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BIODIVERSITY OF THE HONEYBEE *Apis mellifera*, Linne (1758), FROM SOME YUGOSLAV REGIONS
II - ULTRASTRUCTURAL CHROMOSOMAL DIFFERENCES BETWEEN BANAT AND THE SYENICHKO - PESHTERSKI HONEYBEE ECOTYPES

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Starting from the previously formulated hypothesis that amplifications and rearrangements of chromosome regions might be the cause of the biometric chromosomal differences in the analysed samples of honeybees from the Peshter plateau and Belgrade region, comparative ultrastructural chromosome analyses (the distribution of euchromatin and heterochromatin) of these indigenous honeybee ecotypes (the Banat - BET ecotype and Syenichko-Peshterski - SET ecotype) were undertaken.

The ultrastructural chromosome analyses showed marked differences in the G – band distribution on chromosomes 1, 2, 4, 11, 12, 13, 15 and 16 of the SET honeybee ecotype compared to the BET ecotype, thus confirming our previously advanced hypothesis. The chromosomes of the first pair of the SET honeybee ecotype had one heterochromatic (D_2) and one euchromatic (D_3) block more on the p - arm compared to the chromosomes of the same autosomal pair of the Banat honeybee. The chromosomes of the second pair of the SET ecotype had one lighter euchromatic band (B_{1a}) more on the same arm (p - arm). Moreover on chromosomes 4 [two surplus bands: one heterochromatic (C_2) and one euchromatic (C_3) bands], 11 [three surplus bands: two heterochromatic (A_{1a}, A_{1c}) and one euchromatic bands (A_{1b})],, 12 [one surplus heterochromatic band (A_1)], 13 [three surplus bands: one heterochromatic (A_2) and two euchromatic bands (A_1, A_3)], 15 [one surplus heterochromatic band (A_1)], and 16 [one surplus heterochromatic band (A_{1a})] of the SET ecotype, amplifications of euchromatic/ heterochromatic blocks appeared on the q - arm.

Key words: G - chromosome polymorphism, euchromatin, heterochromatin, honeybee, indigenous ecotypes, Syenichko - Peshterski (SET) ecotype, Banat (BET) ecotype.

INTRODUCTION

Biodiversity means the total existing biological diversity, i. e. the natural hereditary ability, and thereby the maintenance of the variability of animal and plant resources (Dempfle, 1990; Hodgers, 1991; Beilharz, 1993; Milner, 1996). According to Torp - Donner and Juga (1997), the precondition for the stability, functioning, mutual interaction and survival of all the existing ecosystems lies in their internal biodiversity.

The fact that honeybee diversity presupposes the existence of not only different agri - ecosystems but also of all terrestial ecosystems, as well as our knowledge of the existence of biometric chromosomal differences between the Syenichko - Peshterski (SET) and Banat (BET) honeybee ecotypes induced us to undertake an investigation of the ultrastructure of the chromosomes (the euchromatin and heterochromatin distributions) of these ecotypes.

The purpose of these studies, was the possible confirmation or negation of the previously proposed hypothesis concerning the observed biometric chromosomal differences in the monitored honeybee ecotypes.

Cytogenetic characterization of the chromosomes of the honeybee *(Apis mellifera mellifera)* was done by Hoshiba and Kusanagi (1978), Hoshiba (1979) and Hoshiba and collaborators (1981), They gave a full description of all chromosomes and stated their biometric characteristics. The first information with regard to the chromosome characterization of honeybee diversity in some Yugoslav regions was published by Popesković and collaborators (1995, 1997). In addition, the papers of Stanimirović et al. (1997 a, 1997 b, 1998 c), contain some interesting data with regard to chromosome polymorphism, selection and behavior of the species *Apis mellifera* originating from Serbia.

MATERIAL AND METHODS

The cerebral ganglia of 80 honeybee praepupae (the Syenichko - Peshterski and Banat ecotypes), with reddish, star-like eyes, nine to eleven days old, were used for the cytogenetic analysis.

Chromosomes from the nervous tissue of the cerebral ganglia were prepared in accordance with the procedure of Imai et al. (1988). The nervous ganglia were kept for 20 minutes at room temperature in colchicine hypotonicity on concave microscopic slides. Then, they were put on ordinary microscopic slides and macerated in the presence of fixative I (60% solution of a mixture of absolute ethanol and glacial acetic acid in the ratio 1:1). Finally, they were fixed with fixative II (a mixture of absolute ethanol and glacial acetic acid in th ratio of 1:1) and fixative III (glacial acetic acid). Such preparations were left at room temperature (20^0C) and a relative humidity of 65% to dry for at least one day.

After this maturation of the preparations, G - banding of chromosomes was done by the method of Ronne (1991), which is a modification and an improvement of those established by Seabright (1971) and Verma (1998).

RESULTS AND DISCUSSION

The results of ultrastructural analyses of chromosomes (G - band polymorphism) of the SET and BET honeybee ecotypes are shown on the original micrographs.

Noticeable differences in the distribution of G - bands on chromosomes were observed. The most significant changes, concerning amplification and chromatic region redistribution, were observed for chromosomes 1, 2, 4, 11, 12, 13, 15 and 16 in the Syenichko - Peshterski ecotype. This variability in the microstructure of the chromosomes can be seen in Figures 1, 2, 3 and 4.

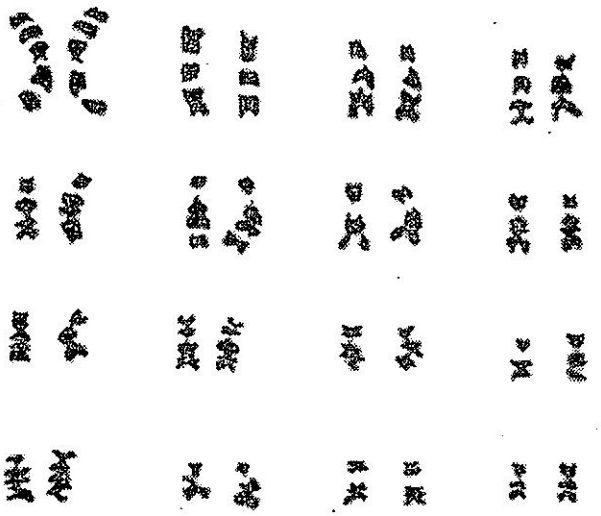

Figure 1. G - bands of the chromosomes of the Banat honeybee ecotype (BET)

In Figures 3 and 4 it can be seen clearly that chromosome 1 of the Syenichko - Peshterski honeybee has an extra heterochromatic (D_2) and euchromatic (D_3) block on the p - arm, compared to the chromosome from the same autosome pair in the Banat honeybee. Moreover, the Sjenicho - Peshterski ecotype has one surplus, paler euchromatic band (B_{1a}) on the p - arm of the second pair of autosomes. On chromosome 4 [two surplus bands: one heterochromatic (C_2) and one euchromatic (C_3)], 11 [three surplus bands: two heterochromatic (A_{1a}, A_{1c}) and one euchromatic (A_{1b})], 12 [one surplus heterochromatic band (A_1)], 13 [three surplus bands: one heterochromatic (A_2) and two euchromatic (A_1, A_3)], 15 [one surplus heterochromatic band (A_1)], and 16 [one surplus heterochromatic band (A_{1a})], amplifications of chromosome blocks occurred on the q - arm of the Syenichko - Peshterski honeybee.

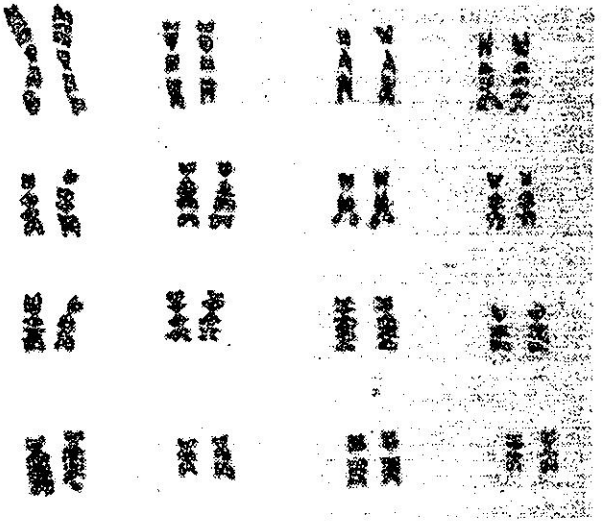

Figure 2. G - bands of the chromosomes of the Syenichko - Peshterski honeybee ecotype (SET)

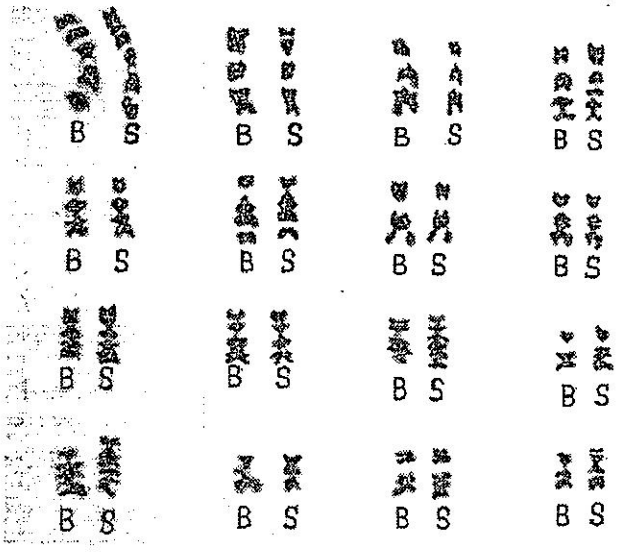

Figure 3. Comparative presentation of G - bands of the chromosomes of the Banat (B) and Syenichko - Peshterski (S) honeybee ecotypes.

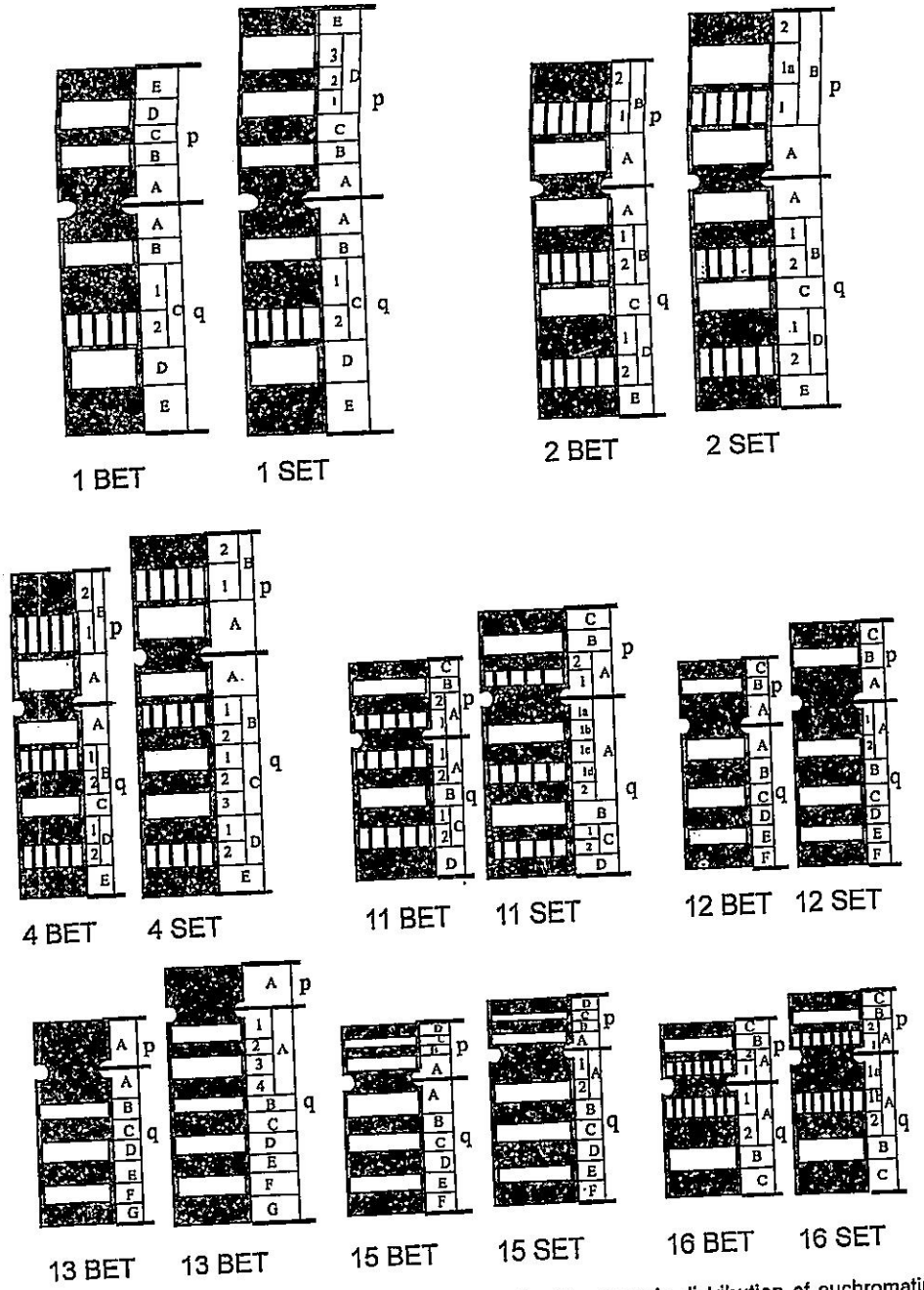

Figure 4. Comparative idiogram of chromosomes with differences in distribution of euchromatin and heterochromatin of Banate (BET) and Syenichko - Peshterski (SET) honeybee ecotypes.

Thus, our results are similar to the results obtained by Popesković et al. (1995, 1997) and Stanimirović et al. 1997a, 1998), who also investigated the variability of chromosome macro- and microstructures of the honeybee from Yugoslavia.

Also, our results for the BET ecotype are in agreement with those of Hoshiba and Kusanagi (1978), Hoshiba (1979) and Hoshiba et al. (1981), with the exception of those pertaining to the G - banding chromosomes of the SET ecotype.

On the basis of the results obtained by biometric analyses of honeybee chromosomes and G - band polymorphism, it can be concluded that the SET honeybee represents an indigenous ecogenotype from the Syenichko-Peshterski area. This ecogenotype has its own specific cytogenetic characteristics, but it also has some impressive adaptive and productive features (Stojanovic, 1992, 1994; Popeskovic et al. 1994, Stanimirović et al. 1997b, Stanimirović et al. 1997c) under the severe conditions of the plateau. The genome of the SET honeybee ecotype is invaluable for the preservation and conservation of animal genetic resources.

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BIODIVERZITET MEDONOSNE PČELE *Apis mellifera,* Linne (1758), JUGOSLOVENSKIH PODRUČJA
II - ULTRAHROMOZOMSKE STRUKTURNE RAZLIKE IZMEĐU BANATSKOG I SJENIČKO - PEŠTERSKOG EKOTIPA MEDONOSNE PČELE

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SADRŽAJ

Ispitivan je hromozomski diverzitet medonosne pčele Peštersko - Sjeničke visoravni (sjeničko-pešterski ekotip - SET) i područja Beograda i okoline (banatski ekotip - BET).

Ultrahromozomske strukturne analize ukazuju na postojanje jasne razlike u distribuciji G - traka na hromozomima 1, 2, 4, 11, 12, 13, 15 i 16 sjeničko-pešterskog ekotipa medonosne pčele u odnosu na Banatski, tako potvrđujući našu ranije postavljenu hipotezu da su amplifikacije i rearanžmani hromozomskih regiona mogući uzrok biometrijskih hromozomskih razlika istraživanih

ekotipova medonosne pčele. Hromozomi prvog para sjeničko-pešterskog ekotipa medonosne pčele na p - kraku poseduju jedan heterohromatinski (D_2) i jedan euhromatinski blok (D_3) više u poređenju sa hromozomima istog autozomalnog para banatske medonosne pčele. Hromozomi drugog para sjeničko-pešterskog ekotipa na istom kraku imaju jednu svetliju euhromatinsku traku više (B_{1a}). Međutim, na hromozomima 4 [u višku dve trake: jedna heterohromatinska (C_2) i jedna euhromatinska (C_3)], 11 [u višku tri trake dve heterohromatinske (A_{1a}, A_{1c}) i jedna euhromatinska (A_{1b})], 12 [u višku jedna heterohromatinska traka (A_1)], 13 [u višku tri trake: jedna heterohromatinska (A_2) i dve euhromatinske (A_1, A_3)], 15 [u višku je jedna heterohromatinska traka (A_1)], i 16 [u višku je jedna heterohromatinska traka (A_{1a})] sjeničko-pešterskog ekotipa, amplifikacije euhromatinskih/heterohromatinskih blokova obavljene su na q - kraku.