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GENETIC VARIATION IN INDIGENOUS TURKEY POPULATIONS IN SOUTH WEST NIGERIA

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ABSTRACT

The study was carried out to assess the genetic variation in indigenous turkey populations in South West Nigeria using seven protein markers: Hemoglobin (Hb), Albumin (Alb), Transferin (Tf), Esterase-1 (Es-1), Alkaline phosphatase (ALP), Carbonic anhydrase (CA), and Esterase-3 (Es-3).. A total of 97 turkey birds from Ibadan, Ijebu Ode and Akure were purposively sampled. Separation of plasma and red cell proteins were determined on cellulose acetate electrophoresis to estimate the genetic variability within and among the populations. The degree of heterozygosity, deviation from Hardy-Weinberg's Equilibrum (HWE), F_{IS}, and F_{ST} values were estimated.

From a total of 7 loci analyzed, six were found to be polymorphic in all the populations sampled while Es-1 was monomorphic. Two codominant alleles which controlled three different genotypes were observed at each polymorphic loci. Deviations from Hardy Weinberg equilibrium and a deficiency of heterozygotes were observed in all the populations. Average heterozygosities ranged between 0.13 and 0.20, heterozygote deficit F_{IT} was estimated at 0.5784 while within breed deficit as evaluated by F_{IS} ranged from 0.38 to 0.56. The fixation index F_{ST} revealed that genetic diversity within the studied population was moderately differentiated. Genetic distance among the populations quantified through calculation of Nei genetic distance ranged from 0.037 - 0.109. The result obtained may be used as an initial guide in defining objectives for future investigations of genetic integrity and developing conservation strategies for Nigeria indigenous turkeys.

Indexing terms/Keywords

Genetic variation; Indigenous turkeys; polymorphism; heterozygosity

Academic Discipline And Sub-Disciplines

Animal Science; Animal Breeding and Genetics

SUBJECT CLASSIFICATION

Animal Breeding and Genetics; Genetic diversity

TYPE (METHOD/APPROACH)

Research article

INTRODUCTION

Nigeria is endowed with an impressive array of indigenous livestock, the potential of which cannot be overlooked considering the huge foreign exchange implication of the importation of improved exotic stock, and the genotype-environment interaction which has lead to considerable loss of fitness of the exotic stock (Ibe, 1990). Indigenous animals are both functionally and genetically valuable because they contain genetic materials which may be harnessed for improvement. The knowledge of their genetic diversity is important as it forms the basis for designing breeding programmes and making rational decisions on sustainable utilization of animal genetic resources (Mwacharo *et al.,* 2005), selection, and development of new breeds with improved resistance to environmental challenges.

Loss of genetic diversity within indigenous livestock populations has been a major global concern and realization of this has led to efforts to study genetic diversity in livestock species in order to provide a basis for conserving these potentially useful germplasms. The loss of genetic variation within and between breeds is detrimental not only from the perspectives of culture and conservation but also utility since lost genes may be of future economic importance (FAO, 2011). Within breed, high rates of loss of genetic variation leads to reduced chances of breed survival due to decreased fitness through inbreeding depression.

The genetic characterization of the domestic animals is part of the FAO global strategy for the management of farm animal genetic resources. This strategy places a strong emphasis on the use of molecular methods to assist the conservation of endangered breeds and to determine the genetic status of breeds. These range from elecrophoretic detection of polymorphism of gene products at structural loci to Deoxyribonucleic acid (DNA) analysis. DNA-based technologies are now the methods of choice for genetic characterization of livestock (Arora *et al.,* 2011) however most diversity and phylogenetic studies are mainly based on microsatellite loci (Erhardt and Weimann, 2007) and single nucleotide polymorphisms (SNPs)

Nigerian indigenous turkeys (*Meleagris gallopavo*) are kept mostly under semi-intensive system, because of their better adaptation to the adverse climatic conditions of the tropical environment and survivability under low management inputs. In spite of their importance, information on blood protein and or enzymes types in Nigerian Indigenous Turkey is scarce, however, these has been reported extensively in other poultry species; in chicken (Ismoyowati, 2008, Johari *et al*. 2008, Guney *et al*. 2003, Das and Deb, 2008, Al-Samarrae *et al.,* 2010, Ige *et al.,* 2013, Yakubu and Aya, 2012, Ajayi *et al.,* 2013), ducks (Azmi *et al.,* 2006; Johari *et al.,* 2012, Zhang *et al.,* 2002; Ismoyowati, 2008 and Okabayashi *et al.,* 1999)

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and chuckars and pheasants (Ugur *et al.,* 2006). Therefore, the present study was aimed at assessing the genetic diversity within Nigerian indigenous turkeys in South West Nigeria

MATERIAL AND METHODS

The experiment was conducted at the Animal Breeding and Genetics Laboratory of the Department of Animal Science, University of Ibadan.

5 ml of blood was collected through wing veins from a total of 97 purposively sampled adult turkeys comprising 28 turkeys from Ibadan, 29 turkeys from Akure and 40 adult turkeys from Ijebu-Ode all in the southern part of Nigeria (Plate 1) into tubes containing heparin as the anticoagulant and kept refrigerated during transportation. Samples were separated into plasma and red cell fractions by centrifugation at 3000 rpm for 15 minutes. Each fraction was subjected electrophoresis on cellulose acetate The buffer system staining and destaining procedures were as previously described by RIKEN (2006)and Akinyemi and Salako, 2012)

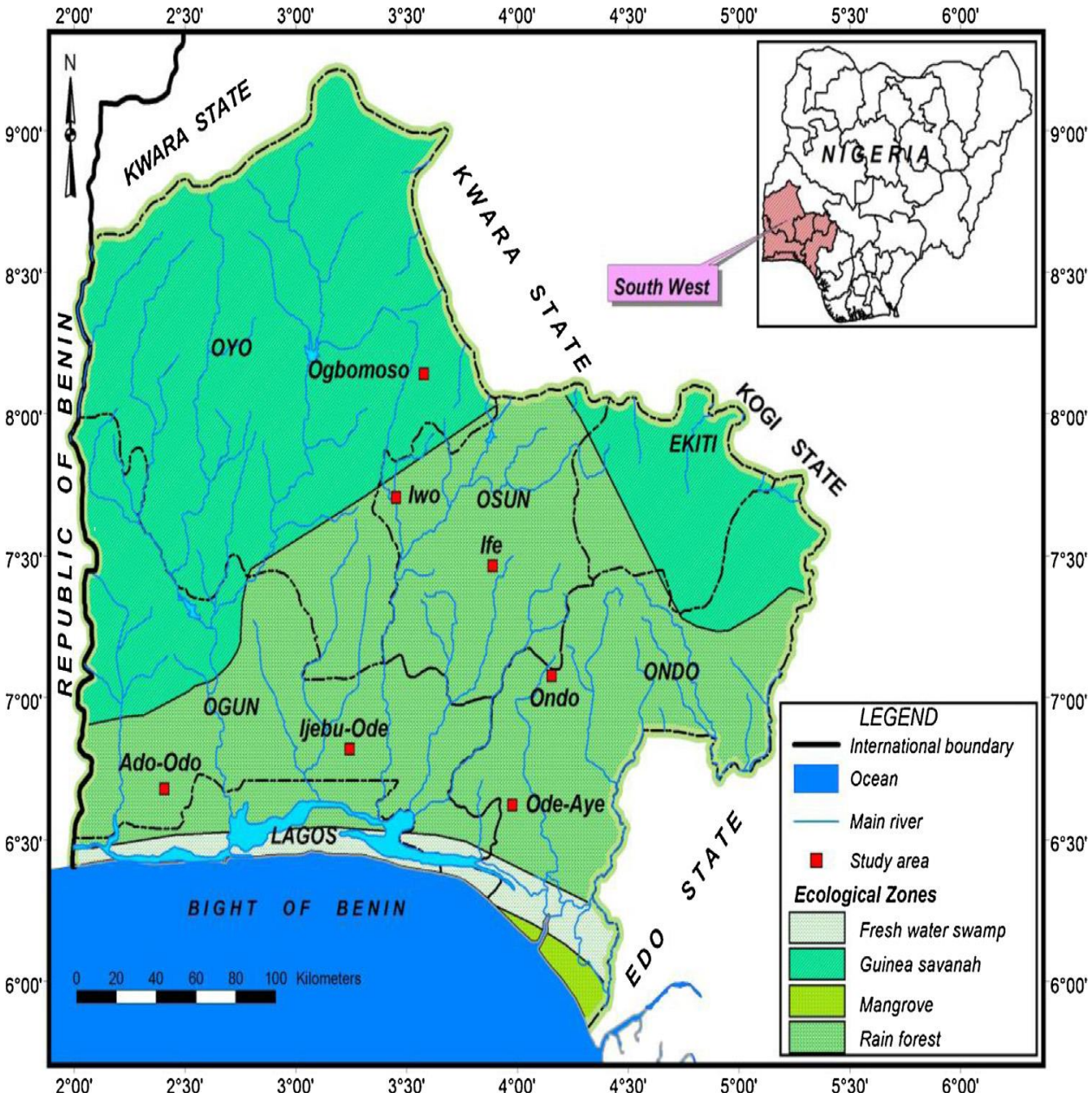

Plate 1: Map of Nigeria highlighting the study area

STATISTICAL ANALYSIS

Allelic variant for each locus were identified in order of increasing mobility after each electrophoretic run. Allele frequencies and genotypic frequencies for each locus in each sample were computed by direct counting and tested for fit to Hardy-Weinberg ratios using goodness of fit test.
In all the investigated loci, the homozygote genotype AA was represented by a single fast band; single slower band was designated as BB homozygote, while the presence of both bands (fast and slow band) was designated as AB heterozygote. Carbonic anhydrase and Alkaline phosphatase genotypes were represented as: homozygote fast (FF), homozygote slow (SS), and heterozygote genotype (FS) respectively.
The observed and expected heterozygosity were calculated according to Nei (1973). The genetic differentiation and fixation indices F_{ST}, F_{IS}, and F_{IT} were analysed according to Nei (1987) by Wright's (1978) F-statistic. All computations were performed using Popgene programme (Yeh *et al.,* 1999). Genetic relationship of the population was determined by construction of dendograms using Tools for Population Genetics Analysis (TFPGA), (Miller, 1997).

RESULTS

Of the 7 proteins and enzymes studied, 6 were found to be polymorphic. Allele and genotypic frequencies are presented in Tables 1 and 2. The CA and Alk phenotypes were designated F and S as previously reported in literature (Das and Deb);with the faster band migrating band designated as F and the slower band S. at the Alp locus, allele F occurred in high frequencies in the three populations studied, allele B was predominant in the Ibadan and Akure populations at the Hb locus. Bands with the slower mobility known as the S allele occurred at a higher frequency at the CA locus while allele B was fixed at the Es-1 in the three populations. Allele B was also predominant at the Tf locus. Deviations of genotypic frequencies from the Hardy-Weinberg equilibrium from the pooled populations are shown in Table 3. Significant deviation from HWE refer to a deficiency of heterozygotes was stronger when the total population was considered. With the exception of Es-1 which was monomorphic, other loci in the pooled population exhibited significant deviations from HWE. F values which indicate loss of variability in populations are presented in Table 4. Fis values were higher in the Akure population suggesting higher levels of inbreeding. Moderately high values ranging from 0.51-0.54 were reported for Shannon Information Index (Lewontin. 1972)..

Table 1: Allele Frequency at seven Allozyme Locus in Nigerian Indigenous Turkey

Locus	5	Ibadan (n=28)	Ijebu-Ode (n=40)	Akure (n=29)	Overall
ALP	F	0.571	0.537	0.689	0.593
	S	0.428	0.463	0.310	0.407
Hb	A	0.267	0.587	0.207	0.381
	B	0.732	0.413	0.793	0.619
CA	F	0.250	0.300	0.241	0.268
	S	0.750	0.700	0.759	0.732
Tf	A	0.250	0.337	0.413	0.335
	B	0.750	0.663	0.586	0.665
Alb	A	0.187	0.182	0.569	0.386
	B	0.813	0.812	0.431	0.613
Es-1	A	****	****	****	****
	B	1.000	1.000	1.000	1.000
Es-3	A	0.393	0.362	0.759	0.489
	B	0.607	0.638	0.241	0.510

ALP= Alkaline phosphatase, Hb= Hemoglobin, CA=Carbonic anhydrase, Alb=Albumin, Tf=Transferin, Es-1=Esterase$_1$, Es-3= Esterase$_3$; A, B, F and S- alleles.z

Table 2: Genotype Frequencies at seven Allozyme Locus in Nigerian Indigenous Turkey

Locus	Genotype	Ibadan (Oyo) (n=28)	Ijebu-Ode (Ogun) (n=40)	Akure (Ondo) (n=29)	Overall
ALP	FF	0.571	0.525	0.690	0.587
	FS	0.000	0.025	0.000	0.010
	SS	0.429	0.450	0.310	0.402
Hb	AA	0.179	0.450	0.172	0.288
	AB	0.179	0.275	0.069	0.154
	BB	0.643	0.275	0.759	0.525
CA	FF	0.143	0.175	0.207	0.175
	FS	0.214	0.250	0.069	0.185
	SS	0.643	0.575	0.724	0.639
Tf	AA	0.143	0.175	0.207	0.175
	AB	0.214	0.325	0.414	0.319
	BB	0.643	0.500	0.379	0.505
Alb	AA	0.214	0.10	0.379	0.237
	AB	0.536	0.175	0.379	0.340
	BB	0.25	0.725	0.241	0.443
Es-1	AA	****	****	****	****
	AB	****	****	****	****
	BB	1.000	1.000	1.000	1.000
Es-3	AA	0.393	0.175	0.758	0.412
	AB	0.000	0.375	0.000	0.154
	BB	0.607	0.450	0.241	0.433

ALP= Alkaline phosphatase, Hb= Hemoglobin, CA=Carbonic anhydrase, Alb=Albumin, Tf=Transferin, Es-1=Esterase$_1$, Es-3= Esterase$_3$, AA, AB, BB, FF, FS and SS- genotypes.

Table 3: Heterozygosity Estimates for All Loci in the pooled population

		Heterozygosity			
Allele	Sample size	Ho	He	Ave.H	HWE
ALP	97	0.000	0.474	0.402	93.96**
Hb	97	0.185	0.474	0.401	36.37**
CA	97	0.185	0.394	0.387	27.64**
Tf	97	0.319	0.448	0.435	8.06**
Alb	97	0.340	0.476	0.431	8.04**
Est-1	97	0.000	0.000	0.000	****
Est-3	97	0.154	0.502	0.435	46.95**

ALP= Alkaline phosphatase, Hb= Hemoglobin, CA=Carbonic anhydrase, Alb=Albumin, Tf=Transferin, Es-1=Esterase$_1$, Es-3= Esterase$_3$, Ho= observed heterozygosity, He= expected heterozygosity, Ave.H= average heterozygosity, HWE=Hardy-Weinberg equilibrium. **p<0.01

Table 4: Average heterozygosities across the populations sampled

Populations	Sample size	Ho	He	Nei Expe	I*	F_{IS}
Ijebu Ode	40	0.20	0.37	0.37	0.53	0.38
Ibadan	28	0.16	0.38	0.37	0.53	0.47
Akure	29	0.13	0.35	0.35	0.51	0.56

Ho= observed heterozygosity, He= expected heterozygosity, Ave.H= average heterozygosity,
***Shannon Information Index (Lewontin, 1972)** F_{IS}

Table 5: F-Statistics and Gene flow for all the Loci studied

Locus	Sample Size	F_{IS}	F_{IT}	F_{ST}	Nm*
Hb	97	0.5664	0.6192	0.1218	1.8022
CA	97	0.5408	0.5424	0.0034	72.4477
Tf	97	0.2710	0.2857	0.0201	12.1634
Alb	97	0.1580	0.2506	0.1099	2.0238
Es1	97	****			
Es3	97	0.7127	0.7500	0.1296	1.6787
Alp	97	0.9823	0.9826	0.0177	13.8622
Mean		0.5468	0.5784	0.0699	3.3285

* Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst

Table 6: Genetic distance D between the populations according to Nei, 1978 (below the diagonal) and geographical distance in (km) (above the diagonal)

	Ibadan	Ijebu Ode	Akure
Ibadan		76.7	178.0
Ijebu Ode	0.042		205.1
Akure	0.037	0.109	

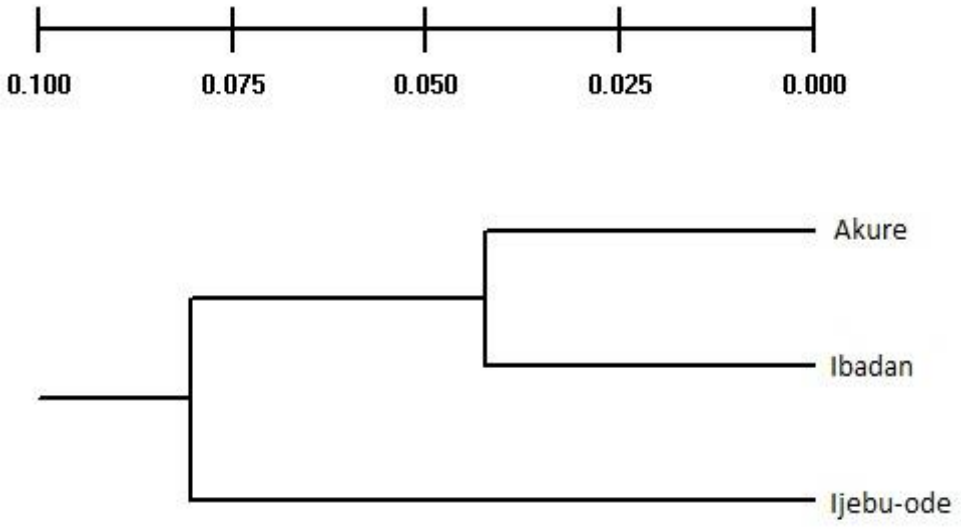

Figure 1: Dendogram of genetic distance between three sampled populations of Nigerian Indigenous Turkey

DISCUSSION

Three hemoglobin genotypes AA, AB and BB reported in this study are consistent with those reported in chukars and pheasants by Ugur *et al.*, (2006). Similar results were reported by (Al-Samarrae *et al.,* 2010; Yakubu and Aya, 2012; and Ajayi *et al.,* 2013 and Ige *et al.*2013), in chickens. The preponderance of allele B observed in this study is consistent with the reports of Ige et al., 2013 and Ajayi et al 2013, however Yakubu and Aya reported a higher frequency of allele A at this locus. This can be explained by the fact that the birds in the study were sourced from a different region of the country and may suggest an adaptive significance.

The observed albumin genotypes (AA, AB and BB) controlled by two codominant alleles Alb^{A} and Alb^{B} in this study agrees with the report of Quinteros *et al.,* (1964) on domestic turkey (M. gallopavo), the Ocellated turkey (M. ocellata) and in the descendants from a Domestic turkey x Ocellated turkey cross. Albumin polymorphisms have also been reported in several poultry species. Esmailkhanian *et al.,* (2000) observed three albumin phenotypes i.e AA, AB and BB in Iranian native poultry breed. Ismoyowati, (2008) reported Alb^{AA}, Alb^{AB}, Alb^{AC}, Alb^{BB} and Alb^{BC} Albumin genotypes in Kampung chicken. Johari *et al*. (2008) also reported the presence of two alleles: A and B in Kedu chickens. Other species such as the Muscovy and Pekin ducks (Azmi *et al.,* 2006 and Johari *et al.,* 2012) have also been reported to exhibit albumin polymorphism.

In this study, observations at the transferin locus showed two different alleles (Tf^{A} and Tf^{B}) and three different genotypes Tf^{AA}, Tf^{AB} and Tf^{BB} in all the populations studied. The frequency of the Tf^{A} was lower than that of Trf^{B} in all the three populations. Transferin polymorphism has been in other species, in chickens (Johari *et al*. 2008; Guney *et al*. 2003) and ducks (Zhang *et al.,* 2002; Ismoyowati, 2008; and Johari *et al.,* 2012). The effect of heterozygous transferrin appears have a biological functional effect on fertility, hatchability and egg production (at least 90 days' production). Chicken with Tf^{A} appears to have delayed sexual maturity while the chicken with the Tf^{B} has the earlier age of sexual maturity (Das and Deb, (2008).

Carbonic anhydrase are zinc containing enzymes that catalyze the reversible hydration/dehydration of carbon dioxide and bicarbonate and thus participates in a variety of biological processes that include acid-base balance, carbondioxide transport and ion exchange. This enzyme has been shown to be involved in the active deposition and dissolution of CaCO3 deposits (Henry and Kromanig, 1985: Serrano et al., 2007). This likely explains the role the enzyme plays in egg shell thickness of several poultry species (Das and Deb, 2008). In this study two codominant alleles CA^F and CA^S were observed. However, the frequency of CA S was consistently higher than F in all the populations studied. Ige et al., 2013 however reported the predominance of CA F in Yoruba and Fulani ecotype chickens, this apparent contradiction may be due to specie differences

Three ALP genotypes (FF, FS and SS) were observed in all the three populations studied which were controlled by two codominant alleles (F and S). The presence of two alleles at this locus has been reported (Singh and Nordskog (1981); Okamoto et al., 1999; Das and Deb 2008). ALP^{FF} has the highest frequency in all the three populations studied followed by the ALP^{SS} while ALP^{FS} had the lowest genotype frequency. Similar trend was also observed for overall population genotypes where 58.76%, 1.03% and 40.21 were identified for ALP^{FF}, ALP^{FS}, and ALP^{SS} respectively suggesting the predominance of the fast allele F. However Singh and Nordskog (1981) reported the prevalence of the slow allele S in some lines of Leghorn chickens. For age at sexual maturity, Das and Deb (2008) reported that the birds with the fast type allele mature about 13 days before birds with the slow type. This enzyme has been reported to be higher in pullets selected for higher production (Singh et al., 1983) suggesting that the enzyme likely play a significant role in sexual maturity.
Esterase-1 was monomorphic for all the samples analyzed, Okamoto *et al.,* (1999) however reported the presence of four alleles (A, B, C and D) at this locus in Laos native chickens with allele B exhibiting the highest frequency in the populations studied.
Two codominant alleles A and B which controlled three different genotypes (AA, BB and AB) were observed at Es-3 locus in this study. Pravakaran, (1985) identified serum esterase electrophoresis variants in five strains of chicken, Das and Deb (2008) reported the presence of four alleles, E/, Es1, Ess and Es° in chickens. The number of alleles obtained in this study could be attributable to the specie differences.
The measure of heterozygosity for each individual locus in the population revealed that, CA has the lowest value (0.3871) and ALP locus value (0.4717) appeared highest (Table 3). The average heterozygosity coefficient for all the studied loci was 0.3661. This relatively high values of heterozygosity indicates that there is sufficient genetic variability within the populations studied. This values reported were higher than those obtained by Okabayashi *et al.,* (1999) in Magelang duck (0.136) and Tegal duck (0.118) but very close to 0.41 average heterozygosity reported by Yakubu and Aya (2012) at the hemoglobin locus in Nigerian indigenous chicken.
Chi-square analysis for the differences between the expected and observed genotype frequencies showed that Tf locus in Ibadan and Akure, Es-3 locus in Ibadan and Alb locus in Ijebu-ode and Akure populations did not significantly deviated from HWE ($P < 0.05$). However, on the overall data set there were significant deviations from HWE in all the 7 studied loci (Table 3). This could be attributed to migration occasioned by the introduction of birds from different sources into the respective populations and selection. (Aminafshar *et al.,* 2008).

F-statistics of the pooled and individual population sampled are presented in Tables 4 and 5 respectively. The heterozygosity deficit (F_{IT}) was estimated at 0.5784 and within breed deficit in heterozygosity as evaluated by F_{IS} ranged between 0.01580 (Alb) to 0.9823 (ALP) having a total mean of 0.5468 for all the studied loci. Within breed differentiation evaluated by F_{ST}, was estimated at 0.0699 with a range of 0.000 (Es-1) to 0.1296 (Es-3). The gene flow values for each of the seven loci studied ranged from 1.6787 for Es-3 to 72.4477 for CA. The mean gene flow over all the studied loci was 3.3285.

The Wright's (1978) fixation index ($F_{ST)}$ value observed ranged from 0.0000 to 0.1296 which indicate that genetic diversity within the studied populations was moderately differentiated (Cavalli-sforza *et al.,* 1981). Observed positive and low values of F_{ST} and F_{IT} showed the deficiency of heterozygotes in the populations.

The genetic distance among the populations studied were small but within the range 0.000 and 0.058 reported by Nei, (1976), for local breeds (Table 6). The distance indicates differentiation among the studied population, the Ibadan population is situated in the same cluster with the Akure population (Figure 1). Correlation between genetic and geographic distance was low (r=0.257) suggesting that migration is not pronounced in the birds found in the region. Further analysis based on molecular markers should be employed to provide meore information on the genetic characterization of the indigenous birds.

Conclusion

The information may be useful as an initial guide in defining objectives for future investigations of genetic integrity and developing conservation strategies for Nigeria indigenous turkeys. This may also pave way for marker-assisted selection in the genetic improvement of these birds.

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Author' biography with Photo

Dr Mabel Omolara Akinyemi earned her BSc., Masters and PhD degrees in Animal Science from University of Ibadan in 2000, 2004 and 2010 respectively. She specialized in Animal Breeding and Genetics with a PhD dissertation that elucidated the morphological and biochemical characterization of three indigenous sheep breeds in Nigeria. Her research focus since graduation is dedicated to generating new knowledge in genetics, genomics and molecular biology that can be applied to improve animal breeding and economic value of indigenous livestock in Nigeria.

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