## Evaluation of morphometric characters of honeybee (Apis mellifera L.) populations in Nigeria

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#### Abstract

Samples of workers of honeybee were collected from 41 colonies in nine localities in Nigeria and analysed using classical morphometry. Measurements of 35 morphological characters of body size, colour and pilosity were taken from 10 workers per colony and the data subjected to one-way analysis of variance (ANOVA) and principal component analysis. Although ANOVA revealed a considerable variation of morphological characters between the sampled localities, principal component analysis indicated that this variation was not sufficient to group the colonies under investigation into geographically separable groups. Based on the agreement between the results of this study and those of previous studies, it is concluded that the honeybees of this area are morphometrically pure populations of sub-Saharan A. mellifera.


Keywords: Apis mellifera, honeybee, African bees, morphometry, Nigeria.

## 1 Introduction

Honeybees are of considerable economic importance, producing products of commercial value, such as honey and wax, and pollinating crops and wild plants. For example, Calderone (2012) estimated the value of honeybees, through pollination of crops, to be US $\$ 11.68$ billion in the United States of America, for the year 2009. Similarly, the contribution of honeybees to the economy of the United Kingdom, through pollination, was estimated at $£ 191.80$ million (Anonymous 2009). The combined production of honey by the top 20 producing countries for 2011 was estimated at 1.26 million metric tonnes valued at US $\$ 3.16$ billion (Anonymous n . d.). In addition, products of honeybees, notably honey, royal jelly, propolis and bee venom, contribute to our well-being through their nutritional and therapeutic properties.

The natural range of the western honeybee, A. mellifera, is western Asia, Africa and Europe: From southern Scandinavia in the north to the Cape of Good Hope in the south, from Dakar in the west to the Urals, Mashhad and the coast of Oman in the East. Geographical isolation and ecological adaptations resulted in the evolution of local populations showing considerable geographical variation, resulting in adaptation to local factors of climate, vegetation, pests and pathogens (Meixner et al. 2013; Ruttner et al. 1978). These adaptations may be lost due to human activities in beekeeping that affect wild honeybees in different ways: competition for floral sources, introduction of exotic genes, pests, parasites and diseases (De la Rúa et al. 2009; Moritz et al. 2005). Therefore an adequate knowledge of the natural diversity of local subspecies and ecotypes is essential for their management and conservation. To protect the biological diversity of local populations of honeybees in their natural habitats, these populations must, first of all, be characterised. One of the standard methods of characterising honeybees is classical morphometry (Meixner et al. 2013). This method uses numeric data resulting from exact measurements of 36 morphological characters of body size, colour and pilosity from which means of colony characters are obtained for statistical analyses (Ruttner 1988). Using this method Ruttner (1988), classified $A$. mellifera into 24 subspecies and four evolutionary lineages. However, whereas the European subspecies have been thoroughly studied, the study of their Asian and African counterparts is still in its infancy, in many places (Meixner et al. 2013). With only 190 colonies, from 91localities, morphometrically analysed (Hepburn \& Radloff 1998), the western part of Africa (Countries in West and Central Africa, from Mauritania and Senegal in the west, to Chad in the east, then south to Namibia, through Zambia) is evidently under-studied. Although a few studies have been carried out, recently, in Nigeria (Ajao et al. 2014; Oyerinde et al. 2012; Yu et al. 2012), they have done little in improving the situation, due to their inadequacy in coverage and/or methodology. Thus, the present study attempted to improve our knowledge of the diversity of the honeybees of this region by analysing 41 colonies from nine localities in Nigeria through classical morphometry. The main purpose of the study was to provide reference data for future studies.

## 2 Materials and Methods

### 2.1 Description of the Area of Study

The area of study (Figure 1) lies approximately within $3^{\circ}$ to $14^{\circ} \mathrm{E}$ and $4^{\circ}$ to $14^{\circ} \mathrm{N}$ and covers the whole of Nigeria. A summary of the important physical features of the area, based on Hepburn \& Radloff (1998), is given below:

The area consists of four climatic zones, namely, equatorial, wet tropical, dry tropical and sahelian. These correspond, approximately, to four zones of vegetation (Figure 1): Tropical rainforest, Guinea savanna, Sudan savanna and Sahel. Sahel is the transitional zone between savanna and desert.

The forest zone is characterized by a very dense vegetation, very rich in species diversity; a very heavy rainfall and a long rainy season (more than half of the year); and a mean annual temperature of about $25^{\circ} \mathrm{C}$ which varies a little. Plants flower throughout the year.

The savannas consist of a mixture of grasslands and woody vegetation; lighter rainfall with a short rainy season (less than half of the year); and a high variation of mean annual temperature $\left(10\right.$ to $\left.15^{\circ} \mathrm{C}\right)$. The density of vegetation, species richness, amount of rainfall and length of the rainy season decrease with the increase in latitude. Annuals flower at the end of the rainy season while trees flower during the dry season.

The Sahel is characterized by scanty rainfall; very short rainy season; very sparse vegetation of short trees and grass; and frequent droughts.

Altitude varies from sea level, through 1200 metres on the Jos Plateau, to about 1800 metres on the Mambila Plateau.

### 2.2 Collection of honeybees

Samples of workers of honeybee were collected from 41 colonies in nine localities (Figure 1 and Table 1). Bees were collected, from wild nests or unmanaged traditional or top-bar hives populated by wild swarms, and preserved in $70 \%$ ethanol. The collection of samples and the morphometric data generated therefrom were stored at the Institut für Bienenkunde in Oberursel (Polytechnische Gesellschaft), University of Frankfurt, Germany.

### 2.3 Morphometric Measurements

Morphometric measurements of 35 characters were taken from 10 bees from each colony according to Ruttner (1988) and Ruttner et al. (1978). Measurements of hair and pigmentation were taken under a Leica dissecting microscope, fitted with an eyepiece graticle, at a magnification of 40 x . Measurements of wings, legs and sternites were taken with the help of a Leica CCD camera connected to a desktop computer, using the measuring program Bee Morphometric, Version 1.02 (Meixner 1994). The details of the variables measured are shown in Table 2.

### 2.4 Statistical Analyses

First, the mean and standard deviation of each of the 35 morphometric characters were calculated for every colony. Then the means for colonies were used to calculate the means and standard deviations for localities. The data were subjected to a one-way analysis of variance (ANOVA) to compare different localities. Tukey HSD tests, on the means of the 35 characters, were used to detect significant differences between the localities. The level of statistical significance chosen was $p=0.05$.

A PCA, using colony means of 35 morphometric characters was run in order to detect any possible clusters. The suitability of PCA was assessed, prior to the analysis, using correlation coefficients of the variables, Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity (Anonymous 2014; Burns \& Burns 2008). Any variable that did not have a correlation with at least one other variable where $r \geq 0.3$ should be removed from the analysis. The KMO measure is used as an index of whether there are linear relationships between the variables. Its value can range from 0 to 1 , with values above 0.6 suggested as a minimum requirement for sampling adequacy. Bartlett's test of sphericity tests the null hypothesis that there are no correlations between any of the variables and, therefore, the variables cannot be reduced to a smaller number of principal components. For a PCA to be feasible the null hypothesis must be rejected: The result of Bartlett's test is used to take this decision. Statistical analyses were carried out with $\mathrm{IBM}^{\circledR}$ SPSS $^{\circledR}$ Statistics Version 20 with additional material from Burns \& Burns (2008) and Anonymous (2014).

## 3 Results

Means of the 35 morphometric characters for the sampled localities are given in Tables 3-6.
Table 3 shows the means of characters of body hair and pigmentation. The length of cover hair ranged from $0.13 \pm$ s.d. 0.00 mm in Yola to $0.19 \pm$ s.d. 0.01 mm in Abeokuta; the width of tomentum from $0.63 \pm$ s.d. 0.05 mm in Okuta to $0.76 \pm$ s.d. 0.02 mm in Umuahia and the pigmentation of the scutellum from $6.02 \pm$ s.d. 0.24 in Sokoto to $7.40 \pm$ s.d. 0.37 in Umuahia. The mean values of the characters of the hind leg are given in Table 4: The bees of Abeokuta had the shortest femur $(2.35 \pm$ s.d. 0.02 mm$)$, tibia $(2.84 \pm$ s.d. 0.00 mm$)$ and metatarsus $(1.82 \pm$ s.d. 0.02 mm ) while all the three characters were longest in the bees of Sokoto (femur $=2.52 \pm$ s.d. 0.03 mm ; tibia $=3.07 \pm$ s.d. 0.04 mm ; metatarsus $=1.93 \pm$ s.d. 0.02 mm ). The width of metatarsus ranged from $1.04 \pm$ s.d. 0.02 mm in Afunori to $1.14 \pm$ s.d. 0.02 mm in Sokoto. As may be seen in Table 5, the longitudinal diameter of tergite 3 varied between $1.92 \mathrm{a} \pm$ s.d. 0.03 mm in Gitata and $2.07 \pm$ s.d. 0.05 mm in Maiadua; while that of tergite 4 ranged from $1.87 \pm$ s.d. 0.06 mm in Afunori to $2.01 \pm$ s.d. 0.05 mm in Maiadua. The longitudinal diameter of sternite 3, on the other hand, was smallest in Abeokuta ( $2.28 \pm$ s.d. 0.03 mm ) and largest in Sokoto ( $2.50 \pm$ s.d. 0.04 mm ). The longitudinal diameter of wax mirror varied between $1.11 \pm$ s.d. 0.01 mm in Abeokuta to $1.26 \pm$ s.d. 0.03 mm in Sokoto. Sternite 6 was longest $(2.35 \pm$ s.d. 0.03 mm$)$ and widest $(2.90 \pm$ s.d. 0.07 mm$)$ in the bees of Sokoto but shortest $(2.15 \pm$ s.d. 0.03 mm$)$ and narrowest $(2.58 \pm$ s.d. 0.07 mm$)$ in those of Abeokuta. The length of fore-wing varied between $7.98 \pm$ s.d. 0.10 mm in Abeokuta and $8.51 \pm$ s.d. 0.13 mm in Sokoto while its width varied between $2.71 \pm$ s.d. 0.04 mm and $2.89 \pm$ s.d. 0.05 mm in Abeokuta and Sokoto, respectively (Table 6).

A one-way ANOVA revealed that means of 23 of the morphometric characters differed significantly $(p<0.05)$ between sampled localities. These were: Length of cover hair on tergite 5 (1), width of the tomentum band on the side of tergite 4 (2), width of the dark stripe between the tomentum and the posterior rim of the tergite (3), length of femur (5), length of tibia (6), length of metatarsus (7), width of metatarsus (8), longitudinal diameter of tergites 3 (9) and 4 (10), longitudinal diameter of sternite 3 (11), longitudinal diameter of plate of sternite 3 (12), transversal diameter of wax plate of sternite 3 (13), longitudinal diameter of sternite 6 (15), transversal diameter of sternite 6 (16), length of fore-wing (17), width of fore-wing (18), five angles of wing venation (E9 (24), G18 (25), J16 (27), L13 (29) and N23 (30)), pigmentation of
scutellum (35) and pigmentation of plates of scutellum (36). However, means of the remaining 12 characters (pigmentation of the second, third and fourth tergites, distance between wax mirrors of sternite 3, the two cubital distances, and angles A4, B4, D7, J10, K19, and 026) did not $(p>0.05)$.

In order to investigate the similarity of the honeybee colonies under study, a PCA, using colony means of seven morphometric characters ( 28 characters were excluded from the analysis during a preliminary PCA due to their failure to meet some conditions) of 10 worker honeybees from each of 41 colonies at nine localities, was run to detect possible clusters. The suitability of PCA was assessed prior to analysis. Inspection of the correlation matrix showed that all variables had the minimum requirement of at least one correlation coefficient greater than 0.3 . The overall Kaiser-Meyer-Olkin (KMO) measure was 0.91 with individual KMO measures from 0.89 to 0.93 , thus meeting the minimum requirement for sampling adequacy. Bartlett's test of sphericity was statistically significant ( $p<.0005$ ), indicating that the data could be appropriately analysed using PCA (Anonymous 2014; Burns \& Burns 2008).

Three principal components, with eigenvalues 5.55, 0.45 and 0.40 each, and accounting for $91.37 \%$ of the total variance, were extracted. As revealed by the scree plot, the first two components carried sufficient variation (85.68\%) between the colonies. A Varimax orthogonal rotation was employed to aid interpretability. There were strong loadings of characters of size on all three components (Table 7).

As may be seen in Figure 2, a scatter plot of the first two principal components did not produce distinct clusters.

## 4 Discussion

As may be seen in Table 8, the mean values of a set of the morphometric characters measured in this study are in general agreement with those reported for the subspecies of $A$. mellifera in subSaharan Africa (Ruttner 1988; Yu et al. 2012), except some values of doubtful validity reported by Ajao et al. (2014) and Oyerinde et al. (2012). This agreement is a confirmation of the correctness of the measurements taken in this study.

Although ANOVA reveals a considerable variation of morphological characters between the sampled localities, principal component analysis suggests this variation is not sufficient in grouping the colonies under investigation into geographically separable groups. As revealed by PCA, the most important morphological variation of the honeybees of this area is size of the body. As may be seen from the scatter plot of the principal components (Figure 2), the colonies form one mixed cluster: In other words, they do not segregate according to the type of vegetation of their origin. This suggests a continuous variation in size, which is not related to the present ecological variation of the area. This observation agrees with that of Ruttner (1988): "Although a phenetic north-south cline was established along the African west coast, no morphometric differentiation has yet been found, in spite of the huge geographic distance and important differences in humidity and altitude."

## 5 Conclusion

Based on the agreement between the results of this study and those of previous studies, it is concluded that the honeybees of this area are morphometrically pure populations of sub-Saharan A. mellifera.

## Acknowledgements

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Figure 1: The area of study (Nigeria) showing sampled localities and variation in vegetation. Inset: A map of Africa showing the location of the area of study (highlighted in grey). Information on vegetation was obtained from Hoyle et al. (1958).


Figure 2. A scatter plot of the first two principal components of PCA, using the colony means of seven morphological characters, of workers of A. mellifera from nine localities in Nigeria. Both components, which carry about $86 \%$ of the total variance, were loaded with characters of body size: Component 1 with longitudinal diameter of wax plate (mirror) of sternite 3 , longitudinal diameter of sternite 3 , transversal diameter of sternite 6 and length of tibia; and component 2 with length and width of fore-wing. The colonies are coded according to the type of vegetation of their origin.

206 Table 1: Localities (in Nigeria) from which samples of honeybee were collected for 207 morphometric analysis (See Figure 2)

| Locality | Latitude <br> $\left({ }^{\circ} \mathrm{N}\right)$ | Longitude <br> $\left({ }^{\circ} \mathrm{E}\right)$ | Altitude <br> $(\mathrm{m})$ | Number of <br> Colonies |
| :--- | ---: | ---: | ---: | :---: |
| Afunori | 13.70 | 13.33 | 283 | 5 |
| Maiadua | 13.18 | 8.23 | 469 | 3 |
| Sokoto | 13.05 | 5.23 | 283 | 5 |
| Okuta | 9.22 | 3.18 | 455 | 5 |
| Gitata | 9.12 | 7.95 | 444 | 5 |
| Yola (Bole) | 9.12 | 12.45 | 232 | 5 |
| Abeokuta | 7.17 | 3.40 | 131 | 4 |
| Nguroje | 6.95 | 11.12 | 1828 | 4 |
| Umuahia (Umudike) | 5.48 | 7.57 | 125 | 5 |

B. Size

5 Femur, length
6 Tibia, length
7 Metatarsus, length
8 Metatarsus, width
9 Tergite 3, longitudinal diameter
10 Tergite 4, longitudinal diameter
11 Sternite 3, longitudinal diameter
12 Wax plate(mirror) of sternite 3, longitudinal diameter
13 Wax plate of sternite 3, transversal diameter
14 Distance between wax plates of sternite 3
15 Sternite 6, longitudinal diameter
16 Sternite 6, transversal diameter
C. Fore-wing

17 Fore-wing, length
18 Fore-wing, width
19 Cubital vein, distance a
20 Cubital vein, distance b
21-31 11 angles of wing venation
(No. 21=A4, 22=B4, 23=D7, 24=E19, 25=G18, $26=\mathrm{J} 10,27=\mathrm{J} 16,28=\mathrm{K} 19,29=\mathrm{L} 13,30=\mathrm{N} 23$, $31=026$ )
D. Colour

32 Pigmentation of tergite 2
33 Pigmentation of tergite 3
34 Pigmentation of tergite 4
35 Pigmentation of scutellum
36 Pigmentation of plates of scutellum
Table 2: List of characters measured for morphometry ${ }^{1}$

| Ruttner No. Character |  |  |
| :---: | :---: | :---: |
| A. Hair |  |  |
|  | 1 | Length of cover hair on tergite 5 |
|  | 2 | Width of the tomentum band on the side of tergite 4 |
|  | 3 | Width of the dark stripe between the tomentum and the posterior rim of the tergite |
| B. Size |  |  |
|  | 5 | Femur, length |
|  | 6 | Tibia, length |
|  | 7 | Metatarsus, length |
|  | 8 | Metatarsus, width |
|  | 9 | Tergite 3, longitudinal diameter |
|  | 10 | Tergite 4, longitudinal diameter |
|  | 11 | Sternite 3, longitudinal diameter |
|  | 12 | Wax plate(mirror) of sternite 3, longitudinal diameter |
|  | 13 | Wax plate of sternite 3, transversal diameter |
|  | 14 | Distance between wax plates of sternite 3 |
|  | 15 | Sternite 6, longitudinal diameter |
|  | 16 | Sternite 6, transversal diameter |
| C. Fore-wing |  |  |
|  | 17 | Fore-wing, length |
|  | 18 | Fore-wing, width |
|  | 19 | Cubital vein, distance a |
|  | 20 | Cubital vein, distance $b$ |
|  | 21-31 | 11 angles of wing venation |
|  |  | (No. 21=A4, 22=B4, 23=D7, 24=E19, 25=G18, |
|  |  | $31=026)$ |
| D. Colour |  |  |
|  | 32 | Pigmentation of tergite 2 |
|  | 33 | Pigmentation of tergite 3 |
|  | 34 | Pigmentation of tergite 4 |
|  | 35 | Pigmentation of scutellum |
|  | 36 | Pigmentation of plates of scutellum |

[^0]Table 3: Means and standard deviations of morphological characters of body hair ( mm ) and pigmentation of 10 worker bees each from 215 (N) colonies from nine localities in Nigeria.

| Locality | N | Hair(1)** | Tom1(2)*** | Tom2(3)* | $\mathrm{Pt2}(32) \dagger$ | $\mathrm{Pt} 3(33) \dagger$ | $\mathrm{Pt4(34)} \mathrm{\dagger}$ | Scut1(35)*** | Scut2(36)*** |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Afunori | 5 | 0.14 a | 0.73 ab | 0.43 | 9.00 | 8.04 | 4.40 | 7.52 d | 5.24 d |
|  |  | 0.01 | 0.03 | 0.03 | 0.00 | 0.09 | 0.89 | 0.28 | 0.40 |
| Maiadua | 3 | 0.15 a | 0.73 ab | 0.44 | 9.00 | 8.00 | 5.30 | 6.75 c | 4.10 bc |
|  |  | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | 2.25 | 0.07 | 0.00 |
| Sokoto | 5 | 0.15 ab | 0.73 ab | 0.43 | 8.96 | 8.00 | 4.00 | 6.02 a | 3.04 a |
|  |  | 0.01 | 0.01 | 0.01 | 0.09 | 0.00 | 0.00 | 0.24 | 0.34 |
| Okuta | 5 | 0.17 ab | 0.63 a | 0.46 | 9.00 | 8.00 | 4.10 | 6.50 abc | 3.74 abc |
|  |  | 0.01 | 0.05 | 0.02 | 0.00 | 0.00 | 0.41 | 0.20 | 0.50 |
| Gitata | 5 | 0.17 ab | 0.70 ab | 0.45 | 8.88 | 7.68 | 4.00 | 6.08 ab | 3.30 ab |
|  |  | 0.04 ab | 0.05 | 0.02 | 0.27 | 0.70 | 0.00 | 0.28 | 0.20 |
| Yola | 5 | 0.13 a | 0.75 ab | 0.42 | 8.96 | 8.00 | 4.02 | 6.62 bc | 3.26 ab |
|  |  | 0.00 | 0.01 | 0.01 | 0.09 | 0.00 | 0.04 | 0.20 | 0.09 |
| Abeokuta | 4 | 0.19 b | 0.72 ab | 0.41 | 9.00 | 8.00 | 4.00 | 6.63 bc | 3.03 a |
|  |  | 0.01 | 0.02 | 0.03 | 0.00 | 0.00 | 0.00 | 0.29 | 0.63 |
| Nguroje | 4 | 0.16 ab | 0.64 a | 0.42 | 9.00 | 8.08 | 4.23 | 7.53 d | 5.23 d |
|  |  | 0.02 | 0.03 | 0.05 | 0.00 | 0.15 | 0.66 | 0.17 | 0.71 |
| Umuahia | 5 | 0.16 ab | 0.76 ab | 0.45 | 8.96 | 7.86 | 3.96 | 7.40 d | 4.58 cd |
|  |  | 0.02 | 0.02 | 0.01 | 0.09 | 0.31 | 0.09 | 0.37 | 0.36 |

*Significant ( $p<0.05$ ); **highly significant ( $p<0.01$ ); ***very highly Significant ( $p<0.001$ ); † not significant ( $p>0.05$ ); according to one-way ANOVA. Means within a column followed by the same letter are not significantly different at $p=0.05$ according to Tukey HSD test. Hair: Cover hair on tergite 5; Tom1: Width of tomentum; Tom2: Width of stripe behind tomentum; Pt2,3 \& 4: Pigmentation of tergites $2,3 \& 4$; Scut 1,2: pigmentation of scutellum and its plates. Numbers in brackets are Ruttner numbers.

Table 4: Means and standard deviations (mm) of characters of the hind leg of 10 worker bees each from ( N ) colonies from nine localities in Nigeria.

| Locality | N | Femur, length(5)*** | Tibia, length(6)*** | Metatarsus, length(7)*** | Metatarsus, width $(8)^{* * *}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Afunori | 5 | 2.37ab | 2.89ab | 1.82a | 1.04a |
|  |  | 0.03 | 0.04 | 0.03 | 0.02 |
| Maiadua | 3 | 2.50 cd | 3.05 cd | 1.91 bc | 1.13 cd |
|  |  | 0.08 | 0.12 | 0.04 | 0.03 |
| Sokoto | 5 | 2.52d | 3.07d | 1.93c | 1.14 d |
|  |  | 0.03 | 0.04 | 0.02 | 0.02 |
| Okuta | 5 | 2.45 cd | 2.98 bcd | 1.86ab | 1.09 bcd |
|  |  | 0.03 | 0.04 | 0.01 | 0.02 |
| Gitata | 5 | 2.45 cd | 2.99 bcd | 1.86ab | 1.11 bcd |
|  |  | 0.03 | 0.02 | 0.04 | 0.03 |
| Yola | 5 | 2.43 bc | 2.94ab | 1.84ab | 1.07 ab |
|  |  | 0.02 | 0.03 | 0.03 | 0.03 |
| Abeokuta | 4 | 2.35a | 2.84a | 1.82a | 1.05 ab |
|  |  | 0.02 | 0.00 | 0.02 | 0.00 |
| Nguroje | 4 | 2.45 cd | 2.96bc | 1.87 abc | 1.08abc |
|  |  | 0.02 | 0.02 | 0.03 | 0.02 |
| Umuahia | 5 | 2.43 bc | 2.94bc | 1.85 ab | 1.08 abcd |
|  |  | 0.04 | 0.05 | 0.04 | 0.02 |

***Very highly significant ( $p<0.001$ ), according to one-way ANOVA. Means, within a column, followed by the same letter are not significantly different at $p=0.05$ according to Tukey HSD test. Numbers in brackets are Ruttner numbers.

Table 5: Means and standard deviations (mm) of characters of the abdomen of 10 worker bees each from ( N ) colonies from nine localities in Nigeria.

| Locality | N | Lt3 <br> $(9)^{* *}$ | $\mathrm{Lt4}$ <br> $(10)^{* *}$ | Lst3 <br> $(11)^{* * *}$ | Lwm <br> $(12)^{* * *}$ | Wwm <br> $(13)^{*}$ | Dwm <br> $(14) \dagger$ | Lst6 <br> $(15)^{* * *}$ | Wst6 <br> $(16)^{* * *}$ |
| :--- | :---: | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Afunori | 5 | 1.94 a | 1.87 a | 2.33 ab | 1.18 ab | 1.92 | 0.31 | 2.22 ab | 2.64 ab |
|  |  | 0.05 | 0.06 | 0.02 | 0.01 | 0.13 | 0.01 | 0.05 | 0.06 |
| Maiadua | 3 | 2.07 b | 2.01 b | 2.46 d | 1.23 bc | 2.05 | 0.33 | 2.29 bc | 2.79 cd |
|  |  | 0.05 | 0.05 | 0.09 | 0.05 | 0.13 | 0.02 | 0.10 | 0.10 |
| Sokoto | 5 | 2.05 ab | 1.99 ab | 2.50 d | 1.26 c | 2.07 | 0.33 | 2.35 c | 2.90 d |
|  |  | 0.08 | 0.07 | 0.04 | 0.03 | 0.07 | 0.01 | 0.03 | 0.07 |
| Okuta | 5 | 2.01 ab | 1.95 ab | 2.44 cd | 1.21 bc | 2.04 | 0.30 | 2.22 ab | 2.78 bcd |
|  |  | 0.03 | 0.03 | 0.04 | 0.03 | 0.05 | 0.03 | 0.03 | 0.06 |
| Gitata | 5 | 1.92 a | 1.87 a | 2.45 cd | 1.22 bc | 2.04 | 0.32 | 2.25 abc | 2.75 bc |
|  |  | 0.03 | 0.03 | 0.04 | 0.04 | 0.10 | 0.01 | 0.08 | 0.07 |
| Yola | 5 | 2.01 ab | 1.95 ab | 2.36 abc | 1.18 ab | 2.03 | 0.30 | 2.21 ab | 2.71 abc |
|  |  | 0.05 | 0.05 | 0.03 | 0.02 | 0.06 | 0.02 | 0.02 | 0.06 |
| Abeokuta | 4 | 1.95 ab | 1.89 a | 2.28 a | 1.11 a | 1.92 | 0.30 | 2.15 a | 2.58 a |
|  |  | 0.10 | 0.10 | 0.03 | 0.01 | 0.05 | 0.01 | 0.03 | 0.07 |
| Nguroje | 4 | 2.02 ab | 1.97 ab | 2.41 | 1.19 bc | 2.01 | 0.30 | 2.26 abc | 2.75 bc |
|  |  | 0.03 | 0.03 | 0.05 | 0.05 | 0.06 | 0.03 | 0.06 | 0.04 |
| Umuahia | 5 | 2.01 ab | 1.95 ab | 2.42 bcd | 1.19 bc | 2.00 | 0.31 | 2.23 ab | 2.71 abc |
|  |  | 0.07 | 0.07 | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 |

*Significant $(p<0.05)$; **highly significant ( $p<0.01$ ); ***very highly significant $(p<0.001)$; $\dagger$ not significant $(p>0.05)$, according to one-way ANOVA. Means, within a column, followed by the same letter are not significantly different between each other at $p=0.05$ according to Tukey HSD test. Lt3, 4: Tergites 3, 4, longitudinal; Lst3: Sternite 3, longitudinal; Lwm: Wax mirror, longitudinal; Wwm: Wax mirror, transversal; Dwm: Distance between wax mirrors; Lst6:
Sternite 6, longitudinal; Wst6: Sternite 6, transversal. Numbers in brackets are Ruttner numbers.

Table 6: Means and standard deviations of characters of the fore-wing of 10 worker bees each from ( N ) colonies from nine localities in Nigeria.

| Locality | N | Fwl $(17)^{* * *}$ | Fww <br> $(18)^{* * *}$ | Cub-a $(19) \dagger$ | Cub-b $(20) \dagger$ | $\mathrm{A} 4(21) \dagger$ | $\mathrm{B} 4(22) \dagger$ | $\mathrm{D} 7(23) \dagger$ | $\mathrm{E} 9(24)^{*}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Afunori | 5 | 8.04 ab | 2.77 ab | 48.90 | 21.10 | 32.13 | 103.63 | 101.18 | 19.61 ab |
|  |  | 0.14 | 0.06 | 4.25 | 1.04 | 0.67 | 1.65 | 1.77 | 0.98 |
| Maiadua | 3 | 8.50 cd | 2.82 abc | 48.40 | 23.00 | 32.63 | 102.45 | 101.40 | 19.40 ab |
|  |  | 0.18 | 0.10 | 3.22 | 1.34 | 1.36 | 3.95 | 2.06 | 0.61 |
| Sokoto | 5 | 8.51 d | 2.89 c | 47.30 | 21.22 | 32.84 | 105.97 | 101.93 | 19.89 b |
|  |  | 0.13 | 0.05 | 4.31 | 0.45 | 0.33 | 2.67 | 4.03 | 0.70 |
| Okuta | 5 | 8.33 bcd | 2.84 bc | 49.18 | 22.46 | 32.36 | 104.37 | 102.17 | 19.08 ab |
|  |  | 0.05 | 0.02 | 3.17 | 0.62 | 0.61 | 3.53 | 1.52 | 0.67 |
| Gitata | 5 | 8.37 cd | 2.83 bc | 50.61 | 21.63 | 32.03 | 103.04 | 101.43 | 18.26 a |
|  |  | 0.16 | 0.03 | 3.14 | 2.19 | 0.85 | 3.92 | 2.71 | 0.75 |
| Yola | 5 | 8.24 abcd | 2.76 ab | 48.04 | 22.74 | 32.89 | 101.08 | 101.46 | 18.92 ab |
|  |  | 0.09 | 0.02 | 3.06 | 0.79 | 1.94 | 4.42 | 1.90 | 1.02 |
| Abeokuta | 4 | 7.98 a | 2.71 a | 46.64 | 21.13 | 31.24 | 102.85 | 100.98 | 18.98 ab |
|  |  | 0.10 | 0.04 | 1.60 | 2.10 | 0.22 | 1.37 | 1.05 | 0.42 |
| Nguroje | 4 | 8.40 cd | 2.86 bc | 46.95 | 21.89 | 32.77 | 104.97 | 100.64 | 18.74 ab |
|  |  | 0.19 | 0.04 | 1.39 | 2.63 | 0.91 | 2.62 | 1.42 | 0.07 |
| Umuahia | 5 | 8.20 abc | 2.77 ab | 47.06 | 20.68 | 33.05 | 102.00 | 101.65 | 18.64 ab |
|  |  | 0.16 | 0.08 | 4.91 | 0.67 | 1.22 | 2.08 | 3.15 | 0.29 |

237 Table 6 continued

| Locality | N | $\mathrm{G} 18(25)$ <br> $* * *$ | $\mathrm{~J} 10(26) \dagger$ | $\mathrm{J} 16(27)^{*}$ | $\mathrm{~K} 19(28) \dagger$ | $\mathrm{L} 13(29)^{* *}$ | $\mathrm{~N} 23(30)^{* * *}$ | $\mathrm{O} 26(31) \dagger$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Afunori | 5 | 94.17 ab | 52.51 | 90.11 a | 79.29 | 16.04 ab | 86.18 | 36.86 |
|  |  | 2.04 | 0.51 | 1.34 | 2.02 | 0.71 | 1.98 | 2.42 |
| Maiadua | 3 | 95.78 b | 53.20 | 93.25 ab | 78.43 | 15.04 ab | 88.06 | 38.94 |
|  |  | 1.45 | 1.41 | 0.87 | 0.54 | 0.79 | 1.17 | 3.43 |
| Sokoto | 5 | 96.45 b | 52.73 | 91.66 ab | 78.46 | 15.21 ab | 87.07 | 38.22 |
|  |  | 1.18 | 1.24 | 1.69 | 0.98 | 0.43 | 2.63 | 1.10 |
| Okuta | 5 | 95.49 b | 51.96 | 93.64 ab | 77.76 | 14.75 a | 88.07 | 37.56 |
|  |  | 2.55 | 2.21 | 1.79 | 0.69 | 0.67 | 1.13 | 2.20 |
| Gitata | 5 | 94.65 ab | 53.53 | 93.11 ab | 79.63 | 15.50 ab | 88.74 | 38.32 |
|  |  | 0.94 | 2.79 | 2.94 | 1.69 | 0.97 | 1.92 | 2.67 |
| Yola | 5 | 96.98 b | 53.84 | 93.96 ab | 78.75 | 16.38 b | 89.06 | 38.25 |
|  |  | 1.72 | 1.33 | 1.99 | 0.89 | 0.48 | 1.31 | 0.86 |
| Abeokuta | 4 | 97.15 b | 53.42 | 92.26 ab | 79.53 | 16.43 b | 86.29 | 38.11 |
|  |  | 0.16 | 0.75 | 1.12 | 0.47 | 0.46 | 0.69 | 0.13 |
| Nguroje | 4 | 91.79 a | 52.35 | 93.40 ab | 80.44 | 15.44 ab | 90.67 | 37.11 |
|  |  | 0.97 | 2.26 | 1.69 | 0.57 | 0.96 | 1.60 | 2.17 |
| Umuahia | 5 | 96.89 b | 54.61 | 94.52 b | 78.35 | 15.37 ab | 90.69 | 39.66 |
|  |  | 1.99 | 2.03 | 2.37 | 1.81 | 0.49 | 1.69 | 2.44 |

$238 *$ Significant ( $p<0.05$ ); **highly significant ( $p<0.01$ ); ***very highly Significant ( $p<0.001$ ) and †not significant $(p>0.05$ ), according to one-way ANOVA. Means, within a column, followed by the same letter are not significantly different at $p=0.05$ 240 according to Tukey HSD test. Measurements of distance are in mm and of angles in degrees. Fwl: Fore-wing, length; Fww: Fore-

Table 7: Rotated component matrix for PCA, with Varimax rotation, of morphometric characters of $A$. mellifera colonies from Nigeria. Major loadings for each character are shown in bold.

| Character (Ruttner numbers) | Rotated Component 1 | omponent C Component 2 | efficients Component 3 | Communalities |
| :---: | :---: | :---: | :---: | :---: |
| Wax plate(mirror) of sternite 3 , longitudinal diameter (12) | 0.831 | 0.42 |  | 0.924 |
| Sternite 3, longitudinal diameter (11) | 0.806 | 0.31 | 0.38 | 0.89 |
| Sternite 6, transversal diameter (16) | 0.728 | 0.48 | 0.35 | 0.881 |
| Tibia, length (6) | 0.624 | 0.35 | 0.61 | 0.882 |
| Fore-wing, width (18) | 0.347 | 0.85 | 0.32 | 0.944 |
| Fore-wing, length (17) | 0.503 | 0.74 | 0.32 | 0.903 |
| Metatarsus, length (7) | 0.306 | 0.32 | 0.88 | 0.972 | from various sources.


| Character | This study | Ruttner (1988) | Ajao et al. <br> $(2014)$ | Yu et al. <br> $(2012)$ | Oyerinde et al. <br> $(2012)$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Cover hair, length | $0.14 \pm 0.01-0.19 \pm 0.01$ | $0.20 \pm 0.02-0.26 \pm 0.04$ |  |  |  |
| Fore-wing, length | $7.98 \pm .0 .10-8.51 \pm 0.13$ | $8.13 \pm 0.19-8.95 \pm 0.37$ | $9.54 \pm 0.01 \dagger$ | $8.42 \pm 0.66$ | $3.43 \pm 0.16 \dagger$ |
| Fore-wing, width | $2.71 \pm 0.04-2.89 \pm 0.05$ |  |  | $3.13 \pm 0.21$ | $1.18 \pm 0.08 \dagger$ |
| Tergites 3+4, | $3.81 \pm 0.06-4.08 \pm 0.05$ | $3.92 \pm 0.10-4.25 \pm 0.21$ | $3.80 \pm 0.28$ |  |  |
| diameter |  |  |  |  |  |
| Angle J16 | $90.11 \pm 1.34-94.52 \pm$ | $86.44 \pm 8.37-96.76 \pm 3.60$ |  |  |  |
|  | 2.37 |  |  |  |  |
| Pigmentation, | $6.02 \pm 0.24-7.40 \pm 0.37$ | $0.35 \pm 0.73-6.77 \pm 0.96$ |  |  |  |
| scutellum |  |  |  |  |  |

$\dagger$ The validity of these values is doubtful because they fall outside the range reported for all subspecies of A. mellifera. For example, in respect of the length of the fore-wing, the smallest value, $8.13 \pm 0.19 \mathrm{~mm}$, was reported for $A$. m. jemenitica and the highest value, $9.33 \pm 0.11 \mathrm{~mm}$, for $A$. m. mellifera (Ruttner 1988). Measurements of distance are in mm and of angles in degrees.

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