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Original article

Both geometric morphometric and microsatellite data consistently support the differentiation of the *Apis mellifera* M evolutionary branch*

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Abstract – Traditional morphometrics, allozymes, and mitochondrial data have supported a close relationship between the M branch subspecies *A. m. iberiensis* and the North African subspecies (A branch). However, studies using nuclear DNA markers have revealed a clear distinction between the latter and the two European M branch subspecies. In help resolve this paradox, we analyzed 663 colonies from six European and African subspecies. A geometric morphometrics approach was applied to the analysis of wing shape, and the results were compared with data of six microsatellite loci. Both data sets were found to be highly consistent and corroborated a marked divergence of West European subspecies from North African ones. This supports the hypothesis that the presence of the African lineage mitotype in Iberian honey bee populations is likely the consequence of secondary introductions, with a minimal African influence within the current Iberian genetic background. Wing geometric morphometrics appears more appropriate than mitochondrial DNA analysis or traditional morphometrics in the screening and identification of the Africanization process.

honeybee / evolutionary branch / wing morphology / geometric morphometrics / microsatellite

1. INTRODUCTION

The honey bee (*Apis mellifera*) is naturally widespread throughout Africa, Europe, and Western Asia. Based on morphometric measurements, different subspecies have been identified and grouped into four major evolutionary branches (Ruttner et al., 1978; Ruttner, 1988): the A (Africa), M (Western Europe), C (South-Eastern Europe), and O (Middle East) branches. This classification was largely supported by mitochondrial studies (Garnery et al., 1992; Arias et al., 1996; Franck et al., 2000), which revealed an additional fifth evolutionary branch, called Y (Yemenitica from Ethiopia; Franck et al., 2001). However, controversy still exists over the differentiation of the M branch and the North African A branch populations, and more specifically, over the relationship between the subspecies *A. m. intermissa* (A branch), *A. m. mellifera* (M branch from North-Western Europe), and *A. m. iberiensis* (M branch from Iberian Peninsula).

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Western Europe and North Africa are contact regions for the A, M, and C evolutionary branches. Using a wide set of morphological characters – related to body and wing distances, ratios and angles, color, and pilosity – Ruttner et al. (1978) highlighted the morphological affinity between the Iberian honeybee, *A. m. iberiensis*, and the North African, *A. m. intermissa*, describing a clinal variation *A. m. intermissa- A. m. iberiensis- A. m. mellifera* subspecies. Based on these results, they hypothesized a progressive transition linking the M branch with the North African A branch via the Iberian Peninsula. Subsequently, Cornuet and Fresnaye (1989) analyzed six of Ruttner's morphological characters in the Iberian bees, and their results supported this idea. However, it must be pointed out that Arias et al. (2006) analyzed 23 morphometric characters, mainly from the wing, and detected a discontinuity between European and North African populations, questioning the previously described close relationship between them.

Regarding genetic data, the North Africa - Europe morphological gradient was supported by a parallel cline at the malate dehydrogenase (MDH) locus from Morocco to France through the Iberian Peninsula, but this cline was not supported by another allozyme marker – phosphoglucomutase (PGM) – (Smith and Glenn, 1995; Arias et al., 2006). Mitochondrial studies of the honey bee populations of the Iberian Peninsula also detected a south-western to north-eastern clinal transition from the African A lineage to West European M mitochondrial lineage (Smith et al., 1991; Arias et al., 1996, 2006; Garnery et al., 1995, 1998a; Franck et al., 1998; Miguel et al., 2007; Canovas et al., 2007). This mitotype cline, together with the high divergence detected between A and M mitochondrial DNA lineages, led Smith et al. (1991) to propose a hybrid origin of Iberian populations after secondary contact between *A. m. intermissa* and *A. m. mellifera*. Nevertheless, studies based on microsatellite loci (Franck et al., 1998, 2001; Garnery et al., 1998b) did not support a hybrid origin for the *A. m. iberiensis* subspecies. On the contrary, they showed a close genetic resemblance between the two European subspecies of the M branch and a clear break between them and all the analyzed Africans subspecies, including *A. m. intermissa*. Recently, a study based on single nucleotide polymorphisms (SNPs; Whitfield et al., 2006) revealed a marked differentiation between all branches described by Ruttner (1988). They detected major differences between the A and M branches, although the differences were smaller than those between the M and C branches. The North African populations were grouped close to the rest of the African populations, far from western European ones.

In short, phylogeographic studies based on traditional morphological data and those based on genetic data are largely consistent in terms of their description of the evolutionary branches of *Apis mellifera*. However, these studies are not in agreement concerning the differentiation between the M branch and North African populations. Moreover, discrepancies among morphological studies, among genetic studies, and between these two empirical approaches abound regarding this matter.

Since its initial development (Bookstein, 1991), geometric morphometrics has been shown repeatedly to have better descriptive and higher statistical power than traditional morphometrics (c.f. Monteiro et al., 2000; Adams et al., 2004). In insects in general (Baylac and Daufresne, 1996; Baylac and Penin, 1998; Klingenberg et al., 2001; Baylac et al., 2003) or in honeybees in particular, geometric morphometric analyses of wing shape have provided many new insights, either into the characterization and identification of populations or lineages (Baylac et al., 2008; Tofilski, 2008), the Africanization process of American populations (Francoy et al., 2008, 2009), or even into the more demanding analyses of heritability (Monteiro et al., 2002) or individual wing asymmetry (Smith et al., 1997; Klingenberg et al., 2001). When molecular and geometric morphometrics results were compared, a close congruence was generally observed, with exceptions being almost always related to differential selective pressures (Marroig and Cheverud, 2004; Hamon and Gibson, 2006; Evin et al., 2008).

The goal of this work was to contribute to resolving the controversy that currently exists regarding differentiation between the A and

M evolutionary branches. To this end, a wide sample of populations from six subspecies of *Apis mellifera* (two for each evolutionary branch A, M, and C) were analyzed, applying two different approaches: geometric morphometrics analysis of wing shape variability and microsatellite analysis.

2. MATERIALS AND METHODS

2.1. Geometric morphometrics: data acquisition and treatment

A total of 663 colonies (one honeybee per colony, preserved in absolute alcohol) from 27 populations of *A. mellifera* were sampled for the geometric morphometrics analysis (Tab. I): 18 from the Iberian Peninsula (*A. m. iberiensis*), five from France and Belgium (*A. m. mellifera*), and four populations of subspecies *A. m. intermissa* and *A. m. major* from branch A and *A. m. ligustica* and *A. m. macedonica* from branch C.

Coordinates of 19 landmarks located on the fore-wing (Fig. 1), identical to those already used by Smith et al. (1997), were measured using a video camera (768 × 512 pixels) connected to an AT-OFG frame-grabber and MeasurementTV software (Updegraff, 1990). Wings were temporarily mounted in distilled water. Water in excess was gently absorbed in order to ensure the flatness of the wing membrane onto the slide. Each wing was measured twice and the two measurements were averaged in order to reduce the measurement error. Raw coordinates of the landmarks were superimposed using a Procrustes generalized least-squares (GLS) superimposition algorithm (Rohlf and Slice, 1990): the sum of squared distances between homologous landmarks of each object and a reference configuration are iteratively minimized by translations and rigid rotations. At each iteration, the reference taken as the mean configuration of the whole superimposed sample is updated. Centroid size, defined as the square root of the sum of the squared distances between the centre of the object and its landmarks (Bookstein, 1991), is eliminated from the superimposed coordinates by ratios. Geometrically, each object is therefore scaled to unit centroid size, centered, and rotated in order to minimize its deviations from a reference object. At the end of the superimposition process, the whole dataset is represented by (1) a vector of centroid sizes, (2) the coordinates of the reference object or consensus, and (3) a matrix of shape parameters that correspond to the projection of the Procrustes superimposed coordinates onto the linear tangent space at the consensus location (Rohlf, 1999). Due to the large number of variates and in order to maximize the power of the analyses, the dimension of the shape space was reduced using principal components, following the approach detailed in Baylac and Friess (2005).

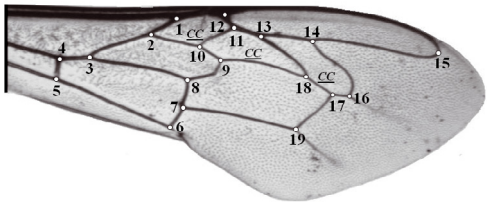

Figure 1. Location of the nineteen landmarks on the fore-wing of honeybee workers considered in the geometric morphometric analysis (CC = cubital cell).

2.2. Microsatellite data compilation

Frequencies of six microsatellite loci (A7, A28, A113, B124, A24, A88) from a total of 36 populations belonging to 11 *A. mellifera* subspecies from A, M, and C branches were compiled from literature reports. For the M evolutionary branch, 18 populations of *A. m. iberiensis* (Miguel et al., 2007) and nine populations of *A. m. mellifera* (Garnery et al., 1998b) were used. For the A evolutionary branch, one population for each of the following subspecies were used: *A. m. adansonii* from Guinea; *A. m. intermissa*, *A. m. major*, and *A. m. sahariensis* from Morocco; and *A. m. capensis* and *A. m. scutellata* from South Africa. For the C evolutionary branch, *A. m. carnica* from Germany, *A. m. ligustica* from Italy, and *A. m. macedonica* from Greece were used (Estoup et al., 1995; Franck et al., 1998). The microsatellite data were previously calibrated against each other using reference samples.

2.3. Statistical analysis

Preliminary Principal Component Analysis did not reveal the presence of outliers. All specimens were therefore used in Multivariate analysis of variance (MANOVAs) and in Canonical Variate Analyses (CVA). Multiple discriminant analyses were used to estimate the classification rates among localities. All classification rates were estimated

Table I. Geographical location and identification codes of the 27 *Apis mellifera* populations analyzed in the morphometric study.

Locality	Country	Geographic region	Coordinates	Branch	Code	n
A. m. iberiensis						
Lisboa	Portugal	South-Western Iberia	38.47N, 9.30N	M	IP-SO-Lis	23
Evora	Portugal	South-Western Iberia	38.60N, 7.54N	M	IP-SO-Evo	26
Sevilla	Spain	South-Western Iberia	37.23N, 5.59W	M	IP-SO-Sev	12
Caceres	Spain	South-Western Iberia	39.28N, 6.22W	M	IP-SO-Cac	11
Granada	Spain	Southern Iberia	37.11N, 3.35W	M	IP-S-Gra	23
Aguilas	Spain	Southern Iberia	37.24N, 1.35W	M	IP-S-Agui	15
Alicante	Spain	Eastern Iberia	38.20N, 0.29W	M	IP-E-Ali	30
Valencia	Spain	Eastern Iberia	39.28N, 0.22W	M	IP-E-Val	24
Segovia	Spain	Central Iberia	40.57N, 4.07W	M	IP-C-Seg	8
Tortosa	Spain	North-Eastern Iberia	40.49N, 0.31E	M	IP-NE-Tor	29
Badalona	Spain	North-Eastern Iberia	41.27N, 2.15E	M	IP-NE-Bad	28
Gerona	Spain	North-Eastern Iberia	41.59N, 2.49E	M	IP-NE-Ger	23
Zaragoza	Spain	North North-Eastern Iberia	41.39N, 0.52W	M	IP-N-NE-Zar	22
Onati	Spain	Northern Iberia	43.05N, 2.30W	M	IP-N-Ona	50
Goizueta	Spain	Northern Iberia	43.12N, 1.45W	M	IP-N-Goi	50
Erronkari	Spain	Northern Iberia	42.50N, 0.55W	M	IP-N-Err	25
Araba	Spain	Northern Iberia	42.51N, 2.41W	M	IP-N-Ara	25
Bizkaia	Spain	Northern Iberia	43.13N, 2.40W	M	IP-N-Biz	25
A. m. mellifera						
Chimay	Belgium	Southern Belgium	50.05N, 4.31W	M	B-S-Bel	25
Savoie	France	Eastern France	45.38N, 6.19E	M	F-E-Sav	25
Bayonne	France	South-Western France	43.29N, 1.28W	M	F-SO-Bay	25
Sabres	France	South-Western France	44.21N, 0.41W	M	F-SO-Sab	25
Foix	France	Southern France	42.57N, 1.36E	M	F-S-Foix	14
Other subspecies						
Forli	Italy	North-Central Italy	44.13N, 2.32E	C	*A. m. ligustica*	25
Chalkidiki	Greece	North-Eastern Greece	40.25N, 23.30E	C	*A.m. macedonica*	25
North Morocco	Morocco	North Morocco	35.14N, 3.56W	A	*A. m. major*	25
South Morocco	Morocco	South-Western Morocco	29.42N, 9.44W	A	*A. m. intermissa*	25

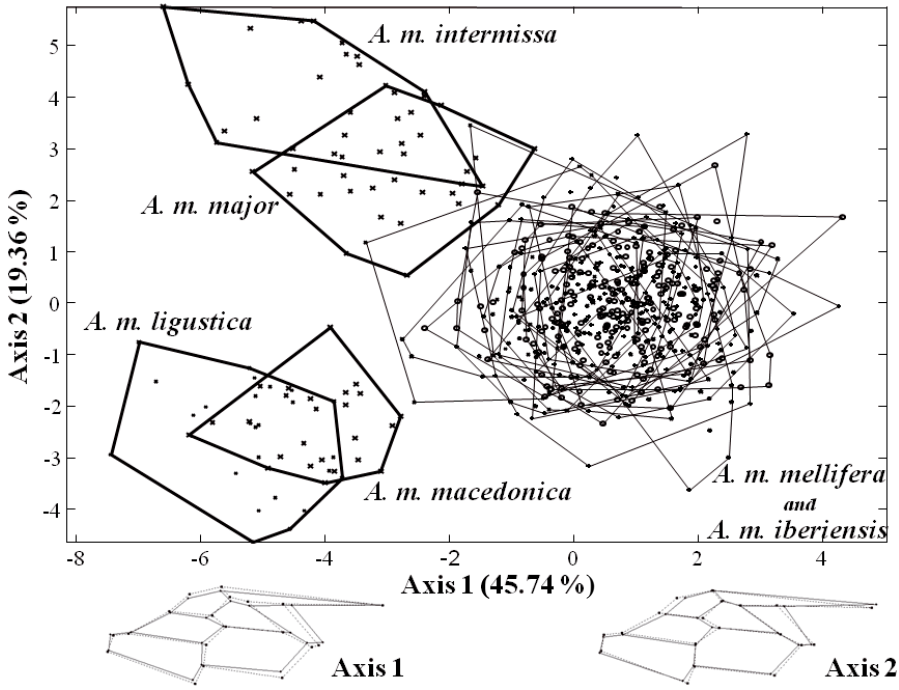

Figure 2. Canonical Variate Analyses of shape (the identification codes of the populations are listed in Tab. I). Shape deformations of the wing along factorial axes 1 and 2 (dotted links for the negative side and solid links for the positive side) were amplified by a factor of 2 for better visualization.

using leave-one-out cross-validations, which provide lower but unbiased estimates (Ripley, 1996). CVA used shape parameters only (i.e., the centroid size parameter was excluded since it never increased significantly the classification rates). Wing deformations along factorial axes were estimated by multivariate regression (Monteiro, 1999) and two extreme shapes were then calculated, one for each axis extremity. Deformations were amplified by a factor of two for better visualizations. We defined a set of links between landmarks in order to help to visualize the overall wing shape. As a rule, the two extreme shapes are drawn using dotted links for the negative side and solid links for the positive side of each axis. To evaluate the morphological relationships among branches A, M, and C, a neighbor-joining (NJ) tree was constructed based on Mahalanobis D2 distances data (Mahalanobis, 1936). All calculations were done using the MATLAB computational environment with the morphometrics toolbox devised by one of us (M.B.).

In order to assess the power of the microsatellites to assign correctly the individuals into their evolutionary lineages and subspecies, two methodologies were used: self-classification of reference data option and a Bayesian approach (Rannala and Mountain, 1997), running Geneclass2 software (Piry et al., 2004). To evaluate the phylogenetic relationship between A, M, and C branches, a NJ tree was constructed on the basis of D_A distance data (Nei et al., 1983), running Populations 1.2.28 software (Langella, 2002). In order to explore the agreement between morphometric and genetic distance matrices, first-order correlations were calculated. Matrix comparison was carried out by Mantel's method, using ZT software (Bonnet and Van de Peer, 2002) and significance was obtained after 10 000 iterations.

3. RESULTS

3.1. Geometric morphometrics

The first canonical axis (Fig. 2, 45.74%) separated M lineage populations from A and C populations; the second CVA axis (19.36%) distinguished A and C population branches.

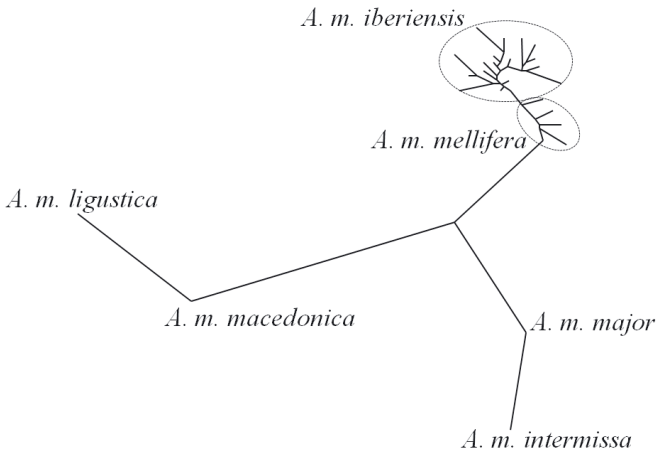

Figure 3. Neighbor-joining tree using the Mahalanobis D2 distance matrix based on geometric morphometric data of the fore wing, using 19 landmarks (see Fig. 1).

The corresponding MANOVA was highly significant (Wilks' Lambda = 0.0144; F = 5.818; $df_{(11)}$ = 520; $df_{(12)}$ = 9902; $P < 10^{-16}$). These CVA patterns are best summarized by a NJ tree calculated from the D2 Mahalanobis distances (Fig. 3): populations from the same evolutionary branch were grouped together, and the three evolutionary branches – A, M, and C – were clearly separated. Moreover, all honeybee populations from the Iberian Peninsula, *A. m. iberiensis*, were grouped within the Western European M cluster, which was distant from the A and C subspecies clusters. D2 distances were significantly higher between Iberian and North African populations (D2 = 27.56) than between *A. m. iberiensis* and *A. m. mellifera* (D2 = 4.70).

Leave-one-out cross-validated misclassification percentages, calculated by multiple discriminant analyses, were remarkably low between branches (A-M = 1.14%; M-C=0.16%). Wing venation differences observed along the first two canonical variate axes are illustrated in Figure 2, whereas pairwise comparisons between M, C, and A lineages are depicted in the Figure 4. On the whole, the differences are spread over the whole wing surface, a result which indicates the lack of any strict location of differences, though evolutionary branches differ by opposite relative proportions of the basal and distal regions of the wing. The lack of any location applies equally to most cells, with the possible exception of the basal cell with three landmarks (4, 5, and 7), which exhibit relatively low variability. All remaining cells contribute to the overall differences. This is particularly evident for cubital cells, which exhibit particularly complex patterns (Fig. 4). Although classical differences between M-C lineages on the cubital cell are clearly retrieved from the geometric morphometric visualizations, it is nonetheless evident that simple ratios such as the cubital index are unable to extract all the pertinent differences contained in the cubital cell landmarks.

3.2. Microsatellite results and correlations between genetic and geometric morphometric data

Misclassification percentages of individuals between branches using six microsatellites were low. Only 1.19% of M individuals were classified into C, and the same percentage was classified incorrectly within the A branch. The percentage of A branch individuals assigned to M branch and C to A branch was 4.8%. Finally, 2.41% of C branch honeybees were

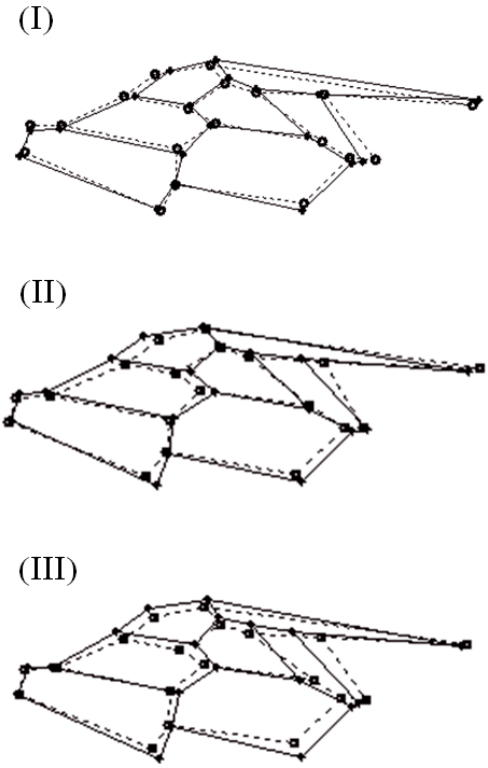

Figure 4. Patterns of fore wing differences between branches. Pairwise comparisons between (I) A-M branches (A solid link and M dotted link), (II) A-C branches (A dotted link and C solid link) and (III) M-C branches (M dotted link and C solid link).

classified within M and 1.80% of A individuals were assigned to the C branch. The genetic distance tree clearly discriminated the three branches (Fig. 5). Populations were clustered according to their branches, with the Iberian populations being grouped within the M branch and separated from *A. m. intermissa*. Moreover, Iberian populations were connected with African ones through French and Belgian *A. m. mellifera* populations. Correlation values between the molecular and morphological distance matrices (the Nei D_A vs. the Mahalanobis D2 distances) were highly significant. When the 27 populations were analyzed together, a correlation of R = 0.9212 (Mantel test, $P < 0.0001$) was obtained. Considering only M branch populations, the correlation value decreased but was still highly significant (R = 0.6566; Mantel test, $P < 0.0001$).

4. DISCUSSION

This study not only clarifies the controversy regarding the relationship between M and A branch subspecies, it also demonstrates the overall agreement between geometric morphometrics and nuclear marker data. Moreover, these results highlight the usefulness of geometric morphometrics applied to wing-shape analysis for the individual classification of honeybees within A, M, or C branches. More complete and formal analyses including more lineages and populations are still needed before being able to conclude about its classificatory value. However, in our present results, it appears more than only marginally better, as already stated by Tofilski (2008).

Geometric morphometric results from this study do not corroborate previous studies using traditional morphometrics (Ruttner et al., 1978; Ruttner, 1988; Cornuet and Fresnaye, 1989). Wing shape analysis through geometric morphometrics does not reflect a clinal transition between the *A. m. intermissa, A. m. iberiensis*, and *A. m. mellifera* subspecies. On the contrary, they show a marked break between North African and Western European populations. This finding is consistent with the conclusions of previous genetic studies using microsatellites and SNPs (Franck et al., 1998; Garnery et al., 1998b; Whitfield et al., 2006) and with the results of Arias et al., (2006) based on classical morphometrics mostly using wing characters. A similar result was also reported by Hepburn and Radloff (1996), though the methodology used (CVA on grouped populations) seems largely questionable when applied to population mixtures and hybrids (Neff and Smith, 1979).

Discrepancies between the results of studies based on traditional and geometric morphometrics, and even between the different quantitative approaches which have been used in the previous characterizations of honeybee diversity, can be ascribed to the choice of different character suites and to the nature of the markers employed: traditional morphological

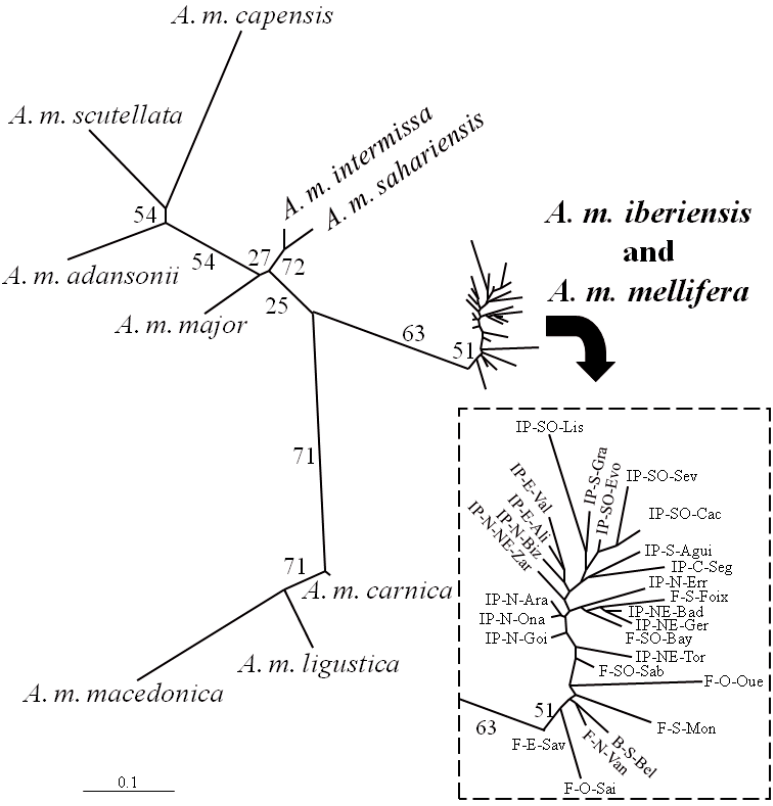

Figure 5. Neighbor-joining tree (bootstrap with 1000 iterations (A7, A28, A113, B124, A24, A88) using D_A distance matrix (Nei et al., 1983) based on data from 6 DNA microsatellite loci of populations of A, M, and C evolutionary branches (Estoup et al., 1995; Franck et al. 1998; Garnery et al., 1998b; Miguel et al., 2007). The identification codes of the populations are listed in Table I.

studies include a number of characters that are environmently sensitive, such as pigmentation, size, and length characteristics, which are not appropriate for phylogeographic or phylogenetic studies. Measurement of color, body size, hair, proboscis, and hind legs were found to be highly correlated with geographic latitudes (Hepburn and Radloff, 1998; Diniz-Filho et al., 1999; Ruttner et al., 2000). Similar correlations exist among other characters and altitude (Mattu and Verma, 1983; Ruttner, 1988; Meixner et al., 1989, 1994). Although different approaches can be carried out in evolutionary studies, neutrality to the environment should be the primary quality of the selected markers (Franck et al., 1998). In this sense, wings of insects in general and honeybees in particular constitute adequate markers to investigate the patterns of evolution (Baylac et al., 2003). Up to now, many publications using geometric morphometrics of Hymenoptera forewings (Smith et al., 1997; Klingenberg et al., 2001; Pretorius, 2005; Francoy et al., 2006, 2008; Baylac et al., 2003) have shown its great interest when investigating asymmetry, population differentiation, hybridization, and species complexes. Roberts (1961) observed high heritability for wing width and cubital index, and Ruttner (1988) reported that wing venation was a rich source for genetic and taxonomic analyses. Diniz-Filho et al. (1999) show that wing angles are correlated to phylogeny rather than to geography, while the reverse was true in characters such as

size or color. Thus, wing characters are more appropriate to resolve the question of phylogenetic branch membership, irrespective of traditional or geometric measurement/analysis method. Finally, three main points explain the greatest statistical and interpretative power of geometric morphometrics (Bookstein, 1991; Adams et al., 2004): (1) the more precise descriptions of forms achieved using homologous locations, (2) the use of coordinates instead of distances, ratios, or angles, leading to more exhaustive descriptions of geometric forms (Bookstein, 1991), and (3) the splitting of forms into size and shape parameters that are optimal sensu (Darroch and Mosimann, 1985; Mosimann and James, 1979; Bookstein, 1990; Dryden and Mardia, 1998). The interest of using data that are independent of (body) size to characterize races was already pointed out by Ruttner et al. (1978). But independence between size and shape is an exception in biology (Gould, 1966; Sprent, 1972; Bookstein et al., 1985). Mosimann's framework offers a proper *modus operandi* to separate size and shapes parameters that results in coherent and powerful statistical analyses of geometric approaches, and it explains also why previous empirical approaches using ratios led frequently to incongruous results.

This study revealed a high consistency between morphological and genetic data. Distance matrices based on both geometric morphometrics and genetic data were found to be highly correlated. Wing-shape data analysis (CVA, NJ tree and the percentage of misclassified individuals) and microsatellite analysis (NJ tree) reflected an almost complete differentiation between the three population groups corresponding to the A, M, and C branches. These observations shed fresh light on previous controversies dealing with the origin and phylogeographic position of *A. m. iberiensis*. Both geometric morphometrics and microsatellite data revealed that *A. m. iberiensis* is close to *A. m. mellifera*, and that both are distant from the African subspecies. All these results reject the hypothesis by Ruttner et al. (1978) of a progressive transition of a chain of races between *A. m. intermissa, A. m. iberiensis*, and *A. m. mellifera* subspecies, as well as the proposal by Smith et al. (1991) of a hybridization process between *A. m. mellifera* and *A. m. intermissa* as an explanation for the origin of *A. m. iberiensis*. In other words, the existence of a close evolutionary relationship between *A. m. iberiensis* and *A.m. intermissa* seems unlikely.

Concerning the presence of African A mitotypes in *A. m. iberiensis*, the differences detected between Iberian and North African A mitotypes, together with the higher mitotype diversity in Iberia, suggest various introduction events of African mitotypes from different origins or an ancient introduction and subsequent differentiation (Garnery et al., 1992, 1998a; Arias et al., 1996; Sheppard et al., 1997; Franck et al., 1998; Miguel et al., 2007; Canovas et al., 2007). Our results of wing shape and nuclear data did not detect this African influence, indicating that the hypothetical African genetic introgressions seem to have been diluted in the Iberian population. Because genetic introgression between populations of the African evolutionary branch and *A. m. iberiensis* was only detected in the mitochondrial DNA of the latter subspecies, the propagation of the A lineage mitotypes may well be related to an environmental advantage (Franck et al., 1998; Garnery et al., 1998b; Canovas et al., 2007). However, the higher swarming tendency of African honeybee queens (Ruttner, 1988) may also have exerted an influence on the propagation of their mitochondrial material into southern Europe. In this sense, it is known that the diffusion of the African mitochondrial genome into the Americas does not necessarily correspond to the Africanization of the nuclear genome (Lobo and Krieger, 1992).

Finally, our morphometric results assigned individuals to their correct evolutionary branch, even if they had an A lineage mitochondrial haplotype and European M branch nuclear alleles. For this reason, the geometric morphometric methodology applied in this study seems more appropriate than the analysis of mtDNA for the screening and identification of the Africanization process of Central and North American honeybee populations. Moreover, this method can be easily automated (Baylac et al., 2008) and therefore it is cost-effective, fast and precise, and provides

information that reflects the genetic background of honeybee populations.

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Les données obtenues à partir de la géométrie morphométrique et des microsatellites se rejoignent pour soutenir la thèse de la différenciation de la branche évolutive M d'*Apis mellifera*.

abeille / branche évolutive / morphologie / aile / microsatellite / morphométrie géométrique

Zusammenfassung – Geometrische Morphometrie und Mikrosatellitendaten unterstützen übereinstimmend die Differenzierung der evolutionären M-Linie von *Apis mellifera*. Westeuropa und Nordafrika sind Kontaktzonen für die evolutionären Linien A, M und C der Honigbiene. Obwohl allgemein Übereinstimmung über die Gültigkeit dieser Linien herrscht, wird die Differenzierung der westeuropäischen Linie M kontrovers diskutiert. Traditionelle Morphometrie und die Analyse von Allozymen und mitochondrialer DNA haben bisher eine enge Verwandtschaft zwischen der der M-Linie zugeordneten Unterart *A. m. iberiensis* und den nordafrikanischen Unterarten der A- (Afrikanischen) Linie unterstützt (Ruttner, 1978; Cornuet and Fresnaye, 1989; Smith et al., 1991; Smith and Glenn, 1995; Arias et al., 1996, 2006). Studien auf der Basis von Mikrosatelliten und Einzelnukleotidpolymorphismen (SNPs) haben jedoch klare Unterschiede zwischen den beiden europäischen Unterarten der M-Linie (*A. m. mellifera* und *A. m. iberiensis*) und den nordafrikanischen Unterarten aufgedeckt (Franck et al., 1998, 2001; Garnery et al., 1998b; Whitfield et al., 2006). Als Beitrag zur Auflösung dieses Widerspruchs haben wir 663 Völker von 6 Unterarten der A, M und C Linie analysiert. Die Flügelform wurde mittels einer geometrisch-morphometrischen Methode analysiert, und die Ergebnisse wurden mit den Resultaten einer Mikrosatellitenanalyse von 6 Loci verglichen. Die morphologischen und genetischen Daten stimmten gut überein. Mit beiden Methoden konnten die drei evolutionären Linien unterschieden werden, und mit beiden Methoden kann eine deutliche Differenzierung zwischen den westeuropäischen Unterarten *A. m. mellifera* und *A. m. iberiensis* und den nordafrikanischen Unterarten abgesichert werden. Die Ergebnisse unterstützen somit eine klare Differenzierung der Unterarten der M-Linie und bestätigen die Hypothese, dass die Präsenz von A-Linien Mitotypen in iberischen Populationen der Honigbiene wahrscheinlich als Folge von sekundärer Einführung betrachtet werden muss. Dabei ist im zurzeit vorhandenen genetischen Hintergrund in Iberien der afrikanische Einfluss minimal. Durch ihre große deskriptive und statistische Aussagekraft führt die geometrische Morphometrie des Flügels bei der Zuordnung von Individuen zu evolutionären Linien zu einer geringeren Anzahl von Fehlklassifikationen. Sie erscheint daher gegenüber der Analyse von mitochondrialer DNA und traditioneller Morphometrie bei der Überprüfung von Populationen der Honigbiene in Zentral- und Nordamerika zum Verfolgen der Afrikanisierung geeigneter.

Honigbiene / Evolutionäre Linie / Flügelmorphologie / Geometrische Morphometrie / Mikrosatelliten

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