TARIM BİLİMLERİ DERGİSİ 2004, 10 (3) 245-249
DOI: 10.1501/Tarimbil_0000000901

The Graphic Evaluation of Morphological Characters in Honey Bees (*Apis mellifera* L.) by Chernoff Faces

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Geliş Tarihi: 31.03.2003

Abstract: Seven parent colonies and 5 offspring colonies of two different Anatolian honey bee (*Apis mellifera anatoliaca*) ecotypes (total 24 colonies) were sampled randomly for morphological analysis. Morphometric measurements were made on 10 characters. A multivariate graphic method (Chernoff faces) was applied to data of parents and offsprings in representing morphometric data. This multivariate graphic technique, "Chernoff faces", could distinguish differences between ecotypes and between parents and offsprings enabling simple and quick visual comparisons.

Key Words: honey bee, *Apis mellifera*, morphometry, Chernoff faces, instrumental insemination

Bal Arısı (*Apis mellifera* L.) Morfolojik Özelliklerinin Chernoff Yüzleri ile Grafiksel Değerlendirilmesi

Özet: İki farklı Anadolu arısı (*Apis mellifera anatoliaca*) ekotipinden 7'şer ebeveyn koloni ve bunların dölleri olan 5'er koloni (toplam 24 koloni) morfolojik analiz için rasgele örneklenmişlerdir. Morfometrik ölçümler 10 morfolojik özellik üzerinde yapılmıştır. Ebeveynlerin ve döllerin morfolojik özelliklerine ilişkin verilerine çok değişkenli grafik yöntemi (Chernoff yüzleri) uygulanmıştır. Bu çok değişkenli grafik tekniği "Chernoff yüzleri" basit ve çabuk görsel karşılaştırmalar sağlayarak ekotipler arasındaki ve ebeveynler ile döller arasındaki farklılıkları ortaya koymuştur.

Anahtar Kelimeler: bal arısı, *Apis mellifera*, morfometri, Chernoff yüzleri, yapay tohumlama

Introduction

The western honey bee (*Apis mellifera* L.) is one of the *Apis* species that has a broad range of distribution and adaptation. Ruttner (1988) distinguished 24 *Apis mellifera* subspecies, which he called geographic races. Geographic races of honey bees can be clearly classified by their morphological characters using biometrical methods. The morphological characters are the most commonly used quantitative characteristics for identification of honey bee races, since they are mainly genetically determined by polygenes and highly constant (Collins 1986). It was first verified by Alpatov (1929) that honey bees preserve all their original morphological characters when transferred to an entirely new environment.

Multivariate analysis of morphology is helpful in evaluating changes in the honey bee genome, and is therefore an effective means of studying the population genetics of honey bees (Oldroyd *et al.* 1991). Beside the intra-species classifications of honey bees, multivariate morphometry provides the tool to control the results of matings (Moritz 1991). Various statistical methods such as principal component analysis, factor analysis, discriminant analysis were applied to morphometric data of honey bee populations by many scientists (Du Praw 1965, Daly and Balling 1978, Ruttner *et al.* 1978, Daly *et al.* 1982, Moritz 1991, Nazzi 1992, Crewe *et al.* 1994, Maul and Hähnle 1994, Kauhausen-Keller and Keller 1994, Hepburn and Radloff 1997, Sheppard *et al.* 1997, Radloff *et al.* 2001).

The graphical representations of these well-known methods such as dendrogram, phenogram, scattergram enable visual comparison of specimens or samples. There are also several other ways for representing multivariate data in 2 dimensions (Johnson and Wichern 1982). "Chernoff faces" developed by Chernoff (1973) is one of these pictorial multivariate graphic representations used to picture 'n' variables on a two-dimensional surface. Yet, it has not been applied to morphometric data of honey bees. "Chernoff faces" are simplified, cartoon-like faces illustrating trends in multidimensional data. Each of several variables is assigned to a facial characters and a face is then created for each situation. The assignment of variables to facial features is done by the investigator. When the investigator is fairly sure 2 or 3 variables are primarily responsible for distinguishing clusters, these variables can be associated with prominent facial characteristics (Johnson and Wichern 1982). In this current study, we aimed to apply a pictorial multivariate representation method, Chernoff faces, to morphometric data of honey bees to provide quick visual comparisons in pure race breeding control.

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Materials and Methods

Experimental design: The study was carried out with colonies from two distinct ecotypes (E1 and E2) of Anatolian honey bee (*Apis mellifera anatoliaca*) kept in Ankara University, Turkey. The Anatolian honey bee ecotypes were obtained from 2 stationary apiaries closed to migratory beekeeping in different locations in Central Anatolia. The sampling localities (the origins of ecotypes) were selected in accordance with the view 'the more primitive the beekeeper, the purer the race' (Ruttner, 1988). Seven colonies for each ecotype were sampled randomly as parental groups (E1-P and E2-P) for morphological assessment. After statistical analysis of morphometric data of parental groups (see statistical analyses), one colony that had least intra-colonial morphometric variance was selected from each ecotype as breeder colonies (E1-BC and E2-BC). Sister queens were reared from E1-BC and from E2-BC and inseminated instrumentally (Moritz, 1989), each with a mixture of 8 mm^3 semen from drones of all colonies of their own ecotypes. The 8 instrumentally inseminated queens from E1-BC and 10 instrumentally inseminated queens from E2-BC were introduced to colonies to establish offspring groups (E1-G and E2-G). Five colonies for each offspring group were sampled randomly for morphological assessment.

Morphometric analyses: Twenty five worker bees from each of parent colony (total 14 colonies) were dissected and 10 morphological characters were measured with ocular micrometer mounted on a stereo-microscope as explained by Ruttner *et al.* (1978). The characters measured on parts of each worker bee are as follows: (1) proboscis length (PLN), (2) fore wing length (FWLN), (3) fore wing width (FWWD), (4) cubital vein, distance a (CUVA), (5) cubital vein, distance b (CUVB), (6) longitudinal diameter of tergite 3 (T_3), (7) longitudinal diameter of tergite 4 (T_4) (8) hind femur length (FELN), (9) hind tibia length (TILN), (10) hind metatarsus length (MTLN). For morphometric analysis of offspring groups, 20 samples of worker bees from each colony were used and the same 10 morphological characters were measured with the same technique.

Statistical analyses: Discriminant analysis procedure was applied to data of E1-P and E2-P to determine intra-colonial morphometric variances and to allocate each individual bee into their respective colonies by discriminant functions computed from character measurements of each worker bee. Discriminant analysis was performed with STATISTICA, version 6.0 (Statistica 6.0 2001).

The data of parental and offspring groups in each ecotype were compared by independent samples t-test (E1-P vs. E1-G and E2-P vs. E2-G). The group means of each morphological character were calculated in order to plot Chernoff faces of parental, breeder and offspring groups which provide visual tool for comparison. To plot Chernoff faces, the first 10 of 20 face characters were assigned to 10 measured morphological characters (Table 1). Chernoff faces were plotted by STATISTICA, version 6.0 (Statistica 6.0 2001).

Results

The descriptive statistics (means and standard errors) of characters for parental and offspring groups in each ecotype are reported in table 2. Significant differences between parental and offspring groups for most of characters in both ecotypes were determined by t-test ($P<0.05$). While there were differences between E1-P and E1-G in PLN, FWLN, FWWD, CUVB, T_3, T_4, FELN and MTLN, no statistical differences between E1-P and E1-G were found in the CUVA and TILN. E2-P differed from E2-G for all characters except for CUVA.

The Chernoff faces plotted based on the means of each morphological character calculated for each breeder colony and for each parental and offspring groups in E1 and E2 are shown in figure 1. Some observations could be made on the Chernoff faces. First, the Chernoff faces were roughly consistent with the results from independent samples t-test; the faces of parents were different from the faces of offsprings. Second, two ecotypes in both generations were different from each other. Third, breeder colonies selected from parental groups appeared not to be good samples of their own groups. Fourth, the faces of offspring groups were nearly similar to the faces of breeder colonies in each ecotype.

Discussion

Chernoff faces suggested that offsprings were not similar to parents. The offspring groups were more similar to breeder colonies than to the parental groups. This

Table 1. Morphological characters measured and their corresponding Chernoff face characters used for plotting

No	Morphological character with abbreviation	Face character
1	Proboscis length (PLN)	Face width
2	Fore wing length (FWLN)	Ear level
3	Fore wing width (FWWD)	Half face height
4	Cubital vein, distance a (CUVA)	Eccentricity of upper ellipse of face
5	Cubital vein, distance b (CUVB)	Eccentricity of lower ellipse of face
6	Longitudinal diameter of tergite 3 (T_3)	Length of nose
7	Longitudinal diameter of tergite 4 (T_4)	Position of centre of mouth
8	Hind femur length (FELN)	Curvature of mouth
9	Hind tibia length (TILN)	Length of mouth
10	Hind metatarsus length (MTLN)	Height of centre of eyes

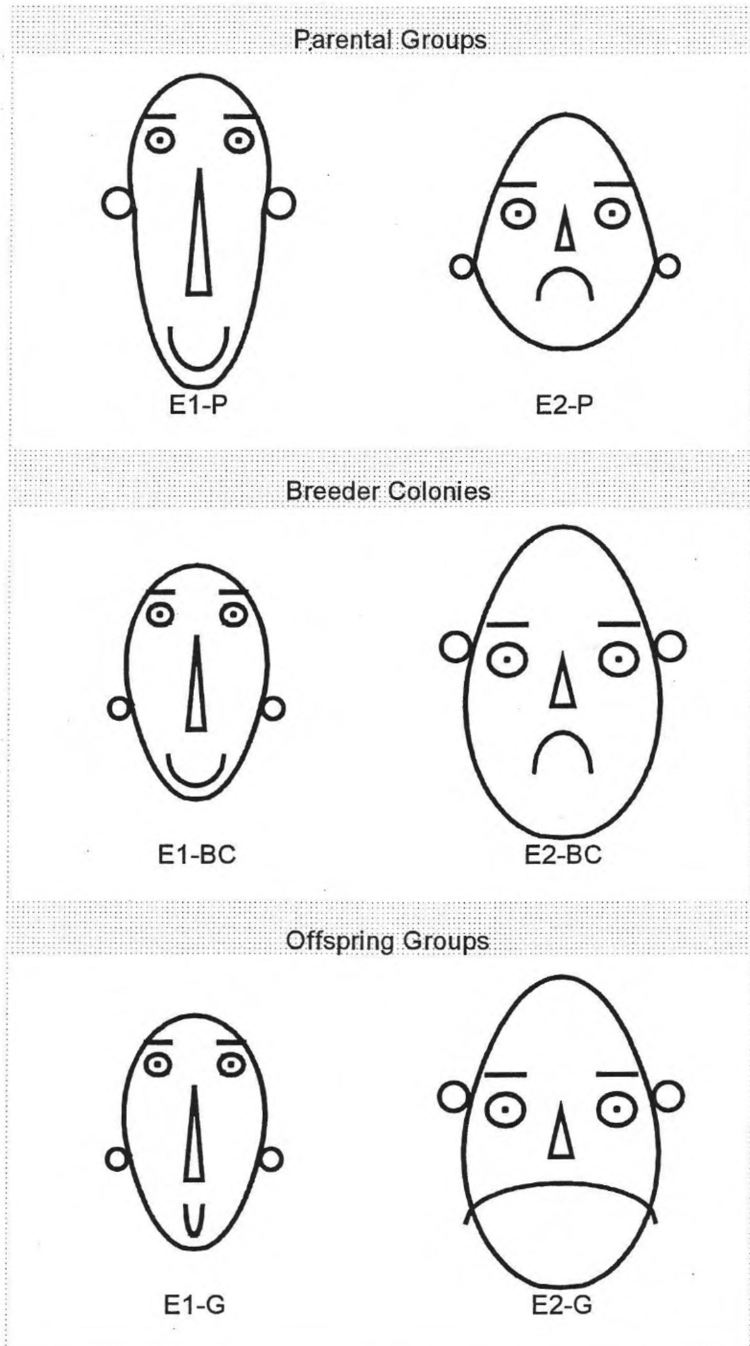

Figure 1. Chernoff faces plotted for parental groups (E1-P and E2-P), breeder colonies (E1-BC and E2-BC) and offspring groups (E1-G and E2-G). Face width: PLN; ear level: FWLN; half-face height: FWWD; eccentricity of upper ellipse of face: CUVA; eccentricity of lower ellipse of face: CUVB; length of nose: T_3; position of centre of mouth: T_4; curvature of mouth: FELN; length of mouth: TILN; height of centre of eyes: MTLN

Table 2. The comparative morphological characters of parental and offspring groups in each ecotype. Measurements are in unit of mm. Means ± s.e. with the same capital letters (for E1) or lower-case letters (for E2) are not different using independent samples t-test.

Characters	E1		E2	
	E1-P	E1-G	E2-P	E2-G
	$\bar{X} \pm S_{\bar{X}}$ (n = 175)	$\bar{X} \pm S_{\bar{X}}$ (n = 100)	$\bar{X} \pm S_{\bar{X}}$ (n = 175)	$\bar{X} \pm S_{\bar{X}}$ (n = 100)
PLN	6.473 ± 0.0061^{A}	6.565 ± 0.0487^{B}	6.482 ± 0.0066^{a}	6.576 ± 0.0382^{b}
FWLN	8.924 ± 0.0103^{A}	9.270 ± 0.0167^{B}	8.916 ± 0.0111^{a}	9.332 ± 0.0129^{b}
FWWD	3.026 ± 0.0051^{A}	3.120 ± 0.0087^{B}	3.002 ± 0.0060^{a}	3.190 ± 0.0086^{b}
CUVA	0.508 ± 0.0035^{A}	0.514 ± 0.0041^{A}	0.507 ± 0.0031^{a}	0.500 ± 0.0041^{a}
CUVB	0.233 ± 0.0015^{A}	0.241 ± 0.0021^{B}	0.240 ± 0.0017^{a}	0.271 ± 0.0024^{b}
T_3	2.158 ± 0.0054^{A}	2.240 ± 0.0048^{B}	2.115 ± 0.0065^{a}	2.223 ± 0.0047^{b}
T_4	2.090 ± 0.0051^{A}	2.166 ± 0.0048^{B}	2.050 ± 0.0063^{a}	2.146 ± 0.0046^{b}
FELN	2.661 ± 0.0037^{A}	2.715 ± 0.0056^{B}	2.621 ± 0.0040^{a}	2.710 ± 0.0066^{b}
TILN	3.184 ± 0.0054^{A}	3.183 ± 0.0088^{A}	3.155 ± 0.0053^{a}	3.233 ± 0.0098^{b}
MTLN	2.073 ± 0.0042^{A}	2.111 ± 0.0061^{B}	2.043 ± 0.0039^{a}	2.108 ± 0.0072^{b}

dissimilarity of offspring groups to parental groups may be due, in part, to the selection of breeder colonies from parental groups on the basis of intra-colony variance, since breeder colonies were not selected for the sizes of their morphological characters. Also, the small sample sizes of parental and offspring groups may account for this divergence of offsprings from parents.

This study showed that the Chernoff faces representation was a decisive and easy tool for discrimination of different honey bee populations in respect of their morphological characters. When the groups are compared by t-test or by other univariate statistical tests, the correlations between characters are not considered or it is assumed that the characters are independent. Since the characters measured are the components of the structure of individuals and are dependent, it is not appropriate to use univariate comparison techniques. The Chernoff faces, as a multivariate technique, both permit accurate univariate comparisons of each character and enable a general outline of characters for comparisons. Therefore, it could be proposed for morphometric evaluations of honey bee populations.

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