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BIODIVERSITY OF THE HONEYBEE *APIS MELLIFERA*, LINNE (1758), FROM SOME YUGOSLAV REGIONS
I - THE BIOMETRIC VARIABILITY OF THE CHROMOSOMES OF THE BANAT AND SYENICHKO - PESHTERSKI ECOTYPES

Z. STANIMIROVIĆ*, D. POPESKOVIĆ*, and D. PEJOVIĆ*

*Department of Biology, Faculty of Veterinary Medicine, Bul. JNA 18, Belgrade, Yugoslavia

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In this paper are presented the results of comparative biometric studies of the chromosomes of two honeybee ecotypes (the Banat ecotype - BET, and the Syenichko - Peshterski ecotype - SET) from some ecogeographically defined Yugoslav regions. They differ significantly in many microgeographic and microclimatic elements, which may be a cause of the genetic (chromosomal) diversity of the studied species.

The biometric analyses indicated differences in the relative chromosome length and centromere index (arm ratio). The greatest differences in the relative lengths of chromosomes were observed between chromosomes 12, 2, 3, 1 and 6 in favour of the SET ecotype, and in chromosomes 15, 14 and 11 in favour of the BET reference ecotype. However, the monitoring of the centromere index revealed the greatest differences between chromosomes 16, 1, 2 and 4.

On the basis of these results we advanced the hypothesis that the observed chromosomal biometric differences are the result of the amplification and rearrangement of chromosome regions of the representatives of the studied honeybee ecotypes, but this requires additional confirmation by ultrastructural analyses of chromosomes.

Key words: honeybee, ecotypes, chromosomes, biometric analysis, centromere index, relative chromosome length, biodiversity.

INTRODUCTION

Biodiversity implies biological heterogeneity. In other words, the entirety of the existing animal and plant genotypes and phenotypes, i.e. the natural hereditary quality, and thereby the maintenance of the variability of animal and plant genomes are involved. (Dempfle, 1990; Hodgers, 1991; Beilharz, 1993;

Milner, 1966). According to Torp - Donner and Juga (1997), biodiversity within all the existing ecosystems is indispensable for their stability, functioning, mutual interaction and survival.

On the basis of the UN programme(1989) pertaining to the protection of the environment, and in cooperation with the European Association for Animal Production, in 1990 the FAO initiated a programme to recording all the breeds and lines of both domestic and other economically important animals. At the Brazil Conference in 1992, the Convention on Biological Diversity was adopted, stating clearly a need for the preservation of biodiversity at the international level (Kišgeci, 1997).

In order to make our own contribution to the above-mentioned global programme, as well as to support the modern concept of the maintenance of biological diversity (Dempfle, 1990; Hodgers, 1991; Beilharz, 1993; Milner, 1996), we undertook an investigation of the diversity of the honeybee in some geographically defined regions of Yugoslavia, characterized by numerous ecogeographical specificities. Thus, we defined two ecotypes as the best adapted to all the ecogeographical characteristics of the regions where the samples were collected. The first ecotype was the Banat ecotype - the best adapted to the lowlands microgeographical conditions of Belgrade and its surroundings (marked as the BET ecotype). This ecotype was taken as the reference ecotype, because its chromosome traits were in total agreement with the results of the cytogenetic analyses of the honeybee samples studied by Hoshiba and Kusanagi (1978), Hoshiba (1979) and Hoshiba et al. (1981). The other ecotype was the Syenichko - Peshterski ecotype (marked as the SET ecotype) - the best adapted to the ecogeographical specificities of the Peshter Plateau.

The genetic characteristics of the honeybee were studied by Hoshiba and Kusanagi (1978), Hoshiba (1979) and Hoshiba et al. (1981). These authors well documented the cytogenetic characteristics of the honeybee describing all the chromosomes of the species *Apis mellifera*. Moreover, Milner (1996) published an interesting and original paper on honeybees, their origins, evolution and diversity. The diversity of the honeybee *Apis mellifera* was also studied by Popesković et al. (1995, 1997). Stanimirović and collaborators (1997, 1998) made a karyological study of some ecotypes of the honeybee from Yugoslavia, pointing out the existence of some significant differences not only in biometric but also in ultrastructural organisation of chromosomes.

MATERIAL AND METHODS

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

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 the 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 the maturation for at least one day, the preparations were stained using the Giemsa technique and then biometric analyses were made.

RESULTS AND DISCUSSION

The results of the coparative analysis of the relative length of chromosomes and arm ratio of the investigated reference BET and SET honeybee ecotypes are shown in Table 1.

Table 1. Comparative analysis of the chromosome variability of two honeybee ecotypes from Yugoslav regions.

No.	Centromere position	Relative chromosome length				Arm ratio			
		BET*	SET*	Δ SET-BET	Q SET/BET	BET*	SET*	Δ SET-BET	Q AI* SET/BET
1.	Metacentric	11.92	12.12	0.20	1.017	1.42	1.78	0.36	1.253
2.	Metacentric	7.73	8.02	0.29	1.037	1.28	1.51	0.23	1.179
3.	Submetacentric	7.37	7.60	0.23	1.031	1.87	1.81	–0.06	0.967
4.	Metacentric	7.03	7.13	0.10	1.014	1.45	1.27	0.18	1.141
5.	Submetacentric	6.94	7.08	0.14	1.020	2.01	2.10	0.09	1.044
6.	Submetacentric	6.53	6.72	0.19	1.029	1.80	1.92	0.12	1.066
7.	Metacentric	6.35	6.51	0.16	1.025	1.25	1.38	0.13	1.104
8.	Submetacentric	6.09	6.06	–0.03	0.995	2.14	2.17	0.03	1.014
9.	Metacentric	5.79	5.82	0.03	1.005	1.18	1.22	0.04	1.033
10.	Submetacentric	5.77	5.69	0.08	0.986	1.78	1.78	0.00	1.000
11.	Metacentric	5.46	5.22	0.24	0.956	1.27	1.45	0.18	1.141
12.	Submetacentric	5.27	5.65	0.38	1.075	2.18	2.26	0.08	1.036
13.	Metacentric	4.99	5.10	0.11	1.022	1.40	1.31	–0.09	0.970
14.	Submetacentric	4.72	4.12	0.60	0.872	1.81	1.98	0.17	1.093
15.	Submetacentric	4.44	3.53	0.91	0.795	2.18	2.26	0.08	1.036
16.	Metacentric	3.67	3.62	0.05	0.986	1.28	1.78	0.50	1.390

BET* Banat ecotype
SET* Syenichko-Peshterski ecotype
AI* Arm indexes (centromere index)

The relative lengths of chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 12 and 13 of the SET ecotype were greater in relation to the equivalent chromosomes of the BET ecotype. However, chromosomes 8, 10, 11, 14, 15 and 16 of the SET ecotype were relatively shorter than those in the BET ecotype, which can be seen clearly in Figure 1. The analyses of the am ratio (centromere index)

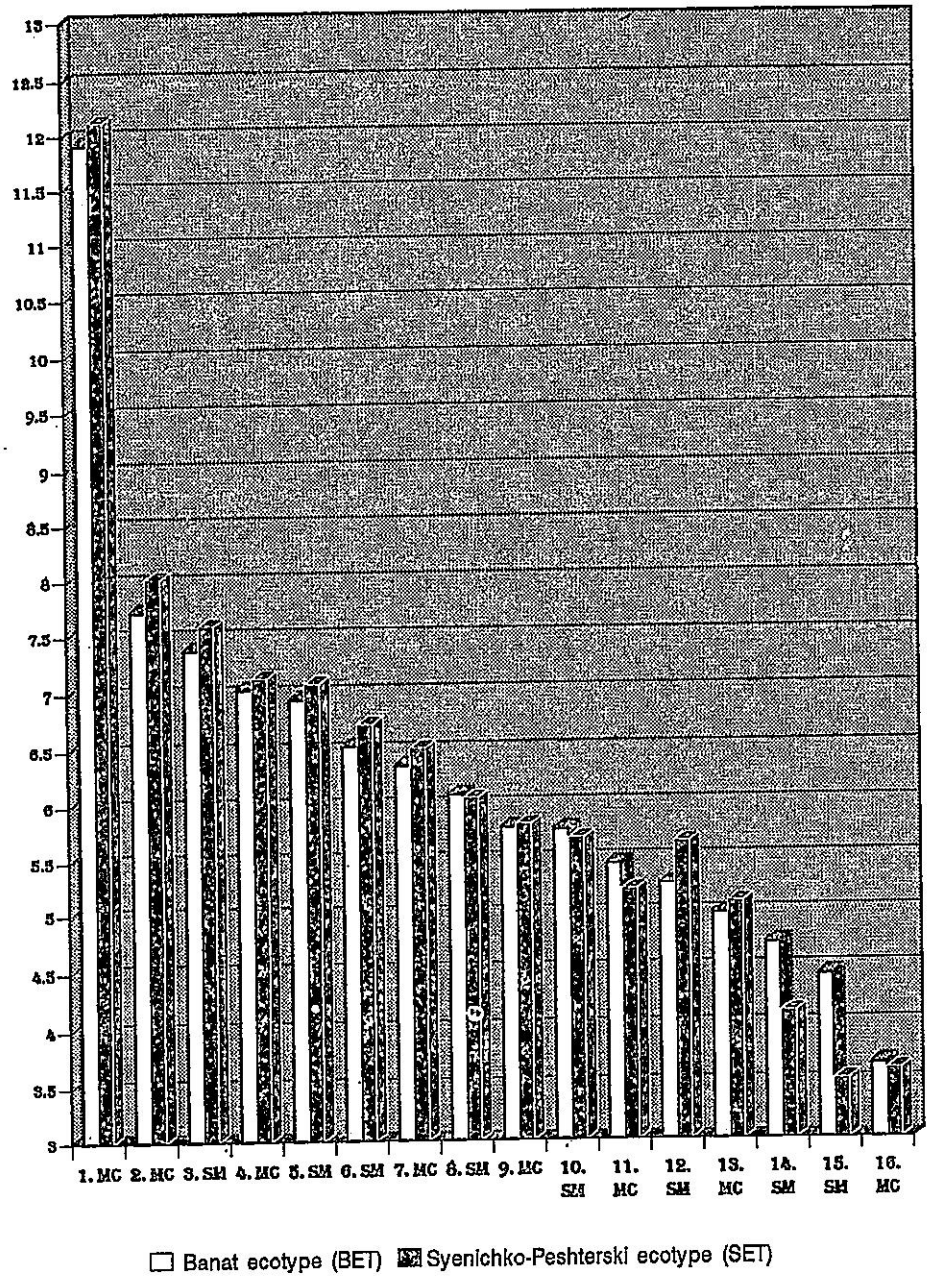

Figure 1. Relative chromosome length of two Yugoslav ecotypes of honeybee *(Apis mellifera)*.

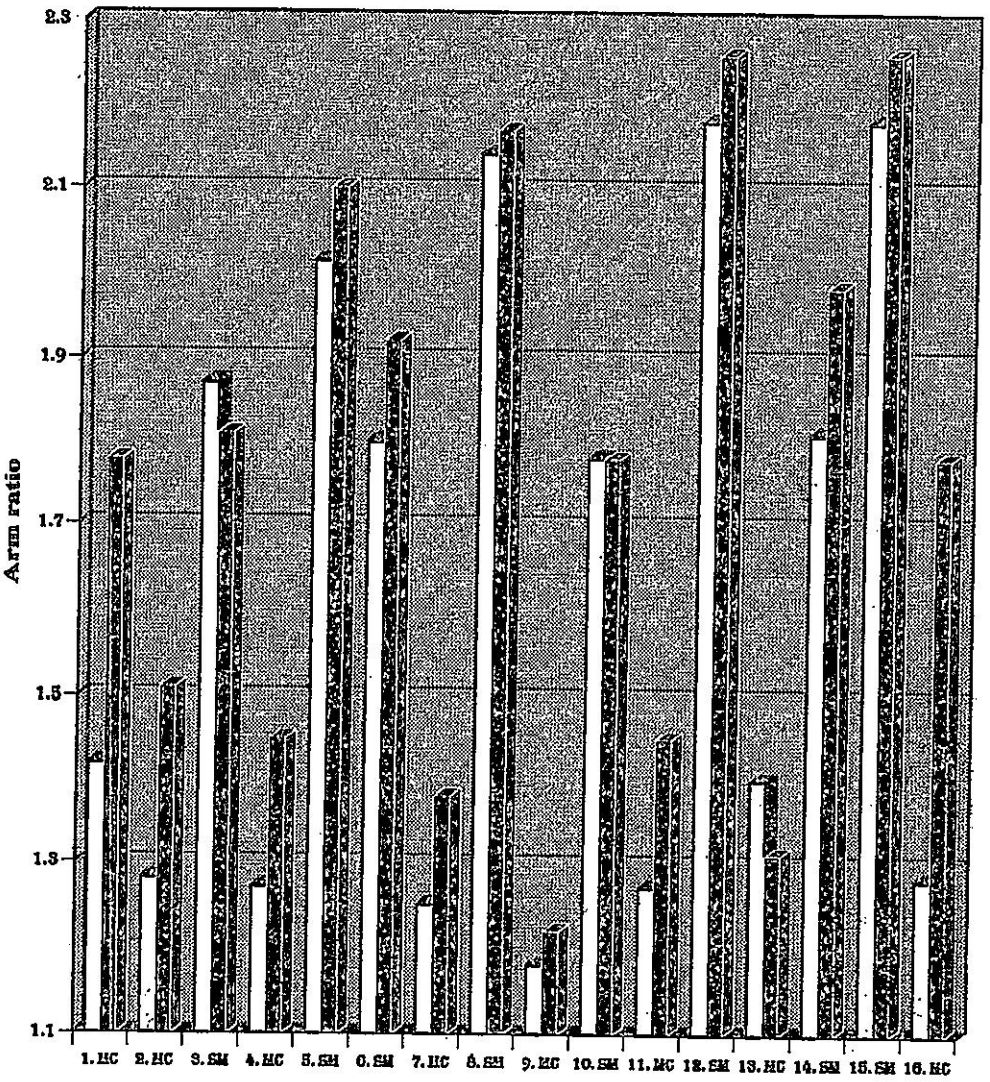

Figure 2. Arm ratio of two Yugoslav ecotypes of honeybee *(Apis mellifera)*

showed the most marked differences for chromosomes 16, 1, 2, 4, 11, 14 and so on as indicated by the Δ values in Table 1. This can also be seen in Figure 2.

These data, indicate clearly that the differences in the relative chromosome lengths are not so marked (Figure 1) as the differences in the centromere index (arm ratio) of the studied honeybee ecotypes (Figure 2). These results prompted us to propose a hypothesis that the marked biometric differences of certain chromosomes might be the result of chromosomal rearrangement and amplifications of chromosomal regions of the SET honeybee ecotype. Our hypothesis was supported by the results of the analysis of the Q value which is the quotient of the studied biometric values for the chromosomes, of the investigated ecotypes. Thus, Table 1 shows clearly the variations in coefficient Q values from the first to the sixteenth chromosome.

A comparison of the results obtained by Hoshiba and Kusanagi (1978), Hoshiba (1979) and Hoshiba et al. (1981), with regard to the relative chromosome lengths and centromere index in the representatives of the subspecies *Apis mellifera mellifera,* with our results for the same parameters in the studied honeybee ecotypes (SET and BET) shows the existence of differences only in the SET ecotype, whereas such differences were not observed between our results for the BET ecotype and the honeybees investigated by Hoshiba and collaborators. These results are in full agreement with our previous biometric analyses of the chromosome set from honeybees sampled in the vicinity of Stalach (hives with honeybees dislocated from the Peshter Plateau), in which there was also a difference in the values for the relative chromosome lengths and centromere index in relation to the Banat honeybee (Popesković et al. 1995 and Popesković et al. 1997).

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BIODIVERZITET MEDONOSNE PČELE *Apis mellifera,* Linne (1758), JUGOSLOVENSKIH PODRUČJA
I - BIOMETRIJSKA VARIJABILNOST HROMOZOMA BANATSKOG I SJENIČKO - PEŠTERSKOG EKOTIPA

Z. STANIMIROVIĆ*, D. POPESKOVIĆ*, D. PEJOVIĆ*

SADRŽAJ

Ispitivan je biodiverzitet medonosne pčele odabranih jugoslovenskih područja (Pešterska visoravan - sjeničko-pešterski ekotip i područje Beograda - banatski ekotip), koja se značajno razlikuju po mnogim mikroekogeografskim elementima, za koje smo predpostavili da mogu biti uzrok genetičkog (hromozomskog) diverziteta ispitivane vrste.

Dobijeni rezultati ukazuju na postojanje biometrijskih hromozomskih razlika praćenih parametara (relativna dužina hromozoma i centromerni index) dva ispitivana autohtona ekotipa medonosne pčele (sjeničko-pešterski - SET i banatski ekotip - BET). Najveće razlike u relativnoj dužini hromozoma uočene su između hromozoma 12, 2, 3, 1 i 6 u korist sjeničko-pešterskog, odnosno 15, 14 i 11 u korist banatskog ekotipa. Međutim, praćenjem centromernog indeksa najuočljivije razlike su bile između hromozoma 16, 1, 2 i 4 kod sjeničko-pešterskog u odnosu na banatski ekotip. Ovi rezultati su nas naveli na hipotezu da su nastale biometrijske razlike posledica amplifikacije ili rearanžmana hromozomskih regiona predstavnika ispitivanih ekotipova medonosne pčele, što je neophodno potvrditi dodatnim ultrastrukturnim analizama hromozoma.