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Original Article

MORPHOLOGICAL DISCRIMINATION OF GREEK HONEY BEE POPULATIONS BASED ON GEOMETRIC MORPHOMETRICS ANALYSIS OF WING SHAPE

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Abstract

Honey bees collected from 32 different localities in Greece were studied based on the geometric morphometrics approach using the coordinates of 19 landmarks located at wing vein intersections. Procrustes analysis, principal component analysis, and Canonical variate analysis (CVA) detected population variability among the studied samples. According to the Principal component analysis (PCA) of pooled data from each locality, the most differentiated populations were the populations from the Aegean island localities Astypalaia, Chios, and Kythira. However, the populations with the most distant according to the canonical variate analysis performed on all measurements were the populations from Heraklion and Chania (both from Crete island). These results can be used as a starting point for the use of geometric morphometrics in the discrimination of honey bee populations in Greece and the establishment of conservation areas for local honey bee populations.

Keywords: ***Apis mellifera*, geometric morphometrics, Greece, morphological discrimination.**

INTRODUCTION

Traditionally, intraspecific taxonomy of the honey bee *Apis mellifera* L. has been based on morphology. Twenty-seven subspecies of *A. mellifera* are currently recognized on the basis of morphometric characteristics (Ruttner, 1988, 1992; Sheppard et al., 1997; Engel, 1999; Sheppard and Meixner, 2003). These subspecies are also described as 'geographic races' because their distributions correspond to distinct geographic areas. Five evolutionary lineages have been characterized based on morphometric, molecular, ecological, ethological, and physiological traits (De la Rùa et al., 2005). Four of these lineages occur in the Mediterranean Basin: African lineage (A), West and North European lineage (M), South-east Europe lineage (C), and Near and Middle East lineage (O) (Garnery et al., 1993; Arias and Sheppard, 1996; Franck et al., 2000, 2001; Miguel et al., 2007; Cánovas et al., 2008).
Traditional morphometrics, so-called classical morphometry, was the only method to describe the diversity of honey bees for a long time (Ruttner, 1988), but biochemical methods have been developed (i.e., allozymes, isoenzymes) (Nunamaker and Wilson, 1982; Badino et al., 1988; Bouga et al., 2005b; Ivanova et al., 2012) for such studies. Molecular markers, such as nuclear DNA (Hall, 1990;

Tarès et al., 1993), mitochondrial DNA (mtDNA) (Moritz et al., 1986; Smith et al., 1989; Smith, 1991; Hunt and Page, 1992; Garnery et al., 1993; Oldroyd et al., 1995; Arias and Sheppard, 1996; Pedersen, 1996; De la Rúa et al., 2000), and microsatellites (Estoup et al., 1993; Garnery et al., 1998), are also used to study genetic variability in honey bees.

Several attempts have been made to simplify morphological determination, such as reducing the number of characters (Dedej and Nazzi, 1994; Cermak and Kaspar, 2000), using a single wing cell (Francoy et al., 2006), or using computer software designed specifically for morphometric measurements (Daly et al., 1982; Batra, 1988; Tofilski, 2004). Dupraw (1964, 1965a, b) was the first to use a set of quantitative characteristics for wing venation and correctly classified many subspecies. In 1978, Daly and Balling successfully applied this method to differentiate Africanized and European honey bees in South America. Later, Daly et al. (1982) began to use digital measurements to investigate the morphometrics of honey bees, significantly reducing the time necessary for measuring, storing, and analyzing the data. Since that time, considerable research focusing on a wide range of problems by using multivariate methods has been published (Rinderer et al., 1990, 1993; Crewe et al., 1994; Quezada-Euán et al., 2003). Steinhage et al. (1997) developed a semi-automated method for wing measurement that reduced analysis time and improved precision in identifying bee species; this method was later improved to an automated system (Steinhage et al., 2001, 2007) with a precision of 99.8% for classifying bee species.

A new method, called geometric morphometrics, was developed based on the coordinates of landmarks located at vein intersections of the wings (Bookstein, 1991; Smith et al., 1997). The fundamental benefits of geometric morphometrics over traditional approaches include the way in which the amount of difference between shapes can be measured (using Procrustes distance), elucidation of the properties of the multidimensional shape space defined by this distance coefficient, the development of specialized statistical methods for studying shape, and the development of new techniques for graphical representation of the results (Bookstein, 1991; Rohlf, 2000; Mendes et al., 2007). The high accuracy in classification indicates that forewings carry sufficient information with which to distinguish the bee groups that are examined. In addition to molecular analysis of mtDNA origin, the methodologies are very informative.

Fig. 1. Sampling localities: [1] Evros; [2] Rodopi; [3] Xanthi; [4] Drama; [5] Kilkis; [6] Thessaloniki 1; [7] Thessaloniki 2; [8] Halkidiki; [9] Elassona; [10] Larissa; [11] Karditsa; [12] Phthiotida; [13] Kerkyra; [14] Paxoi; [15] Lefkada; [16] Ithaki; [17] Kefalonia; [18] Zakynthos; [19] Achaia; [20] Korinthia; [21] Argolida; [22] Messinia; [23] Lakonia; [24] Kythira; [25] Chania; [26] Rethymno; [27] Heraklion; [28] Lasithi; [29] Astypalaia; [30] Chios; [31] Limnos 1; and [32] Limnos 2.

The aim of this investigation was to determine if geometric morphometrics based on forewing venation is a useful tool for the discrimination of Greek honey bee populations and the establishment of conservation areas for honey bee populations that still retain some pure characteristics.

MATERIAL AND METHODS

Sampling

Honey bees were collected from 32 localities (5 non-migratory colonies/ locality, 5 bees/colony = 25 worker bees /locality). The sampling localities were North Greece (Evros, Rodopi, Xanthi, Drama, Thessaloniki 1 and 2, Halkidiki, Kilkis), Central Greece (Elassona, Larissa, Karditsa, Phthiotida) and South Greece (Argolida, Achaia, Korinthia, Lakonia, Messinia), the Aegean islands (Limnos 1 and 2, Chios, Astypalaia, Kythira), the Ionian islands (Kerkyra, Kefalonia, Lefkada, Zakynthos, Ithaki, Paxoi), and Crete island (Heraklion, Lasithi, Rethymno, Chania) (Fig. 1) corresponding to *A. m. macedonica* (North Greece), *A. m. cecropia* (Central and South Greece), Aegean race (near *A. m. adami*, Aegean islands), *A. m. carnica* (Ionian islands), and *A. m. adami* (Crete island) in Ruttner's (1988) morphometrics analysis. The samples consisted of adult workers that were transferred alive to the laboratory and stored in 95% ethanol until processed.

Data acquisition and treatment

The right forewings of honey bees were cut very close to their base and then transferred to 70%, 50%, and 20% ethanol solution for gradual hydration, and finally to distilled water in order to flatten them. Images of the wings were obtained using a video camera mounted on a microscope with

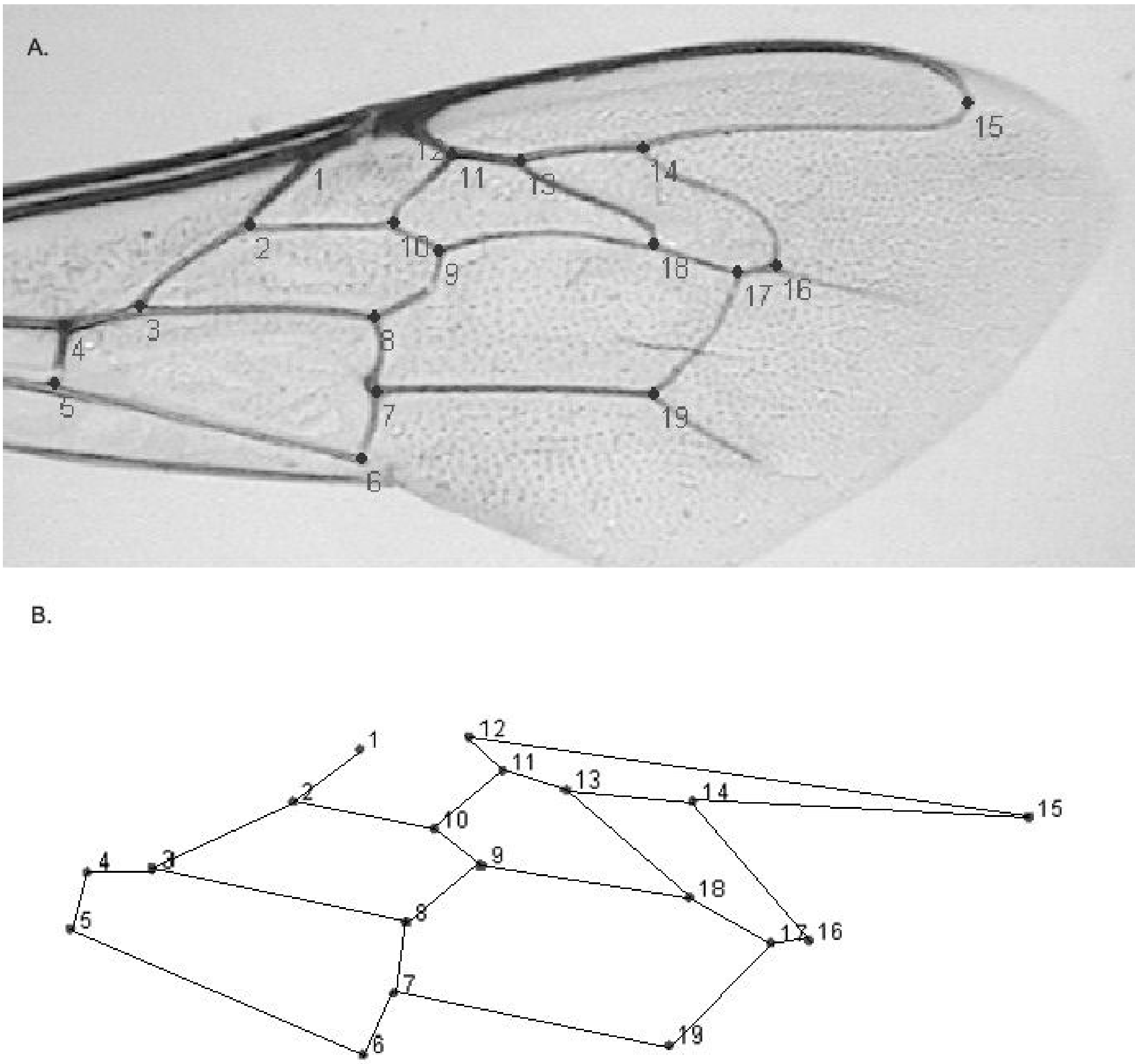

Fig. 2. The shape of the honey bee forewing. A: The coordinates of 19 landmarks located at vein intersections of the honeybee forewing. B: The average shape of the populations studied.

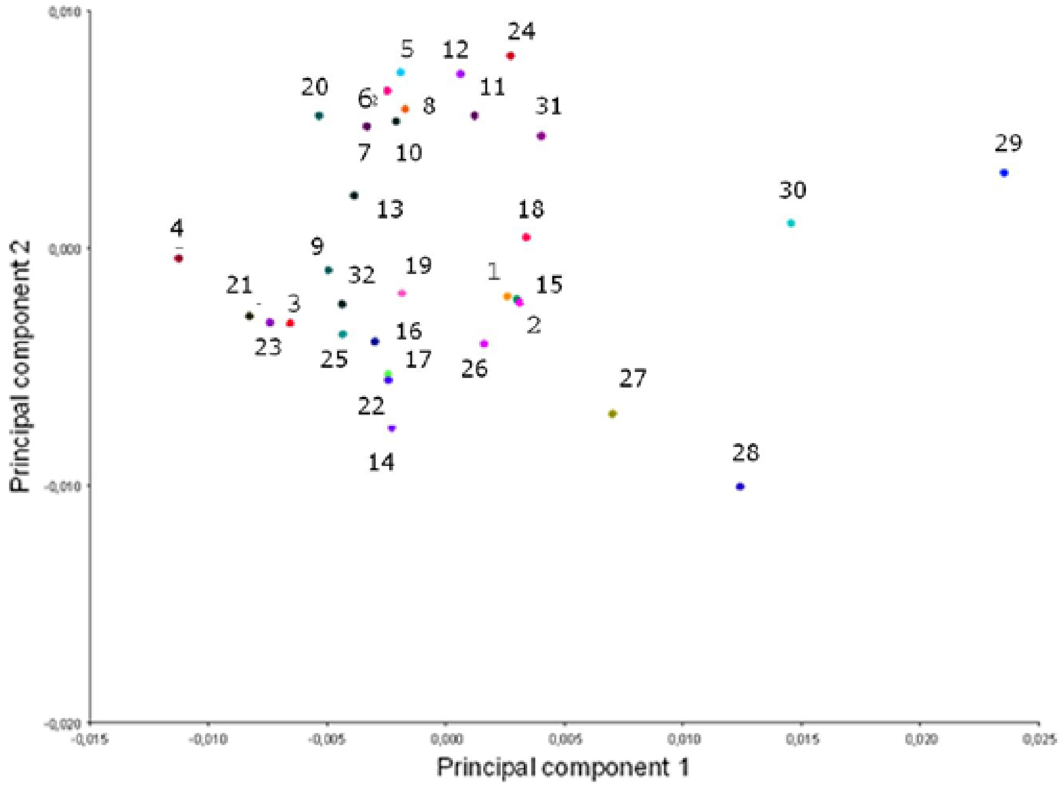

Fig. 3. Principal component analysis based on pooled data from each locality. The sampling localities are given in Fig.1. Aegean island Astypalaia [29] and Chios [30] were the most distant from the other populations, followed by Lasithi [28] and Heraklion [27] on the island of Crete. The total variance was 0.00013461.

a 1x objective. The coordinates of 19 landmarks located at vein intersections (Fig. 2A) were recorded and the two-dimensional x, y Cartesian coordinates digitized using the tpsDig program (Rohlf, 2001). The procedure was performed twice for each wing and the average calculated in order to reduce measurement error (Miguel et al., 2010). The results for each locality were pooled.

The landmark coordinates obtained from tpsDig were used in the MORPHOJ package (Klingenberg, 2011). Alignment was performed using Procrustes fit (Procrustes superimposition) (Dryden and Mardia, 1998). Procrustes analysis (GPA), currently one of the standard methods for analyzing landmark data (Viscosi and Cardini, 2011), was performed. Procrustes distance, a measure of the absolute magnitude of the shape deviation, was calculated (Klingenberg and Monteiro, 2005). Principal component analysis (PCA) was performed based on the spectral decomposition of a covariance matrix created based on the pooled results of each locality. Principal component analysis was also performed with all measurements from each locality. Furthermore, the thin-plate spline method was used to illustrate the transformations of the wing shapes compared to the average shape of the wing. Dendrograms showing the relationships among honey bee populations based on the wing shape were constructed after Fourier transformation of the pooled results of each locality according to the UPGMA method (Sneath and Sokal, 1973) using the NTSYS package (Rohlf, 1990). Canonical variate analyses (CVA) was fianlly performed with all measurements from each locality.

RESULTS

The average shape obtained by the Procrustes fit of the studied populations is shown in Fig. 2B. Procrustes distance indicated that the most distant average shape (0.0064) was found in Astypalaia (Aegean island). PCA of pooled data confirmed the differentiation of the Astypalaia population and also differentiated the populations of Chios, Lasithi, and Heraklion (Fig. 3). The principal component shape changes indicated that the populations from

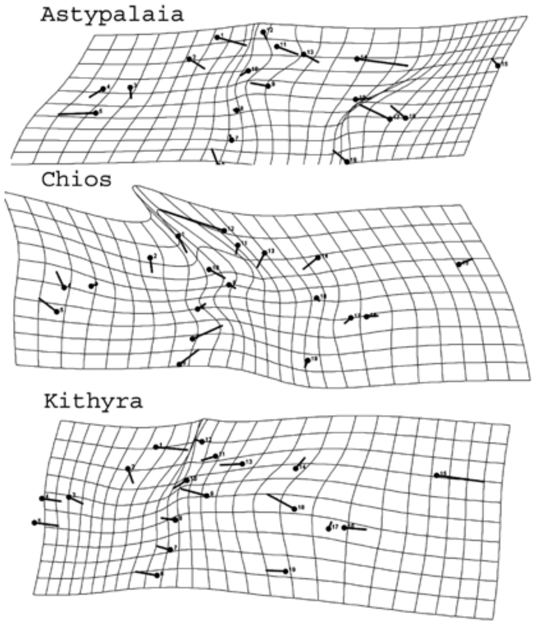

Fig. 4. The average wing shape of honey bees from Astypalaia, Chios, and Kythira and their deviations from the average shape of all populations studied. The numbers show the order of the landmarks according to Fig. 2. The length and direction of the line indicates the movement of the respective landmark from the average shape.

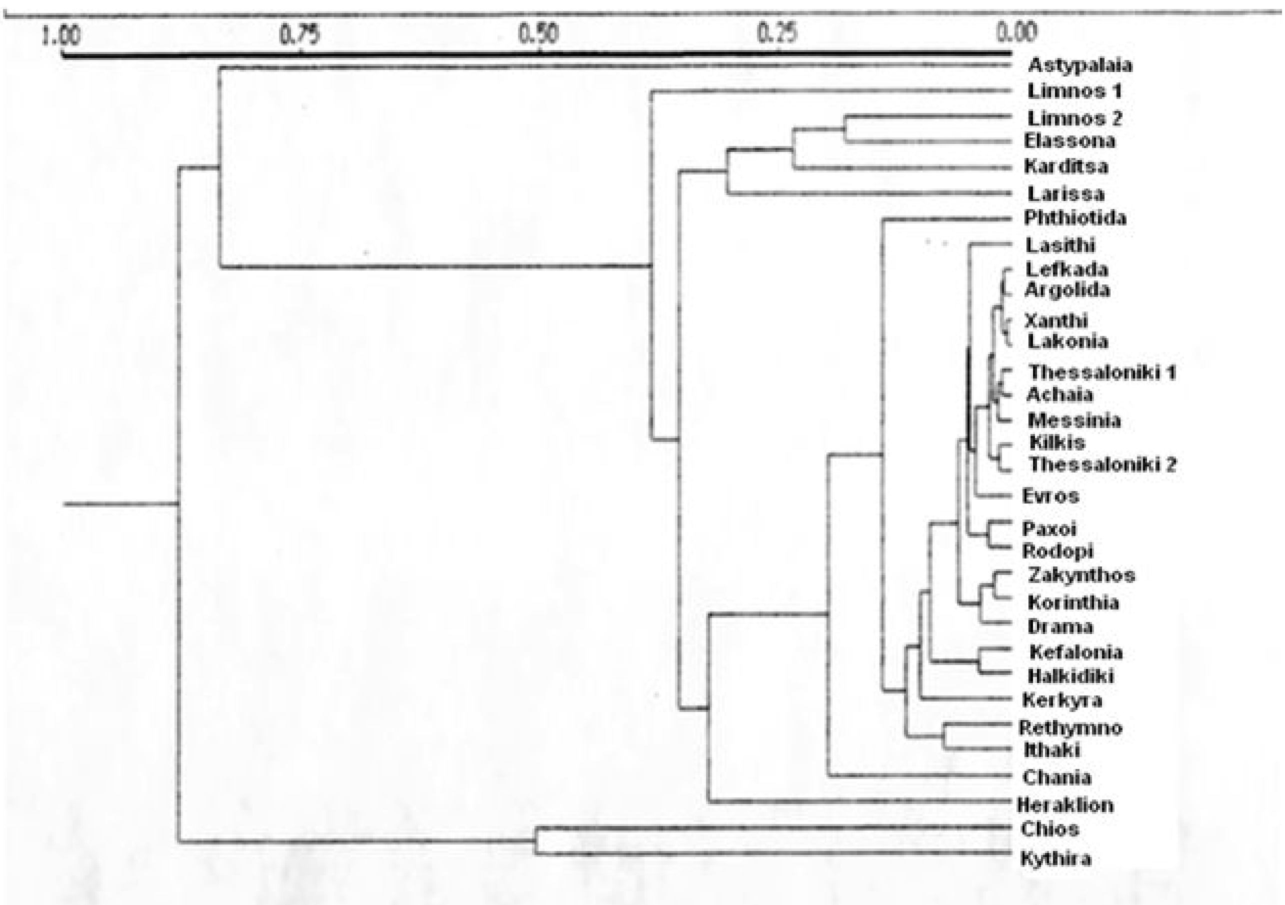

Fig. 5. UPGMA dendrogram based on the pooled results of each locality showing the phylogenetic relationships among the studied honey bee populations. The honey bee populations from Chios, Kythira and Astypalaia islands were discriminated from the rest populations.

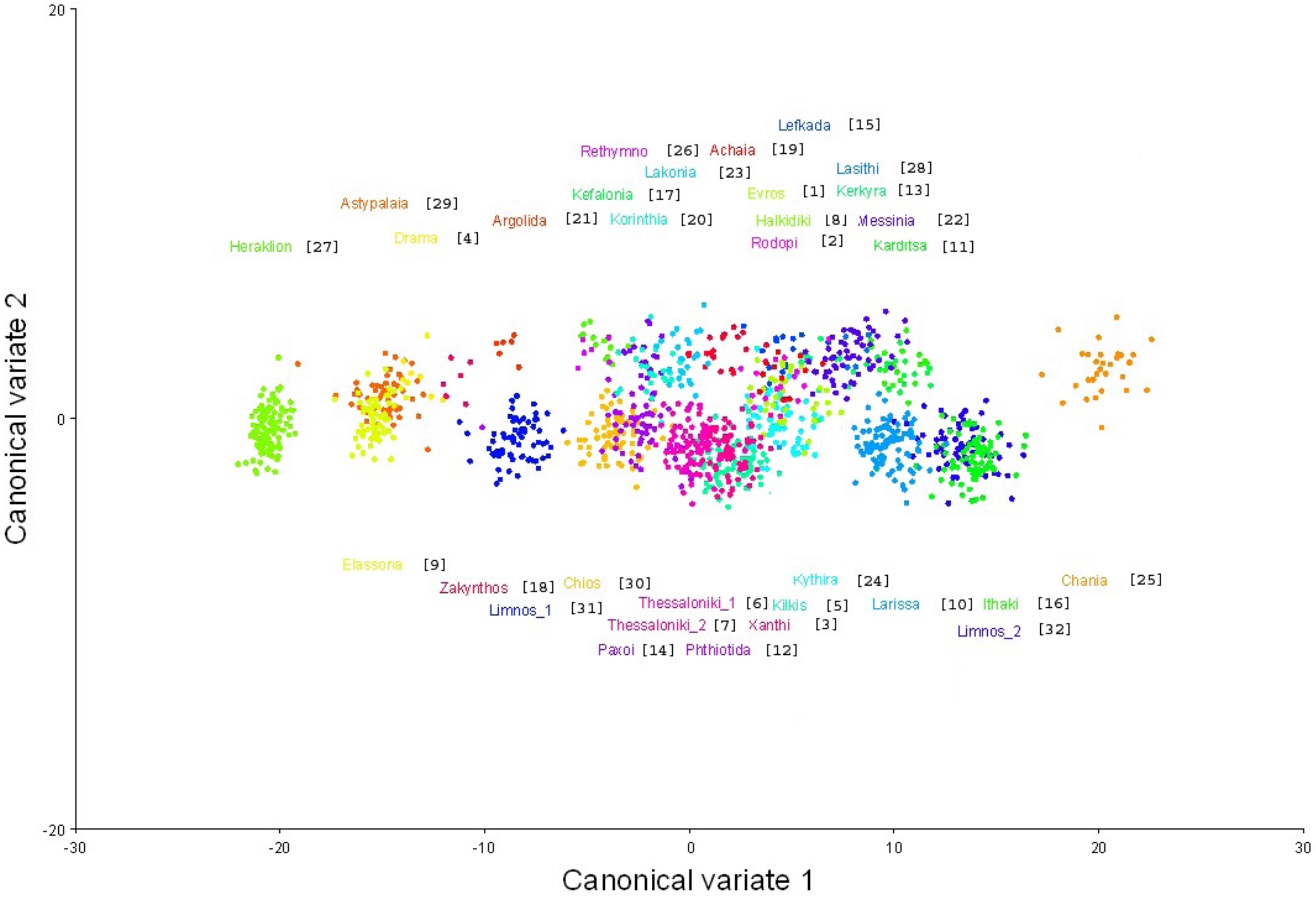

Fig. 6. Canonical variate analysis based on the measurement of all samples from each locality. Some honey bee populations were well differentiated, such as those from Chania and Heraklion (Crete island).

Astypalaia, Chios, and Kythira, all of them Aegean islands, were well separated from the rest of the studied populations. Transformation grids in Fig. 4 show the shape changes as a deformation of the rectangular grid. The total variance based on PCA was 0.00013461.

According to the topology of the UPGMA dendrogram (Fig. 5), there were two main clades initially; Chios and Kythira populations formed the first clade, whereas the other populations studied clustered together in the second clade. However, the Astypalaia population is in a distinct cluster in the second clade and all remaining populations form the second cluster. These results confirm the PCA findings. Looking further in this second cluster of the second clade, one population from Limnos was also differentiated, as well as the populations from Heraklion and Chania (Crete island). Finally, the Larissa population was the only one differentiated from among the populations of Central Greece (Fig. 5).

CVA (Fig. 6) and PCA based on all measurements gave the same results and showed a clearer differentiation among several of the studied populations. The most distant populations in the CVA were the populations from Heraklion and Chania (Crete island).

DISCUSSION

Identification of the species of various groups of bees based on wing features is possible, even in areas like the Mediterranean basin and Europe where many distinct subspecies exist (Kauhausen-Keller et al., 1997). Our study presents the first comprehensive study of honey bee populations in Greece using the geometric morphometrics approach. High morphological variability was found between several honey bee populations in Greece, but a mixture of populations was also found in some localities due to importation and migratory beekeeping. The most distant populations based on principal components shape changes were the populations from Astypalaia, Chios, and Kythira (all belonging to Aegean islands). Similar results were obtained in previous work based on molecular analyses, including mtDNA and isoenzymic analysis (Bouga et al., 2005a, b). Thus, honey bees in some Aegean islands retain distinct characteristics, probably due to reduced beekeeping movements and commercialization. Also, honey bee populations from Limnos were divided in two groups, and this result is similar to the results obtained from mtDNA analysis (Harizanis et al., 2006). Ruttner (1988) had classified the bees of the Aegean islands as an Aegean race (near *A. m. adami*).

A preliminary study on geometric morphometrics analysis of honey bee populations conducted by Hatjina et al. (2002) on a limited number of samples per locality also showed that, although a *macedonica*-like group was predominant in the central part of Greece, some populations originating from the Aegean islands were characteristically different, including the Astypalaia and Limnos islands. The differentiation of Astypalaia is even more interesting considering that different samples collected 10 years later showed the same results. The great number of samples and advanced methodology in this present study support the reliability of the findings.

Furthermore, the UPGMA dendrogram showed that Central Greece populations cluster closely together. Ruttner (1988) also classified the bees from Central Greece as *A. m. cecropia*. However, the population from Larissa was differentiated from the others, similar to previous studies concerning mtDNA analysis (Harizanis et al., 2006; Martimianakis et al., 2011). According to these studies, this population represents a unique haplotype and is characteristically distinguishable from the *A. m. macedonica* hybrids that dominate the mainland. Studying the population structure of the apiaries neighboring the one sampled would be interesting, as well as a comparison of the morphometrics results for today's bees with Ruttner's database.

As in similar studies concerning other countries (Özkan Koca and Kandemir, 2013), the CVA and PCA based on all measurements seemed to be able to detect differences between the honey bee populations from Greece. Based on these analyses, differences were detected among several Greek honey bee populations, and the most distant populations were from Heraklion and Chania (Crete island).

The use of geometric morphometrics in general confirmed what was already known from mtDNA studies; Greek honey bee populations are not classified clearly using only the discrete subspecies referred to in Ruttner's (1988) morphometrics analysis. Great hybridization exists due to beekeeping practices (commercial breeding and migratory beekeeping). However, some important variability still exists. In an attempt to preserve honey bee biodiversity through the establishment of conservations areas, these discrete populations can be used for the reproduction of local populations.

CONCLUSIONS

This study demonstrated that geometric morphometrics analysis of wing shapes can be used to discriminate several honey bee populations from Greece with both Canonical variate analysis and Principal component analysis. The presence of distinct groups was highlighted by honey bee populations from some Aegean islands, such as Astypalaia, Chios, and Kythira, and some localities of Crete island (i.e., Chania and Heraklion). The results are useful for the discrimination and preservation of local populations.

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