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Genetic diversity of four populations of honey bee, *Apis mellifera* (Linnaeus, 1758) from two vegetation zones in Nigeria

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Genetic diversity of four populations of honey bee, *Apis mellifera* from two vegetation zones in Nigeria namely; southwest rainforest and northcentral derived savanna was analysed using fifteen morphometric characters and five microsatellite loci. Discriminant function analysis (DFA) of the morphometric data revealed a considerable variation of morphological characters between the sampled localities while Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) produced overlapping clusters of the populations sampled indicating lack of separation between the various populations. The genetic diversity (FST) revealed low differentiation among populations suggesting that geographic distance was not an impediment to gene flow among populations. The overall FIT value indicated that the four populations have a deficiency of heterozygotes suggesting the presence of inbreeding within populations. The analysis of molecular variance (AMOVA) showed that 91% of the total molecular variance existed within the populations while 9% existed among populations, indicating low inter population genetic variation. It is suggested that there is an apparent loss of genetic diversity in the populations of *A. mellifera* studied in the two vegetation zones of Nigeria. This could have implication for the health and stability of these bee populations.

Key words: Biodiversity, genetic diversity, honey bees, morphometry, microsatellite, population genetics.

INTRODUCTION

The true honey bee, *Apis mellifera* L. is known to be one of the most economically valuable insects because of its honey production and pollinating activities (Lawal and Banjo, 2010). The services of bees and other pollinators to agriculture is estimated to be between $235- $577 billion per year worldwide (FAO, 2018). The *A. mellifera* originated from Africa (Whitfield et al., 2006), and is naturally distributed to Europe and Asia (Howpage, 1991; Nedic et al., 2011). It was introduced into America and Australia by humans (Tunca and Kence, 2011). The species is found on every continent except Antarctica, that is, all the habitats on the planet that contain insect-pollinated flowering plants. Approximately forty-three (43) subspecies based on geographic variations are recognized (Engel, 1999). The subspecies are divided into four major branches, based on work by Ruttner (1988) and confirmed by mitochondrial DNA analysis (Smith, 1991; Garnery et al, 1992; Palmer et al., 2000)

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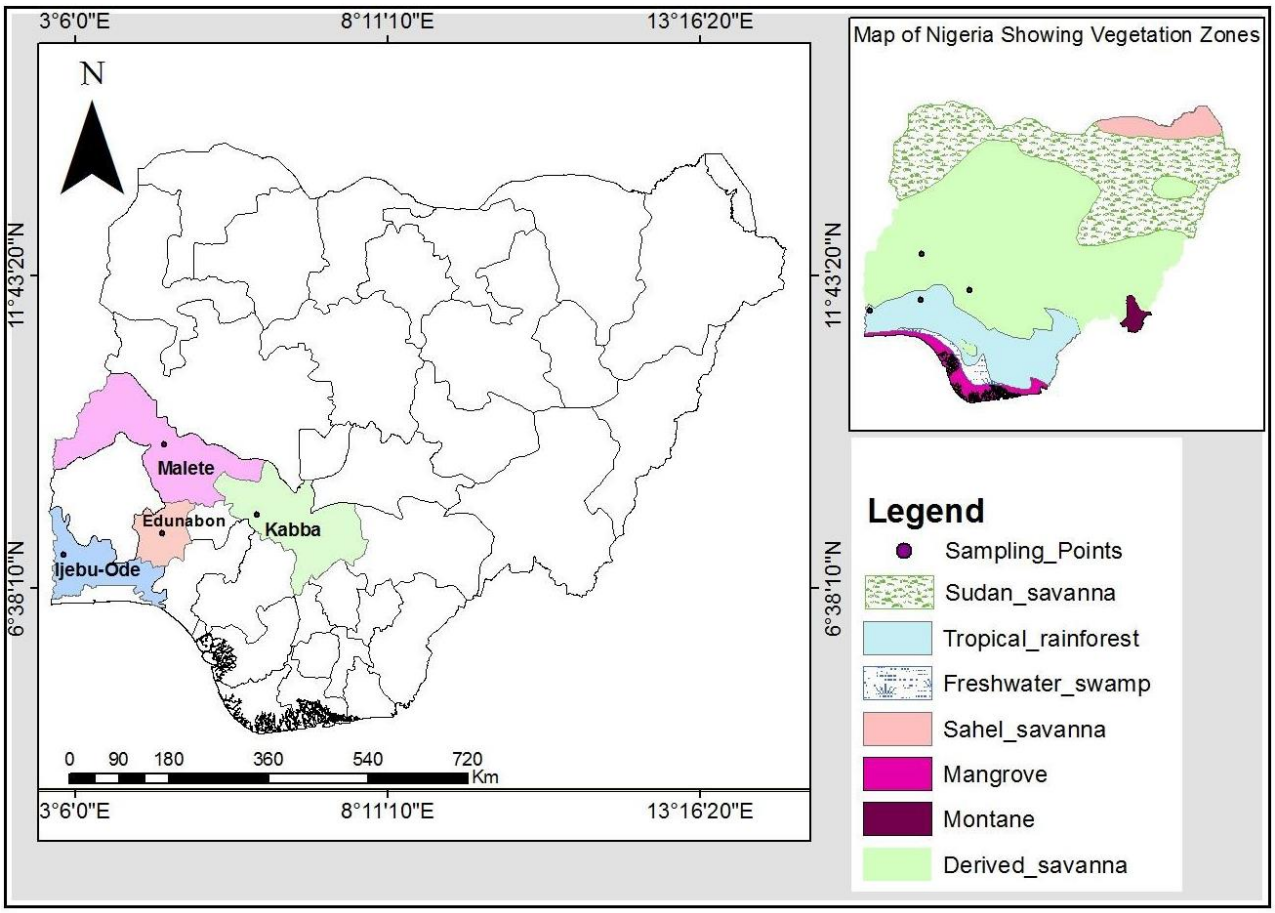

Figure 1. Map showing the vegetation zones and sampling locations of *Apis mellifera.*

and Single Nucleotide Polymorphism (Whitfield et al., 2006).

Recently, honey bee populations all over the world have been reported to be on the decline due to Colony Collapse Disorder (CCD). Akinwande et al. (2013) reported a decline in the number of honey bee colonies from selected apiaries in Southwestern Nigeria. There were 58.34, 44.84 and 40.61 average percentage declines in colony establishment in Lagos, Ogun and Osun States respectively. The presence of pests and diseases, pesticide poisoning, lack of queen rearing, poor hive and seasonal management were suggested to be the major factors responsible for the annual decline in honey bee colony establishment. Oyerinde and Ande (2009) reported that 15.01% of the 2000 installed bee hives in Kwara State, Nigeria had established bee colonies. Some biotic factors were suggested to have been responsible for the rather low bee colonizing record of the state. There is little information about the genetic diversity of honey bee populations in Nigeria. This study provides information on the genetic diversity within and between populations of *A. mellifera* which will be useful for taxonomic re-evaluation of the species for subsequent conservation efforts.

MATERIALS AND METHODS

Sampling

Samples of workers of *A. mellifera* were collected randomly from 28 colonies in two vegetation zones in Nigeria namely: the Tropical Rainforest (Edunabon in Osun State and Ijebu-Ode in Ogun State) and the Derived Savanna (Kabba in Kogi State and Malete in Kwara State). Figure 1 is the map of the study area showing the sampling sites with the map of Nigeria inset. Sampling was carried out in apiaries which do not practice migratory beekeeping, and the hives sampled were stationary during the sampling period. Seventy specimens from each location were sacrificed in ether vapor, and then preserved in 90% ethanol for morphometric studies. Seven specimens from each location were preserved in sample bottles containing 90% ethanol for genomic DNA extraction. Identification was done with a dissecting binocular microscope, using bee identification keys of Michener (2007).

Morphometric analysis

A total of 28 colonies were subjected to morphometric analysis. Ten worker bees per colony were dissected and measured for 15

morphometric characters according to Ruttner et al. (1978) using a dissecting binocular microscope and vernier calliper. The details of the characters measured are shown in Table 1.

Microsatellites analysis

Genomic DNA was extracted from the thorax of seven (7) worker bees per population using the CTAB (Cetyl Trimethyl Ammonium Bromide) method. The isolated DNA was analysed in a thermocycler using five microsatellite primers (A024, A028, A043, A088 and A113) selected according to Genebank which had been previously used by Franck et al. (2001). Polymerase chain reactions were carried out in standardized 10 µL reaction mixture containing 0.1 mM of each deoxyribonucleoside triphosphate (dATP, dCTP, dGTP, dTTP), gel loading buffer, stabilizers, 0.3 µl each of the forward and reverse primer sets, 1.5 mM$MgCl_2$, $(NH_4)_2SO_4$, 0.5 µl Taq polymerase in 1X buffer, 5.9 µl of PCR grade water, and 20 ng total genomic DNA. The mixture was incubated in a GeneAmp PCR thermocycler programmed as follows: 30 cycles each of denaturation, annealing and extension temperature at 95°C for 20 s, 58-60°C for 25 s and 72°C for 45 s respectively and a primer extension temperature of 72°C for 60 s followed by final extension temperature at 72°C for 10 min to complete the amplifications. The amplicons generated were then subjected to electrophoresis on 1.4% Agarose gel and visualized by staining with ethidium bromide.

Statistical analysis

Principal Component Analysis (PCA), Canonical Variate Analysis (CVA), Discriminant Function Analysis (DFA) and Cluster Analysis on morphometric data of the honeybee populations were performed using the software, PAST (Hammer et al., 2006). Data generated from microsatellite studies were analysed using GenAlex 6.502 Software (Peakall and Smouse, 2006, 2012). The total number of alleles, allele frequencies, average number of alleles per locus, observed (Ho) and expected heterozygosity (He) for each population across the loci, were estimated. Analysis of Molecular Variance (AMOVA), fixation indices (FST, FIT and FIS), degrees of heterozygosity and polymorphism, mean gene flow and Hardy-Weinberg Equilibrium (Nei, 1978) were also estimated. Phylogenetic tree was constructed using PHYLIP-3.695 (Felsentein, 2014).

RESULTS

Morphometric studies

The average values, range and standard deviation of all the morphometric characters measured are shown in Table 2. Wide range of sizes (Standard deviation, SD) especially on proboscis length (PL) and right forewing length (RFL) were found in samples from Edunabon and Ijebu-Ode.

Principal Component Analysis (PCA) of the 15 morphometric measurements of *A. mellfera from* the four study areas (Figure 2) showed overlapping of all the four clusters produced. The CVA plot (Figure 3) showed overlapping of clusters of specimens from the different populations studied. Figure 4 shows the respective morphometric characters and their loadings on PC1, which indicated that proboscis length is the main characteristic responsible for variation among the four populations of *A. mellifera* studied (loading, 0.5294), while right forewing length has the second (0.4913) heaviest loading. Proboscises were shown to be longer in the Rainforest than in the Derived Savanna. Similarly, size of tibia and metatarsus were longer in the Rainforest than in the Derived Savanna. Inter-locality variations in wing characteristics were observed which have also been reported by Sharma (1983), and Tahmasebi et al. (2002).

Discriminant Function Analysis (DFA) showed no significant difference between specimens from Edunabon and Ijebu Ode with individuals from both locations overlapping along the discriminant function plot. Also, only 84.3% of the specimens concurred with their *a priori* classification showing that the discriminant function did not recognize any significant difference among the specimens based on their locations. Significant differences were observed between specimens collected from Edunabon and Kabba, Edunabon and Malete, Ijebu-Ode and Kabba, Ijebu-Ode and Malete, and Kabba and Malete with individuals from each paired location clearly separated along the discriminant function plots. Moreover, 98.57, 99.29, 97.14, 93.57 and 98.57% of the specimens concurred with their *a priori* classification. Similarly, DFA showed significant difference between specimens collected from the Rainforest and Derived Savanna zones of Nigeria with individuals from both zones clearly separated along the discriminant function plot. Moreover, most of the specimens (95.36%) concurred with their *a priori* classification. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram revealing the clustering pattern of the specimens of *A. mellifera* across the two vegetation zones is shown in Figure 5.

Microsatellite DNA studies

The mean heterozygosity for the samples across all loci was 50% (Table 3) while the average number of alleles observed (Na) for the total population was 3.450. Unbiased expected heterozygosity (uHe) ranged from 0.830 to 0.997 with a mean value of 0.902±0.118. With a 95% threshold, the percentage of polymorphic loci at the population level was 100%. Observed heterozygosity (Ho) per locus had values of 0.500 each while the expected heterozygosity (He) per locus ranged from 0.770 in locus A043 to 0.836 in locus A024 (Table 3). Shannon's Information Index (I) ranged from 0.624 in locus A024 to 0.862 in locus A028 with an average value of 0.731±0.072.

Genetic diversity parameters based on allelic frequencies are also shown in Table 3. Observed heterozygosity (Ho) per population had values of 0.500 each while the expected heterozygosity (He) per population ranged from 0.766±0.080 for population 4 to 0.838±0.140 for population 3. The mean Ho and mean He were 0.500±0.000 and 0.800±0.146 respectively. The heterozygosity level within a subpopulation (FIS), the

Table 1. List of characters measured for morphometry.

Body part	Character	
Proboscis	Length of proboscis	PL
Antenna	Flagellum length	FL
	Antenna length	AL
Head	Head capsule length	HL
	Head capsule width	HW
Wing	Right forewing length	RFL
	Right forewing width	RFW
	Hind wing length	HWL
	Hind wing width	HWW
Hind leg	Femur length	FEL
	Tibia length	TL
	Tibia width	TW
	Metatarsus length	ML
	Metatarsus width	MW
Thorax	Thorax length	THL

Table 2. The range of mean (R), mean ($\bar{X}$), and standard deviation (SD) of each morphometric variable of *Apis mellifera* from the four populations.

Location	Statistical parameters	Edunabon (N = 70)	Ijebu Ode (N = 70)	Kabba (N = 70)	Malete (N = 70)
PL	R	1.2	0.9	0.6	0.8
	$\bar{X}$	5.682857	5.894286	5.882857	5.58
	SD	0.265925	0.204929	0.086764	0.173289
FL	R	0.6	0.5	0.4	0.2
	$\bar{X}$	2.497143	2.548571	2.455714	2.52
	SD	0.129628	0.123644	0.08277	0.069366
AL	R	0.5	0.5	0.3	0.4
	$\bar{X}$	4.045	4.075714	3.852857	4.011429
	SD	0.1280144	0.10555	0.079348	0.067121
HL	R	0.6	0.4	0.2	0.2
	$\bar{X}$	3.645714	3.554286	3.515714	3.46
	SD	0.1500586	0.069545	0.073496	0.066811
HW	R	0.6	0.4	0.2	0.4
	$\bar{X}$	3.357143	3.318571	3.352857	3.282857
	SD	0.119869	0.090558	0.055746	0.072174
RFL	R	1.1	1	0.7	0.9
	$\bar{X}$	8.534286	8.568571	8.598571	8.584286
	SD	0.259255	0.22876	0.17067	0.210338
RFW	R	0.7	0.3	0.3	0.4
	$\bar{X}$	2.819429	2.865714	2.875714	2.798571
	SD	0.150129	0.099106	0.084159	0.089269

Table 2. cont'd

	R	1.3	0.5	0.7	0.4
HWL	$\bar{X}$	5.811429	5.914286	5.817143	5.841429
	SD	0.248207	0.112012	0.140363	0.094013
	R	0.7	0.4	0.4	0.5
HWW	$\bar{X}$	1.651429	1.63	1.597143	1.63
	SD	0.164839	0.093793	0.10352	0.101224
	R	0.7	0.7	0.2	0.2
FEL	$\bar{X}$	2.29	2.425714	2.37	2.334286
	SD	0.164317	0.109922	0.057357	0.058695
	R	0.5	0.3	0.3	0.4
TL	$\bar{X}$	2.875714	2.88	2.718571	2.675714
	SD	0.120909	0.105775	0.096748	0.106914
	R	0.5	0.4	0.2	0.2
TW	$\bar{X}$	1.104286	1.145714	1.001429	1.031429
	SD	0.119705	0.075538	0.052455	0.052593
	R	1	0.6	0.3	0.3
ML	$\bar{X}$	1.915714	1.98	1.871429	1.94
	SD	0.207564	0.107137	0.081903	0.089118
	R	0.4	0.4	0.2	0.1
MW	$\bar{X}$	1.098571	1.112857	0.982857	0.964286
	SD	0.09999	0.100609	0.048068	0.048262
	R	0.7	0.6	0.5	0.3
THL	$\bar{X}$	3.431429	3.478571	3.437143	3.448571
	SD	0.156541	0.121456	0.115685	0.067551

PL= Proboscis Length, FL= Flagellum Length, AL= Total Antenna Length, HL= Head Length, HW= Head Width, RFL= Right Forewing Length, RFW= Right Forewing Width, HWL= Hind Wing Length, HWW= Hind Wing Width, FEL= Femur Length, TL= Tibia Length, TW= Tibia Width, ML= Metatarsus Length, MW= Metatarsus Width and THL= Thorax Length.

Table 3. Basic indicators of allelic variations across loci and populations.

Pop	% Polymorphism	Parameter	A024	A028	A043	A088	A113	Mean
	100.000	Na	5.000	5.000	5.000	4.000	4.000	4.600 (0.120)
		H_o	0.500	0.500	0.500	0.500	0.500	0.500 (0.000)
Edunabon		H_e	0.890	0.750	0.750	0.750	0.844	0.797 (0.140)
		uH_e	0.857	0.833	0.833	0.857	0.964	0.895 (0.156)
		F	0.438	0.333	0.333	0.333	0.408	0.369 (0.054)
	100.000	Na	4.000	4.000	3.000	2.000	3.000	3.200 (0.240)
		H_o	0.500	0.500	0.500	0.500	0.500	0.500 (0.000)
Ijebu-Ode		H_e	0.844	0.906	0.750	0.750	0.750	0.800 (0.156)
		uH_e	0.964	0.997	0.833	0.900	0.900	0.919 (0.136)
		F	0.408	0.448	0.333	0.333	0.333	0.371 (0.112)
	100.000	Na	3.000	1.000	5.000	3.000	5.000	3.400 (0.142)
		H_o	0.500	0.500	0.500	0.500	0.500	0.500 (0.000)
Malete		H_e	0.861	0.750	0.830	0.861	0.890	0.838 (0.140)
		uH_e	0.964	0.840	0.922	0.964	0.989	0.936 (0.576)
		F	0.419	0.333	0.398	0.419	0.438	0.401 (0.105)

Table 3. Cont'd

	100.000	Na	1.000	1.000	2.000	4.000	5.000	2.600 (0.273)
		H_o	0.500	0.500	0.500	0.500	0.500	0.500 (0.000)
Kabba		H_e	0.750	0.750	0.750	0.750	0.830	0.766 (0.080)
		uH_e	0.833	0.833	0.840	0.857	0.922	0.857 (0.089)
		F	0.333	0.333	0.333	0.333	0.398	0.346 (0.065)
Total		% Polymorphism		Na	H_o	H_e	uH_e	F
Mean	100.000			3.450 (0.214)	0.500(0.000)	0.800(0.146)	0.902(0.118)	0.372 (0.243)

N_a = No. of observed alleles; H_o = Observed Heterozygosity; H_e = Expected Heterozygosity; uH_e = Unbiased Expected Heterozygosity; F = Fixation index; Standard deviation values are shown in parentheses.

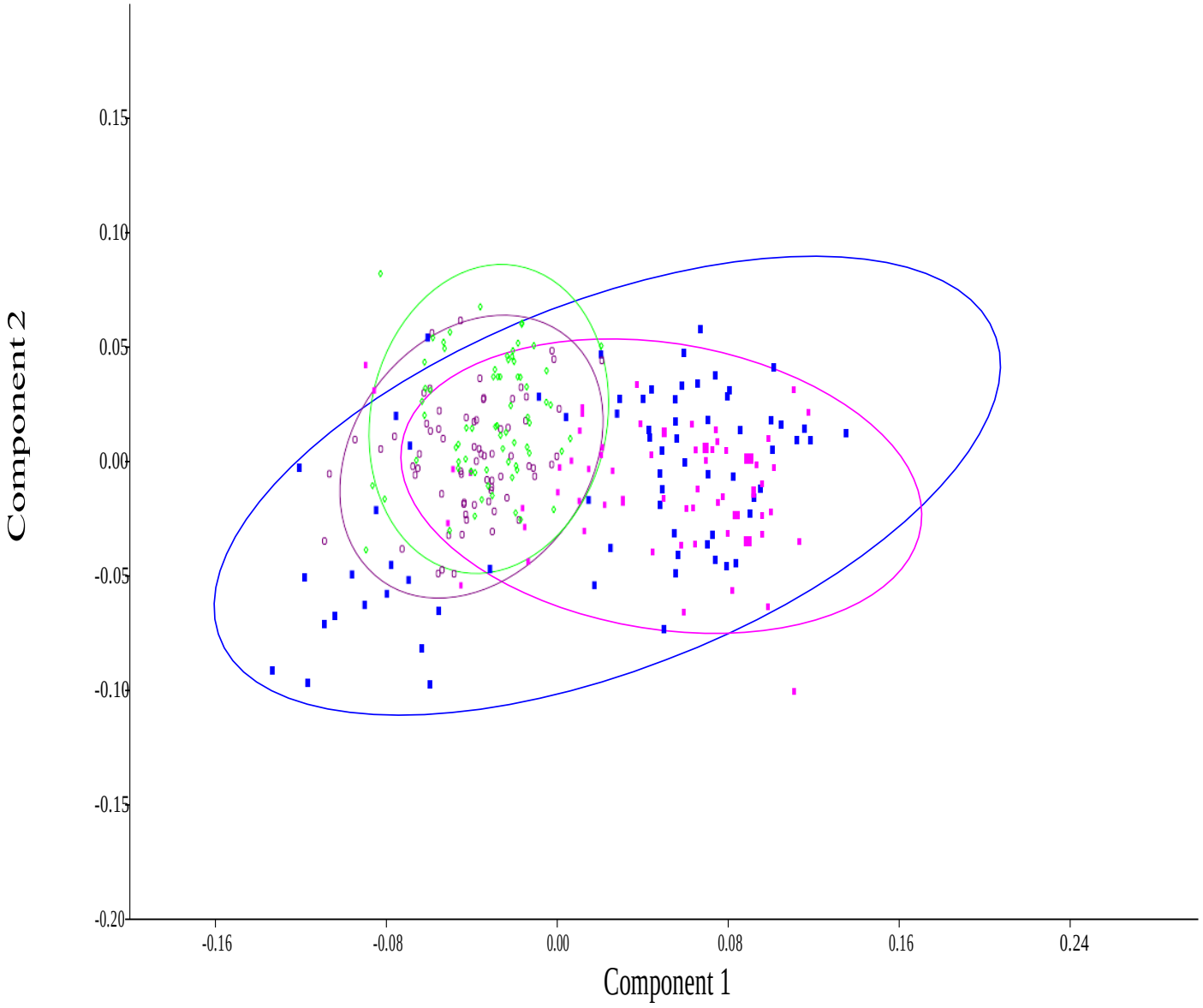

Figure 2. Scatter plot of principal component analysis based on 15 morphological characters of *A. mellifera* from Edunabon (Blue), Ijebu-Ode (Pink), Kabba (Purple) and Malete (Green).

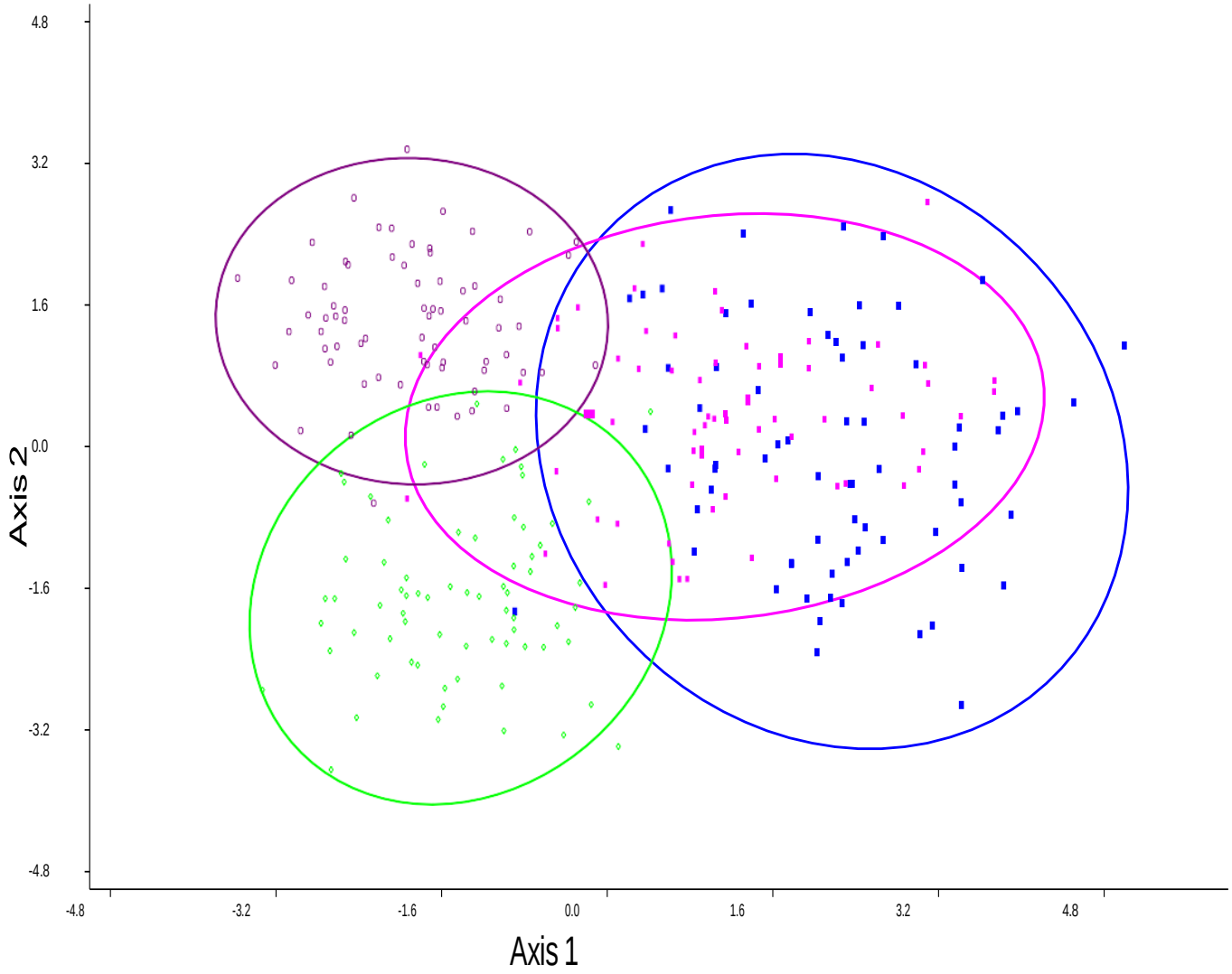

Figure 3. Scatter plot of Canonical Variate Analysis based on 15 Morphological Characters of *A. mellifera* from Edunabon (Blue), Ijebu-Ode (Pink), Kabba (Purple) and Malete (Green).

Table 4. Genetic differentiation across loci.

Locus	FIS	FIT	FST	Nm
A024	0.402	0.494	0.047	5.069
A028	0.366	0.397	0.062	3.782
A043	0.351	0.347	0.060	3.917
A088	0.358	0.391	0.030	8.083
A113	0.396	0.454	0.041	5.848
Mean	0.375 (0.103)	0.407 (0.103)	0.048(0.241)	5.340(0.432)

FIS = Inbreeding coefficient within individuals according to Wright (1978); FIT= Inbreeding coefficient at total sample level; FST = Degree of genetic differentiation of subpopulations; Nm = Mean gene flow; Standard deviation values are shown in parentheses.

heterozygosity level in total populations (FIT) and the degree of genetic differentiation of subpopulations (FST) are presented in Table 4. All 5 loci illustrated deficiency of heterozygotes in the four populations. The mean FIT amounted to 0.407 ± 0.103 (from 0.347 to 0.494) and the mean FIS across loci was 0.375 ± 0.103 (from 0.351 to

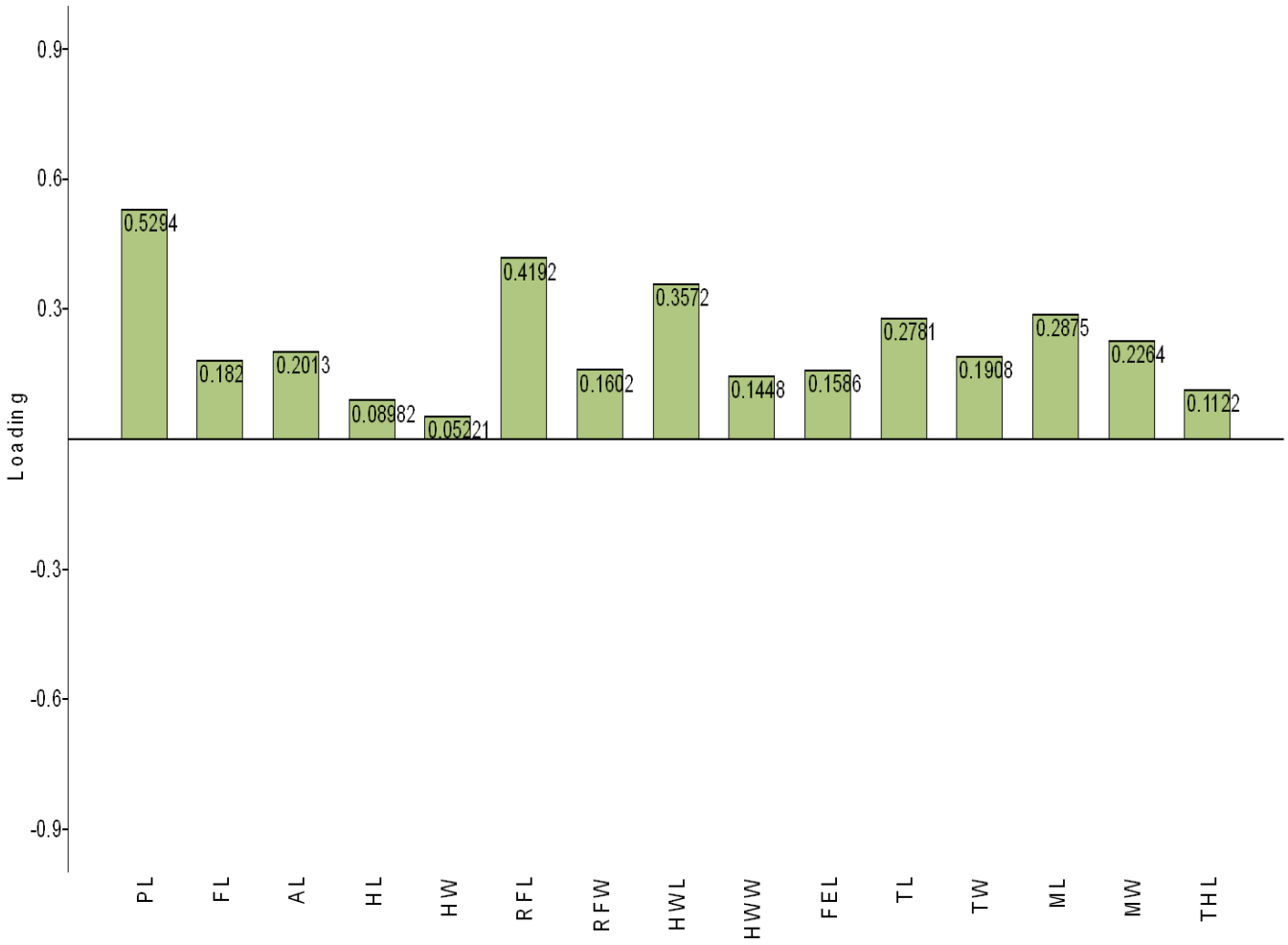

Figure 4. Respective honey bee morphometric characters and their loadings on PC1 of the principal component analysis. PL= Proboscis Length, FL= Flagellum Length, AL= Total Antenna Length, HL= Head Length, HW= Head Width, RFL= Right Forewing Length, RFW= Right Forewing Width, HWL= Hind Wing Length, HWW= Hind Wing Width, FEL= Femur Length, TL= Tibia Length, TW= Tibia Width, ML= Metatarsus Length, MW= Metatarsus Width and THL= Thorax Length.

Table 5. Analysis of Molecular Variance (AMOVA)

Source	df	SS	MS	Est. Var.	% Var.		
Input as codominant genotypic distance matrix for calculation of Φ_{PT}							
						Φ_{PT}	**Nm**
Among Pops	3	7.250	2.417	0.139	9%	0.090*	5.340
Within Pops	24	34.571	1.440	1.440	91%		
Total	27	41.821	-	1.580	100%		

*P(rand>= 0.120); df = degree of freedom; SS = Sum of Squares; MS = Mean of Squares; Est. var. = Estimated variance; % Var. = Percentage variance; ΦPT = Phi PT; Nm = Mean gene flow.

0.402). The fixation coefficients of subpopulations for the loci studied within the total populations, measured as an FST value, varied from 0.030 (A088) to 0.062 (A028), with a mean value of 0.049 ± 0.241. This signified that 4.9% of the total diversity existed among populations while the remaining 95.1% existed within populations. The mean gene flow (Nm) among populations which gives information about genetic divergence or genetic similarity of subpopulations due to gene flow was 5.340 ± 0.432. In other words, gene exchange between

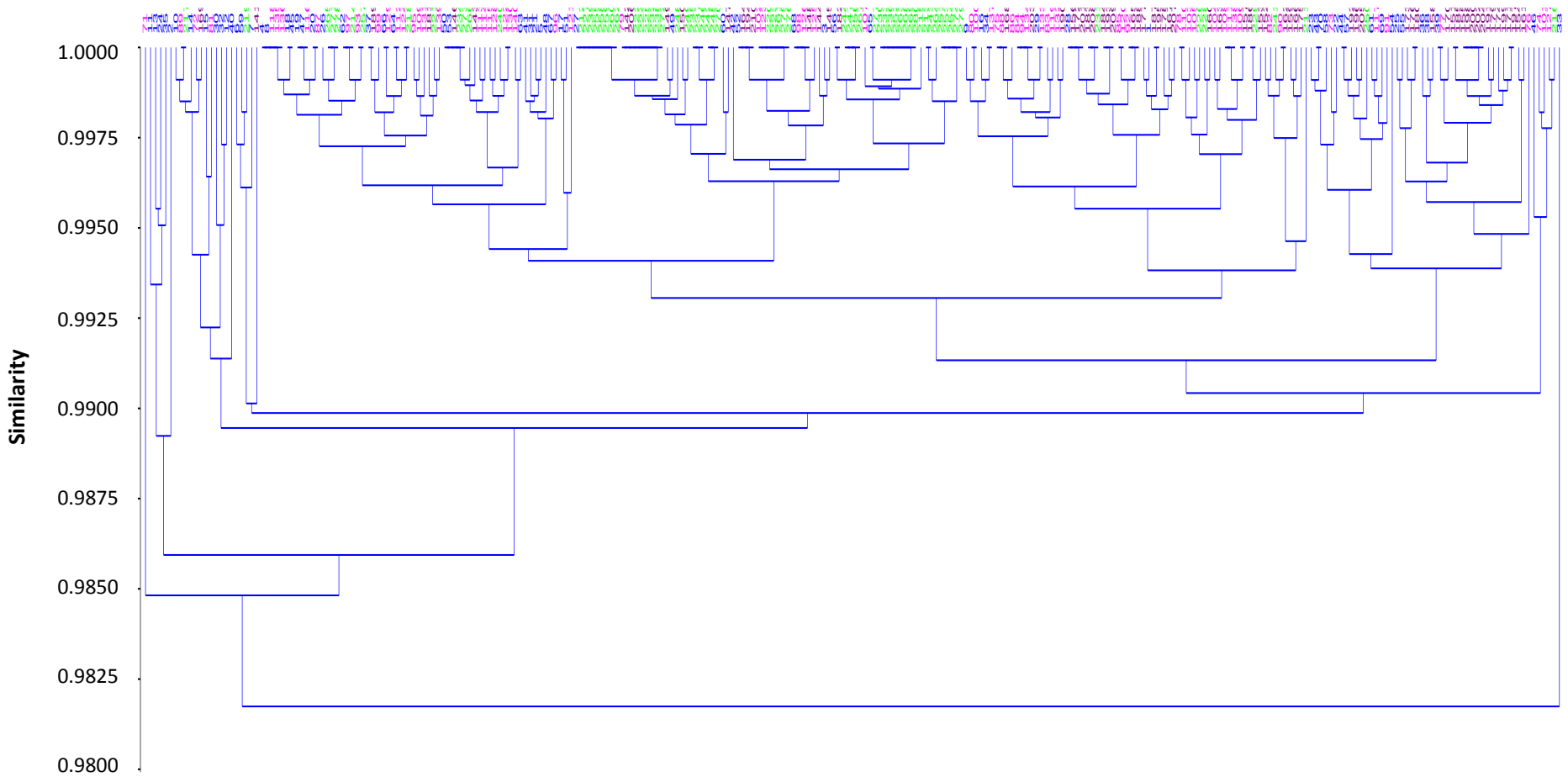

Figure 5. UPGMA Dendrogram showing relationship between the four populations of *Apis mellifera* from Edunabon (Blue), Ijebu-Ode (Pink), Kabba (Purple) and Malete (Green).

populations was low. Analysis of Molecular Variance (AMOVA) showed that 91 % and 9% of the total molecular variance was within and among populations respectively (Table 5). This implies that the populations were not significantly different from each other.

A summary of the test for departure from Hardy-Weinberg (H-W) equilibrium across loci and populations showed that at P <0.05, three (A024, A028 and A113) out of the five loci (60%) studied in the Rainforest and four (A024, A028, A043 and A113) for the Derived Savanna zones (80%) were in H-W equilibrium. The Chi-square ($\chi 2$) test ($P<0.05$) indicated significant departures from H-W equilibrium in many cases (55%). All the deviations were primarily attributed to heterozygotes deficit.

The UPGMA cluster analysis based on Nei's unbiased genetic distances (GD) is shown in Figure 6. The dendrogram separates the four populations into two (2) major clusters with three sub clusters. The first cluster consists of only population 1 while the other cluster consists of populations 2, 3 and 4. Within the second cluster,

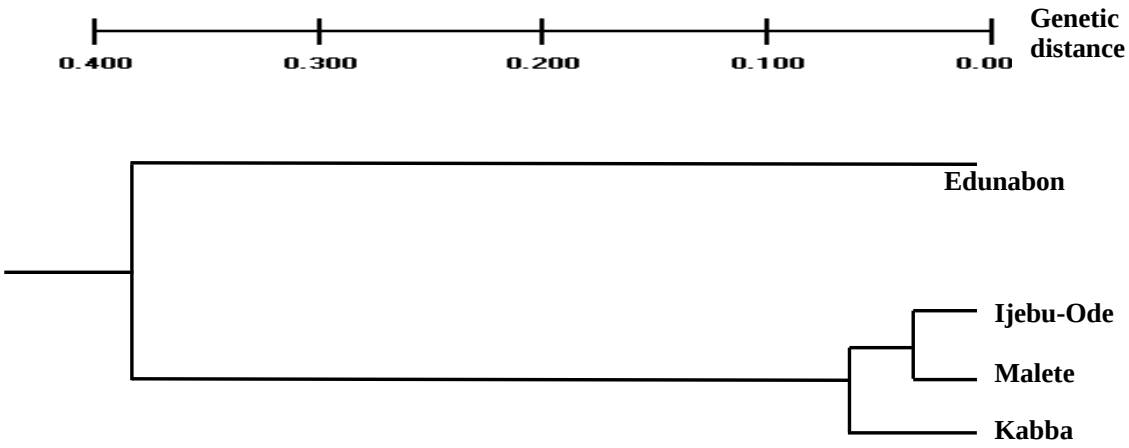

Figure 6. Neighbour-joining dendrogram based on Nei's (1978) unbiased genetic distance showing the genetic relationship among the four populations of *Apis mellifera* from two vegetation zones in Nigeria.

populations 2 and 4 are clustered together.

DISCUSSION

The mean values of the set of morphometric characters measured agrees with those reported for the subspecies of A. mellifera in Nigeria (Dukku, 2016) and sub-Saharan Africa (Ruttner, 1988; Yu et al., 2012), though not in agreement with those reported by Oyerinde et al. (2012) and Ajao et al. (2014), whose values fell outside the range reported for all subspecies of *A. mellifera*. This agreement validates the correctness of the measurements taken in this study. The differences in measured wings characteristics could result from the use of the wings for flight during foraging and thermal regulation of comb. The length of the proboscis was considered a very important character because it showed the geographical variability more than all the other studied characters (Marghitas et al., 2008). Inter-locality variations of the proboscis, tibia and metatarsus are in line with Allen's rule: appendages of the body relatively shorter in the North than in the South (Ruttner, 1988). Although gradual variation was established along the Rainforest-Derived Savanna continuum, no morphometric differentiation has yet been found, in spite of the geographic distance and prominent differences in humidity and altitude.

The Inbreeding coefficient (FIS) value indicated that overall, the four populations had heterozygotes deficit suggesting the presence of inbreeding within populations which could lead to subsequent loss of unexploited genetic potential. The mean gene flow (Nm) among populations, which gives information about genetic divergence or genetic similarity of subpopulations due to gene flow, indicated that there was small genetic differentiation among the populations. In other words, gene exchange between populations was low. AMOVA revealed that most of the variability (91%) was observed in individuals within populations. Measurements of genetic distance (GD) revealed that the Rainforest populations were genetically more diverse (0.600) than the Derived Savanna populations (0.021).

Analysis of the genetic diversity of the *A. mellifera* populations suggests a possible loss of variability. This loss could be attributed to inbreeding depression and/or any of population restructuring, loss of habitat through deforestation, hunting for honey involving killing of wild colonies, natural selection, genetic drift and introduction of exotic honeybees and the parasitic mite, *Varroa destructor* (Akinwande et al., 2013).

Conclusion

The result of this study reveals that *A. mellifera* populations studied are morphometrically similar. There is a need to maintain a healthy level of genetic variability in *A. mellifera* populations, therefore efforts should be made to protect bees from the threats to their abundance, diversity and health. This may be achieved by monitoring and curtailing the effect of inbreeding depression, population restructuring, deforestation, poaching, natural selection, genetic drift and introduction of exotic honeybees and the parasitic mite, *V. destructor.* Also, enforcement of legislations aimed at protecting honeybees, in particular, and the ecosystem in general should be put in place.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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