment it became apparent that local bee populations have developed mechanisms to prove that are far 'superior' of non local genotypes in the course of several years. It is no coincidence that the genotypes with high adaptation to their place of origin, are characterized as 'ecotypes'. It has also been written that different ecotypes have been established according to the availability and diversity of vegetation[16]. As so, the knowledge of the interaction between genotypes and the environment is very important in improvement and breeding patterns of various populations of bees. Is better to improve and develop local populations bees in desired directions such as productivity and resilience to disease, than to be dependent on imported genetic material. Certainly the genotypes which are reproduced and improved for many consecutive years in an environment different from the origin, at the end they will adapt to new living conditions. But the question is: what is the relationship between the final genotype with the original; One thing is certain: that trying to preserve the 'good' characteristics of a population or a genotype and breeding and conserving these characteristics in the natural environment could serve as the largest operation for the populations' evolution.

Preserving and breeding local Greek honey bee populations

According to Ruttner[17], in Greece we had the following bee races: *A. m. carnica* (in Ionian islands), *A. m. macedonica* (Macedonia and Thrace), *A. m. cecropia* (in Central and Southern Greece), *A. m. adami* (in Crete and the Aegean islands). Today because of the many movements and trade, Greece is a country of great hybridization with dominant the Macedonian bee[18] (**Fig. 4**).

Fig. 4 Geographic locations for the four groups of samples distinguished by geometric morphometric analyses. Reprint from Hatjina et al[18].

Recently, in very few areas (some Aegean islands and in Larisa-Central Greece) some populations different from the Macedonian bee have also been found[19,20,21,22,23,24]. Detailed information on methods

16 Louveaux et al, 1966.
17 Ruttner, 1988.
18 Hatjina et al, 2002.
19 Bouga et al, 2004.
20 Bouga et al, 2005a.
21 Bouga et al, 2005b.
22 Martimianakis et al., 2011.
23 Charistos et al., 2014.
24 Hatjina et al., 2004.

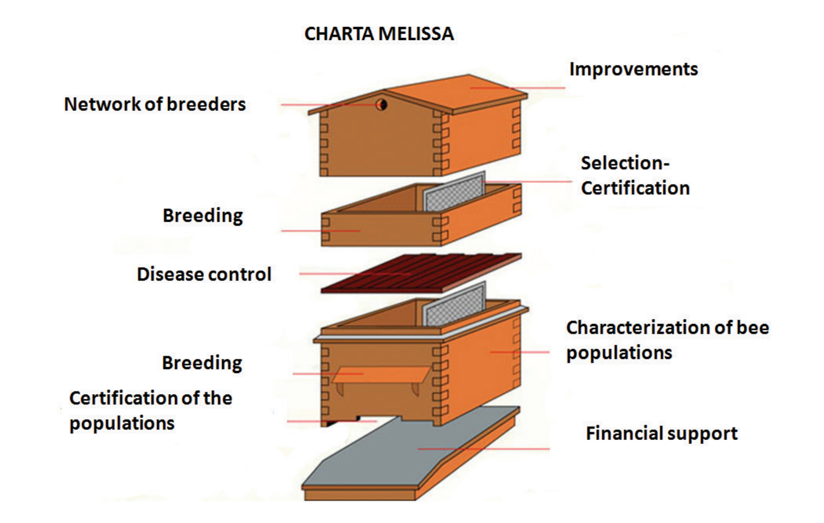

Fig. 5 Graphical description of the program "CHARTA MELISSA" undertaken by the Division of Apiculture -ELGO.

and results for the discrimination of the Greek populations, the reader can find in the previous contribution of this book written by Dr. Maria Bouga.

From ancient times until today beekeeping is for Greece a traditional rural profession. The 'return to Mother Earth' is a very popular message in our times with great success. Today in Greece there are approximately 20,000 registered beekeepers with about 1,400,000 colonies. The 39% of them are professional beekeepers with more than 200 beehives each, and 700,000 total colonies. It is also surprising that Greece holds again the highest density of colonies and apiaries (11.4 colonies per Km^2) according to new research[25]. The total annual honey production in the country is about 15,000 tons, of which 300 tons are exported, mainly in Europe. The average production per hive varies between 10 Kg and 20 Kg[26,9]. The amount of such production is considered too small to cover the financial cost of maintaining the apiaries and to secure livelihoods, when Finland and Germany respectively have at least 30 - 40 Kg per beehive.

Beekeepers often believe that their bees are not productive or do not meet all the requirements and they introduce foreign genetic material with known bee 'excellence' with the main objective to increase production. However the imported foreign queens, even if they are known for their hybrid vigor, create additional hybrids and exhibit adverse effects (mainly aggressiveness and excessive swarming tendency) and the loss of productive benefits after the first generation. The result of the above phenomenon is that the native subspecies are replaced by foreign subspecies and therefore they could be driven to extinction and the Greek honey bee bio-diversity could be greatly reduced.

To solve the problem of productivity and to maintain the genetic diversity many governmental and non-governmental organizations started several improvement and conservation programs of their local honey bee subspecies since the 60s. Greece although it is a very important country in relation to beekeeping, does not yet have an organized system of selection and production of queen bees from the local populations.

A first attempt to maintain and breed the local bee subspecies is the research project undertaken partly by the Apiculture Division of the Institute of Animal Science (Hellenic Agricultural Organization 'DEMETER') (a program under the EC Directive 1234-1207) with the acronym "CHARTA MELISSA" and it is summarized in Figure 5.

The title of the project is "Preservation, improvement and conservation of genetic material of the Greek bee populations - CHARTA MELISSA: The characterization and identification of the Macedonian, Cecropian and Cretan bee through natural and artificial means of fertilization"

The aim of the project is

1. to find the local populations

2. to characterize them (monitor behaviour, development)

25 Chauzat et al., 2013.

26 Papanagiotou, 2010.

3. select and breed them

4. preserve them in their natural place or in a conservation area

No selection or conservation program can be achieved without the strict control of the couplings. Given the fact that the queen bees mate freely on the air, under certain conditions, the control of the couplings is a very important issue. In general, remote areas or small islands are ideal coupling areas but do not exist in countries with a high density of apiaries such as Greece. Possibly the very large crowds of colonies producing drones give a solution but does not ensure complete control. The artificial insemination on the other hand has solved this problem and is widely used not only by establishing breeding centers but also by using instrumental insemination. However this is a technique that requires much time and specialized staff.

As part of this project the implementation of an innovative method was the controlled mating of queen bees, called "The train of virgin queens". With this system we can achieve controlled, still free on the air, matings with precise handling of beehives used for the production of drones and virgin queens (**Fig. 6**). The 'Train of virgin queens' (TVQ) has been applied till now only in New Zealand and it is also known as the 'Joe Horner system'. The method requires a kind of a construction: a) a cabinet, or a cool box, which keeps the temperature at 14-15o C continuously; b) a number of hives bearing the virgin queens of the selected subspecies (each hive could be divided in 2 or 4 mating nuclei); c) a good number (more than 10) of drone producing colonies of the desired subspecies in a very close vicinity of the virgin queens d) a kind of rails, running out of the cool cabinet in the open surface, on which the hives with virgin queens are sliding. The hives bearing the mating nuclei are rolling on rails resembling a train (that is where the name came from), connected to each other with a chain of about 2 m long. Two days before the virgin queens are ready for mating, they are caged in their nuclei with a queen excluder and then they are placed inside the cool and dark cabinet. After the two days in the cool cabinet, in the afternoon, the queens are taken out of the cabinet. The nuclei are sliding on the rails in a way that in consecutive days they will always have exactly the same position, because the rails restrict their position and the chain between the nuclei restricts their distance. There are also some orientation cues around in permanent positions for the bees to facilitate their returning to home. The train of the virgin queens is going out of the cool cabinet for several afternoons and goes in again in the evenings just before dark.

Fig. 6 Views from "The Train of Virgin Queens" as established in the Division of Apiculture in New Moudania Halkidiki: a and b: the nuclei on their rails spread out; c. a close view of a nucleus; d. the drone colonies with the queen excluder to restrict the flying of the drones.

At the same afternoons and only when all available free flying drones have returned to their colony, the drones from the selected colonies are allowed to fly, as they were kept restricted by queen excluder in their colonies. The pressure for mating is strong and the selected queens will eventually mate with the selected drones, as they are the only ones available at that time of day. The exact time of the day need to be defined in order to avoid undesired matings. The 'Train of the Virgin Queens' ensures the mating of several queens at the same time, without much labor. A video on 'The train of Virgin queens' can be found here: https://www.youtube.com/watch?v=V8jXQeScgVg.

The first time, the above system used in Greece, gave a mating efficiency >50%. The system will be tested again, with different subspecies in order to define the time of the day the queens are flying for mating naturally, the times they fly out, the duration of mating flights and the genetic differences among the populations due to the use of this mating system. The system will be tested for both macedonica and cecropia queens.

At the same time we are aware that no system or model is always effective. We need continuous efforts of specialized centers for several years to achieve the best of the results. But it is clear that without a National Selection and Improvement Program of our native bees we will never increase our economic benefit while maintaining our genetic material. In this effort it is absolutely necessary the close collaboration among scientists and beekeepers and State in order to reach the desired result, which is the economic development of industry of apiculture.

BIBLIOGRAPHY

Bouga M, M Tsipi, M Mavroudis, P Harizanis, L Garnery, G Arnold and D. Tselios, 2004. Genetic variation in Greek Honey Bees: molecular and classical morphometrics approach. *First European Conference of Apidology*, Udine, Italy. Proceedings p. 39.

Bouga, M, G Kilias, PC Harizanis, V Papasotiropoulos, S Alahiotis, 2005a. Allozyme variability and phylogenetic relationships in honey bee (Hymenoptera: Apidae: A. mellifera) populations from Greece and Cyprus. *Biochemical Genetics* 43:471-484.

Bouga, M, PC Harizanis, G Kilias, S Alahiotis, 2005b. Genetic divergence and phylogenetic relationships of honey bee *Apis mellifera* (Hymenoptera: Apidae) populations from Greece and Cyprus using PCR - RFLP analysis of three mtDNA segments. *Apidologie* 36: 335-344.

Büchler R, C Costa, F Hatjina, S Andonov, M Meixner, Y Le Conte, A Uzunov, S Berg, M Bienkowska, M Bouga, M Drazic, W Dyrba, P Kryger, B Panasiuk, H Pechhacker, P Petrov, N Kezic, S Korpela, J Wilde, 2014. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera L.* colonies in Europe. *Journal of Apicultural Research*, 53(2): 205-214.

Charistos L, F Hatjina, M Bouga, M Mladenovic, A-D Maistros, 2014. Morphological discrimination of Greek Honey Bee populations based on Geometric Morphometrics analysis of wing shape. *Journal of Apicultural Science* 58(1): 75-84.

Chauzat M-P, Cauquil L, Roy L, Franco S, Hendrikx P, et al. (2013) Demographics of the European Apicultural Industry. *PLoS ONE* 8(11): e79018.

C Costa, R Büchler, S Berg, M Bienkowska, M Bouga, D Bubalo, L Charistos, Y Le Conte, M Drazic, W Dyrba, J Fillipi, F Hatjina, E Ivanova, N Kezic, H Kiprjanovska, M Kokinis, S Korpela, P Kryger, M Lodesani, M Meixner, B Panasiuk, H Pechhacker, P Petrov, E Oliveri, L Ruottinen, A Uzunov, G Vaccari, J Wilde, 2012. A Europe-wide experiment for assessing the impact of genotype environment interactions on the vitality of honey bee colonies: methodology. *Journal of Apicultural Science, 56*(1), 147-158.

Francis R, P Kryger M Meixner, M Bouga, E Ivanova, S Andonov, S Berg, M Bienkowska, R Büchler, L Charistos, C Costa, W Dyrba, F Hatjina, B Panasiuk, H Pechhacker, N Kezić, S Korpela, Y Le Conte, AUzunov, J Wilde, 2014a. The genetic origin of honey bee colonies used in the COLOSS Genotype-Environment Interactions. Experiment: a comparison of methods. *Journal of Apicultural Research* 53(2): 188-204 (2014 a). DOI: 10.3896/IBRA.1.53.2.02.

Francis R, P Kryger M Meixner, M Bouga, E Ivanova, S Andonov, S Berg, M Bienkowska, R Büchler, L Charistos, C Costa, W Dyrba, F Hatjina, B Panasiuk, H Pechhacker, N Kezić, S Korpela, Y Le Conte, A Uzunov, J Wilde, 2014b The genetic origin of honey bee colonies used in the COLOSS Genotype-Environment Interactions. Experiment: a comparison of methods. *Journal of Apicultural Research* 53(2). Online supplementary material.

Fries I, A Imdorf, P Rosenkranz 2006. Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. *Apidologie* 37(5): 564-570.

Hatjina F, **M Baylac, L. Haristos, L Garnery, G Arnold D Tsellios, 2002.** Wing differentiation among Greek populations of honey bee (*Apis mellifera*): a geometric morphometrics analysis*. Poster in the 7th European Entomological Congress, Thessaloniki, October 7-13, 2002.*

Hatjina F, L Haristos, M Bouga 2004. Geometric morphometrics analysis of honey bee populations from Greek mainland, Ionian islands and Crete island. Poster in *Proceedings of the First European Conference of Apidology*, Udine, 19-23 September, 2004 (p. 44).

Hatjina F, C Costa, R Büchler, A Uzunov, M Drazic, J Filipi, L Charistos, L Ruottinen, S Andonov, M Meixner, M Bienkowska, D Gerula, B Panasiuk, Y Le Conte, J Wilde, S Berg, M Bouga, W Dyrba, H Kiprjanovska, S Korpela, P Kryger, M Lodesani, H Pechhacker, P Petrov, N Kezic, 2014. Population dynamics of European honey bee genotypes under different environmental conditions. *Journal of Apicultural Research*, 53(2): 233-247.

Le Conte Y, G De Vaublanc, D Crauser, F Jeanne, JC Rousselle, JM Becard, 2007. Honey bee colonies that have survived *Varroa destructor*. *Apidologie* 38(6): 566-572.

Louveaux J, MAlbisetti, M Delangue, M Theurkauff, 1966. Les modalités de l'adaptation des abeilles (*Apis mellifica* L.) au milieu naturel. *Annales de l'Abeille* 9(4): 323- 350.

Martimianakis S, E Klossa-Kilia, M Bouga, G Kilias, 2011. Phylogenetic relationships of Greek Apis mellifera subspecies based on sequencing of mtDNA segments (COI and ND5). *Journal of Apicultural Research* 50: 42–50.

Meixner MD, C Costa, P Kryger, F Hatjina, M Bouga, E Ivanova, R Büchler, 2010. The role of genetic diversity and vitality in colony losses *Journal of Apicultural Research –Special Edition* 49(1): 85-92.

Meixner M, Roy M Francis, A Gajda, P Kryger, S Andonov, A Uzunov, G Topolska, C Costa, E Amiri, S Berg, M Bienkowska, M Bouga, R Büchler, W Dyrba, K Gurgulova, F Hatjina, E Ivanova, M Janes N Kezic, S Korpela, Y Le Conte, B Panasiuk, H Pechhacker, G Tsoktouridis, G Vaccari , J Wilde, 2014. Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment *Journal of Apicultural Research* 53(2): 215-229.

Papanagiotou E, 2010. Economic Analysis of Greek beekeeping. Aristotle Univ. of Thessaloniki, Agronomy Faculty, p 78.

Parker R, AP Melethopoulos, R White, SF Pernal, MM Guarna, LJ Foster, 2010. Ecological adaptation of diverse honey bee (*Apis mellifera*) Populations. *PLoS ONE* 5(6): e11096.

Pilar De la Rua et al. 2009. Biodiversity, conservation and current threats to European Honey bees. *Apidologie* 40: 263–284.

Ruttner F, 1988. *Biogeography and taxonomy of honey bees*. Springer-Verlag; Berlin, Germany.

Uzunov A, C Costa, B Panasiuk, M Meixner, P Kryger, F Hatjina, M Bouga, S Andonov, M Bienkowska, Y Le Conte, J Wilde, D Gerula, H Kiprijanovska, J Filipi, P Petrov, L Ruottinen, H Pechhacker, S Berg, W Dyrba, E Ivanova, R Büchler, 2014. Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. *Journal of Apicultural Research* 53(2): 248-260.