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Full Length Research Paper

Morphometric and landmark based variations of *Apis mellifera* L. wings in the forest vegetation zone of Nigeria

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There are several different methods of classifying insect. The technique of using wing landmarks and morphometric features to discriminate insect population into species and specify the variations in the varieties within the intra specific group was used to classify *Apis mellifera* kept in beekeeping practice in the forest vegetation zone of Nigeria into morphoclusters. The multivariate morphometric data obtained was analysed with parametric statistic tools of mean, standard deviation and standard error. The distribution and relation between them were subjected to two step cluster analysis. Morphoclusters means were presented in centroids and also the simultaneous confidence intervals (95%) of means values of wing morphometric and landmarks were expressed. Varieties of *Apis mellifera* found in this study formed four distinct morphoclusters based on wing landmarks and morphometric. Osun State recorded morphoclusters 1 and 2 while Ebonyi and Oyo States had morphoclusters 3 and 4 respectively. 20 landmarks that occurred on the forewing of morphoclusters 1, 3 and 4 were the same while the record of 19 landmarks observed in honeybees of morphoclusters 2 was different from the existing records. Based on our findings in this study it is reliable to use the variations on the wing landmarks and morphometric of honeybee workers to group *Apis mellifera* in Nigeria into varieties of existing species.

Keywords: *Apis mellifera*, wing landmarks, morphometric features, cluster analysis.

INTRODUCTION

Taxonomy of insect entails the use of evolutionary relationships to classify insect into appropriate group. There are several different methods of classifying insect. In any of these methods of classification, a taxonomist can be specified in several different ways (Liverpool, 2008). Currently, there are only seven recognized species of honeybee with a total of 44 subspecies (Engel, 1999) though historically, anywhere from six to eleven species have been recognized. The European or Western honeybees are known as *A. mellifera*. *A. mellifera* is the most commonly domesticated species of honeybees. It seems to have originated in Eastern Tropical Africa and spread from there to Northern Europe and eastwards into Asia to the Tien Shan range (Engel, 1999). There are many subspecies that have adapted to the local geographical and climate environment.

The development of tools for identification of insect species is germane, to ascertain the phylogeny relationship and biodiversity of the existing species of insect in a given ecology. Of note is the fact that some studies have used the morphology aspects to identify insect species (Greenberg and Szyska, 1984; Amorim and Ribeiro, 2001) by using morphological criteria, which readily identify some insect specimens to the generic level. In addition, the use of variations in the wing morphology of insect also produced remarkable result in identification of insect species based on different in the wing morphology alone; several authors have successfully used this type of information to discriminate insect population into species and specify the variations in the varieties within the intra specific group (Tofilski, 2004; Steinhage *et al*., 2007; Mendes *et al*., (2007).

The need to determine the variations in the species of

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Table 1. Cluster Distribution of Ecotype *Apis mellifera* in the Forest Zone of Nigeria

Cluster	No	% of Combined honeybee sample	% of Total
1	126	126	28.0%
2	24	24	5.3%
3	150	150	33.3%
4	150	150	33.3%
Combined	450	450	100.0%
Total	450		450

honeybee in Nigeria is crucial as controversy over the species of honeybees in Nigeria has been engendered by apparent discordance of records on species/subspecies of honeybees that are kept in beekeeping practice in the country. Also, the influence of geographical location on the distribution of honeybee species remains elusive in Nigeria. In this study we examined the morphological variations of *Apis mellifera* collected from three different states in the forest vegetation zone of the country in order to determine the differences within the ecotype honeybees in the nation.

MATERIAL AND METHODS

Study Site and Collection of Sample

Random samples of 4500 honeybee workers were collected from 150 colonies situated in the forest vegetation zone of Nigeria. Samples of 30 honeybee workers were collected from 50 colonized hives sited in established honeybee farms in Igbeti (Oyo State), Ishiagwu (Ebonyi State) and Oshogbo (Osun State) of Nigeria. All samples were taken from colonies located in apiaries initiated with captured swarms and unmanaged for queen replacement. Samples of the bee collected were stored separately in 70% ethanol in a small labelled container according to their state of collection. These were used for multivariate morphometric analysis in the laboratory, to determine the variations in the specie of honeybees collected from colonies in the forest vegetation zone of the country.

Morphometric Studies

In the laboratory multivariate morphometric analyses was performed on ten randomly selected samples of honeybee workers obtained from the three states. This was done based on methods used in morphometric analyses of *A. mellifera* (Andere *et al.,* 2008) and use of wing landmarks in classifying bumble bees into races (Aytekin, *et. al.*, 2007).

All measurement was taken with the aid of calibrated hand held digitalised MiScope microscope with magnification range of 40-140x in millimetres and replicated trice. The following variables were measured on the right fore and hindwings of the honeybee samples: The length of the hindwing (LHW), width of the hindwing (WHW), length of the forewing (LFW), width of the forewing (WFW), number of landmarks on the forewing (NLF), number of landmarks on the hindwing (NLH), number of landmarks on the radial cell of the forewing (NLR), length of radial cell (LRC) and width of radial cell (WRC) were the experimental variables. Each morphometric feature measured was carefully recorded per hive and all readings served as raw data for the statistical analysis.

Data Analysis

Data obtained from the multivariate morphometric studies was analysed with SPSS statistic 17 software. The analysis involved parametric statistic tools of mean, standard deviation and standard error. Also, the distribution and relation between them were subjected to two step cluster analysis. Morphoclusters means were presented in centroids and also the simultaneous confidence intervals (95%) of means of wing landmarks and morphometric variables were expressed in charts.

RESULTS

Table 1 showed the morphoclusters distribution of ecotype *Apis mellifera* obtained in the forest vegetation zone of Nigeria. Bees collected from the different states were classified into four distinct morphoclusters based on their wing morphometric features and landmark differences. Morphoclusters 3 and 4 recorded 33% of the overall total of experimental samples while morphoclusters 1 and 2 had 28% and 5.3% respectively. Also, the distribution of the respective morphoclusters in the studied state revealed (Table 2) the occurrence of bees in morphoclusters 1 and 2 in Osun State while

Table 2. Within Cluster Percentage of State of *Apis mellifera* in the Forest Zone of Nigeria

	Ebonyi State		Osun State		Oyo State	
Cluster	**Frequency**	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**
1	0	.0%	126	84.0%	0	.0%
2	0	.0%	24	16.0%	0	.0%
3	150	100.0%	0	.0%	0	.0%
4	0	.0%	0	.0%	150	100.0%
Combined	150	100.0%	150	100.0%	150	100.0%

Table 3. Centroids of *Apis mellifera* Morphoclusters in the Forest Zone of Nigeria

Cluster	LHW Mean[a]	WHW Mean[a]	LFW Mean[a]	WFW Mean[a]	NLF Mean[a]	NLH Mean[a]	NLR Mean[a]	LRC Mean[a]	WRC Mean[a]
1	2.38±.10	.65±.06	3.50±.08	1.17±.06	20.00±.00	5.63±.49	5.00±.00	1.27±.05	0.20±.02
2	2.25±.18	.96±.83	3.28±.49	1.14±.07	19.13±.34	5.42±.50	5.00±.00	1.27±.03	0.19±.01
3	2.48±.10	.66±.07	3.72±.11	1.23±.08	20.00±.00	5.75±.44	5.00±.00	1.34±.06	0.20±.02
4	2.45±.09	.65±.05	3.66±.15	1.18±.06	20.00±.00	5.66±.48	5.00±.00	1.30±.04	0.18±.02
Combined	2.43±.11	.67±.21	3.61±.20	1.19±.07	19.95±.21	5.67±.47	5.00±.00	1.30±.06	0.19±.02
±SEM	.01	.01	.01	.00	.02	.01	.00	.00	.00

[a]=mean +standard deviation, Length of Hindwing (**LHW),** Width of Hindwing (**WHW),** Length of Forewing **(LFW),** Width of Forewing (**WFW),** No of Landmarks on Forewing (**NLF),** No of Landmarks on Hindwing (**NLH),** No of Landmarks on Radial Cell (**NLR),** Length of Radial Cell (**LRC),** Width of Radial Cell (**WRC)**

Ebonyi State and Oyo State had bees of morphoclusters 3 and 4 respectively.

Mean values of the morphometric features examined, as well as, the landmarks observed on the wings of the ecotype *Apis mellifera* were extracted in centroid (Table 3). Morphoclusters 3 recorded the highest mean values in LHW (2.48), LFW (3.72), WFW (1.23) and LRC (1.34) while 0.20mm WRC that occurred in morphoclusters 1and 3 varied with the 0.19mm and 0.18mm recorded in morphoclusters 2 and 4 respectively. The NLR (5) observed on all the radial cell of the forewings were uniform. Also, the means of NLH (5.42) and NLF (19.13) were lowest in morphoclusters 2. The simultaneous intervals (95%) for mean values (Figure 1) showed wider range in data recorded in all the morphometric features, as well as, wing landmarks in morphoclusters 2 except in the NLR (5).

DISCUSSION

Cluster distribution of *Apis mellifera* into four ecotype varieties as a result of wing morphometric features and landmarks variations was indicative of differences in the type of honeybee raised in beekeeping practice in the forest vegetation zone of Nigeria. The relative wing morphometric features and landmarks differences confirm the earlier biodiversity of honeybee species as was reported in earlier study in Nigeria (Hussein, 2000). The order of preponderance of the various bee varieties within the forest vegetation zone of Nigeria was morphoclusters 2< morphoclusters 1< morphoclusters 3= morphoclusters 4.

The mean values recorded in the morphometric analysis of the fore and hindwings in this study disagrees with the range of means of morphometric features of *Apis mellifera* as earlier reported by David (2008). The number of landmarks observed on the hindwings of the four honeybee morphoclusters the zone revealed in this study were quite different with earlier studies on bumble bees (Aytekin, *et. al*., 2007) thus conforming to the variance in the morphometric features of insects of different species. The number of landmarks (approximately 20) that occurred on the forewing of morphoclusters 1, 3 and 4 (Plate 1) were the same with the findings on bumble bees (Aytekin, *et. al*., 2007) while the record of approximately 19 landmarks (Plate 2) observed in honeybees of morphoclusters 2 was different from the existing records. Also the existence of 5 landmarks on all the radial cells of honeybee workers examined conform to the result of using morphometric differences of a singles cell in classifying *Apis mellifera* into racial types (Francoy, *et. al*., 2006).

Of note is the wide variations recorded in nearly all the simultaneous confidence intervals of means of morphometric features and landmarks in morphoclusters 2. This showed that there are large difference in the morphometric and landmarks of honeybees of this

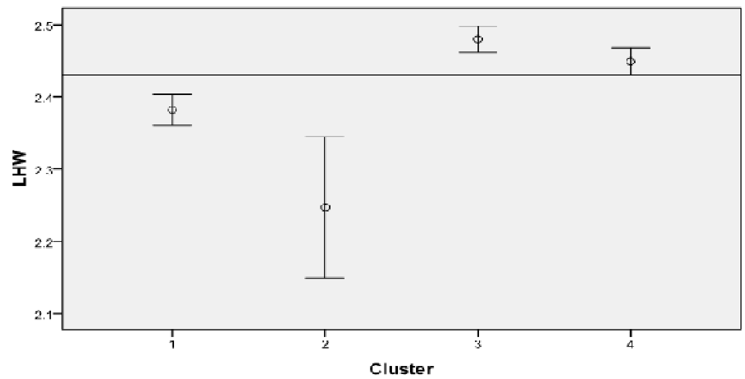

Figure 1a. Within Cluster Variation of Means of Length Hindwing (LHW)

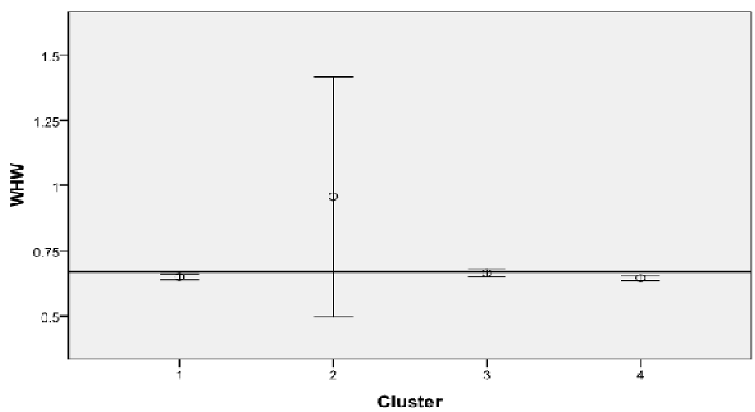

Figure 1b. Within Cluster Variation of Means of Width Hindwing (WHW)

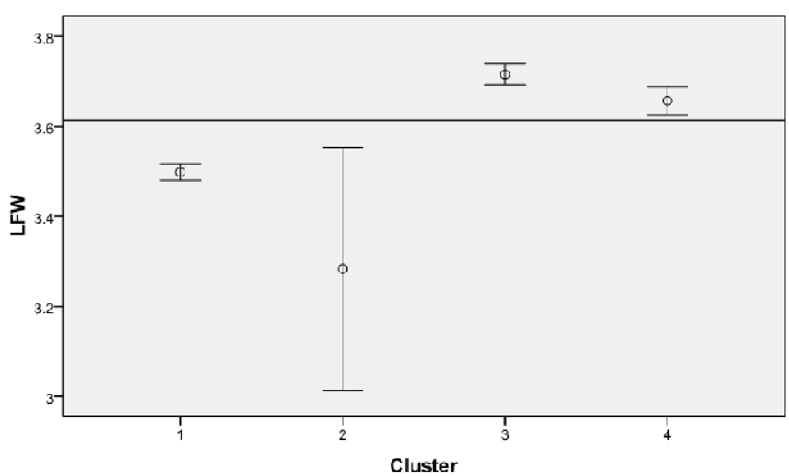

Figure 1c. Within Cluster Variation of Means of Length Forewing (LFW)

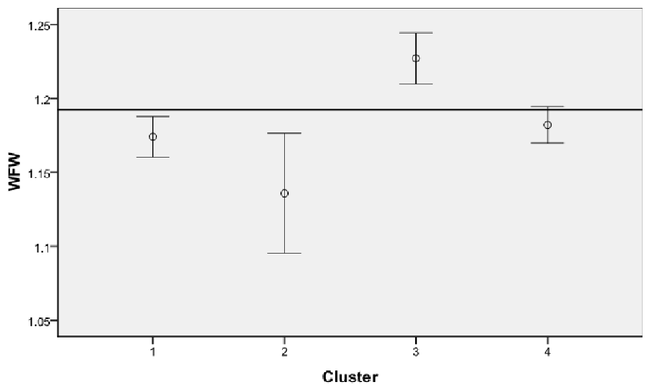

Figure 1d. Within Cluster Variation of Means of Width Forewing (WFW)

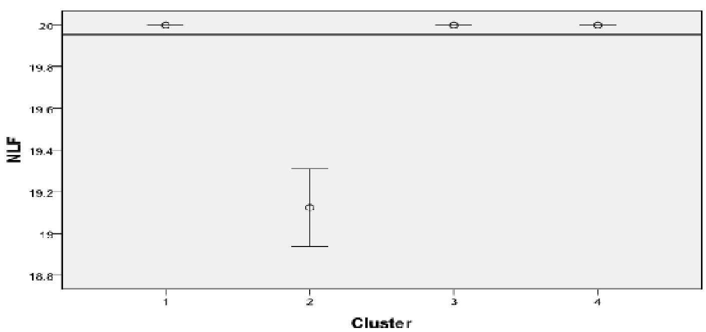

Figure 1e. Within Cluster Variation of Means of Number of Landmarks on Forewing (NLF)

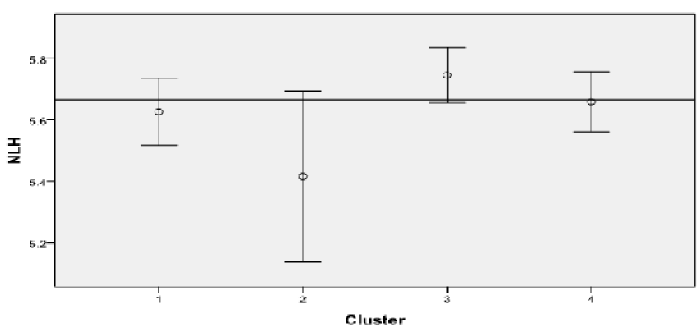

Figure 1f. Within Cluster Variation of Means of Number of Landmarks on Hindwing (NHF)

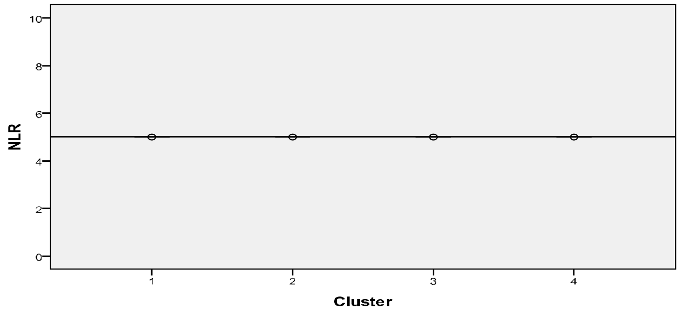

Figure 1g. Within Cluster Variation of Means of Number of Landmarks on Radial Cell (NLR)

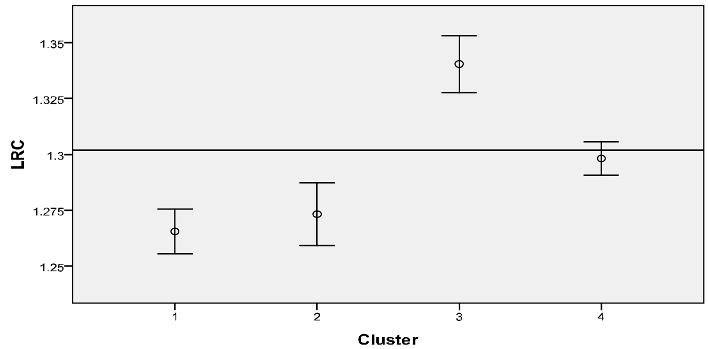

Figure 1h. Within Cluster Variation of Means of Length Radial Cell (LRC)

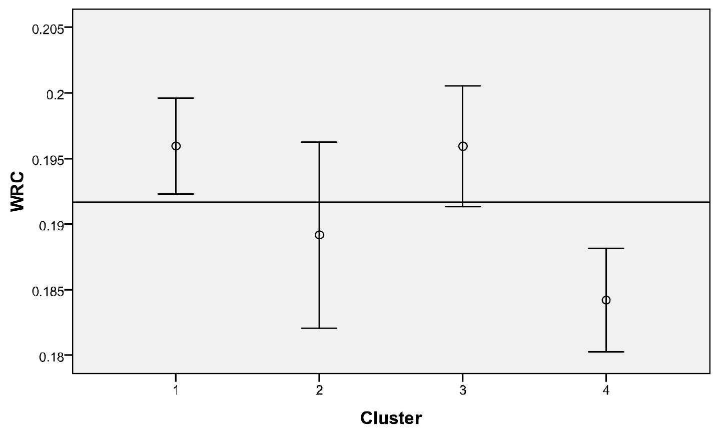

Figure 1i. Within Cluster Variation of Means of Width Radial Cell (WRC)
Figure 1. Simultaneous 95% Confidence Intervals of Means of Mophoclusters Morphometric and Landmarks Variables

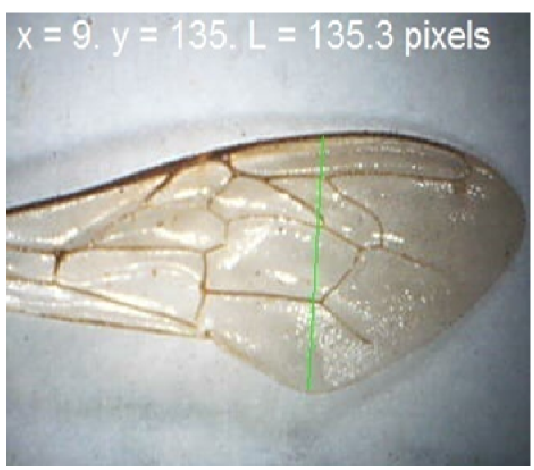

Plate 1. Twenty Landmarks on Forewing

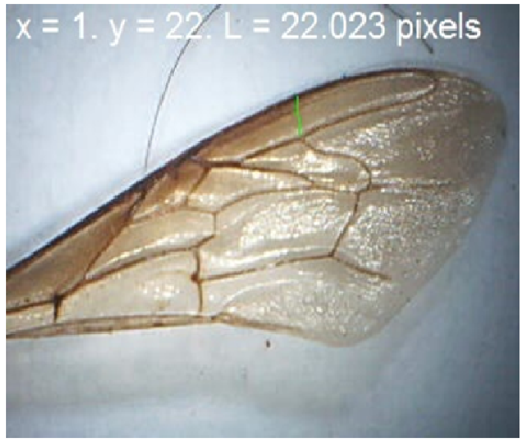

Plate 2. Nineteen Landmarks on Forewing

cluster. Thus, in turn portrays *Apis mellifera* of the morphoclusters 2 as the most diverge out of the four morphoclusters.

CONCLUSION

Based on our findings in this study, it is reliable to use the variations of the wing landmarks and morphometric features of honeybee workers in grouping *Apis mellifera* kept in beekeeping practice in Nigeria into varieties of existing species. Further research into the specific genomic information of the various morphoclusters need be encouraged, as this will go a long way to trace the phylogeny relationship of the different honeybee morphoclusters in the forest vegetation zone of Nigeria.

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