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Multivariate Morphometric Study on *Apis florea* Distributed in Iran

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Abstract: Multivariate Morphometric and Spatial Autocorrelation Analyses were performed to determine the morphometric variation in *Apis florea* colonies representing 13 localities from 4 states on the coastal north-south diagonal in Iran. New morphometric characters (hind wing length, hind wing width, and hind wing angles) were also measured to determine the usefulness of these characters for *Apis florea*. Analysis of variance of new morphometric characters showed that 7 out of the 8 hind wing variables displayed statistically significant differences among populations ($P < 0.05$). The scatter plot from Canonical Variate Analysis revealed that the colonies from north (Ilam, Khuzestan, and Bushehr states) showed an overlapping distribution. The colonies from south (Hormuzgan state) formed a non-overlapping distant cluster. Based on the spatial autocorrelation analysis, 14 correlograms were significant for morphometric characters (According to the Bonferroni criterion). Six characters (CuB, C.Ind, HWW, B4, D7, and I13) showed clinal type correlogram.

Key Words: *Apis florea*, Iran, Morphometry, Multivariate morphometric analysis, Spatial autocorrelation

İran'da Dağılım Gösteren *Apis florea* Üzerine Çok Değişkenli Morfometrik Çalışma

Özet: İran'nın kuzey-güney köşegeni üzerinde bulunan 4 eyaletteki 13 lokasyondan alınan *Apis florea* kolonilerindeki morfometrik varyasyonu belirlemek için çok değişkenli morfometrik analizler ve uzamsal otokorelasyon analizi yapılmıştır. Aynı zamanda *Apis florea* için arka kanat uzunluğu, arka kanat genişliği ve arka kanat açıları gibi yeni karakterlerin uygunluğunu belirlemek için bu karakterlerde ölçülüp analize dahil edilmiştir. Yeni karakterlerin varyans analizi, 8 arka kanat değişkeninin 7'si populasyonlar arasında istatistiksel olarak anlamlı farklılıklar bulunduğunu göstermektedir ($P < 0.05$). Kanonikal Değişken Analizi'nden elde edilen serpilme grafiği Kuzey'deki kolonilerin (Ilam, Khuzestan ve Bushehr eyaletleri) örtüşen dağılım gösterdiğini ortaya koymaktadır. Güney'deki koloniler (Hormuzgan eyaleti) diğer eyaletlerdeki kolonilerden daha uzakta örtüşmeyen küme şeklindedir. Uzamsal otokorelasyon analizinde elde edilen korrelogramlardan 14 korrelogram Bonferroni kriterine göre anlamlıdır. 6 karakter (CuB, C.Ind., HWW, B4, D7 ve I13) klinal tipte korrelogram göstermektedir.

Anahtar Sözcükler: *Apis florea*, İran, Morfometri, Çok değişkenli morfometrik analiz, Uzamsal otokorelasyon

Introduction

The genus *Apis* has been separated into 11 distinct species historically (Engel, 1999) based on morphological, behavioral, and physiological characteristics, and also on the geographic distribution. Although 10 *Apis* species were recognized, the validity of *Apis laboriosa* is still questionable (Arias and Sheppard, 2005). Among these species, *Apis mellifera* L. was the most extensively studied

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honey bee species in the world utilizing their morphology, biochemical variations and DNA-RFLP, microsatellites and mtDNA genes, and DNA sequencing (Ruttner, 1988; Sheppard, 1988; Smith, 1988; Del Lama et al., 1990; Cornuet and Garnery, 1991; Cornuet et al., 1991; Hall, 1991, 1992; Garnery et al., 1992, 1993, 1998; Crozier and Crozier, 1993; Estoup et al., 1995; Sheppard et al., 1996; Franck et al., 2000; Kandemir et al., 2000, 2006). Although the other species had not been studied intensively, recently they were the subject of several investigations (Diniz-Filho et al., 1993; Hepburn et al, 2001; Rinderer et al., 2002; Radloff et al., 2005). Remaining 9 species (*Apis cerana, Apis dorsata, Apis florea, Apis andreniformis, Apis laboriosa, Apis koschevnikovi, Apis binghami, Apis nigrocincta,* and *Apis nuluensis*) were distributed in Southeast Asia and Far East. *A. cerana* and *A. florea* reach to the border of Iran, Pakistan, and Afghanistan (Otis, 1996). Moreover *A. florea* further distributed into Iraq, Saudi Arabia (Whitcombe, 1984), and Jordan (Haddad et al., 2008). In this vast geography, *A. florea* occupies rainforests, savannas, subtropical steppes, and semi-deserts with open-air nest (Hepburn et al., 2005). *A. florea* naturally does not overlap with the distribution of *A. mellifera*. Anthropogenic factors, nowadays, affect the distributional ranges of *Apis* species. Although there was no natural overlapping distribution of *A. mellifera* with the other *Apis* species, currently *A. florea*, *A. cerana*, and *A. mellifera* are found in the same geographical areas.

Based on the multivariate analysis of morphometric data, *A. florea* populations from several countries were investigated regionally (Ruttner, 1988; Mossadegh, 1993; Rinderer et al., 1995; Ruttner et al., 1995; Markmoor and Ahmad, 1998; Tahmasebi et al., 2002; Chaiyawong et al., 2004; Hepburn et al., 2005). The first morphometric research on *A. florea* demonstrated that clinal type of geographical variability exists in *A. florae;* larger bees found in the north and smaller ones in the south (Ruttner, 1988). Besides geography-related variability, climate-related variation was also reported for *A. florea* (Ruttner et al., 1995). In addition, *A. florea* was separated into 2 indistinct morphoclusters in Iran (Tahmasebi et al., 2002) and morphoclusters were changed clinally with latitude in a much wider geography extending from Vietnam and southeastern China to Iran and Oman (~7000 km) (Hepburn et al., 2005).

In recent years, 2 different approaches have been used to evaluate geographic variation in *Apis*. The most common approach is to extend the traditional multivariate approach, showing their results with Principle Component Analysis, Discriminant Function Analysis, or Canonical Variate Analysis (Tahmasebi et al., 2002; Chaiyawong et al., 2004; Hepburn et al., 2005; Radloff et al., 2005; Adl et al., 2007). On the other hand, multiple regression analysis and direct spatial autocorrelation analysis can also be performed by pairing geographic coordinates with character means of local populations (Daly et al., 1991; Diniz-Filho et al., 1993, 2000; Diniz-Filho and Malaspina, 1995; Kandemir et al., 2000). In addition, new size and angle characters have been used in morphometric studies of honey bee populations due to deformations in some of the body parts and usefulness of wing asymmetry (Dedej and Nazzi, 1994; Clarke and Oldroyd, 1996). The geographic variation of *A. florea* has been tried to be explained with multivariate analysis based on characters of fore wing, hind leg, abdomen, wing angles, and the number of hamuli and antenna (Ruttner et al., 1995; Chaiyawong et al., 2004; Hepburn et al., 2005). The geographic variability based on spatial autocorrelation was not studied in *A. florea*.

The objectives of our research were to investigate the extent of morphometric variation in *A. florea* populations distributed in the coastal states of Iran and to determine whether differences could be detected within this species and also to show whether there is a clinal type of variation with respect to latitude and longitude.

Materials and Methods

Apis florea colonies were collected from Hormuzgan, Bushehr, and Khuzestan along the cost of Persian Gulf and from inland state Ilam between 2005 and 2007 (Table 1, Figure 1). *Apis florea* workers were collected from each colony and preserved in 70% ethanol until morphometric examinations were carried out. From each colony, 10-15 worker bees were selected randomly. Body parts (fore wing, hind wing, and hind leg) were mounted on microscope slides. In total 28 morphological characters were measured and hamuli were counted for each worker bee (Ruttner, 1988; Nazzi, 1992). These characters included: fore wing length (FWL), fore wing width (FWW), cubital A (CuA), cubital B (CuB), numbers of hamuli (HAM), femur length (FL), tibia length (TL), metatarsus length (MTL), metatarsus width (MTW), fore wing angles (A4, B4, D7, E9, G18, J10, J16, K19, L13, N23, O26) (Ruttner, 1988), fore wing distance D (WDL), fore wing distance C (WCL) (Nazzi, 1992), hind wing length (HWL),

Table 1. The sampling locations of *A. florea* in Iran (n: number of colonies).

State	Location	n	Coordinates
Ilam	1. Dehloran	21	32.41N 47.15E
	2. Sarollah	3	32.35N 47.22E
	3. Musiyan	9	32.32N 47.22E
	4. Dasht Abas	17	32.29N 47.47E
	5. Ainsole	4	32.10N 47.41E
Khuzestan	6. Dezful	5	32.23N 48.23E
	7. Soush	4	32.11N 48.14E
	8. Ahvaz	22	31.17N 48.43E
	9. Mollasany	3	31.35N 48.53E
Bushehr	10. Bushehr	13	28.59N 50.50E
	11. Hkark	2	29.14N 50.20E
Hormuzgan	12. Bandar Lingeh	6	26.34N 54.52E
	13. Siri Jazireh	6	26.07N 54.26E

hind wing width (HWW), and hind wing angles (HW1, HW2, HW3, HW4, HW5). Hind wing characters were used in the studies for the first time (Figure 2). Size characters were measured under a stereo-microscope with ocular micrometer and angles were measured with a goniometer. Multivariate morphometric analyses were performed on 1667 individual worker bees collected from 115 colonies from 13 localities.

Multivariate statistical analysis (Analysis of Variance-ANOVA, Discriminant Functions analysis-DFA, Canonical Variates Analysis-CVA) was performed with SPSS (SPSS, 2004) and NTSYS-pc version 2.20 (Rohlf, 2004) statistical packages. Regression of morphometric variables on latitude and longitude and the correlation between morphometric variables were computed using SPSS (SPSS, 2004). Spatial autocorrelation analysis was also computed using SAAP (Wartenberg, 1989). Spatial correlograms were defined by Moran's I coefficient for 6 distance classes (Sokal and Oden, 1978) and the significance of correlogram was established using the Bonferroni criterion (Oden, 1984).

Figure 1. Geographical distribution map of the analyzed populations of *A. florea* in Iran.

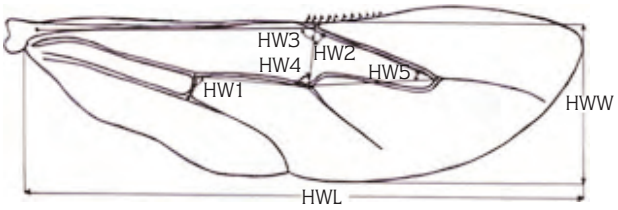

Figure 2. Hind wing of *A. florea* showing the 7 characters examined.

Result

A total of 29 morphometric characters from fore wing, hind wing, and hind leg were used for multivariate statistical analysis. Colony sample means and standard deviations were computed for each morphometric character from 10-15 bees per colony. The mean values and standard deviations of characters are shown in Table 2. *Apis florea* colonies from 4 states on the coastal north-south diagonal in Iran show high levels of morphometric variation. Analysis of variance of morphometric characters showed that 24 out of the 29 morphometric variables displayed statistically significant differences among groups ($P > 0.05$), (Table 3).

Figure 3A-B shows the results of CVA and the colony groupings on a scatter plot. The clustering of 115 *A. florea* colonies based on DFA and CVA showed similar results. The colonies from Ilam, Khuzestan, and Bushehr showed an overlapped cluster, while the colonies from Hormuzgan state formed an apparent non-overlapping cluster with the other states.

Table 2. Mean and standard deviations of morphometric characters in honey bee populations from Iran (n: number of colonies, measurements in mm, angle in degrees).

State Character	Ilam (n = 54)	Khuzestan (n = 34)	Bushehr (n = 15)	Hormuzgan (n = 12)
FWL	6.801 ± 0.089	6.896 ± 0.055	6.859 ± 0.062	6.484 ± 0.028
FWW	2.409 ± 0.044	2.446 ± 0.033	2.434 ± 0.043	2.188 ± 0.014
WCL	0.647 ± 0.007	0.644 ± 0.014	0.646 ± 0.013	0.609 ± 0.003
WDL	1.264 ± 0.011	1.267 ± 0.017	1.270 ± 0.015	1.199 ± 0.017
CuA	0.532 ± 0.010	0.517 ± 0.007	0.555 ± 0.017	0.526 ± 0.001
CuB	0.179 ± 0.002	0.153 ± 0.004	0.169 ± 0.003	0.160 ± 0.007
HWL	4.937 ± 0.073	4.839 ± 0.043	4.842 ± 0.000	4.678 ± 0.024
HWW	1.490 ± 0.037	1.683 ± 0.195	1.466 ± 0.009	1.359 ± 0.018
HAM	11.776 ± 0.088	12.175 ± 0.120	11.868 ± 0.160	11.631 ± 0.004
FL	1.849 ± 0.020	1.838 ± 0.347	1.836 ± 0.013	1.799 ± 0.012
TL	2.313 ± 0.030	2.313 ± 0.114	2.307 ± 0.043	2.251 ± 0.002
MTL	1.427 ± 0.017	1.431 ± 0.241	1.421 ± 0.014	1.379 ± 0.001
MTW	0.642 ± 0.007	0.634 ± 0.204	0.641 ± 0.000	0.628 ± 0.000
A4	31.926 ± 0.264	31.329 ± 0.178	31.736 ± 0.055	33.115 ± 0.117
B4	99.817 ± 0.930	101.765 ± 0.182	97.169 ± 0.081	92.045 ± 0.167
D7	86.174 ± 0.546	87.895 ± 0.443	85.331 ± 0.185	81.097 ± 0.570
E9	19.147 ± 0.233	20.100 ± 0.099	20.038 ± 1.400	18.009 ± 0.491
G18	103.051 ± 1.529	103.655 ± 0.285	104.746 ± 2.483	107.792 ± 0.108
J10	40.061 ± 0.254	40.590 ± 0.317	40.319 ± 0.194	39.131 ± 0.668
J16	116.130 ± 0.887	118.610 ± 0.605	117.822 ± 1.261	112.689 ± 0.411
K19	70.118 ± 0.422	69.794 ± 0.967	69.544 ± 2.253	73.523 ± 0.735
L13	15.094 ± 0.201	14.897 ± 0.146	15.334 ± 0.021	16.117 ± 0.267
N23	76.701 ± 1.355	79.503 ± 1.102	75.050 ± 2.112	74.892 ± 0.819
026	26.342 ± 0.579	26.714 ± 0.554	29.212 ± 0.496	29.755 ± 0.355
HW1	86.808 ± 1.241	88.320 ± 1.054	88.186 ± 0.627	86.287 ± 0.202
HW2	64.507 ± 0.265	64.069 ± 0.295	64.350 ± 0.496	63.528 ± 0.626
HW3	89.424 ± 0.295	90.311 ± 0.102	90.147 ± 0.397	90.407 ± 0.495
HW4	81.567 ± 0.174	80.774 ± 0.308	80.934 ± 0.330	80.075 ± 0.263
HW5	28.909 ± 0.023	29.323 ± 0.105	29.474 ± 0.005	28.951 ± 0.137

Table 3. Test of equality of means of 29 morphometric characters.

Character	Wilks' Lambda	F	df1	df2	Sig.
FWL	0.642	18.740	3	101	0.000
FWW	0.625	20.165	3	101	0.000
WCL	0.791	8.907	3	101	0.000
WDL	0.766	10.280	3	101	0.000
CuA	0.815	7.624	3	101	0.000
CuB	0.778	9.583	3	101	0.000
HWL	0.792	8.815	3	101	0.000
HWW	0.714	13.456	3	101	0.000
HAM	0.860	5.490	3	101	0.002
FL	0.958	1.474	3	101	0.226
TL	0.949	1.820	3	101	0.148
MTL	0.871	4.991	3	101	0.003
MTW	0.965	1.212	3	101	0.309
A4	0.898	3.837	3	101	0.012
B4	0.680	15.822	3	101	0.000
D7	0.668	16.738	3	101	0.000
E9	0.756	10.871	3	101	0.000
G18	0.886	4.322	3	101	0.007
J10	0.929	2.574	3	101	0.058
J16	0.730	12.480	3	101	0.000
K19	0.923	2.802	3	101	0.044
I13	0.824	7.183	3	101	0.000
N23	0.823	7.245	3	101	0.000
O26	0.846	6.127	3	101	0.001
HW1	0.830	6.878	3	101	0.000
HW2	0.951	1.738	3	101	0.164
HW3	0.735	12.128	3	101	0.000
HW4	0.835	6.639	3	101	0.000
HW5	0.845	6.159	3	101	0.001

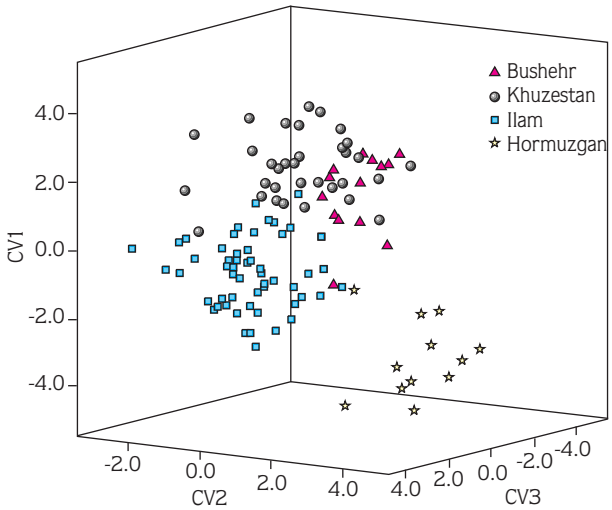

Figure 3A. A scatter plot of 115 *A. florea* colonies from 4 states based on CVA analysis.

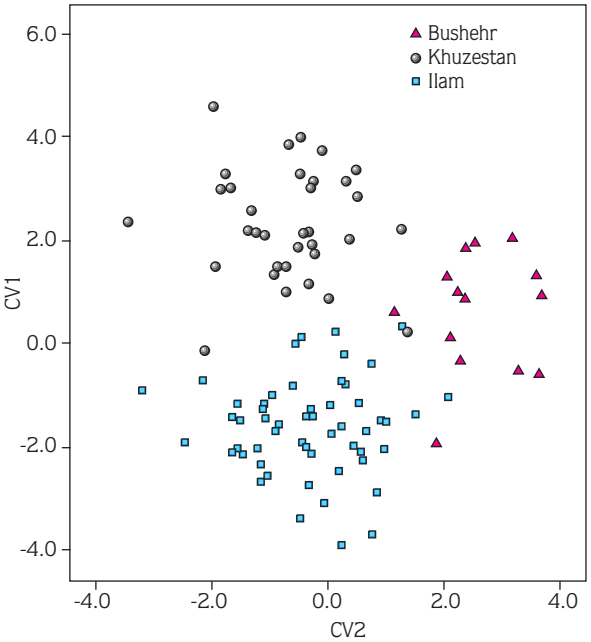

Figure 3B. A scatter plot of 103 *A. florea* colonies (except Hormuzgan colonies) based on CVA analysis.

According to the results of multivariate statistical analysis (DFA) the first, the second, and the third axis explained 53.1%, 39.7%, and 7.2% of the total variation respectively. One hundred percent of the total variation was explained by the first 3 canonical variates. FWW, B4, HWW, and FWL were the variables with the highest loadings on the first canonical axis whereas, HW3, HWL, and HW5 were loaded highly on the second canonical axis. In the third axis, FWW, WCL, and FWL were the variables contributing to the separation of the groups.

Table 4 shows correct classification results of 115 *A. florea* colonies with respect to their original groupings based on the CVA analysis. All the colonies from Hormuzgan state were correctly classified to their assigned groups. Colonies from Khuzestan and Ilam were classified 94% to their pre-assigned groups, whereas Bushehr colonies were classified 93% to their pre-assigned groups. The rate of correct classification of all colonies to their original groups was 95%.

When multiple regression analysis was applied to the morphometric variables using latitude and longitude as independent variables, no correlation with latitude and longitude was found (data not shown). Not all of the morphometric variables turned out to be significantly dependent on latitude and longitude, meaning that there were no relation with morphometrical variables and the geographical variables. According to the spatial autocorrelation analysis, out of 180 Moran's I coefficients calculated for dataset (30 characters 6 distance classes), 51 were significant at 5% or less. Out of 30 correlograms, 14 correlogram were significant according to the Bonferroni criterion (Table 5, Figure 4).

Linear Correlation Analysis between fore wing distance D (WDL) and both wing lengths (fore and hind) and between fore wing distance C (WCL) and both wing widths (fore and hind) were carried out. The associations between fore wing distance D (WDL) and both wing lengths (fore and hind) and fore wing C distance and both wing widths were high and significant ($P < 0.05$). The associations between fore wing distance D (WDL) and both wing lengths were found to be highly significant. The linear correlation between WDL and FWL were 0.848, while correlation between WDL and HWL were 0.816 ($P < 0.01$). Similarly, the associations between fore wing distance C (WCL) and both wing widths (fore and hind) were found to be highly significant. The correlation between WCL and FWW were 0.801, while correlation between WCL and HWW were 0.774 ($P < 0.01$).

Table 4. Classification results [n (%)] of 115 *A. florea* populations based on CVA analysis.

State	Bushehr	Khuzestan	Ilam	Hormuzgan
Bushehr	14 (93)	0 (0)	1 (7)	0 (0)
Khuzestan	1 (3)	32 (94)	1 (3)	0 (0)
Ilam	2 (4)	1 (2)	51 (94)	0 (0)
Hormuzgan	0 (0)	0 (0)	0 (0)	12 (100)

Discussion

In the present study, the extend of morphometric variation was studied with respect to 29 morphometric characters using 115 colonies collected from 4 states in Iran on the coastal north-south diagonal. This study was the most extensive study in terms of area coverage and the number of morphometric characters used. New size and angle characters utilized for the first time for the fore and hind wings. The morphometric variation among 4 states and usefulness of the news characters were discussed.

Distribution of *Apis florea*

Apis florea is another species in the genus *Apis* distributed in Iran, in addition to *Apis mellifera*. Its distribution starts from Qhasr-e-Shirin in Kermanshah (Hashemi, 2004) and spreads to Ilam, Lorestan, Khuzestan, Bushehr, Fars, Hormuzgan, Kerman, Sistan va Baluchestan, and Boyarahmad va Kohgiluye in Iran (Mossadegh, 1993; Ruttner et al., 1995). In addition, Moradi and Kandemir (2005) stated that the distribution may extend to Kurdistan states in the north. The present study covers a vast area extending over 8 parallels, from Hormuzgan (26°) in the south to Ilam (33°) in the north. The multivariate morphometric analyses showed clear groupings with respect to geography.

Geographic variation

Geography-related variation was pronounced in *Apis* by Ruttner, who states that clinal type of geographical variability exists in *Apis florea*, meaning that larger bees found in the north and smaller ones in the south. Tahmasebi et al. (2002) also demonstrated that a western group of larger bees found at a higher latitude (29°-34°) and an eastern group of smaller bees found at a lower latitude (<29°) in Principal Components Analysis in Iran. The finding is in accordance with the Bergman's rule about other animals. However, based on the multiple regression analysis in this study, geography-related

Table 5. Moran's I coefficients for 30 morphometric characters of *A. florea*.

Character	Distance Classes						
	1	2	3	4	5	6	P
FWL	0.04	0.13	0.02	0.15	-0.58*	-0.26	0.082
FWW	0.16	0.17	0.04	0.19	-0.56*	-0.49*	0.094
WCL	0.21	0.29*	-0.55**	0.31*	-0.04	-0.72**	**0.004**
WDL	0.10	0.23	-0.17	0.15*	-0.31	-0.60**	**0.022**
CuA	-0.08	0.07	-0.34	-0.16	0.02	-0.01	0.549
CuB	0.22	0.48**	0.09	-0.43*	-0.17	-0.69**	**0.006**
C.IND	0.18	0.39*	0.16	-0.71**	-0.10	-0.43*	**0.004**
HWL	0.18	0.09	-0.20	-0.01	0.12	-0.68**	**0.005**
HWW	0.07	0.18	0.06	0.03	-0.23	-0.61**	**0.020**
HAM	0.27	-0.34	-0.14	0.06	-0.43	0.07	0.311
FL	-0.10	0.16	-0.22	0.11	-0.05	-0.39	0.350
TL	-0.17	0.05	-0.10	0.19	-0.20	-0.27	0.514
MTL	-0.04	0.15	-0.04	0.12	-0.20	-0.49*	0.111
MTW	-0.13	-0.08	-0.09	-0.03	0.08	-0.24	1.000
A4	0.15	0.15	0.03	0.08	-0.59*	-0.31	0.065
B4	0.39*	0.32*	0.26*	-0.19	-0.47*	-0.82**	**0.000**
D7	0.37*	0.26*	0.18	-0.09	-0.61**	-0.61**	**0.019**
E9	0.20	-0.19	0.00	0.13	-0.62**	-0.02	**0.040**
G18	-0.02	0.06	0.02	-0.26	0.18	-0.43*	0.128
J10	-0.04	0.10	-0.05	0.11	-0.37	-0.25	0.516
J16	0.19	0.05	-0.10	0.14	-0.76**	-0.02	**0.008**
K19	0.22	0.07	-0.36	0.43**	-0.27	-0.58**	**0.029**
I13	0.17	0.21	0.17	-0.07	-0.47*	-0.51*	0.086
N23	-0.16	0.14	-0.05	-0.28	-0.06	-0.09	0.856
O26	0.47**	0.44**	-0.08	-0.48*	0.12	-0.98**	**0.000**
HW1	-0.26	-0.31	0.19	-0.03	-0.10	0.02	0.470
HW2	0.11	-0.35	-0.04	-0.37	0.37*	-0.23	0.103
HW3	0.40*	-0.25	-0.47*	0.31*	-0.17	-0.33	0.073
HW4	0.65**	-0.66**	0.08	-0.26	0.36*	-0.66**	**0.004**
HW5	0.60**	-0.69**	0.07	0.04	-0.88**	-0.35*	**0.001**

*significant at 5% level, **significant at 1% level.

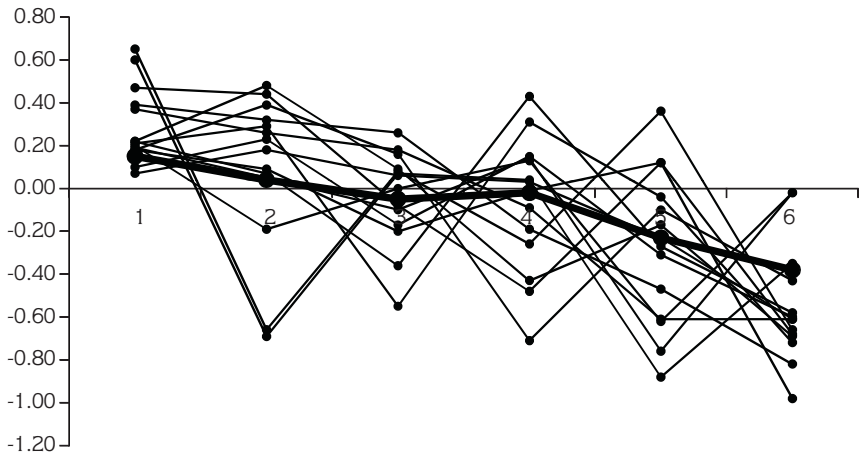

Figure 4. Significant autocorrelograms of *A. florea* morphometric characters (P < 0.05). Bold indicates the average autocorrelation between neighboring localities.

variation was not found in *A. florea* colonies of Iran. On the other hand, *A. florea* colonies from lower latitude (Hormuzgan state) were morphologically distinguishable from higher latitudes (Bushehr, Khuzestan, and Ilam states). Based on morphometric measurements of size characters (expect CuA, CuB, and TL), bees were smaller in the Hormuzgan colonies compared with the colonies of the other states (Table 2). Canonical Variates Analysis also supported morphological differentiation in *A. florea* colonies.

Cubital index was first used for the morphometrical discrimination of *Apis mellifera* subspecies (Ruttner, 1988). Thus, Ruttner (1988) also used the same index for *A. florea* and found 2.89 in southern Iran. In this study, cubital index value varied, ranging between 2.82 (Khuzestan) and 3.30 (Hormuzgan) and there was no relationship between cubital index and the latitude according to multiple regression analysis (Table 5). Besides, 3rd leg length of *A. florea* populations from Hormuzgan (5.429) was similar to *A. florea* populations from southern Iran (5.431) (Table 6).

In order to examine whether the observed variable at one locality is dependent on the values of the neighboring localities, we analyzed the geographic variation. According to spatial autocorrelation analysis, 14 correlograms were significant according to the Bonferroni criterion (Figure 4). Six characters (CuB, C.Ind, HWW, B4, D7, and I13) showed clinal type correlograms indicating positive autocorrelation in short distance classes and negative autocorrelation in long distance classes. For the characters CuB, C.Ind, B4, D7, and I13, a positive autocorrelation existed at the first 3 distance classes (Table 5, Figure 4), followed by negative autocorrelations in the last 3 distance classes. For HWW, a positive autocorrelation exists at the first 4 distance classes, followed by negative autocorrelations in the last 2 distance classes. Rest of the characters showed more or less crazy quilt type correlograms, meaning that there is no pattern between neighboring localities.

Usefulness of new characters

Ruttner (1988) reported that *Apis florea* samples from different regions vary not only in size characters, but also in others, especially wing venations. With this approach, angles of hind wing were first used for multivariate morphometric study of *A. florea* colonies. Analysis of variance of morphometric characters showed that 7 out of the 8 hind wing variables displayed statistically significant differences among populations ($P < 0.05$) (Table 3). In addition, 3 characters of hind wing had high loadings on the second canonical axis in multivariate statistical analysis. This demonstrated that characters of hind wing were valuable for discrimination of colonies.

Beside new hind wing characters (HWL, HWW, HW1, HW2, HW3, HW4, and HW5), fore wing distance D (WDL) and fore wing distance C (WCL) were used in this study. Dedej and Nazzi (1994) reported that WDL and WCL were used instead of fore wing

Table 6. Some characteristic of *A. florea* of different origin (N: number of individuals, measurements in mm).

Location	N	FWL	FWW	Cubital Index	Hamuli	3rd Leg
Ilam	806	6.801	2.409	2.98	11.78	5.558
Khuzestan	354	6.896	2.446	2.82	12.17	5.581
Bushehr	206	6.859	2.434	3.29	11.87	5.564
Hormuzgan	178	6.484	2.188	3.30	11.63	5.429
*South Iran	60	6.706	2.313	2.89	11.37	5.431
*Oman	60	6.516	2.248	3.08	13.20	5.260
*Pakistan	40	6.598	2.316	2.85	-	5.202
*Sri Lanka	80	6.168	2.125	3.50	11.60	5.118
*South India	20	6.252	2.140	3.32	10.50	4.901
*Thailand	60	6.433	2.201	2.94	-	5.269

*adopted from Ruttner, 1988

length and fore wing width for *Apis mellifera* subspecies to estimate the fore wing length and fore wing width. Highly significant correlations between WDL and the length, as well as between WCL and the width, were in agreement with Dedej and Nazzi (1994) ($P < 0.01$). Moreover, correlation analysis to determine the associations between fore wing and hind wing length and between fore wing and hind wing width displayed significant associations between fore wing and hind wing length (0.763), as well as between fore wing and hind wing width (0.905) ($P < 0.01$). These correlations revealed that one can use fore wing - hind wing length and fore wing - hind wing width characters interchangeably.

Beside size dependent morphometric characters, geometric morphometric characters were also applied to the same data set (unpublished data, manuscript in preparation) and similar findings were found on the wing shape of *A. florea*. However molecular tools should also be applied along with size-dependent and size–independent morphometric characters in order to elucidate the population differentiation in *A. florea*.

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