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Discrimination of bee populations in Côte d'Ivoire by geometrical morphometry

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Abstract

A single honeybee subspecies, *Apis mellifera adansonii*, has been described from all of West and Central Africa. The data in the literature concerning the characteristics of honeybees present in Côte d'Ivoire are less numerous than in European subspecies. The present study characterizes the morphological diversity and deepens the knowledge on the local subspecies of honeybees thanks to geometric morphometrics approach based on the configuration of the landmarks located on the wings, from a sample of 1,620 worker bees of the subspecies *Apis mellifera adansonii* collected in the Center, North and West of Côte d'Ivoire. Geometric morphometrics indicates that despite the diversity shapes and colours observed, the collected honeybees constitute a fairly homogeneous group with an absence of geographical differentiation within the colonies.

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Introduction

Bees (*Apis mellifera* (Linnaeus, 1758)) are social insects that play a vital role in maintaining the balance of terrestrial ecosystems and the incomes of rural populations around the world. About 75% of global agricultural production depends on pollinators among which, the most important are bees (Kearns *et al*., 1998; Klein *et al*., 2007). Moreover, because of their very large distribution and due to their haploid, diploid and polyandry character, bees are of scientific interest.

Bees belong to the Apoïdae superfamily, which includes seven families: Colletidae, Andrenidae, Halictidae, Melittidae, Megachilidae, Anthrophoridae and Apidae (Michener, 2000), and were composed of around 20,000 species found worldwide (Rasmont *et al*., 1995; Michener, 2007). However, the best-known honeybees used in apiculture in the world belong to the species *Apis mellifera* comprising 26 subspecies which has a natural distribution throughout Africa, Europe and the Middle East (Segeren *et al*., 1996).

Studies showed that *Apis mellifera adansonii* (Latreille, 1804) is the only subspecies of honeybees found all over West and Central Africa, from Senegal to Congo (Latreille, 1802; Ruttner, 1982). Nevertheless, these studies were based only on a few samples. However, studies done in Benin, West Africa, have shown that *Apis mellifera adansonii* presents two different forms: one yellow, smaller and the other black and larger (Hounkpè *et al*., 2007; Paraïso *et al*., 2011).

In addition, bees found in Benin could be clustered into three groups by phylogenetic analysis (Paraïso *et al*., 2017). This may suggest the existence of other subspecies other than *Apis mellifera adansonii*. Unlike European breeds whose characteristics and geographical distribution are known with precision, the inventory of *Apis mellifera* breeds and the cartography of their geographical distribution are still incomplete in Africa (Morse et Hooper, 1985).

In Côte d'Ivoire, although bees are exploited in the traditional way such as hunting, beekeeping is a rapidly growing activity. Unfortunately, the genetic and morphometric knowledge on honeybees is poorly documented so that beekeepers do not have a good knowledge of the breeds of bees they raise. Whereas, the selection of more productive breeds will help cope with changes in the environment and the emergence of new diseases. Indeed, one of the most important steps for the development and mastery of beekeeping is the knowledge of the characteristics of the exploited species. This requires in advance a deeper knowledge of the races exploited through their characterization. An investigation using traditional morphometrics study has shown the existence of only one subspecies, *Apis mellifera adansonii*, with differentiated local populations on the basis of their morphometric characters (Assielou *et al*., 2019).

The aim of this study was to apply geometric morphometrics as a method for the study of wing shape by using landmarks instead of traditional morphometrics. This could provide insights about the presence of differentiated local populations and confirm the results obtained by traditional morphometrics.

Material and methods

Study zone

The study was carried out at the Entomology Laboratory of "Institut National Polytechnique Félix Houphouët-Boigny (INP-HB)" from June 2015 to February 2017. Bee samples sites were located in the three main representative phytogeographical zones of the country where beekeeping is widespread and is practiced by a large number of professional beekeepers. These zones were the transition zone between forest and savannah in central Côte d'Ivoire, the mountainous zone to the west of the country, and the savannah zone in the northern part of the country (Fig. 1). The places visited are as follows.

Numbers correspond to sample sites. In the forest-savannah transition of the South-Center (C): 1: Kouassi-Kouassikro, 2: Lengbè-Kouassikro, 3: Yobouekro , 4: INP-HB , 5: N'Guessankro , 6: Soungassou , 7: N'Drikro , 8: M'Batto , 9: Singrobo ; the Western mountainous (W): 10: Forona, 11: Oussougoula, 12: Biankouma , 13: Man , 14: Dainé ;

and the Northern savannah (N): 15: Korhogo, 16: Karakoro, 17: Ferkessédougou, 18: Bouna.

- In Central Côte d'Ivoire, the localities sampled are that of Yamoussoukro (Yobouekro and Institut National Polytechnique Félix Houphouët-Boigny (INP-HB)), Kouassi-kouassikro (Kouassi-kouassikro and Lengbe-kouassikro), Toumodi (N'Guessankro), Dimbokro (Soungassou), Tiassalé (Singrobo), Bongouanou (N'Drikro) and M'Batto.
- In the North, these are the localities of Korhogo, Karakoro, Ferkessédougou and Bouna.
- To the West, the localities visited are those of Séguéla (Forona and Oussougoula), Biankouma and Man (Man and Dainé).

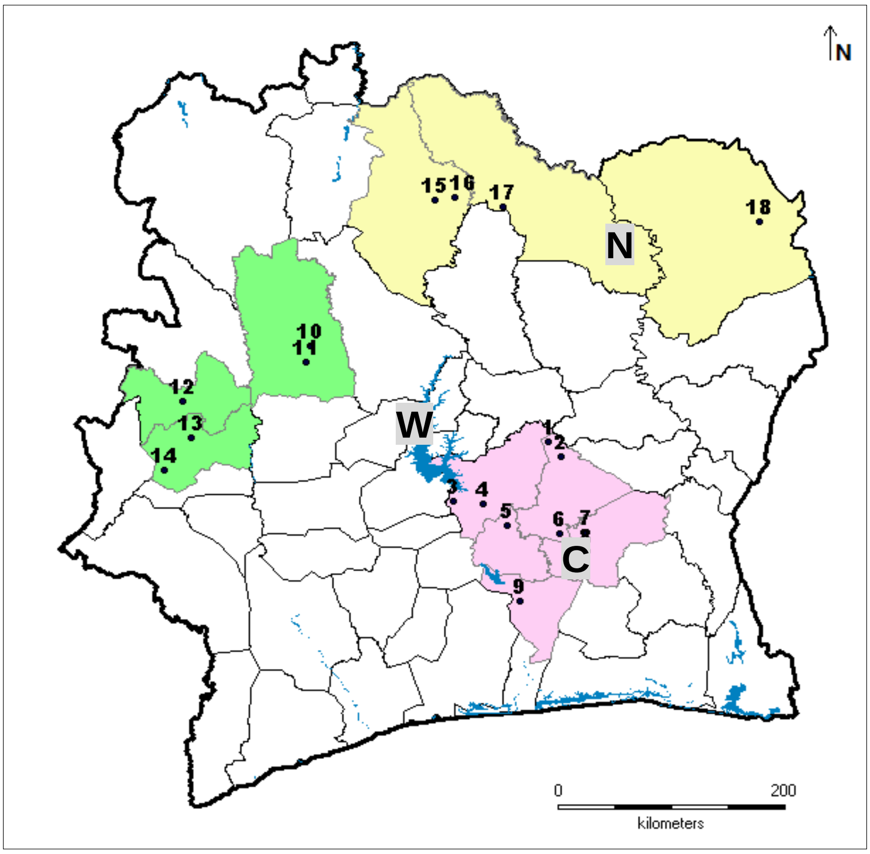

Fig. 1. Localization of the study sites indicating the district where bees were sampled.

Sampling of bees

Bees were collected in the apiaries located in different localities. In each apiary, three to four hives were chosen and 30 worker bees were randomly collected from each hive (Table 1). Male haploid bees were not sampled because they are not representative of the bee population. Thus, beehives were opened and worker bees were taken from the frames avoiding collecting bees from other hives. A total of 1,560 bees were collected for this study. The collected bees were preserved in a solution of ethanol (95%) and then were stored at a temperature of -2°C until they were used (Rúa *et al.*, 2003).

Data collection

In the laboratory, the right forewings of 30 worker honeybees per colony were removed after cutting their base. They were mounted by microscope slides with a drop of distilled water as a mounting medium to avoid deformation (Barour *et al.*, 2011). Digital photos were taken from mounted wings using a DM143 camera and SMZ-168 Motic stereomicroscope. The resulting images were saved in the "jpg" format, which were subsequently converted to the "TPS" format using tpsUtil version 1.65 software. Nineteen landmarks were digitized on forewings by tpsDig version 2.22 software (Fig. 2).

The vein junctions were used as homologous points for geometric morphometrics (Rohlf, 2015). For the reliability of the study, the landmarks used were of type I with the exception of landmark 15 which was type II because of their capacity to discriminate bee populations and their easy positioning (Bookstein, 1991). Then, landmarks were superimposed using a generalized procrustes analysis space (Rohlf, 1998). All non-shape variations of these landmarks such as orientation (or rotation), scale, and size were removed. Finally, digitized landmark outputs were analyzed with the free software MorphoJ version 1.06d (Klingenberg, 2011).

Table 1. Localization of sampling sites, the size of samples and the site geographical coordinates.

Locality	Apiary	Size of the sample		Coordinates	
		Number of Colonies	Number of bees	Longitude	Latitude
West mountainous forest area					
Biankouma	Biankouma	3	90	-7,61877°	7,74079°
Man	Dainé	2	60	-7,547012°	7,171388°
	Man	3	90	-7,547012°	7,44344°
Séguéla	Oussougoula	3	90	-6,642515°	8,070955°
	Forona	3	90	-6,600499°	8,201282°
Northern Savannah area					
Bouna	Bouna	2	60	-3,032777°	9,239722°
Ferkessédougou	Ferkessédougou	2	60	-5,074578°	9,370725°
Korhogo	Korhogo	3	90	-5,611358°	9,42281°
	Karakoro	3	90	-5,4518°	9,447565°
South-central pre-forest area					
Bongouanou	N'Drikro	4	120	-4,407654°	6,654395°
	M'Batto	1	30	-4,409166°	6,493611°
Dimbokro	Soungassou	4	120	-4,622132°	6,633623°
Kouassi-kouassikro	Kouassi-kouassikro	3	90	-4,713597°	7,411008°
	Lengbe-Kouassikro	3	90	-4,606652°	7,283425°
Singrobo	Singrobo	4	120	-4,930403°	6,075834°
Toumodi	N'Guessankro	4	120	-5,034226°	6,701054°
Yamoussoukro	INP-HB	4	120	-5,228529°	6,886443°
	Yobouékro	3	90	-5,4616°	6,9126°
Total	18 apiaries	54 colonies	1 620 bees		

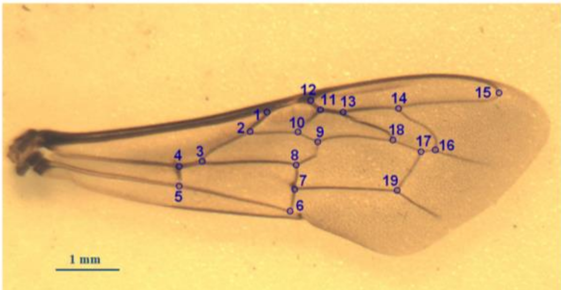

Fig. 2. Location of the nineteen landmarks on Apis mellifera worker forewing.

Statistical Analysis

Statistical analysis was carried out on the procrustes residues, to study the differences in conformation, then on the centroid sizes, to study the differences related to the size itself. The variance of the coordinates of the different landmarks was determined using the tpsRelw software. Multivariate analysis of variance (MANOVA) was used to test the effect of locality and region factors on bee wing shape. Principal Component Analysis (PCA) was used for the representation of procrustes residues in conformation space. Canonical Variate Analysis (CVA) aimed at maximizing differences between groups while minimizing intragroup differences was then performed to compare bee populations. Multivariate analyses were performed with MorphoJ software version 1.06d (Klingenberg, 2011).

Results and discussion

The analysis of shapes in biological research has taken off again for several years with the development of the geometric morphometrics method (Adams *et al.*, 2004; Mitteroecker et Gunz, 2009). It made it possible to respond to the concern, which was to assess the possibility of ecotypic variation in the honeybee populations of Côte d'Ivoire in relation to different phytogeographic conditions. The analyses were based on the technique of landmarks, which is one of the most widespread and widely used morphometric methods in recent years to analyse the forewing shapes of *Apis mellifera*. It refers in particular to comparisons based essentially on the different configurations of landmarks using the fundamental equation: Form = Size + Shape (Needham, 1950).

The variances of the coordinates of the 19 landmarks on the fore wings were determined. It emerges from the analysis that the fifteenth landmark, corresponding to the maximum curvature of the radial vein at the level of the radial marginal cell, shows the maximum variation ($S^2 = 0.000047$).

On the other hand, landmarks 14, 19 and 18 show the second ($S^2 = 0.000046$), third ($S^2 = 0.000032$) and fourth ($S^2 = 0.000031$) respectively the greatest variations. However, landmark 8 exhibited the smallest variation ($S^2 = 0.000014$) (Table 2).

Table 2. Variances at each landmark for aligned specimens.

Landmark	S^2x	S^2y	S^2
1	0.00001674	0.00000744	0.00002418
2	0.00001845	0.00000550	0.00002395
3	0.00002307	0.00000580	0.00002887
4	0.00001635	0.00000574	0.00002209
5	0.00001987	0.00000971	0.00002958
6	0.00001699	0.00001258	0.00002957
7	0.00001211	0.00000753	0.00001963
8	0.00000916	0.00000496	0.00001412
9	0.00002012	0.00000589	0.00002602
10	0.00002419	0.00000304	0.00002723
11	0.00001409	0.00000560	0.00001969
12	0.00001393	0.00000676	0.00002070
13	0.00002542	0.00000450	0.00002991
14	0.00004159	0.00000448	0.00004607
15	0.00003415	0.00001329	0.00004744
16	0.00001505	0.00000456	0.00001961
17	0.00001879	0.00000404	0.00002283
18	0.00002586	0.00000588	0.00003173
19	0.00002085	0.00001171	0.00003256

The results show that the Central and Northern regions are not different in the centroid size of the wings. On the other hand, bees from the West region have significantly smaller centroid sizes ($P < 0.001$) (Fig. 3).

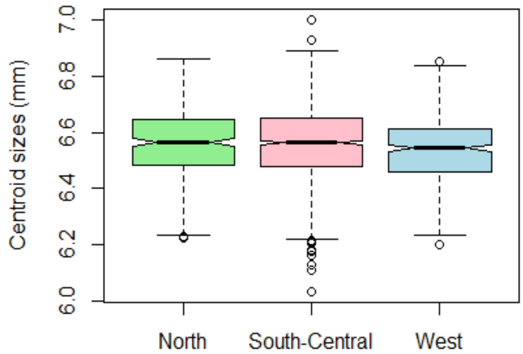

Fig. 3. Graphical representation of the centroid sizes of regional bee populations.

Regarding the shape of the wings, the differences between the regions were tested using one-way ANOVA. The results indicate a significant difference between the procruste coordinates of the 18 regions visited ($p < 0.0001$) (Table 3). There are significant differences in the centroid sizes of bee wings between individuals, colonies and regions. Statistical tests show that the differences observed between bees in the northern and central regions are not statistically different. However, these values differ significantly from those of western bees. The bees collected in the Center of the Côte d'Ivoire occupy an intermediate position between those of the West and those of the North. This would simply result from the size of the Central zone and the larger number of settlements collected in this zone. These results corroborate those of Barour *et al.* (2011) who studied the variability of wing shape in bees *Apis mellifera intermissa* (Buttel-Reepen, 1906) from Algeria. Their work also showed significant differences in wing sizes between the Northeast, North Central and Northwest regions. Such observations were made in Iran on the bee *Apis mellifera meda* (Skorikov, 1929) by Dolati *et al.* (2013).

Table 3. Comparison of the means of the centroid sizes for the factors: "Regions", "Apiaries" and "Colonies".

Factors	Df	F value	Pr (>F)	Signif. Cod
Regions	2	7.417	$2{,}72 \times 10^{-4}$	***
Apiaries	15	12.512	$< 2 \times 10^{-16}$	***
Colonies	36	11.313	$< 2 \times 10^{-16}$	***
Residuals	486			

*Significant difference at * à $P < 0.05$, ** à $P < 0.01$, *** à $P < 0.001$ and ns non-significant difference at $P > 0.05$*

The conformation parameters of the wings were used by the PCA to discriminate bees against the three geographic areas. The graphical eigenvalue jump method was used to determine the number of axes to retain. On the graph, representing the percentages of variance of the axes of the PCA, the break occurs after the second axis (Fig. 4). The analyses were therefore limited to the first two components, which explained 24.21% of the total variability. This explained inertia is low. The PCA plots obtained against the first two factorial planes indicate point clouds in which individuals from different regions cannot be properly differentiated.

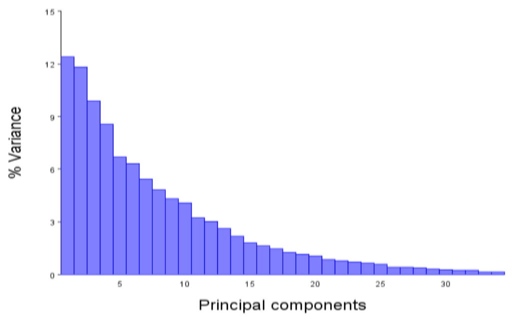

Fig. 4. Graph showing the percentage variance of the axes of the PCA.

PCA showed a dispersion of the points (individuals) around the central part of the two axes (Fig. 5). This indicated that the population of bees from the three phytogeographic zones could not be differentiated into three different populations. The large dispersion of the data could be explained by factors such as measurement errors during the digitization of landmarks such as type 2, which are subject to large variations from one person to another.

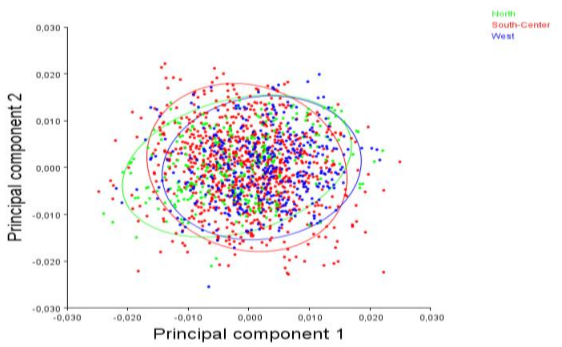

Fig. 5. Scatter Plot of the individuals according to the first two axes of the PCA.

Canonical variate analysis was applied to the data in order to confirm the non-differentiation of the wing shape within the colonies. In this analysis, the total variability explained by the first axes was 66.79% adding the fourth axis lead to 33.20%. Thus, CVA improved the discrimination because it minimized differences within colonies and maximized between colonies. The results of this analysis helped improve discrimination within bee populations. The results show a tendency for the regions to separate (Fig. 6). In fact, bees from the Center occupy an intermediate position between those from the North and those from the West, which are opposite on axis 1. At axis 2, the bees collected in the North and West are at the same level in the axis of the graph and appear opposite to those collected in the Center with a higher position. Overall, the concentration ellipses of individuals from the three geographic areas overlap. These results thus reveal an absence of differentiation of the shape of the wing for the individuals collected in this study. Indeed, the workers of the North and the West appear to be differentiated on the basis of their wing conformations.

The differences observed in the size and shape of the workers' wings between the populations of the three large regions could be explained by factors such as vegetation (food source) and climate (temperature, rainfall ...). However, if the variations are not very clear, this is due to the fact that the sample collection regions are less distant and that there are few natural barriers allowing isolation of bee colonies as in some countries such as Algeria and Brazil. Indeed, the studies by Barour *et al.* (2011) in Algeria based on geometric morphometrics indicated that there are statistically significant differences between centroid sizes and procrustes distances. At this level, the inter-regional variation is greater because of the isolation of the bee populations due to the size of the country and the very rugged relief in places. Likewise, in Brazil, multivariate analysis of wing shape revealed geographic variation in the bee population *Apis mellifera*. This variability could be attributed to the size of the territory as well as to ecological differences (Nunes *et al.*, 2012).

However, the results of the geometric morphometrics analysis did not make it possible to differentiate the bee populations collected in the three major ecological regions of the Center, the North and the West of Côte d'Ivoire based on their wing conformations due to the overlap of individual concentration ellipses. Geometric morphometry therefore allows better separation of the colonies than conventional morphometry. In addition, geometric morphometrics indirectly includes height measurements (Özkan et Kandemir, 2013). These results show that the bees encountered can be used in beekeeping in all regions of Côte d'Ivoire because of their adaptation to all agro ecological zones.

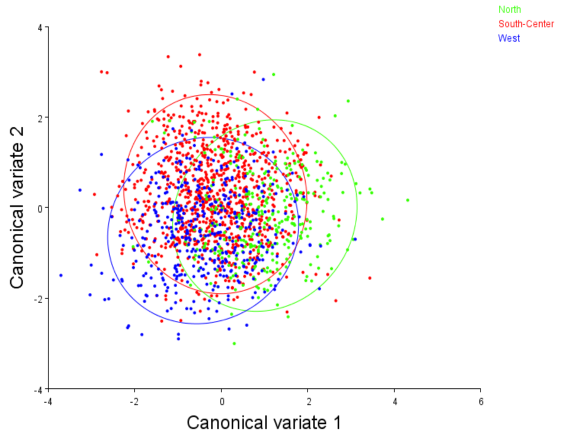

Fig. 6. Scatter Plot of the individuals according to the first two axes of the CVA.

Conclusion

The aim of this study was to assess the biodiversity of honeybee populations in Côte d'Ivoire using geometric morphometrics. The results obtained suggest an absence of differentiation of the colonies from the different geographical areas of the Center, West and North. Indeed, despite the variations observed in the shape of the wings, in the colouring of the abdomen or in the behaviour, the bees collected as part of this study form a homogeneous population.

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