GENETIC DIVERSITY OF HONEYBEE *(APIS MELLIFERA ADANSONII)* IN IJEBU ENVIRONAS REVEALED BY SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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ABSTRACT

Honeybee *Apis mellifera adansonii,* dominant honey producing species in Nigeria was subjected to genetic variability studies using Simple Sequence Repeat (SSR) in other to provide the baseline data in Nigeria. Nine (9) Simple Sequence Repeats (SSR) primers were used to assess the genetic diversity in Two (2) worker bees each collected from 22 colonies found in the four apiaries in Ijebu environs of Ogun State. Data collected were subjected to analysis and results showed that six (6) out of nine primers produced 80 reproducible, polymorphic bands while the remaining three (3) were monomorphic. Gene diversity (H_T) in total population and magnitude of differentiation among populations (*FST*) was 0.430 and 0.340, respectively. Analysis of Molecular Variance (AMOVA) partitioned the total genetic variation as 70% within, 30% among populations. The cluster analysis showed that Ipari-Oke 3 and Odo-Epo 1-8 populations diverged from others which showed they are closer in genetic distances while Ipari-Oke 1 and Odo-Epo 2-5 were newly observed subcluster which represents another subspecies. In conclusion, genetic variations existed amongst the honey worker bees populations in Ogun State.

Keywords: *Apis mellifera adansonii, Genetic Diversity, Honeybee, Ijebu, SSR Markers*

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INTRODUCTION

Bees are a specialized type of flying insects that can be described as a key component of global diversity. They are very much like the wasps, ants and are known for honey production, bee wax, royal jelly and propolis (Woodley *et al.,* 2015). They are a crucial part of ecosystems, pollinating one-third of food consumed by humans, increase the biodiversity of plants species, maintain genetic diversity within plants populations, increase fruit yields and hence support flora and fauna in every level of the food chain (Batra,1995). The Honeybees which belong to the family Apidae are naturally distributed in Africa, Europe and Western Asia (Ostroverkhova *et al.,* 2017). Currently, 28 different subspecies have been recognized within the endemic range with classification based on morphology (Alaux *et al.,* 2019). Many are valuable pollinators in natural habitats and for crops (Akunne *et al.,*2016), *Apis mellifera adansonii* is the most common bee that has been given due attention in Nigeria probably because of its beneficial attributes as a natural agent of pollination and as the most important of all insect pollinators (Fasasi, 2018). It is well adapted to the Nigeria ecological conditions (Abdullahi *et al.,* 2011) and gathers its food freely throughout the year (Fasasi & Afolabi, 2019). The Biodiversity of *Apis mellifera* was first assessed using morphometrics (Sheppard & Meixner, 2003).

Honeybees morphologic structures showed various adaptations for foraging, nectar collection, feeding the queen and the larvae, cleaning brood cells; removing debris, honey and pollen storage and rearing of larvae in cells made from wax secreted by the worker bees. Several works with *Apis mellifera* involving morphologic characters and weight showed that there is a strong influence of the environment in the morphology of the same species (Aboushaara *et al.,* 2012). Furthermore, there is often a positive correlation in some characteristics, for instance, the length of the wing and altitude, the size of pollen basket and hind-leg and the size of the honey stomach and honey production and storage. Such correlation can indicate the importance of the morphologic characteristics in the adaptation of individuals to the environment (Ajao, 2012).

Due to the continuous challenges of morphometric studies, molecular level studies began with the help of allozymes (Allelic form of an enzyme that can be distinguished by gel electrophoresis to observe genetic variation from gene products), mitochondrial DNA (mtDNA) (Hunt & Page 1992, Rinderer *et al.,* 1995) and microsatellites (Strange *et al.,* 2008). Protein and enzyme polymorphism in honeybees proved to be useful in development studies, population genetics and classification (Arias, *et al.,* 2006). Different DNA analysis methods have been applied to clarify the race standard of the local honeybee (Ivanova *et al.*, 2010). Some markers like randomly amplified polymorphic DNA (RAPD) can produce multiple bands fragments. These fragments are usually generated from different regions of the genome and hence multiple loci maybe examined very quickly (Kumar & Gurusubramanian, 2011).

A variety of molecular markers have been used in honeybee as reported by Hunt & Page, (1992), Tunca & Kence, (2006) and Whitfield *et al.,*(2006). However, PCR-based markers are more appropriate for fingerprinting studies such as random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and the inter simple sequence repeat (ISSR). Furthermore, micro satellite markers play an important role in determining genetic diversity and phylogenetic relationships of animals and especially insects (Rahimi *et al.,* 2014) that promotes homeostasis in insect colonies (Oldroyd & Fewell, 2007). Studies have shown that genetically diverse honey bee colonies are more productive, fit, have increased colony growth and are less susceptible to severe infections (Bienefeld, 2016). There has been a paucity of data on genetic studies of honey bees in Ogun State. This work aims to determine the genetic variability of worker bee samples from 22 colonies in some areas in Ijebu environs in Ogun State, Nigeria using SSR markers.

MATERIALS AND METHODS

Study Area:

A total of 44 Samples of honeybees were collected from four different locations of available honeybee farms (Odo-Epo Bee farm 1; Odo-Epo Bee farm 2; Ipari-Oke Bee farm 1 and Bee farm - P S Biomes) in Ijebu environs of Ogun State, Southwestern Nigeria. Consent of approval was sought from the bee farmers from the 4 apiaries before the beginning of the study. A total of 22 colonies were found in the apiaries (Odo-Epo Bee farm 1 (Eight colonies); Odo-Epo Bee farm 2 (Eight colonies); Ipari-Oke Bee farm 1 (Three colonies); Bee farm - P S Biomes (Three colonies). Two (2) worker honeybee samples were taken from 22 colonies in the study area and preserved carefully in well-labelled specimen bottles containing 70% alcohol. These samples were transported to the biotechnology laboratory of Nigeria Institute of Science and Laboratory Technology (NIST), Samonda, Ibadan in Oyo State, Nigeria to undergo molecular analysis which included DNA extraction and amplification.

DNA EXTRACTION:

The DNA of honeybees was extracted using modified Cetyl trimethylammonium bromide (CTAB) extraction procedure according to Murray & Thompson (1980) and DNA was quantified using the ultraviolet spectrophotometer for quality assessment.

DNA AMPLIFICATION:

Nine (9) SSR markers namely LA1, A7, A81, A29, A113, A107, A14, IC1 and B124 (Table 1) were used to amplify the extracted DNA samples. PCR was performed according to Solignac *et al.,* (2003). The amplification products were resolved by electrophoresis in 6.0% polyacrylamide gel with a 1.0 X TBE buffer. After electrophoresis, gels were stained with ethidium bromide solution.

TABLE 1: The List of Primers and Its Sequences

S/N	LOCUS	SEQUENCE OF PRIMERS
1	A81F	5'-GCCGAGTTCTTCGACTCCC-3'
	A81R	5'-GGACTTTGCCAAATGGGTC-3'
2	IC1F	5'-GGTTTGATGCTCGTAAGGG-3'
	ICIR	5'-GGCACCTCTTGCCATCTG-3'
3	A7F	5'-GTTAGTGCCCTCCTCTTGC-3'
	A7R	5'-CCCTTCCTCTTTCATCTTCC-3'
4	A113F	5'-CTCGAATCGTGGCGTCC-3'
	A113R	5'-CCTGTATTTTGCAACCTCGC-3'
5	B124F	5'-GCAACAGGCGGGTTAGAG-3'
	B124R	5'-CAGGATAGGGTAGGTAAGAG-3'
6	A107F	5'-CCGTGGGAGGTTTATTGTCG-3'
	A107R	5'-CCTTCGTAACGGATGACACC-3'
7	A14F	5'-GTGTCGCAATCGACGTAACC-3'
	A14R	5'-GTCGATTACCGATCGTGACG-3'
8	A29F	5'-AAACAGTACATTTGTGACCC-3'
	A29R	5'-CAACTTCAACTGAAATCCG-3'
9	LA1F	5'-GAGAGAGAGAGAGAGACG-3'
	LA1R	5'-ACACACACACACACACAT-3'

DATA ANALYSIS

Polymorphic bands were scored as present (1) or absent (0) for SSR analyses. Percent of polymorphic bands, expected heterozygosity (*He*), gene diversity (Nei, 1973) and Shannon's information indices (*I*) (Lewontin, 1972) were calculated using the following equation:

$I = \Sigma pi \ln pi$

where *pi* stands for the proportion of the *i*th allele in the population.

Pairwise F_{ST} was calculated using POPGENE 1.31 software (Yeh *et al.,* 1999). The total band patterns, and Analysis of Molecular Variance (AMOVA), were carried out using Genalex6 software program (Peakall&Smouse, 2006). UPGMA tree was constructed based on original distance using TFPGA v.1.3 (Miller, 1997). Factorial Analysis was performed using NTSYS v.2.20 software program (Rohlf, 2000).

RESULTS

Nine (9) SSR markers were screened out of which six (6) were polymorphic, hence 6 polymorphic primers were used for the final analysis of honeybees i.e. A81, A29, A113, A107, A14 and IC1 markers amplified 80 polymorphic bands (Figure 1).

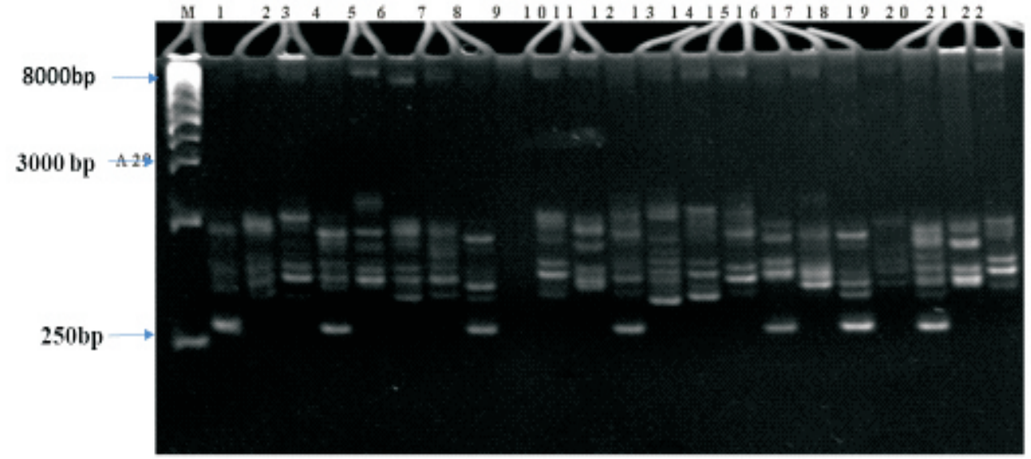

Figure 1: A29 primer plate showing polymorphic bands

The percentage of polymorphic loci ranged between 13% and 54 % in populations of PS Biome2 and Ipari- Oke1 respectively. The proportion of polymorphic bands expected heterozygosity (He), Shannon's information index (*I*) values are given in Table 2. The expected heterozygosity levels for honeybee populations ranged between 0.054 (Odo-Epo 1-8) and 0.19 (Ipari-Oke1). Gene diversity and Shannon's index values for all populations were estimated to be 0.11 and 0.14, respectively.

The most informative primers were A29 and A113, they have the highest number of expressed bands (161 and 130 respectively) in 16 loci. The locations with the highest number of polymorphic expressions were Ipari-Oke 1(48 bands), Odo-Epo1-1 (36 bands) and Ipari-Oke3 (36 bands) while the lowest expression was observed in PS biome2 population (Table 2). Gene diversity (H_T) in total population and magnitude of differentiation among populations (*FST*) was 0.430 and 0.340, respectively. Analysis of Molecular Variance (AMOVA) partitioned the total genetic variation as 70% within, 30% among populations Pairwise *FST* values were given in Table 3, while the highest pairwise *FST* was observed between Ipari-Oke 1 and PS Biome2 (0.552), the lowest was detected between IpariOke2 and Odo-Epo 2-8 populations (0.016).

Table 2: Observed number of bands, percentage of polymorphic bands, expected heterozygosity (H_e) and Shannon's information index (*I*) values for populations studied.

ACC	Observed bands	% Polymorphic	H_e	*I*
Ep 1-1	36	45	0.150	0.180
Ep 1-2	23	29	0.100	0.134
Ep 1-3	32	40	0.160	0.167
Ep 1-4	17	21	0.100	0.109
Ep 1-5	28	35	0.120	0.153
Ep 1-6	31	39	0.140	0.164
Ep 1-8	12	15	0.054	0.084
Ep 1-9	16	20	0.080	0.104
Ep 2-1	30	38	0.134	0.160
Ep 2-2	25	31	0.110	0.142
Ep 2-3	26	33	0.120	0.146
Ep 2-5	19	24	0.080	0.117
Ep 2-6	22	28	0.100	0.130
Ep 2-7	17	21	0.080	0.109
Ep 2-8	26	33	0.110	0.146
Ep 2-9	26	33	0.100	0.146
Ip 1	43	54	0.190	0.201
Ip 2	28	35	0.130	0.153
Ip 3	36	45	0.170	0.180
Ps 1	25	31	0.110	0.142
Ps 2	10	13	0.060	0.074
Ps 3	14	18	0.070	0.094

ACCCODE: Ep 1- Odo-Epo farm 1; Ep 2- Odo-Epo farm 2; Ip – Ipari-Oke farm; Ps – PS Biome

The dendrogram in Figure 3 indicated no unique clusters based on location but Ipari-Oke 3 and OdoEpo-1-8 populations diverged from all other populations. Within superclusters, Odo-Epo and PS Biome formed a subcluster from the populations that shows relativeness in the honeybee samples from both locations. Ipari-Oke1 was observed as an emerging subcluster which tends to represent another subspecies.

The factorial analysis of the worker bee samples classified according to Nei (1972) unbiased genetic distances between populations indicated that the populations of Odo-Epo bee farms 1 and 2 are interrelated but more related to the Ipari-Oke population, while the PS biomes had a distinct population (Figures 4a & b).

Table 3: Pairwise *FST* values among four populations.

	Ep 1-4	Ep 1-9	Ep 1-1	Ep 1-5	Ep 1-6	Ep1-3	Ep 1-8	Ep 1-2	Ep 2-1	Ep 2-7	Ep 2-2	Ep 2-5	Ep 2-6	Ep 2-9	Ep 2-8	Ep 2-3	Ps 3	Ps 1	Ps 2	Ip1	Ip3
Ep 1-9	0.036 ***																				
Ep 1-1	0.296	0.324 ***																			
Ep 1-5	0.18	0.208	0.12 ***																		
Ep 1-6	0.228	0.256	0.068	0.052 ***																	
Ep 1-3	0.252	0.284	0.056	0.084	0.036 ***																
Ep 1-8	0.124	0.088	0.412	0.296	0.348	0.372 ***															
Ep 1-2	0.1	0.124	0.2	0.084	0.136	0.164	0.212 ***														
Ep 2-1	0.212	0.24	0.084	0.036	0.016	0.052	0.328	0.12 ***													
Ep 2-7	0.032	0.02	0.308	0.188	0.24	0.268	0.104	0.108	0.224 ***												
Ep 2-2	0.136	0.16	0.168	0.048	0.1	0.128	0.248	0.036	0.084	0.14 ***											
Ep 2-5	0.048	0.052	0.276	0.156	0.208	0.236	0.14	0.072	0.192	0.036	0.108 ***										
Ep 2-6	0.084	0.108	0.216	0.096	0.148	0.176	0.196	0.016	0.132	0.092	0.052	0.06 ***									
Ep 2-9	0.148	0.168	0.16	0.044	0.096	0.128	0.256	0.048	0.08	0.152	0.024	0.116	0.064 ***								
Ep 2-8	0.148	0.172	0.152	0.032	0.088	0.116	0.26	0.048	0.068	0.156	0.016	0.124	0.064	0.016 ***							
Ep 2-3	0.152	0.18	0.148	0.028	0.08	0.108	0.268	0.056	0.064	0.16	0.024	0.132	0.072	0.032	0.016 ***						
Ps3	0.076	0.04	0.368	0.248	0.3	0.324	0.048	0.164	0.284	0.06	0.2	0.092	0.152	0.212	0.216	0.22 ***					
Ps1	0.136	0.16	0.168	0.048	0.1	0.128	0.248	0.036	0.084	0.14	0.08	0.108	0.052	0.024	0.016	0.024	0.2 ***				
Ps2	0.156	0.124	0.452	0.332	0.384	0.408	0.044	0.252	0.368	0.144	0.284	0.18	0.236	0.296	0.3	0.304	0.084	0.284 ***			
Ip 1	0.396	0.428	0.104	0.224	0.172	0.144	0.516	0.304	0.188	0.412	0.268	0.38	0.32	0.264	0.256	0.248	0.468	0.268	0.552 ***		
Ip 3	0.308	0.336	0.032	0.136	0.08	0.056	0.428	0.216	0.1	0.32	0.18	0.288	0.232	0.18	0.168	0.16	0.38	0.18	0.46	0.088 ***	
Ip 2	0.184	0.212	0.112	0.016	0.044	0.076	0.3	0.092	0.028	0.196	0.056	0.164	0.104	0.056	0.044	0.032	0.252	0.056	0.336	0.216	0.128

Code name: Ep 1- Odo-Epo farm1; Ep 2- Odo-Epo farm 2; Ip – Ipari-Oke farm; Ps – PS Biome

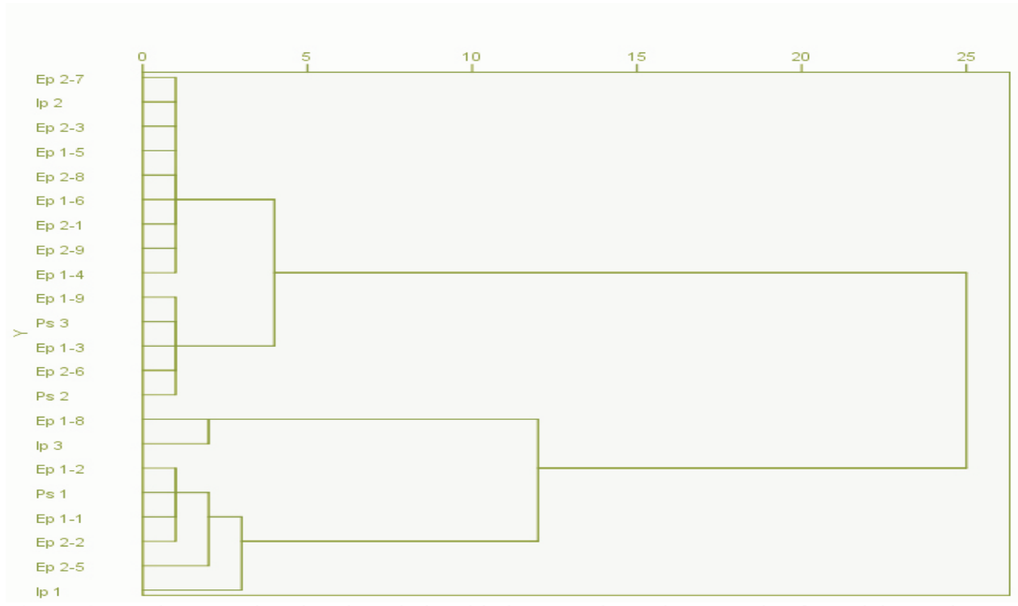

Figure 3: Dendrogram showing the relationship between honeybee samples from Ijebu environ. Code name: Ep 1- Odo-Epo farm 1; Ep 2- Odo-Epo farm 2; Ip – Ipari-Oke farm; Ps – PS Biome

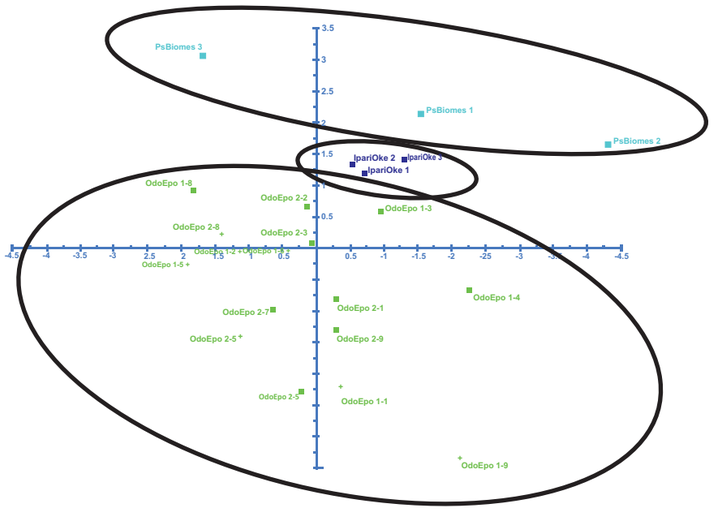

Figure 4a: Factorial analysis of worker honeybees samples based on their locations
Code name: Ep 1- Odo-Epo farm 1; Ep 2- Odo-Epo farm 2; Ip – Ipari-Oke farm; Ps – PS Biome

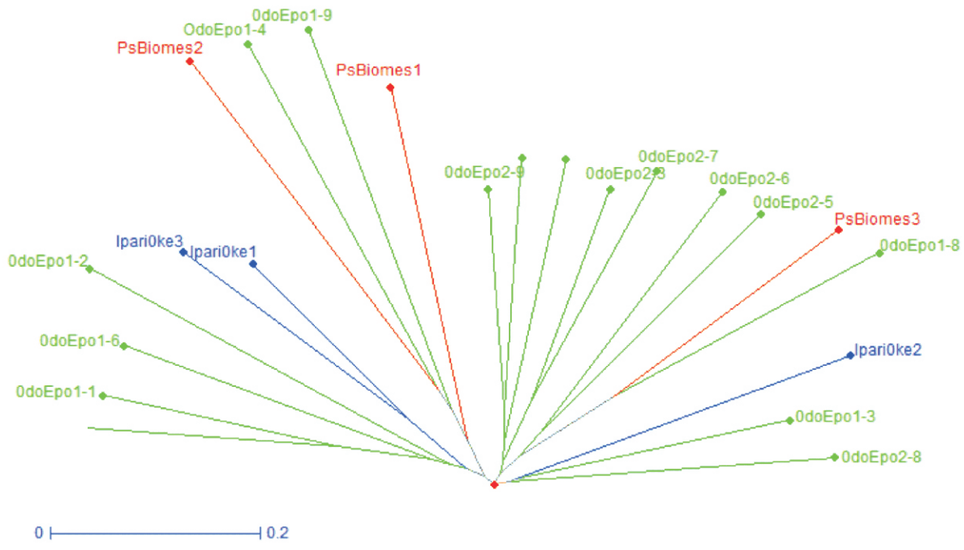

Figure 4b: Factorial analysis of worker honeybees samples based on their genetic diversity.
Code name: Ep 1- Odo-Epo farm 1; Ep 2- Odo-Epo farm 2; Ip – Ipari-Oke farm; Ps – PS Biome

DISCUSSION

Microsatellite markers are locus-specific, inherited as codominant, the mutation in the areas of a microsatellite is abundant, based on PCR and amplifying the genes does not need much DNA with very desirable quality which is useful to perform genetic research such as: determining the genetic mapping, population genetic, phylogenetic, pedigree analysis and even forensic backgrounds (Ghassemi Khademi, 2011).Simple Sequence Repeats (SSR) analysis in worker bee populations *Apis mellifera adansonii* collected from four different locations of bee farms in parts of Ijebu Local Government Area has indicated that the expected heterozygosity (He) levels vary with the various locations. This was also observed in Shannon's index value (I) which indicated some level of diversity in the population.

Coincidentally, I value of Ipari Oke 1 (0.201) and Odo Epo1-3 (0.167) populations (Table 2) were higher than that of the others. When the comparison was made at the population level, Ipari-Oke 1 had the highest gene diversity of 0.190 and Shannon's index, second to that were observed for the Odo-Epo1-3 populations. The highest genetic diversity in Ipari-Oke1 population could have most likely resulted from having favourable climatic and vegetative conditions for extensive queen bee breeding and bee transfer during human settlements and farming activities from many other provinces thereby possible introgressions of genes and interbreeding.

However, the worker bee samples showed distinctiveness in their population and this could be due to the introduction of new alleles through drones from different colonies mating with the queen during the mating season. Also, the migratory pattern of honeybees introduces different genotypes could also contribute to the divergence of genes. This is following Tunca & Kence (2011).

The primer A29 could be said to be the most informative marker and most suitable as all samples were amplified and the alleles were fully expressed.

The dendrogram illustrated that although the worker bee samples belong to a single genetic ancestor, the genetic composition varied considerably among individuals. This may be due to their breeding pattern which includes several drones mating with a queen while on a matting flight, domestication whereby the farmer splitting the hives and a drone brood is introduced to another colony and swarming.

Ipari-Oke3 and Odo-Epo 1-8 populations diverged from all other populations (Figure 3) which showed they were closer in genetic distances than the others, these showed some level of inbreeding and formation of new genes, this is following findings of Rattanawannee *et al.,* (2019) in Thailand honeybee populations. Ipari-Oke 1and Odo-Epo 2-5 were newly observed subcluster which represents another subspecies as they could have contained genes that are not common to others due to the flora they were feeding on, developed beekeeping and imported bee colonies from different origins to this territory so that there are different races of bees in the same area (Ostroverkhova *et al.,* 2017). This, in turn, could have led to the formation of a new subspecies and heterogeneous population. Thus, it indicated that the SSR markers used were effective to discriminate these genetic differences amongst the populations.

The Factorial Component Analysis (FCA) also indicated that Odo-Epo and Ipari Oke honeybee populations were closer than other populations (figure 4). The PS Biome bees had gene pools of different levels of genetic dissimilarities; a similar result was observed on sampled populations of Turkey and Bulgarian bees (Ivanova *et al.,* 2007).

The results of the current study, when compared to that of other genetic studies in bees in Russia (Ostroverkhova *et al*., 2017) and Thailand (Rattanawannee *et al.,* 2019), expressed the same level of heterozygosity and divergence in populations while a lower percentage of polymorphism and average heterozygosity were observed in Turkey (Ozdil *et al.,* 2006; Tunca & Kence, 2011) and Iran (Kence *et al.,* 2009; Rahimi *et al.,* 2016) bees populations using RAPD, mtDNA and microsatellite markers.

CONCLUSION

The genetic composition of worker bees of *Apis mellifera adansonii* in Ijebu environ investigated using simple sequence repeat (SSR) revealed variations in the genetic makeup of the populations, no two individual of the entire population had the same genetic makeup, yet they all belonged to a single genetic linage. The data presented here showed that SSR markers were effective in discriminating honeybee populations, detecting the

variability levels among populations. These results should be considered in conservation plans, particularly about moving of colonies and most importantly introducing bees of foreign origin and distributing queen bees from one centre to all over the country which will homogenize the gene pools of the populations.

This also serves as a baseline for genetic studies in documenting the genetic diversity of the *A. mellifera* 'lineage in Ijebu division of Ogun State using SSR marker. Also, it is suggested that other molecular tools such as ISSR, mtDNA and AFLP be used to access the genetic diversity of the honeybees in the study area to have a comprehensive database and preserve the genetic diversity of *Apis mellifera*.

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