Bee Studies 14(2), 47-53
http://doi.org/10.51458/BSTD.2022.28

RESEARCH PAPER

Molecular Characterization of the Honeybee (*Apis mellifera*) from two Vegetational Zones in Nigeria

Afeez Tope Nasirudeen [1], Michael Olufemi Awodiran [1], Tofunmi Elizabeth Oladimeji [1,*]

[1] Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Article History
Received 26 October 2022
Accepted 22 December 2022
First Online 29 December 2022

***Corresponding Author**
Tel.: +2348033092964
E-mail: toladimeji@oauife.edu.ng

Keywords
Genetic diversity
Polymorphism
Population
RAPD

Abstract

The genetic structure and diversity of *Apis mellifera* from eight populations across two vegetational zones of Nigeria was investigated in order to provide base line information that will enhance its conservation, genetic improvement and sustainable yield. Genomic DNA was extracted from 40 specimens randomly selected from eight colonies in derived savannah and tropical rainforest regions of Nigeria. The specimens were amplified using five Random Polymorphic DNA (RAPD) primers and the amplicons generated were assessed using appropriate statistical indices. Results generated revealed significant genetic polymorphisms among the eight populations of *A. mellifera* ranging from 38.89 to 68.52% with a total of 281 bands amplified from 54 loci in all samples. The derived savannah population (Offa) had a greater amount of genetic diversity than the rest seven (7) populations as revealed by the percentage of polymorphic loci, expected heterozygosity and Shannon information index (I). The genetic structure as revealed by the Neighbour-Joining dendrogram showed that the eight populations studied represent two major genotypic groups with intra-group relationships. Eleyoka and Offa populations were the genetically closest in the first group while Ayetoro-Gbede and Ayegunle-Gbede populations were the genetically closest in the second group. The study suggests that the two major genotypic groups represent two distinct genetic stocks and can thus be managed accordingly.

Introduction

Apis mellifera, also referred to as the western honey bee is a beneficial and economically important insect. The usefulness of honey bees cannot be overemphasized in terms of the role it plays in the pollination of many native plants as well as in the production of essential seed crops, fiber and food crops (Ratcliffe et al., 2011; Awodiran et al. 2021). 80% of all insect pollination are done by honey bees (Sung et al., 2006). The honey bee is also appreciated for its hive products which has both medicinal and economic benefits. The hive products include honey, bee wax, royal jelly, bee venom, propolis, bee bread etc. Some of these products are known for their anti-oxidant properties, wound healing properties, immunity boosting potential and treatment of infection among others. Bees and other pollinators have been known to contribute greatly to the agricultural sector; with an annual estimate of between $235 - $577 billion worldwide (FAO, 2018). *A. mellifera* has suffered a recent population decline due to overexploitation, habitat degradation, poor beekeeping practices, the threat of pests and diseases as well as exposure to non-target or residual pesticide toxicity in the farm (Shaibi & Moritz, 2010; Awodiran et al., 2021). Conservation of genetic diversity is a major way to ensure the sustainable yield of economically important species. The need to preserve the genetic diversity of domesticated species cannot be overstressed. High genetic diversity helps in the adaptability of a population to its environment and promotes the long term health and persistence of that species (Awodiran et al., 2016). The fitness of honey bee colonies is being enhanced by high genetic diversity (Mattila et al., 2008). The honeybee (*Apis mellifera*) has been extensively studied from different perspectives such as ecological, morphometric and genetic studies (Ruttner et al., 2000; Whitfield et al., 2006; Szalanski & McKern, 2007; Tunca & Kence, 2011). Yogesh and Khan (2014) investigated the genetic

diversity and structure of *Apis mellifera* sampled from nine colonies in different regions of India using five Random Amplified Polymorphic DNA primers. Awodiran et al. (2021), studied the genetic characterization of *Apis mellifera* populations from the rain forest and derived savannah zones of Nigeria using morphological and microsatellite markers. Munoz and De La Rúa (2021), reported the genetic structure of honey bee, *A. mellifera* ecotypes and subspecies whose evolutionary lineage is of east European origin using mitochondrial and microsatellite markers. In spite of the widespread knowledge of the numerous benefits of conserving the genetic diversity of domesticated species, there are very few reports on the genetic diversity of arthropod species that have beneficial values (Yogesh & Khan, 2014). Moreover, considering the commercial and economic importance of *Apis mellifera,* there is need for more studies that will provide information on their genetic characterization in Nigeria. In this study, the genetic diversity and structure of *Apis mellifera* from eight populations across two vegetational zones of Nigeria was investigated in order to provide baseline information that will enhance its conservation, genetic improvement and sustainable yield.

Materials and Methods

Sample collection and study areas

Live samples of *Apis mellifera* workers were collected randomly from eight colonies in two vegetational zones of Nigeria namely: the tropical rainforest and derived savannah zone as shown in Table 1 and described in Figure 1. The tropical rainforest samples were collected from two towns in two different states respectively; Osun (Ipetumodu town and Modakeke town), Oyo (Ibadan town and Eruwa town) while the derived savannah zone samples were also collected from two towns in two different states respectively; Kwara (Offa town and Eleyoka town), Kogi (Ayegunle-gbede town and Ayetoro-gbede town). Five specimens were obtained from each colony in eight different apiaries which do not practice migratory beekeeping (i.e. one apiary was sampled in each location). The specimens were sacrificed in ether vapour and preserved in 80% ethanol for genomic DNA extraction. Identification was done with the aid of a dissecting binocular microscope, using identification keys prepared by Michener (2007).

Table 1. Geographical parameters *of the studied Apis mellifera* Populations

Population	Region	Altitude (m)	Latitude (N)	Longitude (E)	Annual Rainfall (mm)	Annual Temperature (°C)
Ayetoro-Gbede	**Derived Savannah**	508	7°59′	6°0′	1200	30
Ayegunle-Gbede	**Derived savannah**	515	7°21′	5°58′	1300	31
Eleyoka	**Derived savannah**	349	8°13′	4°49′	1150	29
Offa	**Derived savannah**	419	8°9′	4°43′	1250	25.7
Ipetumodu	**Rainforest**	252	7°22′	4°30′	1347	26.2
Modakeke	**Rainforest**	264	7°18′	4°16′	1350	25.8
Ibadan	**Rainforest**	230	7°23′	3°55′	1420	23.94
Eruwa	**Rainforest**	252	7°32′	3°27′	1320	26

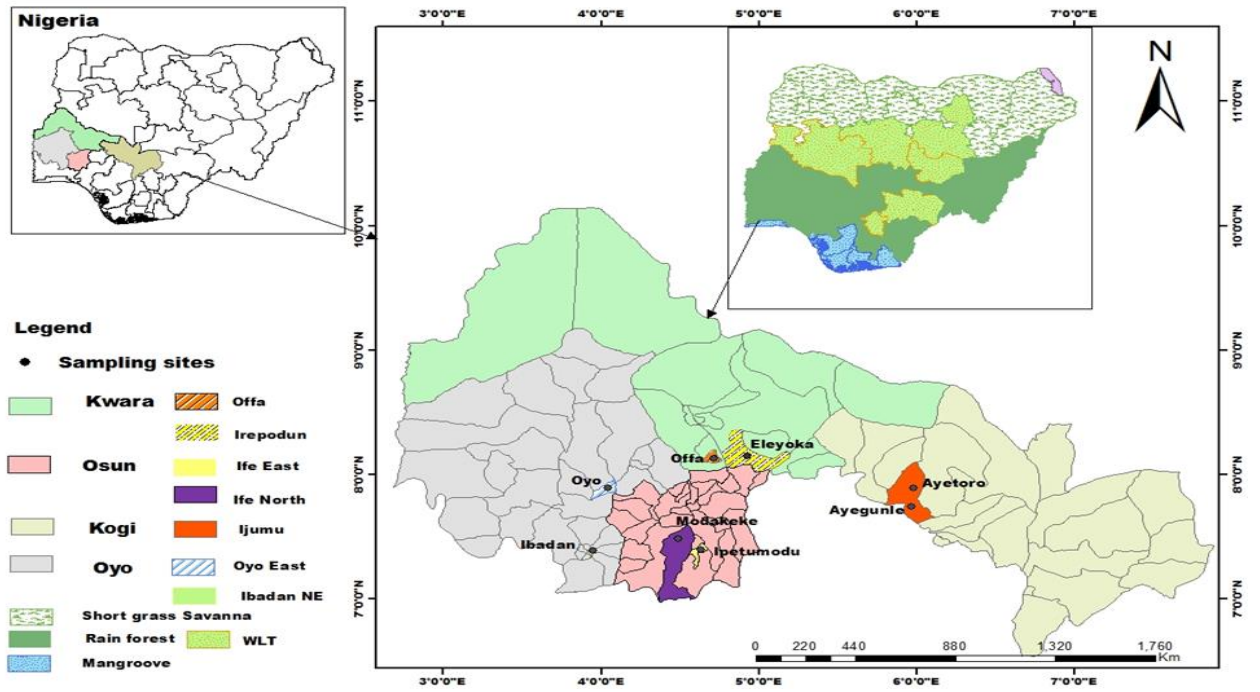

Figure 1. Map showing the vegetation zones and sampling locations of *Apis mellifera*

RAPD/PCR

Whole Genomic DNA of honey bees was extracted from the preserved thorax using CTAB method (Saghai et al., 1984). The concentration of the DNA samples was measured at 260 nm and 280 nm using Nanodrop spectrophotometer. The integrity of the DNA samples was further detected by agarose gel electrophoresis. The genomic DNA was amplified using Random Amplified Polymorphic DNA (RAPD) primers. A total of 10 primers (OPA02, OPA05, OPA07, OPA08, OPAB11, OPAB02, OPA13, OPAB14, OPA15, OPAB08) with different combinations of annealing temperature (37°C - 40°C) were tested, out of which five primers which showed good amplification and exhibited the highest variability were selected for population analysis (Table 2).

The amplification was done according to standard PCR protocols as follows: first denaturation at 95°C for 3 mins, another denaturation at 94°C for 30 secs, annealing for 30 secs, and primer extension at 72°C for 30 secs followed by another extension for 10 mins. Fragment analysis was accomplished by agarose gel electrophoresis. The gel was viewed under UV light, using ethidium bromide as the fluorescent dye. DNA sizing was done using 100 bp DNA ladder (Norgen PCR Sizer). The DNA ladder was loaded along with the gels. The amplified bands were scored as numerical values using GelQuest (GelQuest, 2008).

Table 2. List of RAPD primers used, their sequence and annealing temperature

PRIMERS	SEQUENCE (5'→3')	TM (°C)
OPA 02	TGCCGAGCTG	37.0
OPA 05	AGGGGTCTTG	37.0
OPA 08	GTGACGTAGG	37.6
OPA 13	CAGCACCCAC	37.3
OPAB 11	GTGCGCAATG	36.9

Data and statistical analysis

The data generated was analysed using the Genalex 6.502 (Peakall & Smouse, 2006; 2012). The indices of genetic diversity that were assessed for each population include number of effective alleles (Ne), number of different alleles (Na), analysis of molecular variance (AMOVA), fixation indices, percentage polymorphism, expected heterozygosity, Shannon's Information Index, genetic identity and distance (Nei, 1978). Neighbour-Joining dendrogram was constructed using Evolview (He et al., 2016) and Phylip-3.695 (Felsentein, 2014)

Result

A total of 281 bands were amplified from 54 loci in all samples for the five RAPD primers studied. The derived savannah populations had a total of 145 bands, while the tropical rainforest populations had 136 bands. The amount of genetic diversity, as measured by expected heterozygosity, number of bands, number of private bands, band frequency, and number of locally common bands among the eight populations studied is shown in Figure 2. The Analysis of molecular Variance (AMOVA) (ϕPT =0.001, ϕRT = 0.084, ϕPR = -0.091) indicated that the honey bee populations are significantly different from one another at p = 0.001, but not at greater probability levels (ϕPT is the fixation index). AMOVA also revealed that 92% of the total variation existed within the population, 0% among populations, and only 8% among regions. The number of different alleles (Na), number of effective alleles (Ne), Shannon's information diversity index (I), expected heterozygosity (He), and unbiased diversity values are shown in Table 3. The expected heterozygosity estimate for the honeybee populations ranged between 0.151 (Ibadan) and 0.249 (Offa). Shannon's Information diversity index values for the populations ranged between 0.222 (Ibadan) and 0.371 (Offa). Eleyoka and Eruwa populations had the highest number of bands (39), while the lowest number (27 bands) was recorded in the Ibadan population (Figure 2). Ayetoro, Eleyoka, Offa, and Modakeke populations have one different private band each; Ibadan and Eruwa each have two different private bands while Ayegunle and Ipetumodu have no private bands (Figure 2). Nei's genetic identity, which measures the similarities across all the eight populations, revealed 95% similarity, while the genetic distance, a reciprocal of the genetic identity is 0.05 (Tables 4 & 5). The percentage of polymorphic loci ranged between 38.89 and 68.52%. The lowest and highest percentages were recorded in Ibadan and Offa populations respectively (Table 6). The Neighbour-Joining dendrogram revealed two major genotypic groups with intra-group relationships. The first genotypic group further separates into three distinct clades. The first clade comprises of *A. mellifera* population from Eleyoka, Offa, and Ipetumodu; with Eleyoka and Offa being the closest in the clade. The second clade consists of Ibadan and Eruwa populations while *A. mellifera* population from Modakeke singly represents the third clade. The second major genotypic group comprises of Ayetoro-Gbede and Ayegunle-Gbede populations (Figure 3).

Table 3. Basic indicators of genetic variation across populations for the RAPD data

Population		N	Na	Ne	I	He	uHe	
Ayetoro	Mean	4.000	1.241	1.378	0.332	0.222	0.254	0.031
	SE	0.000	0.132	0.051	0.039	0.027		
Ayegunle	Mean	4.648	1.241	1.378	0.326	0.219	0.246	0.031
	SE	0.066	0.129	0.053	0.040	0.028		
Eleyoka	Mean	4.000	1.352	1.309	0.305	0.196	0.224	0.027
	SE	0.000	0.122	0.043	0.035	0.024		
Offa	Mean	4.815	1.370	1.425	0.371	0.249	0.278	0.030
	SE	0.053	0.128	0.051	0.038	0.027		
Ipetumodu	Mean	3.278	1.278	1.360	0.330	0.219	0.261	0.031
	SE	0.104	0.128	0.046	0.038	0.026		
Modakeke	Mean	4.259	1.204	1.358	0.310	0.208	0.239	0.032
	SE	0.157	0.131	0.053	0.039	0.028		
Ibadan	Mean	3.630	0.889	1.259	0.222	0.151	0.176	0.032
	SE	0.107	0.129	0.049	0.039	0.027		
Eruwa	Mean	3.889	1.389	1.391	0.354	0.235	0.275	0.031
	SE	0.158	0.122	0.048	0.037	0.026		

Na = No. of Different Alleles
Ne = No. of Effective Alleles
I = Shannon's Information Index
He = Diversity' Expected Heterozygosity
uHe = Unbiased Diversity
SE = Standard Error

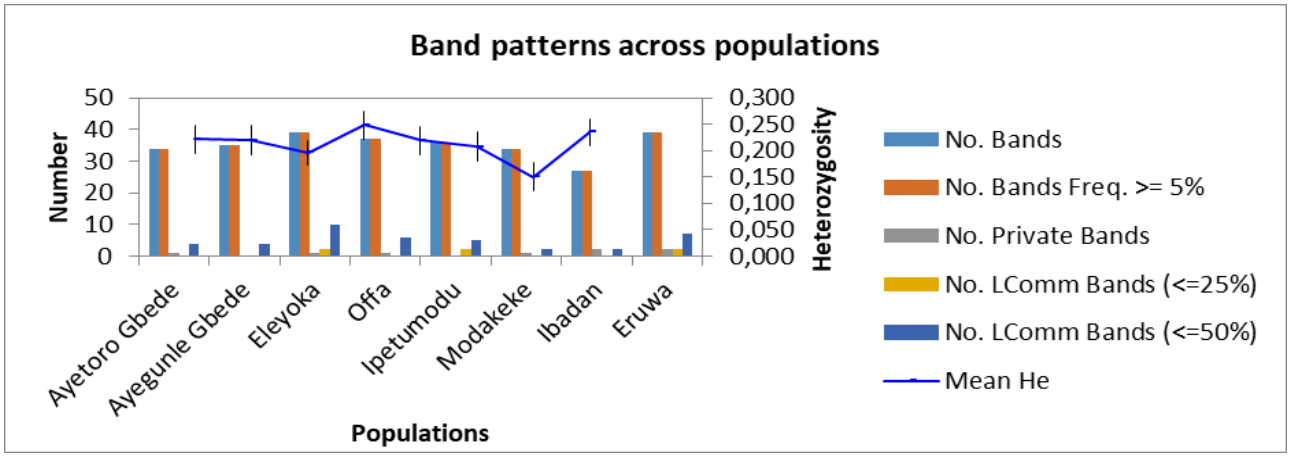

Figure 2. Band patterns and mean diversity of *Apis mellifera* across populations

Table 4: Nei's Unbiased Genetic distance among the eight populations of *Apis mellifera* studied.

Ayetoro	Ayegunle	Eleyoka	Offa	Ipetumodu	Modakeke	Ibadan	Eruwa	
0.000								**Ayetoro**
0.029	0.000							**Ayegunle**
0.076	0.092	0.000						**Eleyoka**
0.005	0.047	0.011	0.000					**Offa**
0.010	0.024	0.067	0.019	0.000				**Ipetumodu**
0.023	0.032	0.113	0.044	0.002	0.000			**Modakeke**
0.037	0.080	0.123	0.065	0.074	0.091	0.000		**Ibadan**
0.010	0.029	0.113	0.044	0.039	0.040	0.063	0.000	**Eruwa**

Table 5: Nei's Unbiased Genetic Identity among the eight populations of *Apis mellifera* studied

Ayetoro	Ayegunle	Eleyoka	Offa	Ipetumodu	Modakeke	Ibadan	Eruwa	
1.000								**Ayetoro**
0.971	1.000							**Ayegunle**
0.927	0.912	1.000						**Eleyoka**
0.995	0.954	0.989	1.000					**Offa**
0.990	0.976	0.935	0.981	1.000				**Ipetumodu**
0.977	0.969	0.894	0.957	0.998	1.000			**Modakeke**
0.964	0.924	0.884	0.937	0.928	0.913	1.000		**Ibadan**
0.990	0.972	0.894	0.957	0.962	0.961	0.939	1.000	**Eruwa**

Table 6: Percentage Polymorphism across the Populations

Population	Polymorphism (%)
Ayetoro Gbede	61.11%
Ayegunle Gbede	59.26%
Eleyoka	62.96%
Offa	68.52%
Ipetumodu	61.11%
Modakeke	57.41%
Ibadan	38.89%
Eruwa	66.67%
Total Mean	59.49%
SE	3.22%

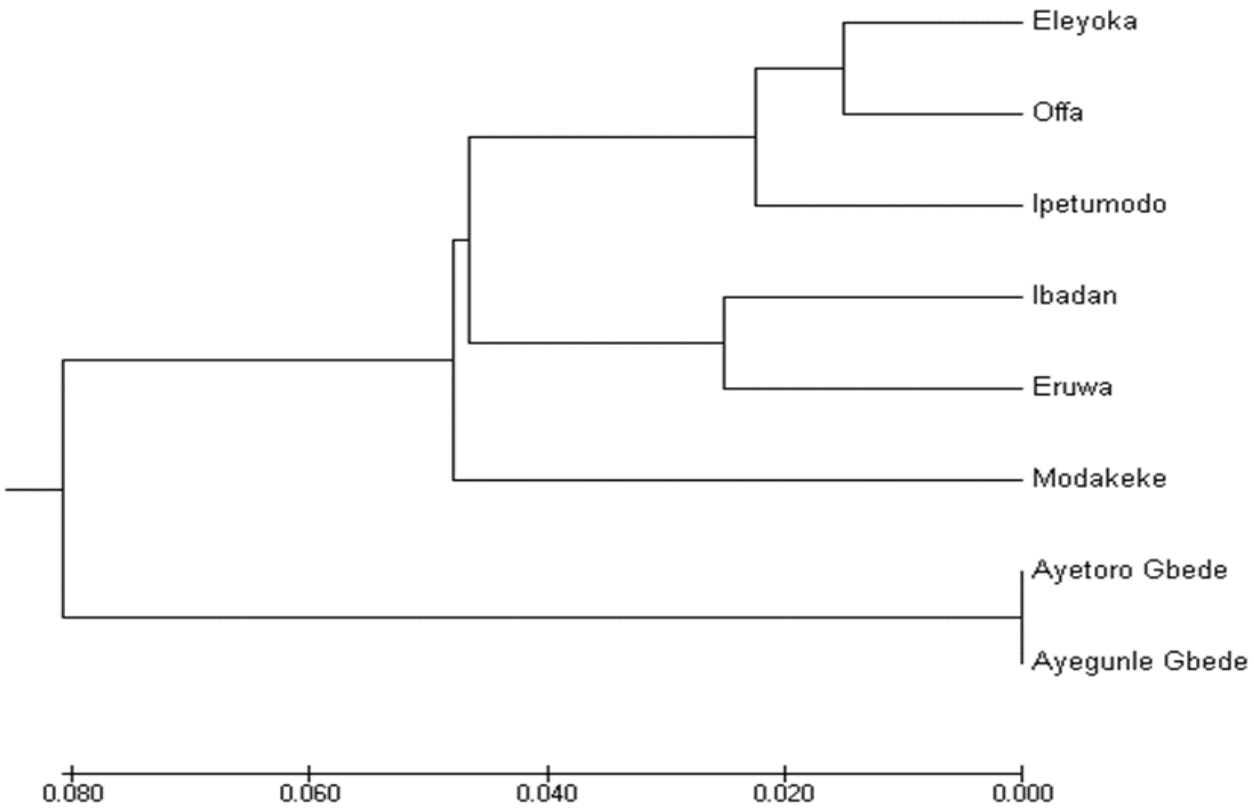

Figure 3. Neighbour-joining dendogram based on Nei's (1978) unbiased genetic distance showing the genetic relationship among the eight populations of *Apis mellifera* from two vegetation zones in Nigeria

Discussion

Amplification of five RAPD-PCR primers was used in this study to comparatively estimate genetic diversity and structure in eight populations of *Apis mellifera.* The result of which revealed significant genetic polymorphisms among the eight populations of *A. mellifera* from derived savannah (Ayegunle-Gbede, Ayetoro-Gbede, Offa, and Eleyoka) and rainforest (Ipetumodu, Modakeke, Eruwa, Ibadan) zones of Nigeria. The derived savannah population (Offa) had a greater amount of genetic diversity and allelic richness than the rest seven (7) populations as shown by indicators such as the number of alleles (Na), the number of effective alleles (Ne), percentage of polymorphic loci (P%), expected heterozygosity and Shannon information diversity index (I). Gene heterozygosity which is a suitable parameter for investigating genetic diversity had values that ranged from 0.151 to 0.249 with an average value of 0.212. This implies that the studied populations of *A. mellifera* can be said to be moderately diverse. The expected heterozygosity values observed in this study are comparable to those reported in other studies on *A. mellifera*. Tunca and Kence (2011), reported expected heterozygosity levels ranging between 0.035 and 0.175 estimated from *A. mellifera* L. populations in Turkey using RAPD markers. Qamer et al. (2021), also reported heterozygosity values ranging from 0.254 to 0.320 in honey bees, *Apis dorsata* collected from two districts in Pakistan using the RAPD marker. Awodiran et al. (2021), however, reported unbiased expected heterozygosity (uHe) values ranging from 0.830 to 0.997 with an average value of 0.902±0.118 in *A. mellifera* L. populations from 28 colonies in the tropical rainforest and the derived savanna zones of Nigeria. The values were estimated from five microsatellite DNA primers. High heterozygosity estimates in a population of a species may be due to long-term selection for adaptation, and the introduction of different strains from several viable populations constituting the parent stock. Expected heterozygosity estimate from a RAPD marker refers to the loci that show evidence of more than one allele (i.e. polymorphic loci). A high level of average heterozygosity in a population is presumed to correlate with high levels of polymorphisms at loci with consequential significance for adaptive response to environmental changes (Kotzé & Muller, 1994). The mean unbiased expected heterozygosity (uHe), a better expression of gene diversity reported in this study was 0.244. The overall fixation index value (ϕPT), which is a good estimate of the genetic differentiation of populations, was very low (0.001). This is an indication that the eight honey bee populations (colonies) studied are genetically similar. This is corroborated by the high value estimated from the genetic similarity matrix. The highest genetic similarity estimate (0.998) was recorded between the pair of Ipetumodu and Modakeke populations (both of Rainforest zones). This is expected because the two populations are the geographically closest, followed by Ayetoro-Gbede and Offa (both of derived savannah zones) with a similarity value of 0.995. The lowest genetic similarity estimate (0.884) was recorded between Eleyoka and Ibadan populations. Genetic distances (GD) according to Nei (1978) were calculated for each pair of populations. The lowest GD (0.002) was found between Ipetumodu (rainforest) populations and Modakeke (rainforest) populations, while the highest GD (0.123) was found between Eleyoka (derived savannah) populations and Ibadan (rainforest) populations. Nei's genetic identity, which measures the similarities across all eight populations, revealed 95% similarity, while the genetic distance, a reciprocal of the genetic identity is 0.05. This is consistent with the findings of Thorpe and Sole-Cava (1994), who reported that most same-species populations have a genetic similarity index that is above 0.85. The genetic structure as revealed by the Neighbour-Joining dendrogram showed that the eight populations studied represent two major genotypic groups with intra-group relationships. Eleyoka and Offa populations were the genetically closest in the first major group while Ayetoro-Gbede and Ayegunle-Gbede populations were the genetically closest in the second group. The study suggests that the two major genotypic groups represent two distinct genetic stocks and can thus be managed accordingly.

Conclusion

The study therefore revealed that RAPD markers can be used as a simple and cost-effective alternative relative to other advanced markers in providing base-line information on the genetic characterization of *A. mellifera* populations. Meanwhile, conservation efforts should be geared towards increasing the inherent genetic diversity in the studied *A. mellifera* populations in order to enhance its sustainable yield.

Ethical Statement

Not applicable.

Funding Information

No external funding was received for this study.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Author 1: Conducted field and laboratory work, acquisition of data and analysis,

Author 2: Conceptualized and supervised the study; critically reviewed the manuscript

Author 3: Led manuscript writing/wrote original draft

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