Wing differentiation among Greek populations of honey bee (Apis mellifera): a geometric morphometrics analysis

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Introduction

- According to Ruttner (1988) three distinct honey bee races should exist in Greece: *macedonica* in the Greek Macedonia and Thrace regions, *cecropia* in Thessalia, Peloponnese and Kyklades islands, and *adami* restricted to Crete. However, bees of Ionian islands were assigned to *carnica* race, while nothing was mentioned about the rest of Aegean islands. Since the work of Ruttner though, many uncontrolled bee introductions took place in Greek mainland and islands, the results of which are not known.
- Geometric morphometrics using homologous landmarks (Bookstein, 1991) has been shown in many occasions (Rohlf and Marcus, 1993) to be more powerful than traditional morphometrics. Together with molecular (mtDNA, isoenzymes and microsatellite) analyses it is now largely employed in evolutionary studies to explore intraspecific variation at the population level.
- The present study is only part of a larger Platon project between Greece and France involving phylogeographic investigations of the Greek honey bee populations.

Materials and Methods

- A sample of 497 bees was col-lected from 3 mainland locations and 10 locations of Aegean islands (Fig 1, sample sizes are indicated for each location). Left wings of bees were described using the coordinates of nineteen (19) landmarks located at vein intersections (Fig. 2).
- Landmark coordinates were superimposed using a Gene-ralized Least-Squares (GLS) Procrustes Superimposition (Rohlf and Slice, 1990): specimens are centered, nor-malized to unit centroid-size (Bookstein, 1991) and iteratively rotated to minimize the sum-of squared distances between each location and its sample mean. Centroid size was analyzed separately by ANOVA or together with shape parameters (i.e. Procrustes residuals). Shape differences were analyzed by multivariate analysis of variance (MANOVA), Canonical Variate Analyses (CVA) and Multiple Discriminant Analyses. Shape patterns along the canonical axes were estimated by multivariate regression (Monteiro, 1999). All calculations were performed with Matlab and R functions.

Figure 1. Sample sizes and locations.

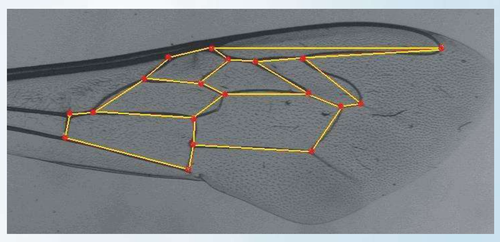

Figure 2. Landmark locations (red dots). Links (yellow lines) are only used to visualize the overall wing venation and shape.

Results

I. Size differences: the ANOVA of the log of centroid sizes was highly significant ($F = 3{,}06$, $df = 12/484$, $p = 0{,}0004$), though no clear geographic patterns could be identified (Figure 3).

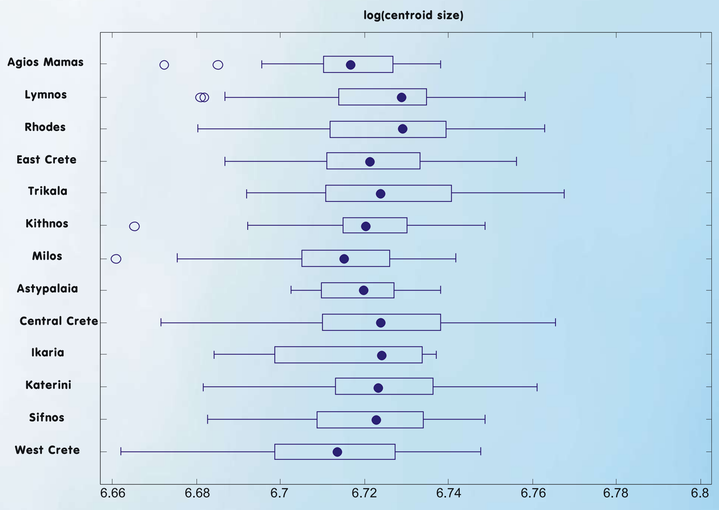

Figure 3. Box-plots for centroid size distributions and differences between locations.

II. Shape differences: The MANOVA was highly significant (Wilks' Lambda = 0,0519, $F = 3{,}76$, $df = 408/5154$, $p < 10^{-4}$. Projections onto the first canonical plane (59,7% of variance, Fig.4) clearly separated three locations (Rhodes, Astypalaia and Ikaria, here called Rhodes-like group, see also Fig. 5) from the rest, which could be characterized as a *macedonica*-like group. Onto the first axis, specimens from Limnos lied in between these two groups. Specimens from Crete also tend to lie in the middle of the plane, but with a greater variability than Limnos ones. Apart, from Crete and Limnos specimens, no clear geographic patterns could be retrieved within this *macedonica*-like group (Fig.5). The third (8,1 %) and fourth (7,8 %) axes (not shown) only partly separated locations within the Rhodes-like group.

All specimens from the Rhodes group were characterized by having smaller wing cells apart the distal and median one, which is larger (see bottom of Figure 4, blue links). Limnos and Crete specimens both had wings of a smaller width and with a longer anterior cell.

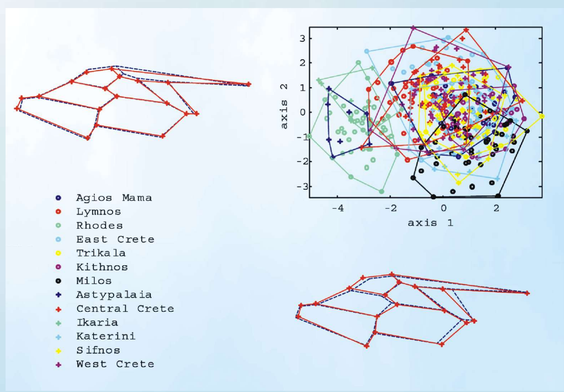

Figure 4. Canonical variates analysis of shape (Procrustes GLS residuals). Shape variability is depicted along each canonical axis: blue links are shapes on he negative side of the axis, red ones are for those on the positive side.

Figure 5. Geographic locations for the four groups of samples distinguished by morphometric analyses.

Fig. 6: Neighbor-joining tree of Mahalanobis D^2 distances between locations.

III. Genetic distances: A neighbor-joining tree of the Mahalanobis D^2 distances clearly confirmed and summari-zed these results (Figure 6). The three Rhodes-like populations clearly segre-gated from the *macedonica*-like group. Crete specimens lied at the bottom of the *macedonica* group while Limnos specimens lied between these two main groups. All shape patterns were free from any allometric component. Inclusion of centroid size in the analyses did not improved the significance of results.

IV. Classification results: Leave-one-out cross-validated classification results, using multiple discriminant analyses are summarized in the Table I. They confirmed the gap between Rhodes-like group and *macedonica*-like one, as well as the partial separation of Limnos and Crete specimens from the latter.

Table I. Classification results using multiple discriminant analyses. Anbiased classification percentages were estimated using leave-one-out cross-validations (only the main groups delineated by morphometric analyses are indicated).

from	to	Rhodes-like	*macedonica*-like		
			Limnos	Crete	Others
Rhodes-like	Rhodes	94,0	2,0	4,0	
	Astypalaia	100,0			
	Ikaria	87,5		12,5	
***macedonica*-like**	Limnos	2,0	54,0	26,0	18,0
	Crete	5,0	12,1	43,5	39,4
	Others	0,8	5,0	15,0	79,2

Conclusions

- The present results clearly show that two main groups of bees are to be found within the sampled area. The largest group may be related to the *macedonica* bee race since most samples came from its previously known distribution rage.
- The three islands which fall apart (Rhodes, Astypalaia and Ikaria) belong to a well differentiated group with a clear geographic relationship and no relationships with *macedonica* bee. This reminds the island population segregation exemplified by mtDNA and isoenzyme analysis (Bouga 2002) Any interpretation though, should take into account the closeliness of anatolica bee, existing in the costline of Turkey.
- Samples from Crete appear to be of very mixed origin. This result is far from expected since bees from Crete were assigned to a distinct *adami* race (Ruttner). The fact that we used only wing shape may explain this difference. But repeated introduction of both *macedonica* and *ligustica* bees in Crete could be an alternative explanation. In this case one can hypothesize that foreign bees introduction could result in a large introgression putting the *adami* race into question.
- Finally, the overall distinctiveness of the Limnos specimens, which appear partly distinct from the *macedonica* specimens and distinct but very close with Rhodes specimens, raises some questions. Answers may come from molecular (mtDNA and microsatellite) analysis, which will provide some lights into its genetic structure and relationship.

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