Apidologie 31 (2000) 343–356

Original article

Genetic and morphometric variation in honeybee (*Apis mellifera* L.) populations of Turkey

Irfan KANDEMIR, Meral KENCE, Aykut KENCE*

Department of Biology, Middle East Technical University, 06531 Ankara, Turkey

(Received 18 December 1998; revised 8 October 1999; accepted 22 October 1999)

Abstract – Six enzyme systems were studied to determine the genetic variability in honeybee populations in Turkey. Ten morphometric characters were also measured to determine the extent of morphometric variation. Out of six enzyme systems, four were found to be polymorphic with 16 allozymes. The average heterozygosity was calculated as 0.072 ± 0.007. Morphometric and electrophoretic variables were equally effective in discriminating honeybee populations. European and Anatolian honeybees were separated on the first axis, and Anatolian honeybees were further separated along a second canonical axis. The observation of rare alleles in isoenzymes, detection of high genetic diversity and the presence of four known subspecies support the argument that Anatolia has been a genetic center for honeybee populations in the Near East.

***Apis mellifera anatoliaca* / *A. m. caucasica* / *A. m. meda* / *A. m. syriaca* / population genetics / genetic variability / morphometry / electrophoresis / Turkey**

1. INTRODUCTION

Ruttner [30] claimed that southwest Asia is a zone of high morphological diversification and evolution for honeybees. Many clearly distinct races have evolved within this region, which includes a diversity of habitats. Asia Minor, including Anatolia, appears to be the genetic center for these honeybee subspecies according to the multivariate statistical analysis of morphometric data [30]. Honey bee races in this region include the subspecies *Apis mellifera anatoliaca, A. m. caucasica, A. m. meda,* and *A. m. syriaca,* which were considered by Ruttner [30] to form a basal branch (*O*) of the species. Another subspecies that is found in the European part of Turkey, i.e., Thrace, may be *A. m. carnica,* which belongs to the branch *C* of Ruttner's classification.

* Correspondence and reprints
E-mail: aykut@metu.edu.tr

Migratory beekeeping has become widespread in Turkey within the last 20–30 years. Thousands of colonies are overwintered in the Mediterranean and Aegean regions, and then moved to central and eastern Anatolia during the summer and fall. These practices might promote the gene flow between different races, and result in homogenization of the gene pool of Anatolian honeybees.

Despite the apparent importance of Anatolia in the evolution of honeybees, very little work has been done on the morphological and genetic diversity of Anatolian honeybees [14, 37]. In this study, we aimed to determine the extent of morphometric and genetic variation of honeybees distributed widely across Turkey. Ten morphometric variables were measured, and electrophoretic variation was studied in six enzyme systems.

2. MATERIALS AND METHODS

Honeybee samples were collected in 1994–1996 between March and September in Turkey. Samples were taken from 77 different locations in 36 provinces from different geographic regions of Turkey. Turkey is divided into seven geographic regions differing both in climatic conditions and in geological structure. Sampling was carried out mostly from small apiaries which do not practice migratory beekeeping, and the hives sampled were stationary during the March-September sampling period. Requeening of colonies was mostly natural, although some beekeepers reported that occasionally queens had been purchased for some colonies. In all cases we attempted to sample colonies that had no history of management for requeening. Special care was taken to sample from localities that were not frequented by migratory beekeepers. Approximately 3 000 worker bees were collected, and were put into small plastic bottles, which were labeled; the insects were fed either with honey cake (honey and powdered sugar [1:1]) or with 'Turkish delight' (water + saccharose + starch), and brought live to the laboratory. Honeybees were dissected, the thoraces were ground, and the homogenates were kept frozen until needed for electrophoresis.

Forewings and hind legs were mounted on a microscope slide for morphometric analysis. Microscope slides of legs and wings were projected onto a TV screen, and measurements were taken. In the present study, ten morphometric characters were measured, i.e., four for the hind legs, four for the forewings (according to Ruttner [30]), and an additional two forewing characters, distance *c* and distance *d* as determined by Nazzi [20].

Six enzyme systems (esterase: 3.1.1.1; hexokinase: 2.7.1.1; malate dehydrogenase: 1.1.1.37; malic enzyme: 1.1.1.40; phosphoglucomutase: 2.7.5.1; and phosphoglucose isomerase: 5.3.1.9), known to be polymorphic in *A. mellifera*, were utilized as biochemical markers. Starch-gel electrophoresis, gel and sample preparation and experimental conditions have been reported previously [14]. All allozymes were designated by using relative mobilities, with the most common allozyme used as standard (relative mobility: 100). Gene frequencies, enzyme heterozygosities and population heterozygosities were calculated according to Nei [22], using BIOSYS [39]. Goodness-of-fit of genotypic frequencies according to Hardy–Weinberg expectations were tested by the χ^2-test [38]. Multivariate statistical analyses were applied to both morphometric and electrophoretic data using SYN-TAX V [28]. A phenogram of samples from seven geographic regions was constructed using the Mahalanobis distances among centroids of groups in discriminant function by UPGMA in NTSYS-PC 1.70 [29]. Regressions of morphometric and electrophoretic variables on latitude and longitude were computed using SYSTAT-7.0 [19].

3. RESULTS

The mean values of the characters measured and standard errors have been shown in Table I, together with the number of hives and the total number of individuals in each province.

Analysis of variance (ANOVA) of the data showed a high heterogeneity among honeybee populations. Out of 19 variables (10 morphometric and 9 electrophoretic), 11 displayed significant heterogeneity. Mdh^{65} and Mdh^{100} gene frequencies and distance *d*, wing length variables were found to be highly heterogeneous ($P < 0.001$).

Out of the six enzyme systems assayed, four were found to be polymorphic and two exhibited invariant banding patterns (Tab. II, and Figs. 1–4). The populations of honeybees in Turkey were found to be in Hardy-Weinberg equilibrium with respect to all polymorphic enzymes, except the *Pgm* enzyme system in the majority of southern honeybee populations, where deviations were in favor of heterozygotes. Out of 77 sampling localities, in 26 of these, there were significant deviations in favor of *Pgm* heterozygotes (17 localities; $P < 0.001$; 2 localities; $P < 0.01$; 7 localities; $P < 0.05$).

3.1. *Pgm* locus

The *Pgm* locus exhibited four alleles, Pgm^{45}, Pgm^{63}, Pgm^{75} and Pgm^{100} according to their relative mobilities in the present study. The frequency of the most common allele (Pgm^{75}) ranged between 0.500–0.976 in 35 polymorphic locations. A significant linear relationship was revealed by the regression of the *Pgm* allele (Pgm^{75} and Pgm^{100}) frequencies on latitude. This is the first report of such a relationship published in the literature. The distribution of allele frequencies where the rare alleles are pooled is given in Figure 1.

3.2. *Est*-3 locus

The *Est*-3 locus exhibited three alleles, Est^{70}, Est^{100}, Est^{130}, as reported previously for Czechoslovakian honeybees by Sheppard and McPheron [35], and in central Anatolian honeybees by Kandemir and Kence [14]. These alleles correspond to Est^{S}, Est^{M}, and Est^{F} respectively, in *A. m. ligustica* [2] and in Greek honeybees [3]. The frequency of the most common allele at the 22 polymorphic locations ranged between 0.853 and 0.995. Esterase was fixed for the Est^{100} allele in 14 locations in the northern and eastern provinces. Generally, there was a north to south differentiation in esterase allele frequencies. This conclusion was also confirmed by a significant linear relationship between the frequency of Est^{70} and latitude. The distribution of allele frequencies where the rare alleles are pooled is given in Figure 2.

3.3. *Hk* locus

In the *Hk* locus, five alleles (Hk^{77}, Hk^{87}, Hk^{100}, Hk^{110} and Hk^{120}) were found in different honeybee populations in Turkey. The frequency of the most common allele in the 19 locations where this enzyme was polymorphic ranged between 0.707–0.990. In Thrace, samples were monomorphic for the *Hk* locus. The majority of samples taken from the Black Sea region were fixed for the Hk^{100} allele, whereas hexokinase was polymorphic in southern Anatolia. There was also a north to south differentiation in the hexokinase allele frequencies, shown by a significant linear relationship between Hk^{110} gene frequencies and latitude. The distribution of allele frequencies where the rare alleles are pooled is shown in Figure 4.

3.4. *Mdh* locus

Four alleles (Mdh^{65}, Mdh^{87}, Mdh^{100} and Mdh^{116}) were found for this enzyme in the

Table I. Arithmetic means and standard errors of 10 morphometric variables from 36 provinces in Turkey.

Locations	No. of hive	No. of bees	Cubital A	Cubital B	Distance C	Distance D	Wing length	Wing width	Metatarsus length	Metatarsus width	Femur length	Tibia length
Adana	13	90	0.559 ± 0.007	0.252 ± 0.005	0.855 ± 0.006	1.868 ± 0.018	8.717 ± 0.056	2.850 ± 0.020	2.000 ± 0.027	1.164 ± 0.005	2.899 ± 0.039	2.468 ± 0.017
Afyon	3	14	0.501 ± 0.001	0.250 ± 0.009	0.840 ± 0.003	1.850 ± 0.020	8.719 ± 0.027	2.962 ± 0.036	2.109 ± 0.025	1.191 ± 0.007	2.757 ± 0.012	2.433 ± 0.002
Amasya	2	21	0.533 ± 0.013	0.245 ± 0.022	0.863 ± 0.000	1.903 ± 0.000	8.778 ± 0.008	2.962 ± 0.062	1.987 ± 0.067	1.188 ± 0.002	3.088 ± 0.025	2.435 ± 0.062
Antalya	22	129	0.523 ± 0.006	0.222 ± 0.005	0.839 ± 0.005	1.878 ± 0.011	8.959 ± 0.052	2.900 ± 0.020	2.072 ± 0.010	1.201 ± 0.004	2.918 ± 0.037	2.517 ± 0.026
Artvin	18	151	0.554 ± 0.005	0.258 ± 0.005	0.902 ± 0.004	1.941 ± 0.008	9.210 ± 0.028	3.034 ± 0.010	2.077 ± 0.066	1.215 ± 0.005	3.130 ± 0.023	2.521 ± 0.014
Balikesir	15	106	0.532 ± 0.009	0.248 ± 0.007	0.863 ± 0.005	1.950 ± 0.013	9.003 ± 0.046	3.000 ± 0.016	1.918 ± 0.019	1.182 ± 0.009	2.970 ± 0.026	2.587 ± 0.017
Bingöl	7	116	0.526 ± 0.010	0.237 ± 0.007	0.870 ± 0.004	1.841 ± 0.012	8.830 ± 0.024	2.995 ± 0.014	2.059 ± 0.010	1.203 ± 0.005	2.721 ± 0.021	2.437 ± 0.024
Bolu	20	125	0.535 ± 0.008	0.249 ± 0.007	0.881 ± 0.006	1.933 ± 0.011	9.056 ± 0.033	3.040 ± 0.016	2.050 ± 0.030	1.187 ± 0.008	2.914 ± 0.028	2.515 ± 0.039
Diyarbakir	3	17	0.510 ± 0.012	0.241 ± 0.016	0.864 ± 0.028	1.807 ± 0.045	8.603 ± 0.088	2.960 ± 0.013	2.072 ± 0.043	1.177 ± 0.022	2.799 ± 0.019	2.479 ± 0.049
Edirne	15	149	0.544 ± 0.009	0.238 ± 0.005	0.850 ± 0.005	1.948 ± 0.012	9.019 ± 0.032	3.003 ± 0.015	2.018 ± 0.017	1.196 ± 0.008	2.877 ± 0.024	2.459 ± 0.021
Elazig	7	55	0.535 ± 0.010	0.257 ± 0.006	0.867 ± 0.004	1.934 ± 0.010	8.845 ± 0.072	2.974 ± 0.018	2.003 ± 0.016	1.203 ± 0.007	2.835 ± 0.045	2.374 ± 0.038
Eskisehir	12	84	0.566 ± 0.007	0.243 ± 0.004	0.894 ± 0.004	2.003 ± 0.005	9.152 ± 0.028	3.033 ± 0.013	2.024 ± 0.018	1.199 ± 0.007	3.031 ± 0.014	2.593 ± 0.012
Hatay	36	271	0.537 ± 0.005	0.220 ± 0.003	0.826 ± 0.004	1.853 ± 0.008	8.696 ± 0.029	2.794 ± 0.010	1.991 ± 0.010	1.180 ± 0.006	2.850 ± 0.014	2.421 ± 0.010
Isparta	8	59	0.556 ± 0.012	0.252 ± 0.005	0.896 ± 0.009	1.970 ± 0.016	9.251 ± 0.070	3.023 ± 0.024	2.045 ± 0.017	1.184 ± 0.010	2.937 ± 0.041	2.512 ± 0.035
Mersin	9	63	0.539 ± 0.009	0.242 ± 0.004	0.859 ± 0.006	1.891 ± 0.018	8.966 ± 0.071	2.915 ± 0.014	1.988 ± 0.034	1.190 ± 0.008	2.911 ± 0.042	2.482 ± 0.015
Izmir	4	28	0.569 ± 0.009	0.247 ± 0.008	0.879 ± 0.005	1.941 ± 0.024	9.130 ± 0.034	3.008 ± 0.023	1.924 ± 0.014	1.194 ± 0.015	2.997 ± 0.015	2.461 ± 0.010
Kars	7	63	0.545 ± 0.011	0.279 ± 0.003	0.870 ± 0.008	1.918 ± 0.008	8.999 ± 0.023	2.949 ± 0.012	1.988 ± 0.018	1.170 ± 0.015	3.138 ± 0.030	2.509 ± 0.011
Kastamonu	16	126	0.508 ± 0.007	0.259 ± 0.005	0.866 ± 0.004	1.878 ± 0.008	8.877 ± 0.024	3.009 ± 0.009	2.053 ± 0.014	1.185 ± 0.005	2.717 ± 0.024	2.361 ± 0.024
Kayseri	7	35	0.561 ± 0.014	0.248 ± 0.005	0.880 ± 0.006	1.904 ± 0.008	9.211 ± 0.038	2.977 ± 0.026	2.028 ± 0.018	1.208 ± 0.012	2.820 ± 0.025	2.468 ± 0.026
Kirklareli	27	183	0.613 ± 0.006	0.228 ± 0.005	1.128 ± 0.017	2.166 ± 0.005	9.253 ± 0.018	3.381 ± 0.066	2.329 ± 0.015	1.480 ± 0.016	3.473 ± 0.026	3.240 ± 0.043
Konya	12	93	0.549 ± 0.005	0.236 ± 0.006	0.861 ± 0.005	2.070 ± 0.013	9.249 ± 0.029	2.992 ± 0.014	2.042 ± 0.009	1.185 ± 0.006	2.946 ± 0.022	2.551 ± 0.014
Manisa	16	111	0.524 ± 0.007	0.241 ± 0.005	0.857 ± 0.006	1.950 ± 0.008	9.066 ± 0.032	2.934 ± 0.015	1.962 ± 0.015	1.179 ± 0.004	2.945 ± 0.015	2.549 ± 0.016
K.Maras	4	12	0.605 ± 0.003	0.258 ± 0.029	0.869 ± 0.016	1.947 ± 0.036	9.001 ± 0.102	2.914 ± 0.017	1.973 ± 0.028	1.189 ± 0.029	2.853 ± 0.037	2.469 ± 0.030
Mugla	6	84	0.527 ± 0.012	0.220 ± 0.007	0.834 ± 0.005	1.894 ± 0.014	9.112 ± 0.084	2.893 ± 0.011	2.061 ± 0.014	1.194 ± 0.006	3.069 ± 0.026	2.532 ± 0.019
Sinop	6	42	0.560 ± 0.007	0.236 ± 0.011	0.926 ± 0.008	2.007 ± 0.009	9.315 ± 0.062	3.104 ± 0.012	2.014 ± 0.010	1.217 ± 0.004	2.996 ± 0.025	2.609 ± 0.025
Sivas	16	112	0.506 ± 0.003	0.225 ± 0.005	0.857 ± 0.005	1.994 ± 0.007	8.991 ± 0.025	3.014 ± 0.009	2.066 ± 0.011	1.222 ± 0.011	2.877 ± 0.007	2.501 ± 0.010
Trabzon	2	16	0.567 ± 0.020	0.248 ± 0.005	0.948 ± 0.008	1.993 ± 0.007	9.142 ± 0.065	3.032 ± 0.002	2.087 ± 0.073	1.235 ± 0.038	2.935 ± 0.042	2.665 ± 0.002
S. Urfa	2	14	0.512 ± 0.008	0.227 ± 0.007	0.833 ± 0.010	1.617 ± 0.017	8.032 ± 0.035	2.752 ± 0.005	1.950 ± 0.003	1.145 ± 0.025	2.643 ± 0.003	2.338 ± 0.052
Usak	5	35	0.563 ± 0.014	0.220 ± 0.004	0.861 ± 0.005	1.924 ± 0.011	9.231 ± 0.031	2.997 ± 0.015	1.993 ± 0.012	1.191 ± 0.004	2.813 ± 0.010	2.398 ± 0.009
Van	19	122	0.530 ± 0.007	0.219 ± 0.004	1.026 ± 0.015	1.917 ± 0.013	9.099 ± 0.059	3.273 ± 0.073	2.089 ± 0.018	1.306 ± 0.011	3.037 ± 0.032	2.957 ± 0.061
Yozgat	4	65	0.560 ± 0.006	0.255 ± 0.006	0.884 ± 0.016	1.983 ± 0.038	9.195 ± 0.105	3.029 ± 0.057	2.048 ± 0.019	1.231 ± 0.014	2.911 ± 0.005	2.563 ± 0.006
Zonguldak	15	104	0.513 ± 0.010	0.212 ± 0.005	1.075 ± 0.007	1.984 ± 0.010	9.225 ± 0.024	3.232 ± 0.028	2.230 ± 0.014	1.354 ± 0.014	3.287 ± 0.027	3.056 ± 0.012
Karaman	6	50	0.541 ± 0.011	0.243 ± 0.006	0.861 ± 0.006	1.954 ± 0.008	9.243 ± 0.036	2.985 ± 0.021	1.977 ± 0.012	1.186 ± 0.008	2.882 ± 0.005	2.561 ± 0.009
Bartin	5	32	0.549 ± 0.009	0.221 ± 0.009	0.918 ± 0.009	1.977 ± 0.018	9.035 ± 0.079	3.055 ± 0.030	2.069 ± 0.026	1.201 ± 0.017	2.874 ± 0.041	2.605 ± 0.024
Ardahan	18	105	0.544 ± 0.005	0.252 ± 0.004	0.899 ± 0.003	1.937 ± 0.006	9.134 ± 0.017	3.045 ± 0.012	2.039 ± 0.012	1.224 ± 0.005	3.193 ± 0.019	2.531 ± 0.011
Igdir	4	26	0.543 ± 0.008	0.235 ± 0.016	0.943 ± 0.042	1.929 ± 0.019	9.094 ± 0.053	2.958 ± 0.003	2.058 ± 0.048	1.188 ± 0.045	3.157 ± 0.020	2.486 ± 0.043

Table II. Allele frequencies of four polymorphic enzymes from 36 provinces in Turkey.

Locations	No. of hive	No. of bees	PGM 45	PGM 63	PGM 75	PGM 100	HK 77	HK 87	HK 100	HK 110	HK 120	MDH 65	MDH 87	MDH 100	MDH 116	EST 70	EST 100	EST 130
Adana	13	90	–	–	0.717	0.283	–	–	0.922	0.050	0.028	–	–	1.000	–	0.044	0.956	–
Afyon	2	21	–	–	0.857	0.143	–	–	0.929	0.071	–	–	–	1.000	–	–	1.000	–
Amasya	3	14	–	–	0.750	0.250	–	–	1.000	–	–	–	–	1.000	–	–	1.000	–
Antalya	22	129	–	0.016	0.930	0.054	–	–	0.884	0.008	0.109	–	–	1.000	–	0.085	0.915	–
Artvin	18	151	–	–	0.752	0.248	0.007	–	0.993	–	–	0.003	–	0.997	–	–	1.000	–
Balikesir	15	106	–	–	0.934	0.066	–	–	1.000	–	–	0.009	–	0.991	–	–	1.000	–
Bingöl	7	116	0.004	–	0.513	0.483	–	0.009	0.707	–	0.184	0.004	–	0.996	–	0.073	0.927	–
Bolu	20	125	–	–	0.756	0.244	0.008	–	0.992	–	–	0.016	–	0.984	–	–	1.000	–
Diyarbakir	3	17	–	–	0.794	0.206	–	–	1.000	–	–	–	–	1.000	–	0.147	0.853	–
Edirne	15	149	0.017	–	0.919	0.064	–	0.010	0.990	–	–	0.258	–	0.735	0.007	0.013	0.977	0.010
Elazig	7	55	–	–	0.973	0.027	–	–	0.982	–	0.018	–	–	0.982	0.018	0.018	0.982	–
Eskisehir	12	84	–	–	0.887	0.113	–	–	1.000	–	–	–	–	1.000	–	–	1.000	–
Hatay	36	271	–	–	0.504	0.496	–	–	0.926	0.024	0.050	–	–	1.000	–	0.015	0.985	–
Isparta	8	59	–	–	0.856	0.144	–	–	0.763	0.237	–	–	–	1.000	–	0.068	0.932	–
Mersin	9	63	–	0.016	0.746	0.238	0.008	–	0.968	0.024	–	–	–	1.000	–	0.032	0.968	–
Izmir	4	28	–	–	0.929	0.071	–	–	1.000	–	–	–	–	1.000	–	0.018	0.982	–
Kars	7	63	–	–	0.730	0.270	–	–	0.984	0.016	–	–	–	0.889	0.111	–	1.000	–
Kastamonu	16	126	–	–	0.905	0.095	–	–	1.000	–	–	0.048	–	0.952	–	0.044	0.956	–
Kayseri	7	35	–	–	0.914	0.086	–	–	0.986	0.014	–	0.014	–	0.986	–	0.014	0.986	–
Kirklareli	27	183	–	–	0.691	0.309	–	–	1.000	–	–	0.363	–	0.637	–	0.005	0.995	–
Konya	12	93	–	–	0.973	0.027	0.005	–	0.844	0.151	–	–	–	0.957	0.043	0.129	0.871	–
Manisa	16	111	–	0.005	0.784	0.212	–	–	1.000	–	–	–	0.005	0.991	0.005	0.068	0.932	–
K. Maras	4	12	–	–	1.000	–	–	–	1.000	–	–	–	–	1.000	–	–	1.000	–
Mugla	6	84	–	0.006	0.673	0.321	–	0.006	0.869	–	0.125	0.012	0.006	0.982	–	0.054	0.946	–
Sinop	6	42	–	–	0.976	0.024	–	–	1.000	–	–	–	–	1.000	–	0.012	0.988	–
Sivas	16	112	–	–	0.942	0.058	–	–	0.906	0.094	–	–	–	1.000	–	0.036	0.964	–
Trabzon	2	16	–	–	0.938	0.063	–	–	1.000	–	–	–	0.031	0.969	–	–	1.000	–
Urfa	2	14	–	–	0.500	0.500	–	–	1.000	–	–	–	–	1.000	–	–	1.000	–
Usak	5	35	–	0.014	0.657	0.329	–	–	0.986	0.014	–	–	–	1.000	–	–	1.000	–
Van	19	122	–	0.008	0.922	0.070	0.004	–	0.980	0.016	–	0.012	–	0.984	0.004	0.037	0.963	–
Yozgat	4	65	–	–	0.831	0.169	–	–	1.000	–	–	0.038	–	0.931	0.031	0.038	0.962	–
Zonguldak	15	104	–	–	0.971	0.029	–	–	1.000	–	–	0.014	–	0.986	–	0.014	0.986	–
Karaman	6	50	–	–	0.830	0.170	–	–	1.000	–	–	–	–	1.000	–	0.130	0.870	–
Bartin	5	32	–	–	0.953	0.047	–	–	1.000	–	–	–	–	1.000	–	–	1.000	–
Ardahan	18	105	–	–	0.800	0.200	0.033	0.005	0.962	–	–	0.005	–	0.995	–	–	1.000	–
Igdir	4	26	–	–	0.750	0.250	–	–	1.000	–	–	–	–	1.000	–	–	1.000	–

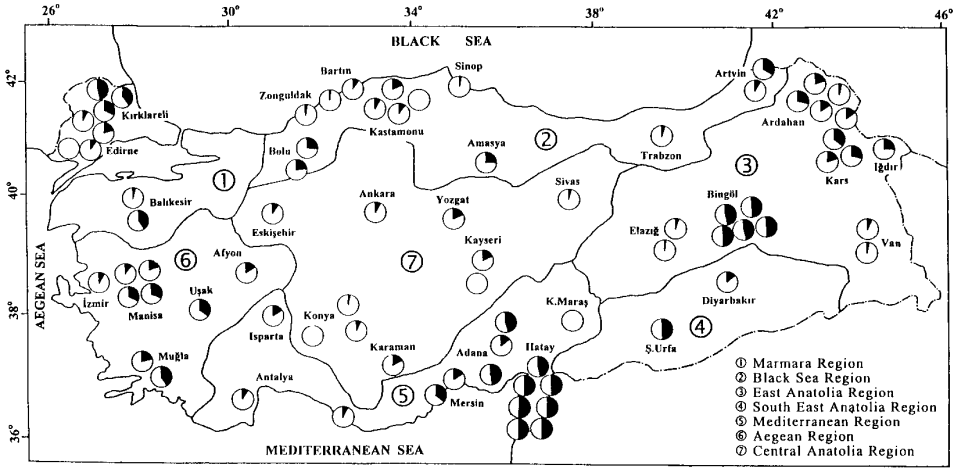

Figure 1. The distribution of Pgm allele frequencies in Turkey (○: common allele, ●: rare alleles).

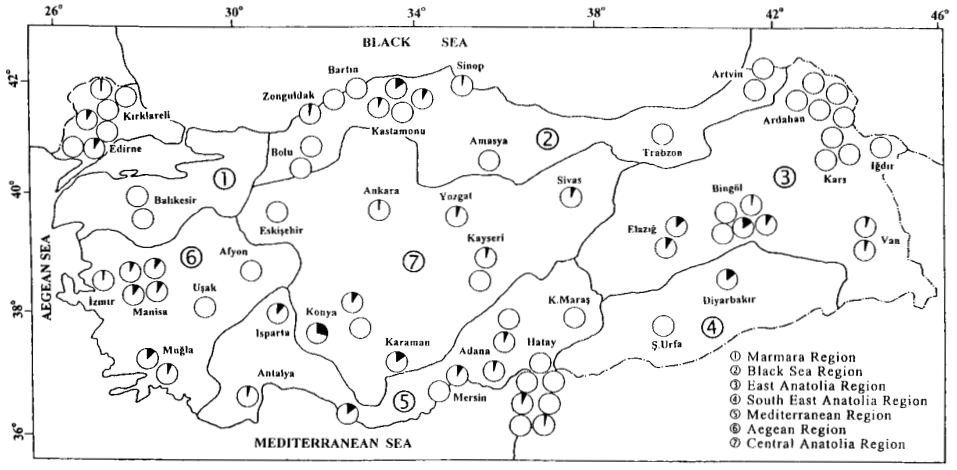

Figure 2. The distribution of Est allele frequencies in Turkey (○: common allele, ●: rare alleles).

present study. The frequency of the most common allele ranged between 0.637–0.997 in the 18 locations where this enzyme was polymorphic. Mdh^{65} is the most frequent of the rare alleles in Thrace, whereas Mdh^{116} is the most frequent rare allele in northeast Turkey. The Mdh^{65} allele, which is infrequent in honeybees in Africa [21] and Anatolia ([14]; and the present study), is common in *A. m. ligustica* [34] and *A. m. carnica* [35]. The distribution of allele frequencies where the rare alleles are pooled is given in Figure 3.

3.5. *Pgi* and *Me* loci

Phosphoglucose isomerase (*Pgi*) and malic enzyme (*Me*) were invariant in

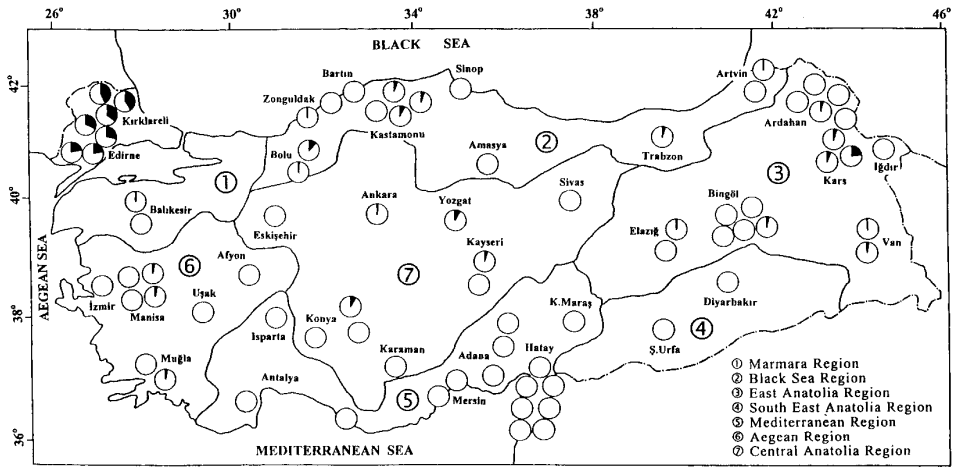

Figure 3. The distribution of Mdh allele frequencies in Turkey (○: common allele, ●: rare alleles).

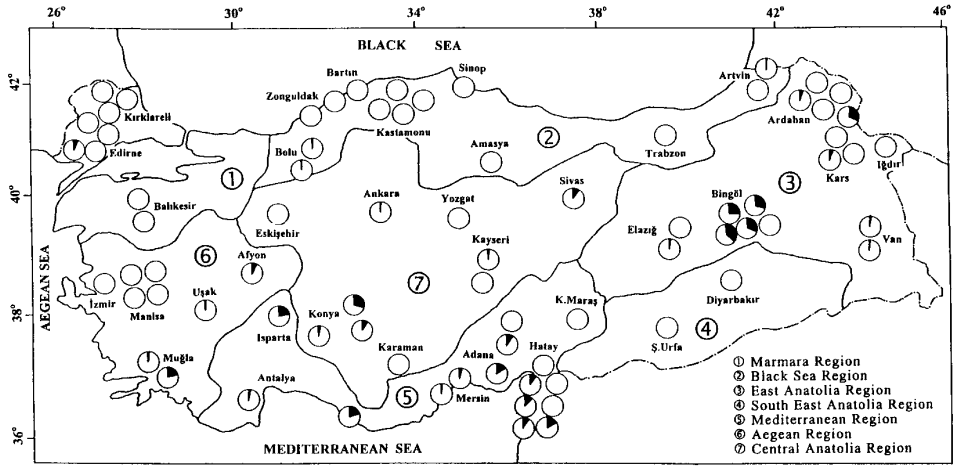

Figure 4. The distribution of Hk allele frequencies in Turkey (○: common allele, ●: rare alleles).

Turkish honeybee populations. The *Pgi* locus was previously studied in Turkey [14], and no genetic variability was detected.

In this study, the heterozygosities of locations for enzyme loci ranged between 0.012–0.186. Overall average heterozygosity for Turkish honeybees was calculated as 0.072 ± 0.007. This is the highest mean heterozygosity reported in *A. mellifera* to date. Pamilo et al. [27] and Sylvester [40] reported the mean heterozygosity for European honeybees as 0.010 and 0.012, respectively. Later, Sheppard [31] estimated the mean heterozygosity of *A. mellifera* from 23 European honeybee colonies as 0.038; he also noted that the mean heterozygosity was rather low in other *Apis* species, except for *A. florea* (mean heterozygosities of

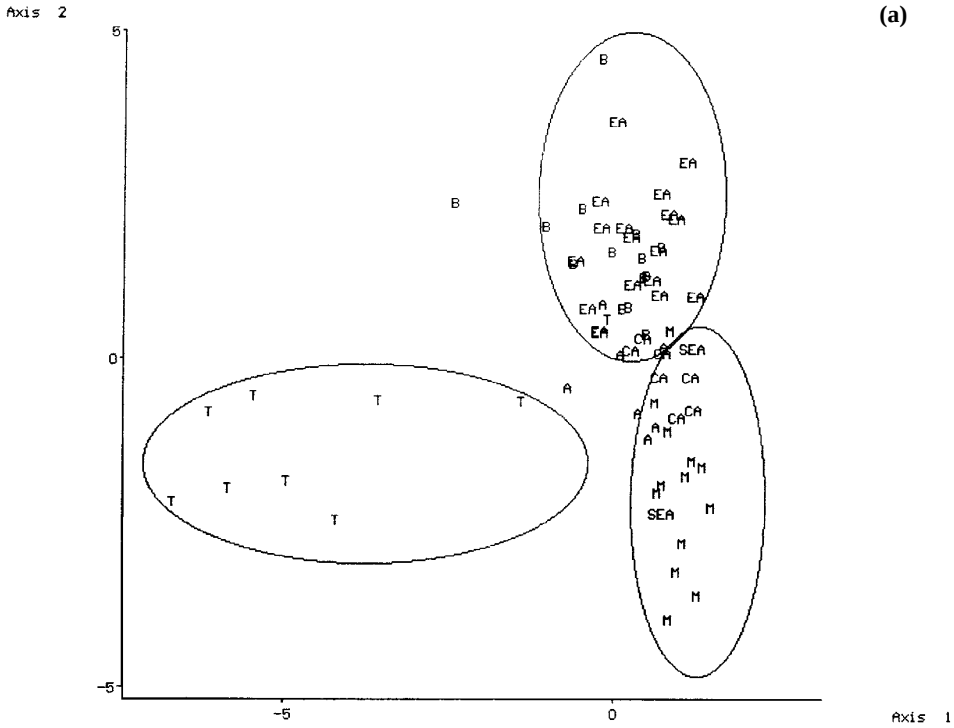

Figure 5. The result of discriminant function analysis of samples from the seven geographic regions in Turkey (**a**), excluding Thrace (**b**). **T**: Thrace; **B**: Black Sea; **EA**: east Anatolia; **CA**: central Anatolia; **M**: Mediterranean; **A**: Aegean; **SEA**: south-east Anatolia.

A. cerana, A. dorsata, A. florea were 0.004, 0.003 and 0.049, respectively).

3.6. Discriminant function analysis

Honeybees were allocated to the seven geographic regions of Turkey according to the geographic position of sampling locations. A multiple discriminant function analysis was carried out on the data collected from the samples taken from the seven geographic regions by combining the gene frequencies from electrophoresis and the measurements of morphometric variables. The three axes obtained in the multiple discriminant function analysis explained 87.86% of the total variation. The proportions of variation explained by the first, second, and third axis were 35.54, 32.26, and 20.06% respectively. Two major groups were discriminated by the discriminant function analysis (Fig. 5a). The first group included the honeybees from Thrace. In this group, the main variation was along the first canonical axis. The second group consisted of Anatolian honeybees (honeybees from the Asian part of Turkey), which varied mainly along the second canonical axis. Cubital A, cubital B, Pgm^{75}, Pgm^{100}, Mdh^{65}, Est^{70}, Est^{100} were the variables with the highest loadings on the first canonical axis, whereas cubital A, cubital B, distance *c*, Pgm^{75}, Pgm^{100}, Mdh^{65}, Mdh^{100}, Est^{70}, and Est^{100} were loaded highly on the second canonical axis. In the third canonical axis, wing length, Pgm^{75}, Pgm^{100}, Est^{70}, and Est^{100} were the variables contributing to the separation of the groups.

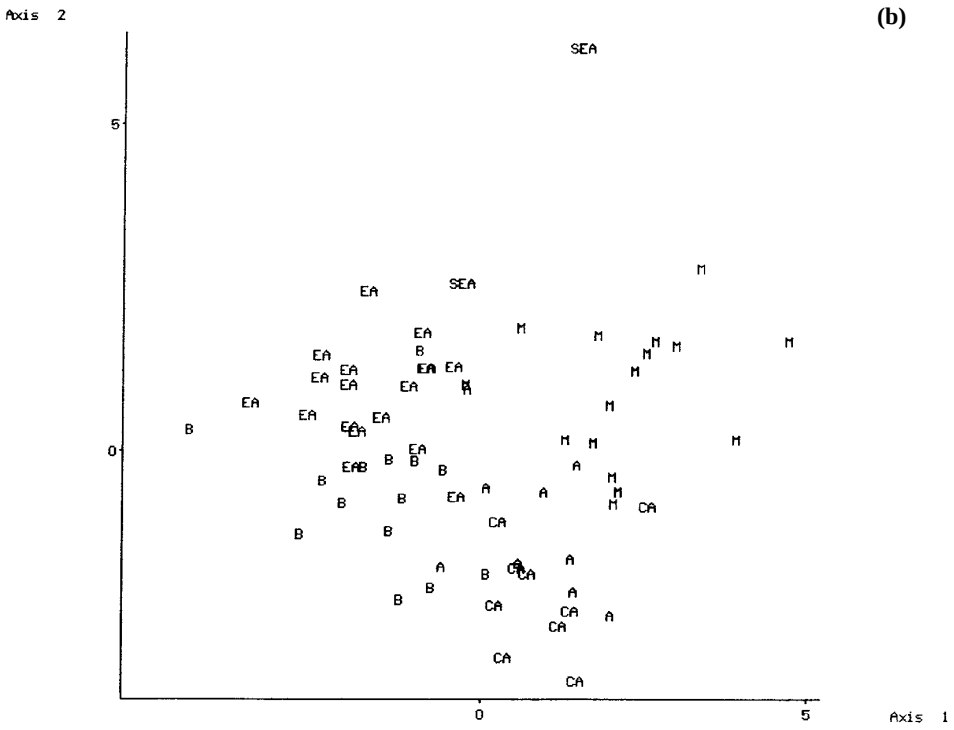

Figure 5. (Continued).

When Anatolian honeybees alone were subjected to discriminant function analysis on the basis of six geographical regions, distinct clusters of the sampling provinces, representing southeastern Anatolia, the Mediterranean region, central and eastern Anatolia were formed. Samples from the Aegean region could not be distinguished from those in central Anatolia, and samples from the Black Sea overlapped with the those from eastern Anatolia to a large extent (Fig. 5b). Three axes explained 89.68% of the total variation when honeybee populations of Thrace were excluded from the analysis. The variations explained by the three axes were 47.60, 33.80 and 9.28% respectively. Cubital A, cubital B, distance *c*, Pgm^{75}, Pgm^{100}, Hk^{110}, Mdh^{65}, Mdh^{100}, Est^{70} and Est^{100} were the variables that had the highest loadings on the first canonical axis. Cubital A, Pgm^{75}, Pgm^{100}, Hk^{100}, Mdh^{65} and Mdh^{100} had high loadings on the second axis. In the third canonical axis, cubital A, distance *c*, Pgm^{75}, Pgm^{100}, Hk^{100}, Mdh^{65}, Mdh^{100}, Est^{70} and Est^{100} were the variables contributing to the separation of the groups.

When a phenogram of honeybees from the seven geographic regions was constructed using Mahalanobis distances, the Black Sea and east Anatolian samples clustered very closely. The Aegean and central Anatolia formed a group, and this group together with Mediterranean samples made up a larger cluster. Thrace and southern Anatolian samples remained as distinct units within this phenogram (Fig. 6).

When multiple regression analysis was applied to the morphometric and electrophoretic variables using latitude and longitude as independent variables, eight out of 10 morphometric variables turned out to

be significantly dependent on latitude (cubital B, distance *c*, distance *d*, wing length, wing width, metatarsus width, femur and tibia length). Cubital B was also significantly dependent on longitude. Among the 12 gene frequencies, six of them showed a significant relationship with latitude (Pgm^{75}, Pgm^{100}, Hk^{110}, Mdh^{65}, Mdh^{100}, and Est^{70}). Mdh^{65} and Mdh^{100} were also significantly dependent on longitude (Tab. III).

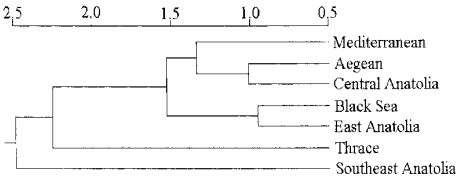

Figure 6. UPGMA phenogram of populations from seven geographic regions based on Mahalanobis distances among centroids of groups in discriminant function analysis.

4. DISCUSSION

Honeybees in Turkey show a high level of morphometric variation. Of the ten characters studied, five (distance *c*, distance *d*, wing length, wing width and metatarsus width) were found to be significantly different between localities ($P < 0.05$).

Table III. Results of multiple regression analysis of morphometric and electrophoretic variables on latitude and longitude.

	Y-Intercept (a)	Latitude regression coefficient (b)	*P*-value	Longitude regression coefficient (b)	*P*-value
Morphometric variables					
Cubital A	1.53	0.00	0.79	0.00	0.41
Cubital B	0.29	0.01	0.02*	0.00	0.01*
Wing C	0.98	0.04	0.00*	0.00	0.48
Wing D	4.59	0.03	0.01*	–0.01	0.26
Wing length	22.49	0.13	0.00*	–0.02	0.22
Wing width	3.57	0.14	0.00*	–0.01	0.65
Metatarsus length	5.08	0.02	0.06	0.00	0.40
Metatarsus width	2.75	0.02	0.00*	–0.00	0.71
Femur length	5.89	0.07	0.01*	0.01	0.49
Tibia length	5.65	0.06	0.04*	–0.01	0.36
Electrophoretic variables					
Pgm 63	0.07	–0.00	0.09	–0.00	0.67
Pgm 75	–0.26	0.03	0.00*	–0.00	0.20
Pgm 100	1.21	–0.03	0.00*	0.00	0.17
Hk 77	–0.21	0.01	0.23	–0.00	0.33
Hk 100	0.69	0.01	0.47	0.00	0.96
Hk 110	0.32	–0.01	0.02*	–0.00	0.54
Hk 120	0.23	–0.01	0.09	0.00	0.09
Mdh 65	–0.48	0.02	0.00*	–0.01	0.00*
Mdh 100	1.55	–0.02	0.00*	0.01	0.00*
Mdh 116	–0.07	0.00	0.60	0.00	0.09
Est 70	0.42	–0.01	0.00*	–0.00	0.26
Est 100	0.45	0.01	0.08	0.00	0.98

The significant regressions of morphometric and electrophoretic variables on latitude and longitude display a structured pattern in the distribution of populations. The spatial nature of this pattern is most likely the result of evolutionary forces acting on the honeybee populations. This hypothesis was supported by a spatial autocorrelation analysis conducted to further determine relationships among honeybee populations of Anatolia [15]. Morphometric variables that showed significant regressions on latitude also had high loadings on the first axis in the principal component analysis [15]. This axis, known as the size axis [13], allows us to conclude that the size of the honeybees increases with increasing latitude. Daly et al. [8] showed similar clinal geographic variation in morphometric characters in feral colonies of California. Based on the UPGMA phenogram, the differentiation in honeybees in Turkey has been maintained, despite extensive migratory beekeeping.

According to Ruttner [30], *A. m. anatoliaca* is distributed throughout central Anatolia, the Aegean, the Mediterranean, and a large part of the Black Sea region. *A. m. meda* is distributed in southeastern Anatolia, *A. m. caucasica* in northeastern Anatolia, and *A. m. carnica* in Thrace. This assessment of subspecies distribution is largely supported by electrophoretic data and our morphometric assessments on a reduced number of characters in honeybee populations. However, the small set of samples from southeastern Anatolia form a distinct cluster that appears to belong to *A. m. syriaca*, based on values of CI, wing length, and body size [11]. Similarly, honeybee populations in the Mediterranean region, isolated from the rest of the Anatolian population by the Taurus mountain range, appear to form another distinct cluster. Further studies are needed to determine the taxonomic status of these honeybee populations by including additional samples and a full morphometric analysis.

The honeybees of Turkey were separated into two groups, the European and the Anatolian, by discriminant function analysis. Kirklareli and Edirne honeybee populations (in Thrace) had the highest Mdh^{65} gene frequencies, with the highest loadings on the first canonical axis. We observed that the Kirklareli honeybee population also had the highest tibia length. Anatolian honeybees were separated along the second canonical axis, with distance *c*, tibia length, Pgm^{75} and Pgm^{100} variables with high loadings. The Black Sea and east Anatolian samples had the highest, whereas the Mediterranean samples had the lowest distance *c* values, which separated these two groups on the second axis.

Of the 36 provinces from which the samples were taken, only one province (K. Maras) was fixed for the Pgm^{75} allele based on a very small sample size (12 worker bees). In all other provinces, *Pgm* showed a high degree of polymorphism. Besides Pgm^{100} and Pgm^{75}, two additional rare alleles (Pgm^{45} and Pgm^{63}) were observed. There were strong deviations from Hardy–Weinberg equilibrium in a number of provinces for *Pgm* ($P < 0.001$). Hatay and S. Urfa samples showed the most extreme deviations. In Hatay, 269 out of 271 individual honeybees were heterozygous for $Pgm^{75/100}$; in S. Urfa, all samples (14 individuals) were heterozygous for the same alleles. Bingöl, Kirklareli, and Elazig also showed some degree of deviations in favor of heterozygotes.

Kandemir and Kence [14] found four alleles (Hk^{87}, Hk^{100}, Hk^{110}, and Hk^{120}) in central Anatolian honeybee populations; and in the present study we found an additional allele, Hk^{77}, which has not been reported previously. Out of seven alleles at the *Mdh* locus (Mdh^{55}, Mdh^{65}, Mdh^{80}, Mdh^{87}, Mdh^{100}, Mdh^{116} and Mdh^{133}), reported by various authors in different honeybee populations [1–3, 5, 6, 12, 16, 17, 21, 25, 26, 32–35], five of them (Mdh^{65}, Mdh^{87}, Mdh^{100}, Mdh^{116}, and Mdh^{133}) have been observed in honeybee populations of Turkey [14]. The frequency of the Mdh^{65} allele in

Turkey has been found to be highly reduced; and in southern and southeastern Anatolia, the *Mdh* locus has become invariant (Mdh^{100}). This relationship has also been seen in the significant linear regression of Mdh^{65} and Mdh^{100} on latitude and longitude. This type of clinal variation has been reported by Nielsen et al. [23] in *Mdh* allozymes in Europe, California, and Brazil with the suggestion that selection may be involved in many clines. There is some evidence that fitness differing in *Mdh* genotypes may occur, as recent studies have shown differences in temperature optima [7] and differential oxygen consumption during hovering [4].

One important result regarding the electrophoretic analysis is the observation of a large number of rare alleles (Tab. II). The existence of rare alleles in a population suggests that there has not been a recent bottleneck for the population. The observations of rare alleles, the presence of four subspecies and the detection of high genetic diversity as reflected in the high heterozygosity support the argument that Anatolia has been a genetic center for honeybee populations in the Near East.

This study is the most extensive survey yet made of the electrophoretic and morphometric variation in honeybee populations in the Near East. However, extended studies including additional morphometric characters and samples from surrounding countries (Syria, Iraq, Iran, Georgia and Armenia) would certainly need to include a more complete picture of the genetic variability in the Middle East and Asia.

Résumé – Variation génétique et morphométrique des populations d'abeilles domestiques (*Apis mellifera* L.) en Turquie. Selon l'analyse statistique multivariée des données morphométriques, l'Asie mineure semble être un centre de diversification génétique pour les races d'abeilles domestiques qui peuplent cette région, mais peu d'études ont été consacrées à la diversité génétique et morphométrique des abeilles d'Anatolie.

Six systèmes enzymatiques ont été étudiés pour déterminer la variabilité génétique des populations d'abeilles en Turquie : enzyme malique (*Me*), phosphoglucomutase (*Pgm*), estérase-3 (*Est*), hexokinase (*Hk*), phosphoglucose isomérase (*Pgi*), malate déshydrogénase (Mdh). Dix caractères morphométriques ont été mesurés pour déterminer l'étendue de la variation morphométrique : huit caractères selon Ruttner [30] (indice cubital A et B, longueur et largeur de l'aile antérieure, longueur du fémur, longueur du tibia, longueur et largeur du métatarse) et deux caractères selon Nazzi [20] (distances *c* et *d* de l'aile antérieure) (Tabs. I et II). La majorité des variables morphométriques et enzymatiques ont des relations linéaires significatives quand on fait une régression sur la latitude et la longitude (Tab. III).

On a trouvé que quatre des six systèmes enzymatiques étaient polymorphes et présentaient 16 isozymes. L'hétérozygosité moyenne était de 0,072 ± 0,007. Les deux types de données, morphométriques et électrophorétiques, ont été utilisées pour discriminer les populations d'abeilles turques. Les abeilles européennes et les abeilles d'Anatolie sont discriminées par le 1er axe canonique, et les abeilles d'Anatolie se séparent le long du 2^{e} axe. Les variables morphométriques ont été aussi efficaces que les variables électrophorétiques pour discriminer les populations d'abeilles.

Un résultat important concernant l'analyse électrophorétique est la présence d'un grand nombre allèles rares (Figs. 1 à 4). L'existence d'allèles rares dans une population suggère qu'il n'y a pas eu de goulot d'étranglement récent. L'observation d'allèles rares, la présence de quatre sous-espèces connues et la mise en évidence d'une diversité génétique élevée démontrée par la forte hétérozygosité confirment l'argument selon lequel l'Anatolie a été un centre de diversification génétique pour les populations d'abeilles domestiques au Proche-Orient.

***Apis mellifera anatoliaca* / *A. m. caucasica* / *A. m. meda* / *A. m. syriaca* / génétique population / variabilité génétique / morphométrie / électrophorèse / Turquie**

Zusammenfassung – Genetische und morphometrische Variation in türkischen Honigbienenpopulationen (*Apis mellifera* L.). Zur Bestimmung der genetischen Variabilität der Honigbienenpopulation in der Türkei wurden sechs Enzymsysteme (Malat-Enzym, Phosphoglucomutase, Esterase-3, Hexokinase, Phosphoglucoseisomerase, Malatdehydrogenase) untersucht. Zur Bestimmung morphologischer Variationen wurden zehn morphometrische Charaktere (Cubitalader A und B, Abstand *c* und *d*, Länge und Breite des Flügels und des Metatarsus, Länge des Femur und der Tibia) vermessen. Die meisten morphometrischen und elektrophoretischen Variablen zeigten signifikante lineare Beziehungen in der Regression auf die geographische Breite und Länge (Cubital B, Abstand c und d, Flügellänge und -breite, Breite des Metatarsus, Länge von Femur und Tibia, Pgm^{75}, Pgm^{100}, Hk^{110}, Mdh^{65}, Mdh^{100} und Est^{70}). Bei vier von sechs Enzymsystemen wurden Polymorphismen mit insgesamt 16 Isoenzymen gefunden. Die mittlere Heterozygosität wurde zu 0,072 ± 0,007 berechnet. Zur Unterteilung der türkischen Bienen wurden sowohl die morphometrischen als auch die elektrophoretischen Daten verwendet. Europäische und anatolische Honigbienen waren auf der ersten canonischen Achse unterschiedlich, die anatolischen Bienen spalteten sich entlang der zweiten Achse weiter auf. Morphometrische und elektrophoretische Variablen waren gleich gut zur Unterscheidung der Honigbienenpopulationen geeignet.

Ein wichtiges Resultat der elektrophoretischen Analyse war die groβe Zahl seltener Allele. Dieser Befund legt nahe, dass in der jüngeren Populationsentwicklung kein Flaschenhals aufgetreten ist. Der Befund seltener Allele, die Anwesenheit von vier bekannten Subspezies, sowie die durch die hohe Heterozygosität angezeigte groβe genetische Diversität stützen die Auffassung, dass Anatolien ein Entwicklungszentrum der Honigbienenpopulationen des Nahen Ostens dargestellt hat.

***Apis mellifera anatoliaca* / *A. m. caucasica* / *A. m. meda* / *A. m. syriaca* / Populationengenetik / genetische Variabilität / Morphometrie / Elektrophorese / Türkei**

ACKNOWLEDGMENTS

The authors wish to thank to all who assisted in the collection, preparation, and measurement of the samples. Thanks are due to the Ministry of Agriculture and Rural Affairs of Turkey for providing assistance during the collection of samples, and to the beekeepers. This work was supported by grant VHAG-1077 from the Turkish Scientific and Technical Research Council to Meral Kence.

REFERENCES

[1] Badino G., Celebrano G., Manino A., Population structure and Mdh-1 locus variation in *Apis mellifera ligustica*, J. Hered. 74 (1983) 443–446.

[2] Badino G., Celebrano G., Manino A., Longo S., Enzyme polymorphism in the Sicilian honeybee, Experientia 41 (1985) 752–754.

[3] Badino G., Celebrano G., Manino A., Ifantidis M.D., Allozyme variability in Greek honeybees (*Apis mellifera* L.), Apidologie 19 (1988) 377–386.

[4] Coelho J.R., Mitton J.B., Oxygen consumption during hovering is associated with genetic variation of enzymes in honeybees, Funct. Ecol. 2 (1988) 141–146.

[5] Contel E.P.B., Mestriner M.A., Martins E., Genetic control and developmental expression of malate dehydrogenase in *Apis mellifera*, Biochem. Genet. 15 (1977) 859–876.

[6] Cornuet J.M., The MDH system in honeybees of Guadeloupe, J. Hered. 70 (1979) 223–224.

[7] Cornuet J.M., Oldroyd B.P., Crozier R.H., Unequal thermostability of allelic forms of malate dehydrogenase in honeybees, J. Apic. Res. 41 (1995) 45–47.

[8] Daly H.V., Hoelmer K., Gambino P., Clinal geographic variation in feral honeybees in California, USA, Apidologie 22 (1991) 591–609.

[9] Del Lama M.A., Mestriner M.A., Pavia J.C.A., Est-5 and Pgm_1: new polymorphisms in *Apis mellifera*, Rev. Brazil. Genet. 8 (1985) 17–27.

[10] Del Lama M.A., Figueiredo R.A., Soares A.E.E., Del Lama S.N., Hexokinase polymorphism in *Apis mellifera* and its use for Africanized honeybee identification, Rev. Brazil. Genet. 11 (1988) 287–297.

[11] Ftayeh A., Meixner M., Fuchs S., Morphometrical investigation in Syrian honeybees, Apidologie 25 (1994) 396–401.

[12] Gartside D.F., Similar allozyme polymorphism in honeybees (*Apis mellifera*) from different continents, Experientia 36 (1980) 649–650.

[13] Jolicouer P., Mosimann J.E., Size and shape variation in the painted turtle: a principal component analysis, Growth 24 (1960) 339–354.

[14] Kandemir I., Kence A., Allozyme variation in a central Anatolian honeybee (*Apis mellifera* L.) population, Apidologie 26 (1995) 503–510.

[15] Kandemir I., Genetic and morphometric variation in honeybee (*Apis mellifera* L.) populations in Turkey, PhD diss., Middle East Tech. Univ., Ankara, Turkey, 1999.

[16] Lobo J.A., Del Lama M.A., Mestriner M.A., Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L.), Evolution 43 (1989) 794–802.

[17] Meixner M.D., Sheppard W.S., Dietz A., Krell R., Morphological and allozyme variability in honeybees from Kenya, Apidologie 25 (1994) 188–202.

[18] Mestriner M.A., Contel E.P.B., The P-3 and Est loci in the honeybee *Apis mellifera*, Genetics 72 (1972) 733–738.

[19] Munyan J., SYSTAT 7.0 for Windows, Standard Version, SPSS Inc., 1997.

[20] Nazzi F., Morphometric analysis of honeybees from an area of racial hybridization in northeastern Italy, Apidologie 23 (1992) 89–96.

[21] Ndiritu D.W., Mutugi N., Ndung'u S., Variation in malate dehydrogenase allozymes among honeybee populations in Kenya, J. Apic. Res. 25 (1986) 234–237.

[22] Nei M., Molecular Evolutionary Genetics, Columbia Univ. Press, 1972.

[23] Nielsen D., Page Jr P.E., Crosland M.W.J., Clinal variation and selection of MDH allozymes in honeybee populations, Experientia 50 (1994) 867–871.

[24] Nunamaker R.A., Wilson W.T., Some isozymes of the honeybee, Isozyme Bull. 13 (1980) 111–112.

[25] Nunamaker R.A., Wilson W.T., Comparison of Mdh allozyme patterns in the African honeybee (*Apis mellifera adansonii* L.) and the Africanized populations of Brazil, J. Kans. Entomol. Soc. 54 (1981) 704–710.

[26] Nunamaker R.A., Wilson W.T., Haley B.E., Electrophoretic detection of Africanized honeybees (*Apis mellifera scutellata*) in Guatemala and Mexico based on malate dehydrogenase allozyme patterns, J. Kans. Entomol. Soc. 57 (1984) 622–631.

[27] Pamilo P., Varvio-Aho S.L., Pakkarinen A., Low enzyme gene variability in Hymenoptera as a consequence of haplodiploidy, Hereditas 88 (1978) 93–99.

[28] Podani J., SYN-TAX-PC Computer Programs for Multivariate Data Analysis in Ecology and Systematics, Version 5.0, Scientia Publ., Budapest, 1993.

[29] Rohlf F.J., NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, Version 1.70, Exeter Software, New York, 1992.

[30] Ruttner F., Biogeography and Taxonomy of Honeybees, Springer-Verlag, Berlin, Heildelberg, 1988.

[31] Sheppard W.S., Genetic variation and differentiation in honeybees (*Apis*), Ph.D. diss., Univ. Illinois at Urbana-Champaign, IL, 1986.

[32] Sheppard W.S., Comparative study of enzyme polymorphism in United States and European honeybee (Hymenoptera: Apidae) populations, Ann. Entomol. Soc. Am. 81 (1988) 886–889.

[33] Sheppard W.S., Berlocher S.H., Enzyme polymorphism in *Apis mellifera* from Norway, J. Apic. Res. 23 (1984) 64–69.

[34] Sheppard W.S., Berlocher S.H., New allozyme variability in Italian honeybees, J. Hered. 76 (1985) 45–48.

[35] Sheppard W.S., McPheron B.A., Genetic variation in honeybees from an area of racial hybridization in western Czechoslovakia, Apidologie 17 (1986) 21–32.

[36] Sheppard W.S., Soares A.E.E., DeJong D., Shimanuki H., Hybrid status of honeybee populations near the historic origin of Africanization in Brazil, Apidologie 22 (1991) 643–652.

[37] Smith D.R., Slaymaker A., Palmer M., Kaftanoglu O., Turkish honeybees belong to the east Mediterranean lineage, Apidologie 28 (1997) 269–274.

[38] Sokal R.R., Rohlf F.C., Biometry, Freeman W.H. and Co., San Francisco, CA, 1981.

[39] Swafford D.L., Selander R.B., BIOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics, J. Hered. 72 (1981) 281–283.

[40] Sylvester H.A., Allozyme variation in honeybees (*Apis mellifera* L.), Ph.D. diss., Univ. California, Davis, CA, 1976.