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Study of the Morphometric Diversity of the Population of Honeybees (*Apis Mellifera*) In the North-East Algeria

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Abstract

A biometric study was conducted on domestic worker bees coming from three 03 sites (Tahir, Al-Ancer and Ziama) of the Algerian north-east located in the wet moderate bioclimatic stage (Province Jijel). On each site, 23 bees were taken randomly, that is to say a total of 69 individuals. For each bee, 15 morphological characters were measured.

The statistical analyzes show a very highly significant effect concerning the length of the tongue, the length and width of the anterior wing and the posterior wing, the length of the nervure B of the cubital index, the cubital index (Ratio A/B), the length of the metatarsus, femur, tibia, the sternite 1 and 3, and coloration ($P \leq 0.001$), and significant concerning the width of the metatarsus ($P \leq 0.05$), and non-significant concerning the length of nervure A of the cubital index ($P \geq 0.05$).

Keywords: Domestic bee; Biometric study; Statistical analysis; Algerian north-east

Introduction

Pollinating insects play a key role in cultivated ecosystems [1,2]. Among them, the Bees who belong to the order of Hymenoptera, the superfamily of Apoidea which forms the widest and specialized group [3,4]. This specialization is the result of a long co-evolution between bees and angiosperms group which have begun after synchronous evolutionary radiation in the mid-Cretaceous [5,6]. Today, there are 11 bee families with about 700 genera and 20,000 living species [7-10]. Its abundance and density are higher in the temperate zone due to genera: Apis which is characterized by a highly social behavior and comprises four species: *Apis dorsata*, *Apis florea*, *Apis cerana*, and *Apis mellifera* (honey bee) (Linné, 1758) (Apidae), that is exploited by the Man in beekeeping for approximately 7000 years [3,11,12].

In Algeria, 02 breeds have been identified: The first one, *Apis mellifera intermissa* (Tellian bee) described by Buttel-Reepen (in Ruttner), it is a breed of north Africa found in northern Algerian Sahara and Libya to Morocco [13-15]. The second breed, was successively described by Baldensperger and also by Haccour [16,17]. *Apis mellifera sahariensis* is found in the south of Morocco and Algeria [14]. The studies aiming to know and characterize morphologically and biologically the breed which populates the Algerian territory are of a great interest in so far as they constitute the basis of any work of selection. It can be obtained using many morphological characters used in systematic. We therefore consider biometrics as a working method to highlight the purity of race or on the contrary, the hybrid character of the selected colonies. Several works were realized in certain regions of Algeria [18-23]. Our present study at 03 sites in the province of Jijel aims to complement and deepen the knowledge of Algerian bee populations.

Materials and Methods

Equipment for collection of samples and the measurement of studied morphological characters

The bees come from healthy apiaries. Shaking the frame covered with bees on top of a small plexiglass hive (6 × 8 × 10 cm) and transported to the laboratory where they were transferred in a freezer at -20°C waiting to be measured. Morphometric measurements of 15 characters on a sample of 69 bees are performed using a binocular magnifying glass (Carl Zeiss, GERMANY) and that of coloration is carried out under a microscope (OLYMPUS OPTICAL, JAPAN) equipped with an ocular micrometer calibrated with objective (X10) [21,23].

Description of the area of study

The sampling of worker bees of unknown ages has been carried out at the level of 03 stations (Tahir, Al-Ancer and Ziama) in the Province of Jijel; located at the extreme north-east of the Algerian coast during the beginning of the winter period (December 2014) **(Figure 1)**. This region is located in the

bioclimatic floor wet temperate and characterized by a type of diverse vegetation [24].

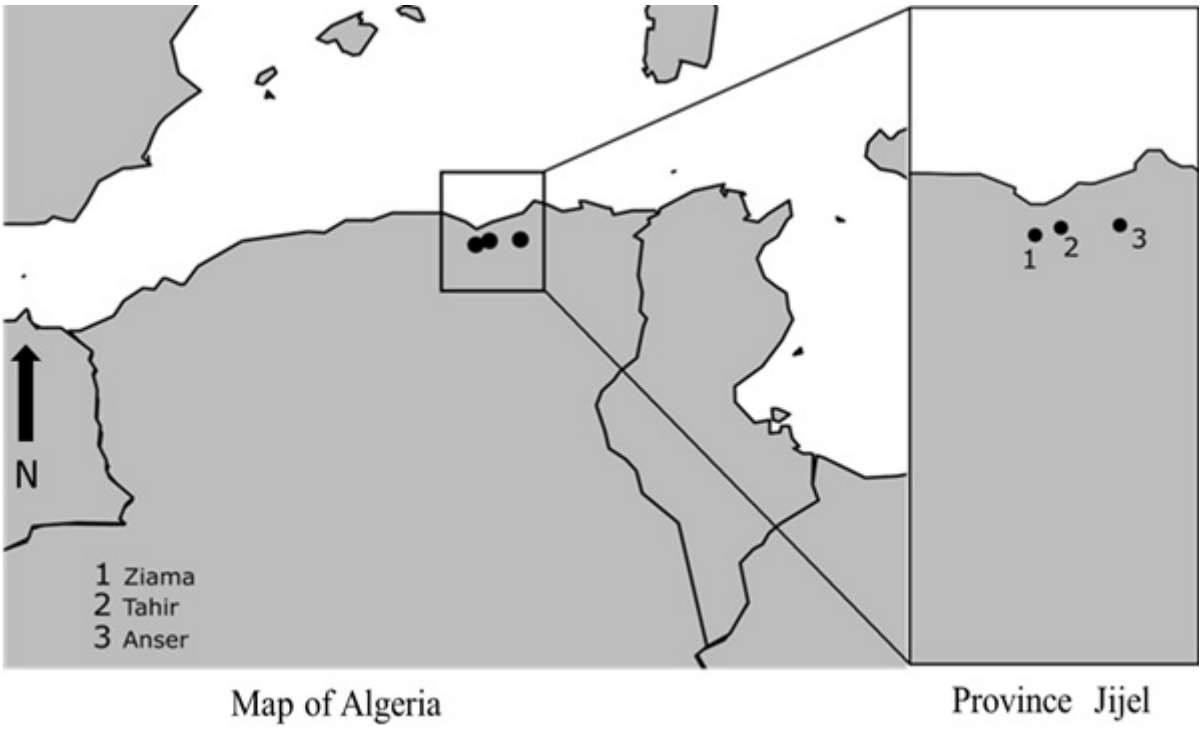

Figure 1 Geographical situation of the study sites in the Province of Jijel.

In each apiary, it was randomly sampled 23 bees, a total of 69 individuals **(Table 1)**. All resident bees in hives of type "Langstroth". It has been verified that they were not object of transhumance and diseases such as it has been advocated by Cornuet et al. and Leporati et al. [25,26].

Table 1 Sites, geographic coordinates, number of worker bees measured per apiary of sampling.

	Sites	Geographical coordinates	Effective
1	Tahir	36°46'N 5°53'E	23
2	El- Ancer	36°48'N 6°10'E	23
3	Ziama	36°40'N 5°28'E	23
Total	3 sites		69

Morphological characters studied and methods of measurement

The measures carried out on the workers relate to 15 characters which have been selected on the basis of their biological significance; these characters contribute much in the production of honey and wax.

The settings selected are: length of the tongue, length and width of the anterior wing, length and width of the posterior wing, length of the nervure A and B, the cubital index (ratio A/B), length and width of the metatarsus, length of the femur, length of the tibia, coloration, and length of the sternite 1 and 3.

The bees as well chosen are dissected one by one. The articles are collected and assembled between blade and cover glass in a few drops of a middle of mounting (glycerine gel) [27,28].

Statistical Analysis

For each studied site we have calculated the means, the standard deviations and the extreme values of each variable.

A comparative study of the means between the 3 sites for each variable (character) was carried out by using the analysis of variance to one classification criterion fixed template (ANOVA) [29,30].

The comparison of 3 sites, between them, for all 15 variables was performed by the analysis of variance multivariate (MANOVA) while using three statistical tests namely: Wilk's Lambda-Lawly Hotteling and Pillai's Trace. However none of these tests is considered as the most powerful and none of them can't be recommended a preferential manner [29].

According to Huberty, the test Wilk's is the most commonly used. This analysis method is an extension of the univariate analysis, to several variables [31].

We used also the analysis in main component (ACP), in order to look for eventually the groups of apiaries homogeneous and the groups of variable. This method was applied to the matrix of the correlation of the averages of the dimension data (nxp) with

n=3 sites and p=15 variables in order to study the structure of bee colonies.

All statistical analyses were performed using MINITAB Software (Version 16, Penn State College, PA, USA).

Results

Description of biometric data

The mean ± standard deviations (SD) and the two extreme values calculated for 03 sites are shown in **Table 2**.

Table 2 Statistical parameters of 15 morphological characters for the whole of 03 sites.

Morphological characters	Site	M ± s	X min	X max
Length of the tongue (X1) in mm	1	8.92 ± 0.78	7.55	9.99
	2	8.16 ± 0.40	7.33	8.66
	3	8.76 ± 0.73	6.88	9.55
Length of the anterior wing (X2) in mm	1	13.56 ± 0.28	12.88	13.99
	2	12.72 ± 0.21	12.22	13.11
	3	13.28 ± 0.18	12.88	13.55
Width of the anterior wing (X3) in mm	1	4.58 ± 0.10	4.44	4.66
	2	4.29 ± 0.10	4.22	4.44
	3	4.45 ± 0.06	4.44	4.66
Length of the posterior wing (X4) in mm	1	9.64 ± 0.16	9.33	9.77
	2	9.19 ± 0.16	8.88	9.33
	3	9.57 ± 0.15	9.33	9.77
Width of the posterior wing (X5) in mm	1	2.62 ± 0.08	2.44	2.66
	2	2.51 ± 0.07	2.44	2.66
	3	2.61 ± 0.06	2.44	2.66
Length of the nervure A (X6) in mm	1	8.10 ± 0.52	7.77	8.88
	2	8.15 ± 0.54	7.77	8.88
	3	8.25 ± 0.56	7.77	8.88
Length of the nervure B (X7) in mm	1	4.34 ± 0.31	3.33	4.44
	2	3.66 ± 0.52	3.33	4.44
	3	3.66 ± 0.52	3.33	4.44
Cubital index (X8)	1	2.08 ± 0.20	1.94	2.58
	2	2.50 ± 0.34	1.94	2.95
	3	2.53±0.33	1.94	2.95
Length of the metatarsus (X9) in mm	1	3.33 ± 0.12	3.11	3.55
	2	3.12 ± 0.06	3.11	3.33
	3	3.24 ± 0.12	2.88	3.33
Width of the metatarsus (X10) in mm	1	1.62 ± 0.05	1.55	1.66
	2	1.59 ± 0.05	1.55	1.66
	3	1.63 ± 0.04	1.55	1.66
Length of the femur (X11) in mm	1	3.87 ± 0.16	3.55	3.99
	2	3.71 ± 0.09	3.55	3.77
	3	3.86 ± 0.12	3.55	3.99
Length of the tibia (X12) in mm	1	4.62 ± 0.10	4.44	4.88
	2	4.38 ± 0.09	4.22	4.44
	3	4.49 ± 0.11	4.22	4.66
Coloration (X13) in mm	1	1.47 ± 0.08	1.27	1.56
	2	1.37 ± 0.10	1.13	1.49
	3	1.47 ± 0.10	1.27	1.63
Length of the sternite1 (X14) in mm	1	4.11 ± 0.32	3.55	4.66
	2	4.82 ± 0.34	3.55	4.66
	3	4.30 ± 0.18	3.99	4.66
Length of the sternite 3 (X15) in mm	1	3.40 ± 0.17	3.11	3.77
	2	3.13 ± 0.07	3.11	3.33
	3	3.33 ± 0.11	3.11	3.55

X min : minimum value, X max : maximum value Site1: Tahir, Site 2: El-Ancer, Site3: Ziama

Analysis of the variance (ANOVA)

The test results of analysis of the variance univariate applied to each of the 15 variables measured, for all 3 sites. Sampling show that there are very highly significant differences concerning the length of the tongue, the length and width of the anterior wing and the posterior wing, the length of the nervure B of the cubital index, the cubital index (Ratio A/B), the length of the metatarsus, femur, tibia, the sternite 1 and 3, and coloration ($P \leq 0.001$), is significant concerning the width of the metatarsus ($P \leq 0.05$), and non-significant concerning the length of the nervure A of the cubital index (**Table 3 and Figure 2**).

Analysis of the variance multivariate or MANOVA

The results of the analysis of variance multivariate are shown in **Table 4**. We distinguish the values of Fobs with associated probabilities, and this for each of the test for equality of vectors of the averages of all the characteristics of 03 sites sampled. For this comparison Inter-apiaries, the three tests (Wilk's Lambda, Hotelling-Lawley and Pilla's Trace) of MANOVA lead to the rejection of legality of the vectors averages for all 03 sites with a very highly significant difference.

Analysis in main components (ACP)

The eigenvalues of the correlation matrix, so the percentages and the cumulative percentages of variation explained by each of the components main for all of the data of the matrix (nxp) (**Table 5 and Figure 3**).

Table 3 Inter-site comparison of medium sized relating to 15 biometric variables of 03 sites sampling (ANOVA).

Morphological characters	ddl	SCE	CM	Fobs	P
Length of the tongue	2	7.3023	3.6512	8.27	0.001***
Length of the anterior wing	2	8.4152	4.2076	78.46	0.000***
Width of the anterior wing	2	0.95257	0.47628	52.97	0.000***
Length of the posterior wing	2	2.7229	1.3614	54.40	0.000***
Width of the posterior wing	2	0.177817	0.088909	15.20	0.000***
Length of the nervure A	2	0.2500	0.1250	0.43	0.655NS
Length of the nervure B	2	6.9998	3.4999	16.21	0.000***
Cubital index	2	2.9164	1.4582	16.05	0.000***
Length of the metatarsus	2	0.51053	0.25527	21.21	0.000***
Width of the metatarsus	2	0.022096	0.011048	4.13	0.021*
Length of the femur	2	0.38299	0.19150	10.96	0.000***
Length of the tibia	2	0.65796	0.32898	27.74	0.000***
Coloration	2	0.16426	0.08213	8.20	0.001***
Length of the sternite 1	2	2.6348	1.3174	14.90	0.000***
Length of the sternite 3	2	0.87541	0.43770	27.35	0.000***

ddl: degrees of freedom; SCE: sum of squared deviations; CM: the means square Fobs: value F of Fisher; P: threshold of the signification. P≤α=0.001: (***) difference very highly significant, P≤α=0.05: (*) difference significant, P≥α=0.05: (NS) non-significant

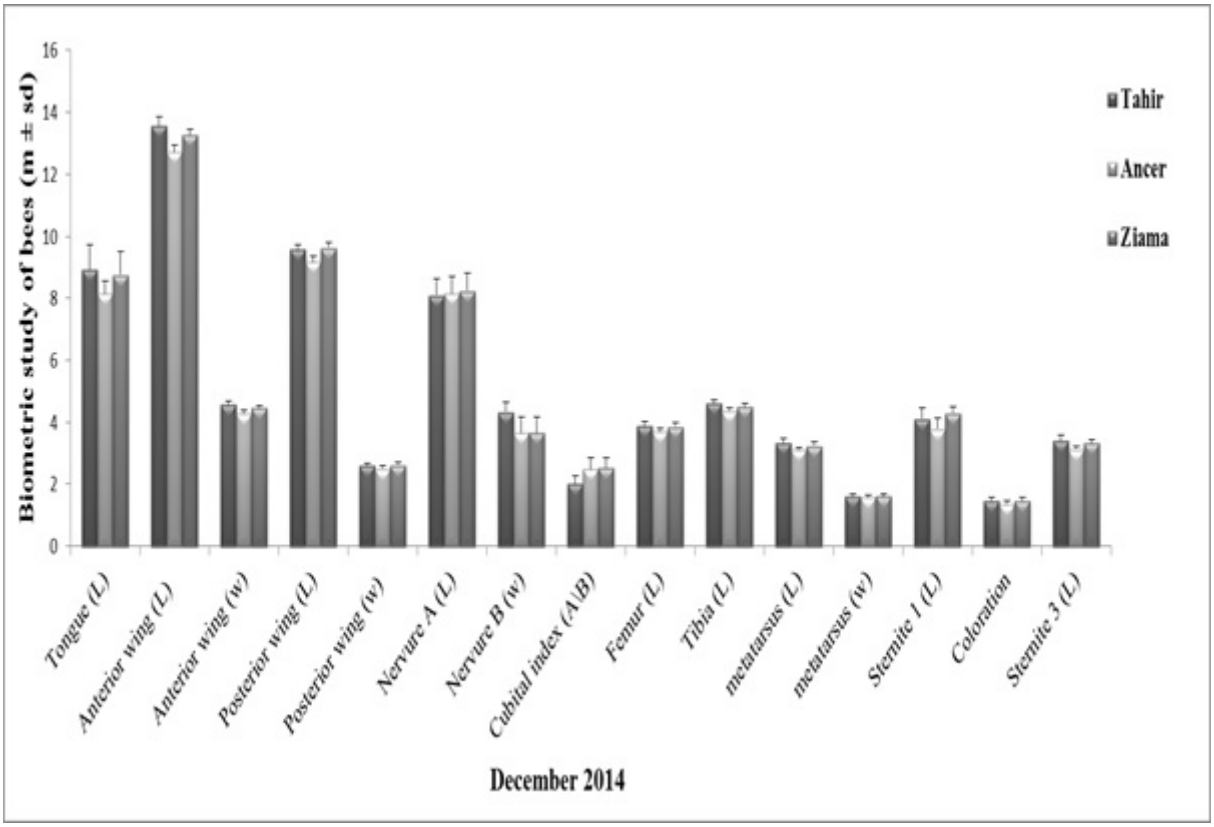

Figure 2 Morphometric study of the bees at the level of the three sites during the winter (December 2014) (m ± s, n=23), (L: Length, w: width).

Table 4 Comparison between sites, vectors of the means of 15 different morphometric variables using MANOVA.

Test	Observed values of the test	Fobs	P
Wilk's	0.08051	8.751	0.000***
Lawley-Hotelling	6.12724	10.416	0.000***
Pillai's	1.34569	7.267	0.000***

Fobs: value F of Fisher; P: threshold of the signification, P≤α=0.001: (***) difference very highly significant

Table 5 Eigen values and percentages of total variation explained by the four main components and the cumulative percentages.

	CP1	CP2	CP4	CP4
Eigenvalue	5.7974	1.7872	1.3438	1.0928
Percentage %	38.6	11.9	9	7
cumulative percentage %	38.6	50.6	59.5	66.8

CP : Main component

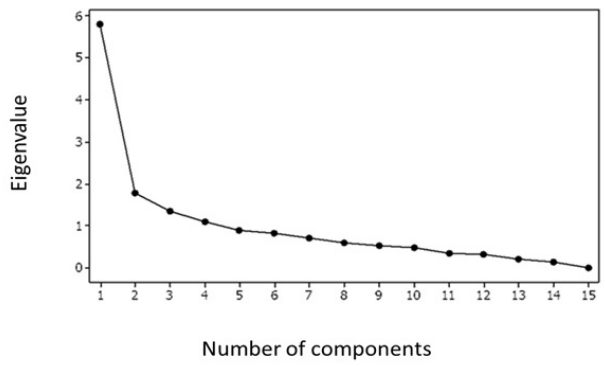

Figure 3 Cone diagrams of main components and eigenvalues.

We note that the first component CP1 explains himself 38.6% of the total variation of the initial variables, the first two component explain the all 50.6%; the first three component explain together 59.5%; and finally the fourth first component explain together 66.8 %. So, these four components or synthetic indices summarize the better the information given by the 15 initials biometric variables. From the 5 the components are less useful and correspond to eigenvalues less than unity (<1).

Table 6 presents the correlation matrix of the initial variables with the first four principal components. The correlation analysis of the variables with the first component CP1 shows that all the variables : length of the tongue (X1), length and width of the anterior wing (X2, X3), length and width of the posterior wing (X4, X5), length of the nervure A (X6) and B (X7), length and width of the metatarsus (X9, X10), length of the femur (X11), length of the tibia (X12), coloration (X13), and length of the sternite 1 and 3 (X14, X15) are positively correlated with the first main component, With the exception of variable (8) which represents the cubital index. The second components CP2 are correlated positively with length of the anterior wing (X2), Width of the posterior wing (X5), Length of the nervure A (X6), Cubital index (8), Length of the metatarsus (X9), Length of the femur (X11), Coloration (X13), Length of the sternite1(X14), Length of the sternite 3 (X15). The third components CP3 are correlated positively with 10 variables: length of the tongue (X1), length and width of the anterior wing (X2, X3), length and width of the posterior wing (X4, X5), length of the nervure A (X6) and B (X7), cubital index (8), width of the metatarsus (X10) length of the tibia (X12).

The study of the correlations of the 15 initial variables relative to axes 1 and 2 of ACP watch that the variable (X7) represents the length of the nervure B is clearly positively correlated according to axis 1, while the variables (X1, X2, X3, X4, X6, X9, X10, X12) corresponding to length of the tongue, length and width of the anterior wing, length of the posterior wing, the length of the nervure A, length and width of the metatarsus length of the tibia get closer to 0.

Finally, the fourth components CP4 correlated positively with length of the tongue (X1), length of the posterior wing (X4), Length of the nervure A (X6) and B (X7), length of the tibia (X12), coloration (X13), and length of the sternite 1 and 3 (X14, X15).

Table 6 Correlation matrix of the initial variables with the first four principal components.

Variables	PC 1	PC2	PC3	PC4
(X1)	0.211	-0.036	0.112	0.291
(X2)	0.379	0.027	0.077	-0.097
(X3)	0.348	-0.000	0.106	-0.255
(X4)	0.335	-0.017	0.006	0.000
(X5)	0.263	0.240	0.274	-0.288
(X6)	0.024	0.293	0.694	0.179

(X7)	0.230	-0.536	0.229	0.093
(X8)	-0.205	0.630	0.088	-0.012
(X9)	0.227	0.225	-0.098	-0.069
(X10)	0.291	-0.032	0.077	-0.133
(X11)	0.282	0.044	-0.139	-0.169
(X12)	0.158	0.003	0.129	0.721
(X13)	0.186	0.161	-0.409	0.159
(X14)	0.197	0.229	-0.319	0.347
(X15)	0.315	0.154	-0.182	0.006
PC : initial components (X) : Morphological characters				

The variable (X8) represent cubital index is negatively correlated according to axis 1. On the other hand, the group formed by variables (X5, X11, X13, X14) are positively correlated according to axis 1 and 2, are respectively width of the posterior wing, length of the femur, coloration, length of the sternite 1 and 3 (**Figure 4**).

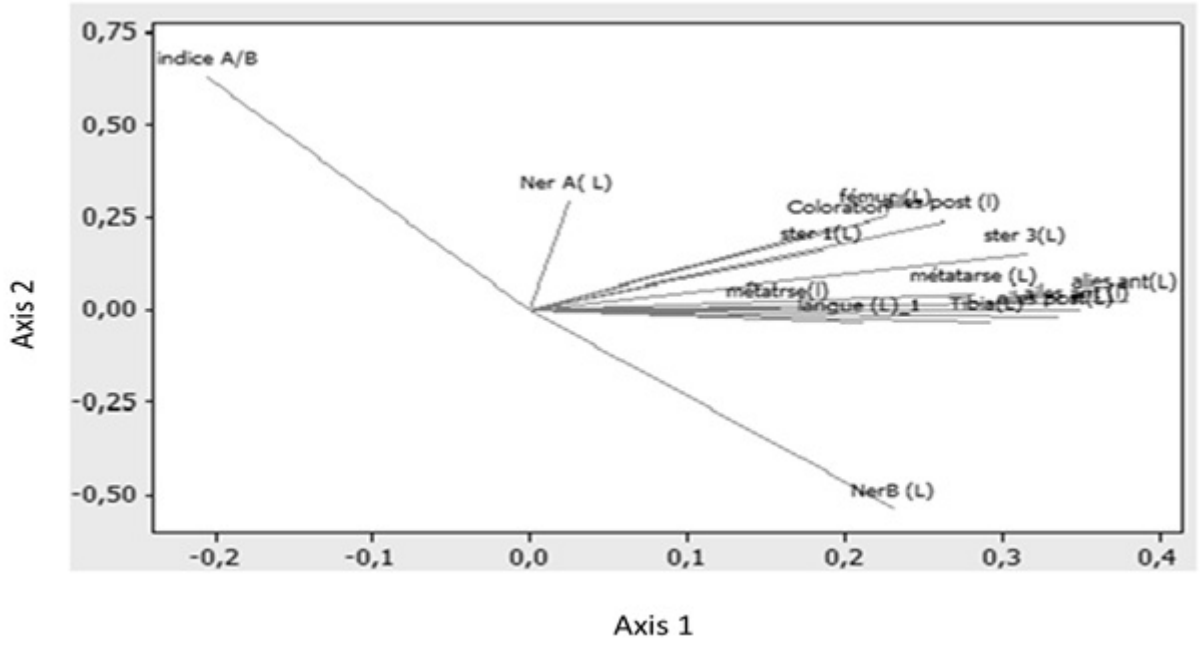

Figure 4 Graphical representation of the correlations of the 15 initial variables on the plan composed of axis 1 and 2 of ACP.

Discussion

The biometric analysis and statistical analysis that we performed on samples of bees North-east Algeria used to define the position of this breed compared to others within the *Apis mellifica* from the point of view morphological

The description of the data by the univariate statistical method revealed that the bee of North-east Algeria is large compared to that of Marocco, Tunisia and the south Algerian [23] for most morphological characters [23,25,32-34].

Our morphometric results obtained compared with those obtained by several study especially, Ruttner, and Cornuet et al. on the Moroccan bees, or Grissa et al. on the Tunisian bees, finally Achou and Bendjedid and Achou on the Algerian bees [21,23,25,32,33].

The mean length of the anterior wing obtained is 13.56 mm (S1), 12.72 mm (S2) and 13.28 mm (S3). It is clearly very high compared to those found respectively by Ruttner, Achou and Bendjedid and Achou which are of the order of 9.19 mm, 8.58 mm and 7.24 mm [21,23,32].

The mean width of the anterior wing is 4.58 mm (S1), 4.29 mm (S2) and 4.45 (S3). It is greater than those found by Ruttner which is of the order of 3.08 mm, Achou 3.05 mm and by Bendjedid and Achou which is 2.39 mm [21,23,32].

The mean value of the width of the yellow band (length of the nervure A is of 8.10 mm (S1), 8.15 mm (S2) and 8.25 mm (S3). It

is much higher respectively to those made by Cornuet et al. and Grissa et al. which are of the order of 0.544 mm and 0.563 mm, Achou which remain of a same order 0.50 mm and by Bendjedid and Achou who is from 0.41 mm [21,23,25,34].

The mean value of the width of the yellow band length of the nervure B obtained is 4.34 mm (S1) and 3.66 mm (S2, S3). This value is also higher compared to those found by Cornuet et al. or by Grissa et al. which are of the order of 0.222 mm and of 0.249 mm respectively the observed values by Achou is 0.210 mm, but by Bendjedid and Achou is from 0.176 mm [21,23,25,34].

The mean value of the width of the yellow band (Coloration) of the three stations studied equals at 1.37 mm and 1.47 mm. It is higher than that given by Achou and Bendjedid and Achou which are of the order of 0.40 mm and 0.45 mm, Grissa et al. who is from 0.19 mm [21,23,34].

This differentiation of the size is due to the high richness of the vegetation and the moderate climate of North-east Algeria. Thus, this bee has a body more large to browse the long distances in search of its food.

We can assign this differentiation to the existence of a north-south gradient for some morphological characters. In effect, one of the first examples of the North-South gradient has been provided for the bees by a number of russian authors, as Chochlov, Michailov, Aplatov and Skorikov in Ruttner, who have found that the length of the tongue decreased gradually from north to south [35-38]. In our country, to the north-east, the length of the tongue is of 6.146 mm, it is therefore noted, based on our result and the value of 8.92 mm (S1), 8.16 (S2) and 8.78 (S3), a similarity of the length of the north to the south [19].

This large size in the Length of the tongue of bees, at the levels of sites presented in this study, is an adaptation to several types of flowers on which the bees feed. Indeed, this would lead to admit the existence of a parallel gradient of the average depth of the corollas of species melliferous. The hypothesis, advanced by Ruttner, we seems more likely [39]. It consists to admit that the environmental rules of Bergmann and Allen set for vertebrates to warm-blood apply here on bees [40]. These rules stipulate that the races cold climates tend to be bigger that the races of the same species living in warm climates (rules of Bergmann).

The comparison between the 3 apiaries for each of the 15 characteristics perform with the analysis test of univariate variance reveals that there exist between the three apiaries the differences very highly significant for the average of all the morphometric variables except for the width of the metatarsus shows a significant differentiation and non-significant for the length of the nervure A of the cubital index. These results are confirmed with the analysis of the variance multivariate (MANOVA) which shows that differences was very highly significant between the three apiaries and this for the majority of the 15 characteristics taken in consideration simultaneously.

This variation inter-apiary cannot be attributed to eco-climatic factors only, given that the bees live in the same biotope. We can be attributed to the size of the cells of the brood, because it is at this level that takes place the embryonic development of bees, consequently, the size of the bees may be affected [19,41-43]. In addition, the parasitism, in particular ectoparasites (for example: Varroa destructor) have an effect on the phenotype of bees [39,44,45].

The principal component analysis applied to our bee colonies made it possible to retain the first four components which together account for 66.8% of the total variation. The first component to it only takes into account 38.6% of variability. These four component or synthetic indices summarize the better the information brought by the 15 variables biometrics.

According to our results, we find that the values obtained are closer to the giant bee (Apis dorsata) and this is declared by Ruttner and that goes back to the importation of the species by the beekeeper, but we have not yet confirmed, because we are trained to do molecular biology [39].

Conclusion

In conclusion, our results indicate that morphometry study of the Algerian bees in three different sites can be used as a modern program for selection and determination of the different breeds. As results, the most studied characteristics revealed similar in the three tested sites.

Competing Interests

The authors declare that they have no competing interests.

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