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Genetic Diversity of West African Honey Bee (*Apis mellifera* adansonii Latreille, 1804) from Rural and Urban Areas of Kwara State, North-Central Nigeria

Oluyinka A Iyiola*[1], Olukayode J Adelaja[2], Rahmat D Aderemi[1], Soliu Aliu Fulani[1], Ademola E Alaba[1,3], Seun F Adejuwon[1], Olayinka S Yusuf[2], Lateefat B Bello[2], Adedayo O Oduola[2]

[1]Cell Biology and Genetics Unit, Department of Zoology, University of Ilorin, P.M.B 1515, Ilorin Kwara state, Nigeria, [2]Entomology Unit, Department of Zoology, University of Ilorin, P.M.B 1515, Ilorin, Kwara state, Nigeria, [3]Department of Biological Sciences, Ajayi Crowther University, Oyo, Oyo State, Nigeria

***Correspondence**:
iyiola.oa@unilorin.edu.ng
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A B S T R A C T

Over one third of the world's crops– including fruits, vegetables, nuts, spices, and oilseed– require insect pollination, and human reliance on pollination services by honey bees (*Apis mellifera*) to promote these crops continues to rise due to increasing demands from growing human populations. Identifying the effects of urbanization on genetic diversity on this pollinating insect is important in the field of bioscience. This study aimed to investigate genetic diversity of *A. mellifera* in Kwara State, Nigeria, using the random amplified polymorphic DNA (RAPD) marker. Thirty honey bees were simultaneously collected from both rural and urban regions in Kwara state, Nigeria. Samples were morphologically identified using standard methods, genomic DNA isolated and amplified using five RAPD primers. Data collected were analysed using PyElph, ARLEQUIN, and GeneAlEx version 6.501 software. The results showed that the DNA fragment sizes produced per primer varied from 200 to 3000 bp. Percentages of polymorphic loci amplified by each primer varied from 17.33 to 33.33%. Analysis of unbiased Nei genetic distance values showed that Agbede (rural) and Adewole (urban) showed the highest value of unbiased genetic distance (0.073), while Amoyo (rural) to Idofian (urban) exhibited the lowest value (0.027). Dendrogram analysis revealed genetically close relationships among the sampled *A. mellifera* populations. The low level of genetic polymorphisms observed among the honey bee populations in the two regions indicated that there is genetic relatedness among them. This study concluded that RAPD marker is a useful method for understanding population genetic structure of the African honey bees. These results can be used as baseline information for future genetic diversity assessment of honey bees in Nigeria with larger samples. It is therefore recommended that there is a need to safeguard the genetic diversity of *A. mellifera* to prevent extinction or gradual loss of diversity.

Keywords: *Apis mellifera*, RAPD-PCR, Honey bee, Pollination, Nigeria

INTRODUCTION

Honey bees (*Apis mellifera*) are popular social insects known for constructing perennial colonial nests from wax. They produce production honey which makes their hives attractive to various animals, including honey badgers, bears, and human hunter-gatherers (1, 2). *A. mellifera* plays a pivotal role in pollination services. They are particularly vital for reproduction of numerous flowering plants and economically important agricultural crops because of pollination services they provide (3). The diversity within the honey bee community is astonishing, with the *Apis* genus encompassing nine distinct species. Among them, the Western honey bee (*A. mellifera* Linnaeus,

1758) stands out as the most well-known and widely domesticated species, used not only for honey production but also for crop pollination (2).

A. mellifera is not unaffected by the environmental modifications resulting from human actions. Human-induced changes, including habitat disruption, climate variations, and pesticide application, have profoundly influenced global honey bee communities (4, 5). Particularly, urban expansion exerts a significant strain on ecosystems, disturbing natural habitats, diminishing the diversity of bee populations, and leading to the rise of species that adapt to urban settings (6). Inappropriate use of pesticides to control pest on the farms has negative impacts on the survival of honey bees (7). Moreover, climate change on the other hand offers a more severe threat to honey bee survival and biodiversity (8).

In recent years, there has been growing concern over the potential decline in honey bee populations due to a wide range of environmental pressures and anthropogenic activities. This decline has profound implications on both agricultural systems and the ecological integrity of wild plant communities (9). With the world's population steadily shifting toward urbanization, this issue becomes even more pertinent. Genetic diversity study will be useful and valuable for understanding gene flow and mutation rates among honey bee populations. In addition, genetic diversity study will reveal locally adapted honey bee for the purpose of improving the quality. Different molecular-based approaches have been used to characterize honey bees (10-12). Random amplified polymorphic DNA (RAPD) has been used to investigate genetic diversity of honey bee populations (12-16). However, there is paucity of information on the population genetic structure of *A. mellifera* in Kwara state, Nigeria. Therefore, the objective of this study was to investigate population genetic diversity of *A. mellifera* in rural and urban areas of Kwara state, North-Central Nigeria. The results from this study will hopefully contribute to current understanding of the genetic variability of honey bees in the North-Central Nigeria. This could provide the basis for further studies on the genetic characteristics and evolutionary relationship of *A. mellifera* species in Nigeria.

MATERIALS AND METHODS

Study Area

The study area was Kwara, a state in the North-Central Nigeria with the capital is Ilorin. The study was carried out in three different local government areas, namely Ilorin south, Ilorin west and Ifelodun. One rural and one urban sampling regions were selected randomly from each area making six different sampling locations in total. Global positioning system (GPS) was used to determine the sampling locations, and the data are stated in Table 1.

Honey Bee Sample Collection

Thirty adult honey bees from selected colonies were collected in six sampling locations covering both rural and urban regions of Kwara state, North-central Nigeria. Bowl traps and aerial nets were also used for sample collections. Personal protective equipment such as hand gloves, nose mask, eye glasses and laboratory coats were worn for field collection activities. Five samples of *A. mellifera* were obtained from each of the three rural areas namely Osin Aremu, Agbede, and Amoyo. These samples were labelled R1A, R1B, R1C, R1D, R1E for Osin Aremu; R2A, R2B, R2C, R2D, R2E for Agbede; and R3A, R3B, R3C, R3D, R3E for Amoyo, totaling 15 samples from the rural areas. Similarly, from the urban regions, five samples of *A. mellifera* were collected from each of the three urban areas, namely Adewole, Tanke, and Idofian. These were labelled U1A, U1B, U1C, U1D, U1E for Adewole; U2A, U2B, U2C, U2D, U2E for Tanke; and U3A, U3B, U3C, U3D, U3E for Idofian, also making a total of 15 samples from the urban region. Samples were then preserved in 10mL of 99% ethanol and kept at -80^{0}C until DNA isolation.

Table 1. GPS coordinate of the study communities from the six sampling locations

Local Government Area	Sample collection locations	Settlement	Coordinates
Ilorin South	Tanke (pipeline)	Urban	Lat 4°N 37' 4.792" & Long 8°E 27'25.2"
Ilorin South	Agbede	Rural	Lat 8°N 27'38.28" & Long 8°E 27'25"
Ilorin West	Adewole	Urban	Lat 8°N 29'6.114" & Long 4°E 30'561"
Ilorin West	OsinAremu	Rural	Lat8°N 25'45.576" & Long 4°E 34'22.758"
Ifelodun	Idofian	Rural	Lat 8°N 23'00.056" & Long 4°E 43'00"
Ifelodun	Amoyo	Urban	Lat 8°N 24'31.056" & Long 4°E 37'39.966"

Honey Bee Insect Identification

The collected *A. mellifera* samples were identified to the species level using insect identification keys using a dissecting binocular microscope and vernier caliper as described in previous studies (13, 14). Honey bee samples were then preserved in 70% ethanol and kept at −20°C until needed for molecular genotyping. Five randomly selected honey bees from each location were subjected to DNA extraction and genotyping using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) assay.

DNA Extraction and RAPD-PCR Amplification

Genomic DNA was extracted from each honey bee using Bioline Isolate II Genomic DNA Kit catalogue No: BIO-52067 from Meridian Bioscience, USA and the presence of DNA fragment was confirmed by running the extracted DNA on agarose gel electrophoresis. The DNA was then stored at −20°C until needed for RAPD-PCR. The octamer OPA 3, OPA

4, OPA 5 and OPA 9 RAPD primers used in this study was previously described by Omar *et al.,* (17) and OPA 11 was previously described by Al-Otaibi *et al.,* (18). Sequences of these primers are listed in Table 2.

Table 2. Oligonucleotide sequence of the RAPD primers used

Primer Name	Base Sequence
OPA 3	5′ AGTCAGCCAC 3′
OPA 4	5′ AATCGGGCTG 3′
OPA 5	5′ AGGGGTCTTG 3′
OPA 9	5′ GGGTAACGCC 3′
OPA 11	5′ CAATCGCCGT 3′

The RAPD mixtures were processed in 50 µL reactions with 0.2 mM of each dNTP, 0.3 µM primer, 1 U of Taq DNA polymerase (Invitrogen), 10× buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), and 20 ng of honey bee genomic DNA. The PCR conditions included initial denaturation of 1 cycle at 94°C for 2 min, then 35 cycles of the following: final denaturation at 94°C for 1 min, annealing at 36°C for 1 min, initial extension at 72°C for 2 min, and one cycle of final extension at 72°C for 10 min. The amplified products were run on 1.5% agarose gel in 0.5× TBE buffer for 90 min at 80 volts, stained with SYBR Safe (Invitrogen), visualized under UV illumination, photographed and documented. A 1 kb DNA ladder was used as the gene ruler.

Molecular Data Analysis

DNA bands on the agarose gel were marked as '1' or '0' indicating presence or absence of the band, respectively. The band intensity was not taken into consideration. The data collected were analysed using PyElph, ARLEQUIN, and GeneAlEx version 6.501 software. The presence or absence of unique, shared, and polymorphic bands was used to generate similarity coefficients. Degree of polymorphism, Nei's genetic distance, allele frequencies, average number of alleles per locus, observed (Ho) and expected heterozygosity (He) for each honey bee population across the loci were determined. The GeneAlEx version 6.501 software was used to calculate estimated variance among population, within population and the hierarchical genetic structure of the honey bee populations. Analysis of Molecular Variance (AMOVA) was used to estimate molecular variability between and within honey bee populations. Similarity coefficients were then used to construct a dendrogram manually by unweighted pair group method using arithmetical averages (UPGMA). The dissimilarity index of the populations was calculated as the mean of all pair wise comparison of the banding pattern in the honey bees (16, 17).

RESULTS

The thirty *A. mellifera* collected from six different geographical locations in Kwara state, Nigeria were identified to be morphologically similar, and RAPD-PCR results showed that each RAPD primer produced a combination of unique and common fragment patterns. The results of the DNA agarose gel electrophoresis of *A. mellifera* using five RAPD-DNA primers are shown in Figure 1. This showed that the amplified DNA generated varied number of polymorphic bands.

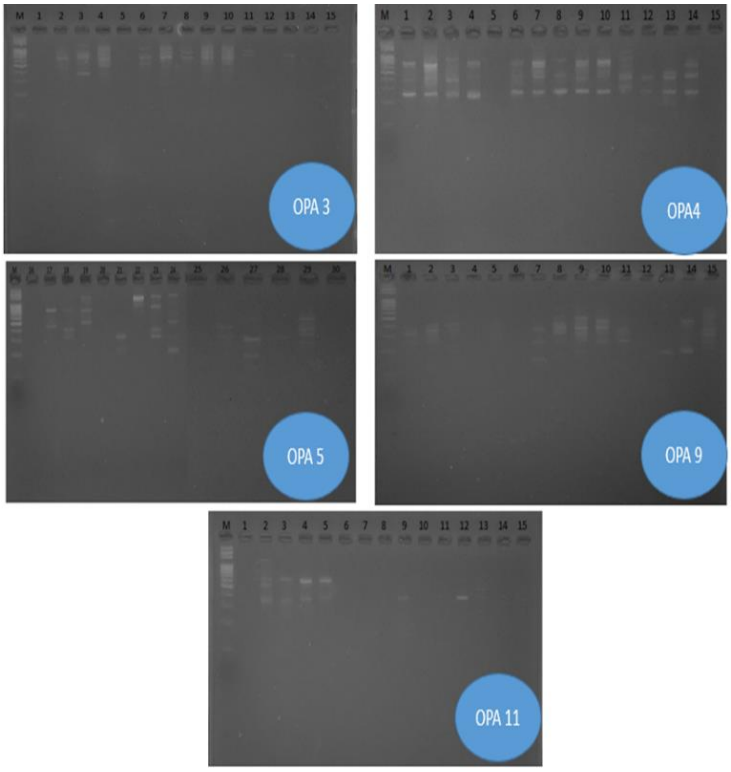

Figure 1. Electrophoresis of RAPD-PCR products on a 1.5% agarose gel using five octamer primers (OPA 3, 4, 5, 9, and 11) reveals PCR amplicons from *Apis mellifera* DNA samples. 'M' denotes the 1kb ladder, used for estimating the sizes of the bands. Lanes 1-15 correspond to the honey bee DNA samples. Primers OPA 3 and OPA 9 generated amplicons for samples R1A-R2E, while OPA 4, OPA 5, and OPA 11 were applied to samples U2A-U3E. The polymorphic bands observed range from 200 bp to 3000 bp

Table 3. Observed number of bands, percentage of polymorphic bands, expected heterozygosity (*He*), Shannon's information index (*I*) values and their standard errors for populations studied

Populations	Observed No of bands	Polymorphic bands (%)	He	I
Agbede	31	37.33	0.111	0.175±0.018
Osin Aremu	26	33.33	0.116	0.176±0.020
Amoyo	20	25.33	0.076	0.118±0.017
Tanke (Pipeline)	27	32.00	0.112	0.168±0.020
Adewole	18	21.33	0.074	0.112±0.017
Idofian	16	17.33	0.049	0.078±0.014

He=Expected Heterozygosity; *I*= Shannon Information Index

Table 3 shows the percentages of polymorphic bands and expected heterozygosity values. In total, these results revealed low level of genetic polymorphism and expected heterozygosity with the highest and lowest polymorphic bands recorded in Agbede and Idofian sampling locations, respectively. The total number of DNA bands across all the six populations was 158. Shannon information index was also low in all the six populations, this suggests the possibility of gradual loss of genetic diversity.

Across all six populations sampled, a total of 138 bands were generated, of which only 55 were locally common bands. The analysis of DNA bands using RAPD primers revealed the greatest number in Agbede with a total of 31, while Idofian had the fewest at 16. When considering private bands, which are unique to a single population, the highest count was observed in both Osin Aremu and Tanke, each with 4, in contrast to Idofian, which had the minimum with just 1. In the case of locally common bands—those found in 50% or fewer of the populations—the number was most significant in Agbede with 16, whereas Adewole had the least, with 4 (Figure 2).

The estimated molecular variance among *A. mellifera* samples from the six different locations in Kwara state, Nigeria are as shown in Table 4. The estimated molecular variance was higher within each of the six populations than among all the six populations. There was no molecular variance among regions, which showed no evidence of genetic polymorphism on the basis of whether or not the *A. mellifera* were collected from urban or rural sampling locations. Estimated molecular variance among populations was 34% indicating that there was low molecular variance among populations. The results of AMOVA revealed that most of the genetic variability (66%) was observed within populations. There was no molecular variance among populations with respect to the location of samples collections. This implies that samples from urban and rural areas do not differ genetically on the basis of locations alone and that there was low inter-population genetic variation.

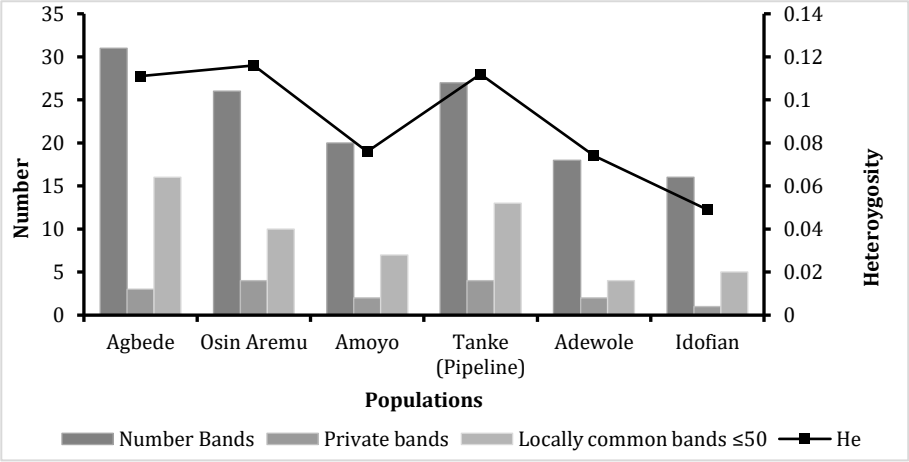

Figure 2. The bands patterns across the six populations (three rural and three urban)

Table 4. Summary of analysis of molecular variance (AMOVA) among and within the sampled honey bee populations

Source	Degree of Freedom	Sum of Square	Means of Square	Estimated Variance	Molecular Variance (%)
Among Regions	1	29.867	29.867	0.000	0
Among Populations	4	184.87	46.217	6.695	34
Within Populations	24	305.80	12.742	12.742	66
Total	29	520.53		19.437	100

Analysis of pairwise population by Nei genetic distance provided insights into the genetic relationships among the six populations of *A. mellifera* sampled (Table 5). The highest genetic distance was observed between Tanke (urban) and Adewole (urban), representing the most genetically distant populations, with a genetic distance (Nei D) of 0.092. However, Amoyo (rural) and Idofian (urban) were identified as the most genetically related populations with the lowest genetic distance value of 0.036. Additionally, pairwise population Nei genetic identity assessments revealed a striking uniformity in genetic identity values among all six populations, regardless of their sampling locations. Each population exhibited genetic identity values between (0.929-0.984), indicating high level of genetic similarity shared by populations within and across rural and urban regions.

Table 5. Pairwise population Nei Genetic Distance, Unbiased Nei Genetic Distance, Nei Genetic Identity and Unbiased Nei Genetic Identity among the six populations

Regions	Population 1	Population 2	Nei D	Neiu D	Nei I	Neiul	Sample Size 1	Sample size 2
Rural-Rural	Agbede	OsinAremu	0.038	0.017	0.968	0.984	5	5
Rural-Rural	Agbede	Amoyo	0.064	0.049	0.938	0.952	5	5
Rural-Rural	Osin Aremu	Amoyo	0.040	0.021	0.961	0.979	5	5
Rural-Urban	Agbede	Tanke (Pipeline)	0.051	0.032	0.950	0.968	5	5
Rural-Urban	Osin Aremu	Tanke (pipeline)	0.053	0.030	0.949	0.970	5	5
Rural-Urban	Amoyo	Tanke (Pipeline)	0.069	0.053	0.934	0.948	5	5
Rural-Urban	Agbede	Adewole	0.088	0.073	0.915	0.929	5	5
Rural-Urban	Osin Aremu	Adewole	0.072	0.053	0.931	0.948	5	5
Rural-Urban	Amoyo	Adewole	0.058	0.046	0.943	0.955	5	5
Urban-Urban	Tanke (Pipeline)	Adewole	0.092	0.076	0.912	0.927	5	5
Rural-Urban	Agbede	Idofian	0.083	0.071	0.920	0.931	5	5
Rural-Urban	Osin Aremu	Idofian	0.065	0.050	0.937	0.951	5	5
Rural-Urban	Amoyo	Idofian	0.036	0.027	0.965	0.973	5	5
Urban-Urban	Tanke (Pipeline)	Idofian	0.080	0.068	0.923	0.935	5	5
Urban-Urban	Adewole	Idofian	0.066	0.057	0.936	0.945	5	5

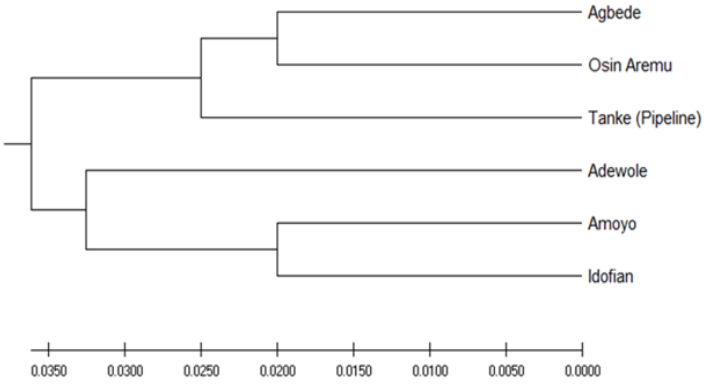

Figure 4. Population phylogenetic tree among the six locations including rural and urban using unweighted pair group method using arithmetical averages (UPGMA) shows an evolutionary distance

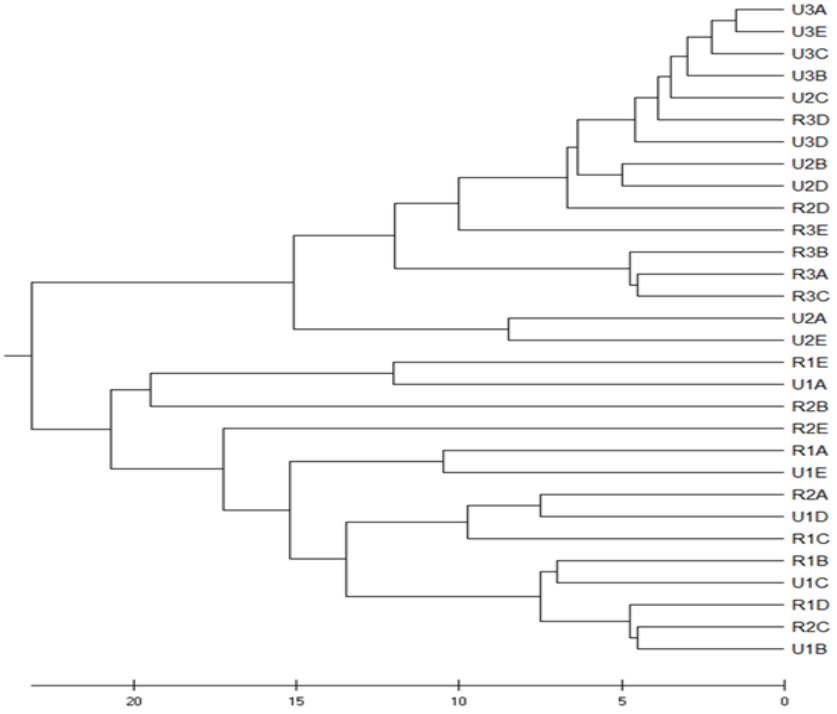

Figure 5. Unweighted pair group method with average dendrogram based on Nei's (Nei, 1972) genetic distance, summarizing data on *A. mellifera* individuals according to RAPD analysis. Urban honey bees (U1A-E, U2A-E, U3A-E) and rural honey bees (R1A-E, R2A-E, R3A-E)

DISCUSSION

Molecular markers are useful tools for assessing genetic variations and resolving species identities. Information on genetic diversity and relationships among honey bees in rural and urban areas in Nigeria is currently very limited. Among the molecular markers, RAPD is increasingly being employed in genetic studies because it is simple, highly reproducible and requires a small amount of genomic DNA (17-19). In addition, RAPD requires no prior sequence information for the fingerprinting of species genomes, and it has been used extensively for estimating genetic variations at the population level and among closely related species (16). RAPD has also been used in the investigation of genetic diversity and relatedness among different organisms (15-18).

The honey bees from the rural and urban populations exhibited low levels of genetic variation. The observed low genetic diversity was also reported in similar studies (12, 20). This may be due to inbreeding between rural and urban honey bees. Similar trend has been reported in the analysis of the genetic diversity of another organism using RAPD (21). In the urban region sampled, there was no distinction in the level of genetic polymorphism. This is surprising because normally, urbanization may subject the honey bee populations to serious pressure due to habitat change. This may drive pollinator genetic diversity and pollination as previously suggested (22). It is therefore surprising that the pattern of genetic diversity was low in *A. mellifera* populations obtained from both rural and urban locations.

Analysis of the molecular variance in *A. mellifera* populations suggests a gradual loss of genetic variability. This loss could be attributed to population restructuring, habitat destruction through bush burning, illegal mining activities, climate change, deforestation, massive destruction of honey bee wild colonies due to hunting for honey, natural selection and genetic drift. Similarly, the high genetic identity and low level of genetic polymorphism observed in this study may also be because these honey bee populations, despite being collected from different locations, are probably from similar origin. This implies that there is evidence of low inter-population genetic variation. Similar result was also reported on study of genetic variation of honey bee, *Apis dorsata* populations using RAPD markers in Saudi Arabia (12). Another plausible reason for the observed low genetic diversity may be as a result of consistent and uninterrupted gene flow and non-random mating that have existed over a long time within the *A. mellifera* populations in both rural and urban samples. This may put the *A. mellifera* populations at a risk of gradual decline and or total extinction.

Genetic distance is the degree of genetic or genomic difference between species or populations or the measure of divergence between species or between populations within a species. Genetic similarity measures the degree of relationship between species or between populations within a species. In this study, genetic similarities obtained from RAPD data showed that all the *A. mellifera* populations showed genetic similarity between individual samples from rural and urban areas. This may be probably due to unrestricted migration and movement by dispersal of honey bees in the studied areas. Thus, the evidence of low polymorphism suggests low divergence between the sites among all the honey bee populations sampled. Investigating the extent of relatedness among *A. mellifera* populations is vital in conservation genetics (23).

Cluster analysis based on Nei similarity coefficients using UPGMA grouped all the thirty honey bees into two main clusters and exhibited a high level of genetic relatedness. Interestingly, the six populations (three rural and three urban) may possibly have similar genetic identity and possibly common evolutionary relationship. This probably suggests that the *A. mellifera* individuals from rural and urban regions are from a common source. It is likely that some *A. mellifera* in rural areas might have migrated to urban areas and vice-versa, but no evidence of genetic exchange has occurred. This may indicate low adaptability among the honeybee populations and make the sampled population susceptible to gradual loss of genetic diversity. Loss of genetic diversity may have been probably induced by environmental factors and or human activities such climate change, bush burning, land clearing for farming and pollution. Previous study has suggested the possibility of enhanced natural immunity among genetically diverse honey bees. This is because genetically diverse populations may have lower prevalence of diseases than genetically similar populations (24). It could be suggested that the data presented in this study showed that RAPD markers were effective in discriminating honey bee populations. This is because the low level of genetic variation was also reported in a previous study where mitochondrial and nuclear DNA markers were used to investigate genetic structure of *A. mellifera carnica* bees obtained from Slovenia (25). In another separate study where mitochondrial DNA marker was used in *A. mellifera pomonella*, there was evidence of shared phylogeographic history within subspecies studied (26). Summarily, this study showed that RAPD markers were effective in discriminating genetic variability between and among honey bee populations in both rural and urban areas.

This study concludes that honey bee populations from rural and urban regions exhibit relatively low genetic variability, which may be due to the fact that the sampled population shared a common ancestral relationship. There is therefore an urgent need for an integrated conservation approach of *A. mellifera* to prevent further genetic diversity loss. These results can be used as baseline information for future genetic diversity and conservation study of honey bees in Nigeria.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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