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GENETIC RELATIONSHIP AMONG NIGERIAN WEST AFRICAN DWARF GOAT POPULATIONS USING BLOOD PROTEIN POLYMORPHISM

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Abstract

This investigation was carried out to study the genetic relationship among three populations of Nigerian West African Dwarf (WAD) goat using blood protein markers. Blood samples were obtained from a total of one hundred and forty adult animals of both sexes, and their protein polymorphism was investigated using cellulose acetate electrophoresis in three loci (haemoglobin, transferrin and carbonic anhydrase). Based on the estimated allele frequencies, the populations were characterized by their genetic variability using heterozygosity (H), a number of alleles (Na) and genetic distance (D) among the populations. The estimates of heterozygosity were 0.48, 0.47, 0.39 and 0.46 for Ogun, Ondo, Osun and Oyo populations respectively. Two alleles were reported for each of the population (A, and B in both haemoglobin and transferrin while F and S for carbonic anhydrase). The closest genetic relationship was found between the Ogun and Oyo WAD goat populations (D=0.011), while population from Ondo and their Osun counterparts were more genetically distance (D=0.066). The relationships among the four populations were consolidated by the dendrogram that emanated from their genetic distances. The present information may be exploited in formulating appropriate management in conservation strategies and improvement of Nigerian WAD goat populations.

Key Words: Protein polymorphism, genetic distance, heterozygosity, West African Dwarf goat

Introduction

Domestic animal biodiversity is the complex result of environmental and genetic effects leading to the differentiation of morphological, physiological and productive traits which are vital to all production systems as it provides the raw materials for breed improvement, and for adaptation to changing circumstances (Ceriotti *et* al., 2003). Characterization of Farm Animal Genetic Resources (FAGR) encompasses all activities associated with the identification, quantitative and qualitative description, documentation of breed populations in their natural habitats and production systems to which they are or are not adapted (Gizaw *et* al., 2011). Genetic resources of livestock are threatened by erosion. The situation is most alarming for developing countries, where industrial breeds often replace local breeds that have unique disease resistance and adaptations to the local environment (Ajmone-Marson, 2010). In the absence of information about the genetic attributes of each breed for a breeding programme, development of local breeds is often ignored in favour of the introduction of germplasm from exotic breeds, about which more information is generally available. Characterization of breeds both at the level of animal phenotypes and their interaction with production systems and at the genetic level is essential (FAO, 2011). DNA-based technologies are now the methods of choice for genetic characterization of livestock (Arora *et* al., 2011), however several alternative assays, such as protein/allozyme polymorphism, remains tremendously useful, especially in developing countries, because of its utility, ease, cost, amount of genetic information accessed and simplicity of data interpretation (Rege and Okeyo, 2006). Additionally, the use of blood protein polymorphism in animal genetic diversity studies should not be underplayed, more so that genetic research in Africa is less fully developed as in Europe (Gifford-Gonzalez and Hanotte, 2011) and other developed countries. Mwacharo *et* al. (2002) reported that for populations whose genetic status is unknown, protein polymorphism may be used first to verify the degree of genetic relationship and then prioritize breeds to be analysed using sophisticated tools like microsatellites. Analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among breeds and in phylogenetic studies (Imumorin *et* al., 1999; Menrad *et* al., 2002; Nyasamba *et* al., 2003; Ibeagha-Awemu and Erhardt, 2004a; Camoglu and Elmaci, 2005). Information on blood proteins has also been used to study the genetic relationships among farm animals (Ibeagha-Awemu and Erhardt, 2004b; Esharatkhah *et* al., 2007; Sun *et* al., 2009; Shahrbabak *et* al., 2010). Unfortunately previous studies on the variation among WAD goats were based mainly on morphological differences (Salako and Ngere, 2002; Yakubu and Akinyemi, 2010; Yakubu and Ibrahim, 2011). Morphological traits are however complex in their mode of transmission and are influenced by the environment thereby underestimating the true levels

of genetic variation. Additionally, morphological characteristics do not necessarily correspond to the genetic characteristics of blood protein and non-protein polymorphisms (Tsunoda et al., 2010). Biochemical variants of different proteins may present higher accuracy procedures for a better measurement of genetic variation in goat breeds because of their polymorphism and simple mode of inheritance. Therefore, the present study aimed at determining genetic diversity among the Ogun, Ondo, Osun and Oyo populations of WAD goat at haemoglobin, transferrin and carbonic anhydrase loci.

Materials and methods

Animals

Blood samples were collected from a total of 140 adult WAD goats of both sexes by jugular venipuncture. 20 animals were randomly selected from each sampling area located using the grid method. Sampling areas include Ijebu- Ode and Ado – Odo, (Ogun state), Ondo, (Ondo state), Ile –Ife, Osogbo and Iwo, (Osun state), and Ibadan, (Oyo state).

Laboratory analysis

The analysis was done in the Animal Breeding and Genetics Laboratory of the Department of Animal Science, University of Ibadan, Nigeria. Blood samples were collected from the jugular vein of the animals into tubes containing heparin as anticoagulant and kept refrigerated during transportation using iced packed. Plasma and erythrocyte samples were separated from the heparinized whole blood by centrifugation. After centrifugation, red cells were washed three times in saline solution (0.155M NaCl), and lysed with a fourth fold volume of distilled H_2O to release haemoglobin. Separate aliquots of plasma and erythrocytes were stored at 4°C until they were analysed.

Gel electrophoresis was carried out on cellulose acetate strips to analyse inherited biochemical differences in the red blood cells and plasma. The procedures involved the use of three blood protein loci were analysed – Haemoglobin (Hb), Transferrin (Tf) and Carbonic anhydrase (Ca). This involved the use of Tris EDTA Borate pH8.6 for haemoglobin, Tris Glycine pH 8.4 for transferrin and Sodium Acetate Trihydrate pH 5.6 for Carbonic anhydrase, as described by RIKEN (2006). The resultant gel was stained with Red Ponceau stain to visualize the protein bands.

Statistical analysis

Tools for Population Genetic Analyses (TFPGA) software was used to generate the genetic distance according to Nei's (1972), the allele frequencies for protein and enzyme polymorphisms were determined, observed and expected heterozygosity, and drawing UnPaired Group Method of Algorithm (UPGMA) dendrograms illustrating the relationship between the three genetic groups.

Results and discussion

The distribution of the genotypes, the observed alleles and their frequencies are presented in table 1 and 2, respectively. A total of 6 alleles were observed in the investigated loci. All loci sampled were found to be polymorphic. All the loci produce two alleles each and each of the allele observed is present in all the populations sampled. Hb^B and CA^S occurred at high frequency among the four WAD goat populations. Tf^A was the most frequent in Oyo while Tf^B was the most frequent in Osun population. Mean heterozygosities in the four WAD goat populations were 0.482, 0.474, 0.392 and 0.457 in Ogun, Ondo, Osun and Oyo State population respectively.

Table 1. Distribution of the genotypes in WAD goat population from Ogun, Ondo, Osun and Oyo

	Ogun	Ondo	Osun	Oyo
Haemoglobin				
Hb^{A+}	NA	0.30	0.02	0.10
Hb^{AB}	0.65	0.20	0.17	0.25
Hb^{B+}	0.35	0.50	0.81	0.65
Carbonic anhydrase				
CA^{F+}	0.28	0.20	0.15	0.05
CA^{FS}	0.30	0.10	0.51	0.85
CA^{S+}	0.43	0.70	0.34	0.10
Transferrin				
Tf^{A+}	0.05	NA	0.02	0.30
Tf^{AB}	0.90	1.00	0.87	0.55
Tf^{B+}	0.05	NA	0.11	0.15

NA -- Not available

Table 2. Allele frequencies of blood protein variants in WAD goat population from Ogun, Ondo, Osun and Oyo State.

Locus	Allele	Ogun	Ondo	Osun	Oyo
Hb	Hb^A	0.33	0.40	0.10	0.23
	Hb^B	0.68	0.60	0.90	0.78
CA	CA^F	0.43	0.25	0.41	0.48
	CA^S	0.58	0.75	0.59	0.53
Tf	Tf^A	0.50	0.50	0.45	0.58
	Tf^B	0.50	0.50	0.55	0.43

Hb -----> Haemoglobin, CA ----> Carbonic anhydrase, Tf ----> Transferrin.

Genetic distance estimates (derived from allele frequencies) between each pair of populations are shown in Table 3. The dendrogram showed that WAD goat populations from Ogun and Oyo are more closely related compared to those from Osun and Ondo States (Figure 1). The predominance of allele B at the haemoglobin locus in this study agrees with earlier works (Clarke et al., 1989; Zanoti Cassati et al., 1990, Bunge et al., 1990; Mwacharo et al., 2002; Boujenane et al., 2008; Shahrbabak et al., 2010) that Hb^B is generally the most occurring allele in most goat breeds.

Table 3. Genetic distance values among the four populations of WAD goat. Nei's Original Measures of Genetic Identity (Above Diagonal) and Genetic distance (Below Diagonal)

pop ID	Ogun	Ondo	Osun	Oyo
Ogun	****	0.9777	0.9727	0.9892
Ondo	0.0225	****	0.9358	0.9475
Osun	0.0277	0.0664	****	0.9815
Oyo	0.0109	0.0539	0.0187	****

In Nigeria, Hb^B type has a very high frequency in WAD goat of the forest zone. This predominance may be due to the decreased haematocrit values, lower blood viscosity and higher availability of water associated with Hb^B blood types compared to Hb^A types, which seems to be of adaptive significance in habitats characterized by the aridity of the climate such as the northern zone of Nigeria. This is buttressed by the reports of Tsunoda et al. (2006) that Hb^A allele has a high affinity for oxygen and is important for survival in mountain areas at altitudes over 3000 m. A high frequency of CA^S in the entire populations of WAD goats sampled agrees with Awobajo et al. (2016) that CA^S is generally the most occurring allele in WAD goat breed.

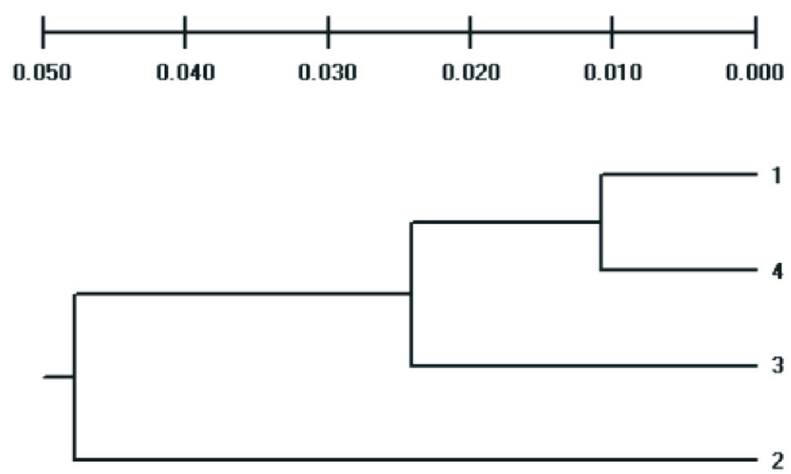

Figure 1. Dendrodram of the relationships among the four WAD goat populations.

Allele A was the most frequent in Oyo population while allele B was more prevalent in population from Osun at transferrin locus. Literature on the use of cellulose acetate electrophoresis method for transferrin analysis is scarce, thus the results obtained can be verified by future researchers. Table 4 shows the measurement of the heterozygosity among the four populations of WAD goats. Gene diversity indicated by H_E ranged from 0.19 for Hb in Osun Population to 0.52 for Tf in Ondo population, with the mean of 0.44 ± 0.09 which was lower than 0.54 reported by Muema *et al.* (2009), who employed the use of microsatellites. However, this value is similar to 0.44 reported by Salako *et al.,* (2007) for Nigerian WAD goat and higher than 0.38 reported for Nigerian sheep (Akinyemi and Salako 2012), indicating a higher genetic variability in Nigerian WAD goat at the three investigated blood protein loci compared to sheep. However, the current estimates in Ogun, Ondo, Osun and Oyo States populations fall within the recommended average heterozygosity of between 0.3 and 0.8 in a population (Takezaki and Nei, 1996) for markers to be useful for measuring genetic variation.

Table 4. A measure of Heterozgosity amoung thr four populations of WAD goat

Locus		Ogun n=40	Ondo n= 12	Osun n= 53	Oyo n= 20	Sample size n=125
Hb	H_E	0.4443	0.5053	0.1878	0.3577	0.3441
	H_O	0.6500	0.2000	0.1698	0.2500	0.3415
Car2	H_E	0.4949	0.3947	0.4868	0.5115	0.4860
	H_O	0.3000	0.1000	0.5094	0.8500	0.4634
Trf	H_E	0.5063	0.5217	0.5003	0.5013	0.5019
	H_O	0.9000	1.0000	0.8679	0.5500	0.8400
All Loci	H_E	0.4819 ± 0.0330	0.4739 ± 0.0691	0.3916 ± 0.1767	0.4568 ± 0.0860	0.4440 ± 0.0869
	H_O	0.6167 ± 0.3014	0.4333 ± 0.4933	0.5157 ± 0.3491	0.5500 ± 0.3000	0.5483 ± 0.2599

Nei's standard genetic distances obtained were within the range reported by Nei (1976) for local breeds. WAD goat population from Oyo is closer to those of Ogun population than Osun and Ondo populations. Populations from Osun and Oyo were closer than any other population except that of Ogun and Oyo. This was corroborated by the phylogenetic tree depicting the relationships among the WAD goat populations. The present findings were however contrary to the report of Awobajo et al., 2015 based on microsatellite DNA polymorphism where WAD goat populations from Osun and Ondo were the closest followed by the populations from Ogun and Oyo. The discrepancy observed may be a reflection of the varying efficiency of biochemical markers and DNA microsatellites in determining the relationship among breeds.

Conclusion

The estimates of heterozygosity obtained using information from haemoglobin, transferrin and carbonic anhydrase loci were within the range required for measuring genetic variation among livestock. However, there is the need for further characterization employing more blood protein loci in a larger population and complement this with DNA microsatellite information for sustainable management and conservation of animal genetic resources in the studied area.

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