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Characterization of genetic potential of the
honeybee *Apis mellifera carpatica* using molecular
techniques in order to conserve its biodiversity

Unique report in 2007

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-December, 2007-

Introduction

The project entitled **“Characterization of the genetic potential of the honeybee *Apis mellifera carpatica* using molecular techniques in order to conserve its biodiversity”** it aims for a single stage in 2007, according to following objectives: scientific study of intra-specific diversity of bee *Apis mellifera carpatica* from Transylvanian Basin and collection and preservation of biological material (*Apis mellifera carpatica*) from Transylvania and establish working protocols. Classification of contemporary races of bees around the world by multivariate methods was first made by DuPraw (1964, 1965) and later improved by Ruttner (1988). The classification of F. Ruttner's (1992) includes 25 sub-species (races) of the honeybees. Must be noticed that within sub-species have been formed various populations and ecotypes. Their emergence is more salient issue for their identification. Moreover, in the work process is making up the taxon selection (lines and ecotypes within breeds) which also must be identified (Crițov, 2003). Morphometric measurements method was the major impetus for classification and alozymic analysis. DNA diversity has contributed to the division races and ecotypes of the genus *Apis*.

The emergence of genetic engineering, molecular genetics and biotechnology and extremely rapid developments has led to the development of beekeeping. They have a special role in the explosion of data accumulated on the structure, expression and modeling artificial genetic material in bees worldwide. Bee races are primarily a result of natural selection and climatic conditions in which they live (climate, floral). Adaptation to different climatic conditions led to the spread of races throughout the world. Races of honeybees are natural, geographical, adapted and efficient in the area where they are formed. Ultrasound breeds are the best bees for ecological areas of an area (Rinderer, 1986). The specific climatic conditions and growing our country was formed *Apis mellifera carpatica* Foti, also called Romanian Carpathian bee. Amazing progress of cell and molecular biology allow a detailed study of genetics including bees.

APIS MELLIFERA CARPATICA ON POPULATION ISSUES

Some races of honeybees Iran-Mediterranean group, one who lives in the Carpathian-Danube area Romania, is considered Carpathian ecotype of *Apis mellifera* (Foti, 1962). Intertwining influences of Mediterranean climate, the West, warm and humid, continental, eastern, dry and high temperature amplitudes, carried on a very varied terrain, from lowland to mountain ridges, have created an accumulation of ecological factors favorable development of certain ecotypes of the race. Native bees, adapting to specific conditions in the temperate continental climate Carpathian-Danube space, constituted a separate population. In this population, the literature of our country, after many studies show that there are distinguished five ecotypes:

- Western Plain ecotype
- Transylvanian ecotype
- mountain ecotype
- Moldova ecotype
- Steppe ecotype

These ecotypes can be distinguished by some morphological characters: trunk length, body length, tibia and tarsus length, wing length earlier, cubital index. First Biometric study of the Romanian honeybees is made by Fisteag I. (1937) who finds that mountain bees from the area north of the country, have generally dark trunk and short legs and big body. In bees of southern and lowland situation is the opposite in terms of size. Later, following the results of various researchers in this field, related to geographical discretion, it is noted that as we descend further south, tongue and feet sizes increase and decrease body size. Measurements made on the bee *Apis mellifera carpatica* from Romania during 1989-1996, on 16 morphometric characters conclude that characters reveal consistency. Samples of bees which we worked were taken from apiaries located in isolated hearths and not made Pastoral beekeeping. Bee "Yellow Banat" was at one time a well-enhanced population in the Romanian honeybee population. Southern part of Banat is a hilly area with a climate and basic sub-Mediterranean honey variety, provided favorable conditions for breeding bees, which are a valuable material for selection, were subjected to more research in order to obtain lines with capacity high production. Ruttner (1975) recognizes the "Banat honeybee " (say no race or population, but simple " Banat bee") undeniable that life, especially for its yellow color, although it is completely different from *A.m. ligustica* (which also has yellow) (www.eurohonig.com).

The knowledge about the origin and evolution of honeybees over time is limited; what is indisputable is known that their existence was closely related to plant biology and succession of polleniferous-nectarous plants. Improving bees in Romania, as in other animal species, seeks to raise productivity indices increase profitability beekeeping operation. Improvement works are carried out in three main directions: selection within the breed or population, the crossing between breeds and crosses between inbred lines of the same race or population.

During the years 1960-1970 there were made some imports of foreign breeds. Inquiries were made about the effectiveness of the production of hybrids between races Mârza E. (1965, 1965b) to cross the Italian bee (*Apis mellifera ligustica*, Figure 3) in the Carpathian honeybee (*Apis mellifera carpatica*). Hybrids resulting from crosses have yielded good results in the first generation, but the resulting decrease in subsequent generations, even well below where it started. 70 years have been massive imports of Italian bees in order to achieve higher production. But proved inadequate to the conditions of our country and dropped them. Traces of the crosses are visible today, with many yellow bees in the hive (Figure 1, 2).



Figura 1: Tergit III and IV to Carpathians honeybees



Figura 2: Romanian honeybees (*Apis mellifera carpatica*)



Figura 3: Italian honeybees - *Apis mellifera ligustica*



Figura 4: Buckfast Hybrid

In the last decade due to intense pastoral stupăritului practiced in Romania, research showed a trend towards the homogenization of bees in different parts bio-apicole. ICDA with ANARZ will conduct a comprehensive program of identification and nomination of bio-apicole apiaries in each area of Romania, which will be preserved genetic material to be exploited by local programs and growing selection of queens. National Program for Improvement of Romanian bee, *Apis mellifera carpatica* prohibit imports or introducing any other ways of bees belonging to other races of bees, as this may lead to irreversible degradation of the native genetic stock. Literature shows that such situations existed in many countries, including Germany, Poland, France etc. (www.eurohonig.com).

As a result of Romania's EU accession, there is the possibility that foreign beekeepers, who have other breeds or hybrids of bees (eg Buckfast) (Fig. 4) to enter in our country the idea of pastoral practice around many areas, in many countries. This raises the risk of damaging the biodiversity of honeybee Romanian ecotype. Any underestimation of the problem is dangerous for the economy but for beekeepers importers, and even for global sustainable development. For now, what is certain is that Romanian honeybee *A.m. carpatica* is best suited to local conditions, therefore most productive. Convention on Biological Biodiversity, signed by Romania, provides conservation of breeds of domestic animals and FAO conducted systematic activity in this regard. The most effective and least expensive is the active conservation, namely the conservation of populations in service. This is consistent with the scientific requirements for improving the bees in Romania.

Article 28 of the Livestock Act (2002) also states that "for national genetic heritage protection and development of animal husbandry in accordance with national programs to improve, all imports of breeding animals must be advised by the Ministry of Agriculture, Forestry and Rural Development from Romania".

Art 40 mentions that breaching the provisions of this law entails minor administrative liability, civil or criminal, as appropriate. In all EU countries have subsidy schemes to preserve local breeds of animals. Furthermore, careful study of local racial diversity populations for conservation is required.

ISSUES ON MEASURES SAMPLING OF HONEYBEE MORPHOMETRIC

Exterior honeybee is basically genetically determined, but not least by the environment. Exterior honeybee characteristics, genetically determined characteristic of each race is constantly in hand. Instead it may change depending on seasonal

variability, size of cells for juveniles, the quantity and quality of feed for fry. Honeybee samples collected from different localities in Transylvania. Bees collected from apiaries are traditional and as far as possible be avoided areas where pastoral practice beekeeping. It is very important as it tried to avoid mixing genes between different populations in Romania.

Flying range of the queen and drones for mating is about 2 km, but can reach 10-20 km and lowland regions. In a flight Suite, queens mate on average with 7-8 drones, which can sometimes reach up to 17, and he implicitly heterospermic phenomenon (Mărghitaş, 2005). These influencing factors should be considered in the sample was taken, but unfortunately it is very difficult for many beekeepers beekeeping pastoral practice. Sampling will be done during the swarming ended. Significant differences were observed in the data measurements taken during swarming. It is recommended that harvesting be done in the first days of September, so surely we can get young bees (Ruttner, 1988).

Another criterion to be considered is the predominant color of the combs of the family. November combs have a yellowish-white, but serve to increase as new generations of bees become brown and then black, as a result of the skin resulting from năpârlirii larvae and nymphs. New cell walls are from 0.35 to 0.40 mm and 0.80 mm in the old. Old cells are elongated by bees. Body mass of bees decreased from 125 mg / head in the third generation at 107 mg / head after 38 generations (Mărghitaş, 2005), which might affect the morphometric data. *Larvae* feeding in large colonies are made by bees with proboscis long and by several nurses, thus receiving a greater quantity of food, which has a positive effect on juvenile development and the bees from the brood size. Compared with those in weaker colonies, they are not fed so often by nurse bees. Moreover, growth of juveniles and season influence the quality and quantity of food.

Samples collected correspond to a single family. This requirement can be accomplished when young bees harvest (those that are in good body condition and have perishable body). Young bees inside the hive will yield. Sampling directly from the flowers is not a recommended method because we do not know the origin of these honeybees and are already old, and some have spent piliferous. Typically, after 21 days, bees are foraging for pollen, nectar and water activity that runs until the age of 35-40 days, when they die. Bees which are the samples can be killed in any form so long as there are no measurements of trunk. Trunk length is a very important character that shows geographical variability more accurately than all other characters. Obtaining accurate measurements of jet bee requires killing it with hot water, steam or air using cyanide bottle. With any other method of slaughter, the language remains in a position which allows its measurement (Ruttner 1988).

Samples preserved in liquid fixative best use of fluid Pampell: acetic acid 10 parts, 20 parts formaldehyde, ethanol 30 parts, 100 parts water. Ruttner noted that using this fixing fluid, after decades of acetic acid and chitin soaked individuals dissection is becoming increasingly difficult. For this reason acetic acid was replaced with 70% ethanol. As a fixative is recommended to use any ethanol alone or mixed with acetic acid (Ruttner, 1988).

SAMPLING OF HONEYBEES

Basically we started from the idea that each district will collect bees in three places, but this has not been achieved because most beekeepers operating in Sibiu

Buckfast hybrid bee or pastoral practice. For other counties tried encompassing a significant area for each county, and lots are not close by each other by 50 km. To achieve bees morphometric measurements were stored in ether vapor, and for genetic analysis were placed in ethanol 70%. For both determinations was taking the same family of bees.



Figure 5. Sampling Points

Localities in which sampling was carried out are: Unirea (County Alba), Cut (County Alba), Cristian (County Sibiu), Bradu (County Sibiu), Vânători (County Mureș), Rupea (County Brașov), Rupea Gară (County Brașov), Făgăraș (County Brașov), Cristian (County Brașov), Arcuș (County Covasna), Zagoni (County Covasna), Poian (County Covasna), Păuleni Ciuc (County Harghita), Zetelaka (County Harghita), Suseni (County Harghita), Borsec (County Harghita), Râștolița (County Mureș), Petelea (County Mureș), Bălăureni (County Mureș), Cuci (County Mureș), Uriu (County Bistrița-Năsăud), Livezile (County Bistrița-Năsăud), Fiad (County Bistrița-Năsăud), Leordina (County Maramureș), Tisa (County Maramureș), Baia Sprie (County Maramureș), Medieșu Aurit (County Satu Mare) Doba (County Satu Mare), Petri (County Satu Mare), Săcueni (County Bihor), Camăr (County Sălaj), Românaș (County Sălaj), Bădești (County Cluj), Băișoara (County Cluj) (Fig. 5). All these biological samples will be subjected to morphometric analysis and all other analysis provided in the project plan.

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