Phenotypic and genotypic variability between two Nigerian indigenous goat population

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In order to estimate the future breeding potential of a livestock breed, it is necessary to estimate the level of genetic diversity within the breed. Thus, this study was conducted to determine the phenotypic and genotypic variability within the West African dwarf (WAD) goats and a non-descript goat population. The WAD goats were obtained from Bodija market Ibadan, Oyo state, while the non-descript goats were obtained from the Animal Science Departmental Farm in Zaria, Kaduna State. Random collection of tissue samples was carried out on each goat population using an all flex ear punch tissue sample collector and aliquoted into plastic tubes containing the all flex preservative. DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya. DNA was extracted from the tissue cells using the Pure Link™ Genomic DNA-minikit according to the manufacturer's specifications and protocol and 25 microsatellite markers as recommended by FAO/ISAG were used for genotyping. Twenty three of the 25 microsatellite markers used in this analysis had four or more alleles. The mean Shannon index (I), observed (H_o) and expected heterozygosity (H_e) and inbreeding coefficient (F_{is}) for the WAD goats were 1.568, 0.584, 0.679 and 0.167, respectively. For the non-descript goats, the mean Shannon index, observed and expected heterozygosity and inbreeding coefficient (F_{is}) were 1.607, 0.678, 0.721 and 0.041, respectively. The microsatellite markers used in this study showed their suitability for analysis of genetic variability in this population as demonstrated by the high mean Shannon index. This study has shown that these two goat populations are significantly different phenotypically and genetically. Also, both populations showed significant deviations (P<0.01) from Hardy-Weinberg expectations.

Keywords: Nigerian Indigenous goats, phenotypic traits, microsatellite markers, heterozygosity

Introduction

Developing countries such as Nigeria hosts a vast majority of indigenous farm animal genetic resources (over 90%), that unfortunately have not being improved to respond to global food security needs (FAO, 1999). One of the primary goals in the management of animal populations is to maintain their genetic diversity at a high level and their inbreeding at a low level

(Fernández *et al.*, 2005). To estimate the future breeding potential of a livestock breed, it is necessary to estimate the level of genetic diversity within the breed. For this purpose genetic materials are used (Saitbekova *et al.*, 1999; Dasmahapatra *et al.*, 2008). From genetic material, microsatellite markers are commonly used to describe the genetic polymorphism (Saitbekova *et al.*, 1999; Luikart *et al.*,

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2001; Naderi *et al.*, 2007; Agha *et al.*, 2008). Microsatellite markers are widely used for the analysis of genetic variability within and between breeds due to their polymorphism, distribution throughout the genome and efficacy of genotyping (Barker *et al.*, 1997; Chenyambuga *et al.*, 2004, Okpeku *et al.*, 2011). There are several studies on genetic variability of goats, based on microsatellite markers, such as Swiss breeds (Saitbekova *et al.*, 1999), Chinese indigenous populations (Li *et al.*, 2002; Li *et al.*, 2004), Indian domestic goats (Rout *et al.*, 2008), goats from Europe and middle east (Canon *et al.*, 2006). Very scanty studies have been carried out on the genetic diversity of Nigerian indigenous goats (Adebambo *et al.*, 2011; Okpeku *et al.*, 2011; Ojo, 2014; Yakub *et al.*, 2014).

Materials and methods

Tissue samples from 80 unrelated individual goats from the two goat populations were used for this study. The WAD goats were obtained from Bodija market in Oyo State, while the non-descript goats were sampled from Animal Science Departmental Farm, Ahmadu Bello University, Samaru, Zaria. Eighty goats were randomly sampled from both sexes. Tissue samples were collected using an all flex ear punch tissue sample collector and aliquoted into plastic tubes containing the all flex preservative. The DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya. The DNA extraction from the tissue cells was carried out using the Pure Link™ Genomic DNA minikit according to the manufacturer's specifications and protocol. Twenty five microsatellite markers were used in this study as recommended by the International Society for Animal Genetics and Food and Agricultural Organization (FAO, 2011).

Polymerase Chain Reaction (PCR) Conditions

The PCR typing was carried out on 50ng of the genomic DNA in a 10µL reaction, comprising 10pM each of fluorescently labeled PCR primers, 1µL of PCR buffer (Amersham) 20µM each of dCTP, dGTP, dTTP and dATP, 0.5 units of Taq polymerase per reaction and 1.5-3.0 µL of MgCl₂ depending on the primer. The mixture was cycled on the Hybaid Omnigene thermal cycler with cycling conditions of initial denaturation at 95°C (4 minutes), annealing at 50-65°C for 45seconds and final extension at 72°C (15 minutes) for 35 cycles.

Multiplexing was carried out following the recommendations of the ISAG/FAO panel. Genotyping was done using the ABI 3700XL automated capillary sequencer using Liz 500-350 internal lane size standard to size fractionate all amplified products. Allele size calling and binding was done with the aid of Gene Mapper 3.5 (Applied Bio systems).

Statistical analysis

The allele frequencies, observed and expected heterozygosities, genetic distance as well as genetic differentiation were computed using Gen Alex 6.5 genetic analysis statistical package (Peakall and Smouse, 2012).

Results and discussion

The results of descriptive statistics of the phenotypic traits (body length, height at withers, and chest girth) of the WAD and the non-descript goat populations are revealed in Table 1. The analysis of variance showed significant ($P < 0.01$) differences in all the measured traits. The non-descript goats showed higher means and variances in all the traits considered compared to the WAD goats. The significant differences observed between

the goat breeds suggest a direct relationship between the extent of morphometric divergence and geographical separation. This is in accordance with the results of Brown *et al.* (2006) that temporal and spatial variation in weather, plant productivity and subsequent nutrition could

have major impacts on physical growth of animals. Also, the results obtained from the coefficients of variation as well as variance could be attributed to breed differences, influence of environmental factors on the measured parameters especially the width measurements.

Table 1: Descriptive statistics of the phenotypic traits of West African dwarf (WAD) and a Non-descript goat population

Trait		WAD Goat	Non-descript goat Population
BL	Mean±s.e	52.97±0.84 ^b	59.90±0.56 ^a
	S ²	42.71	6.20
	CV	12.34	4.16
HW	Mean±s.e	55.88±0.84 ^b	59.20±0.75 ^a
	S ²	41.87	11.12
	CV	11.58	5.63
CG	Mean±s.e	55.87±0.94 ^b	62.65±0.66 ^a
	S ²	52.49	8.66
	CV	12.97	4.70

BL- body length, HW- height at withers, CG- chest girth.

s.e –standard error; S²- variance; CV- coefficient of variation^{a, b, c}

^{ab}Means in the same row with different superscripts are significantly different at P<0.05

Table 2 shows the number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity and fixation index for (I) the WAD goat population. The markers used in this population were highly informative with allelic numbers ranging from 3 for ILSTS005 to 19 for BM6444. The highest value obtained for the effective alleles in this population was 8.571 (SRCSP 9) while the least was 1.116 (ILSTS 005). The observed heterozygosities (Ho) ranged from 0.027 (MAF 65) to 1.000 (SPS 113), while the expected heterozygosity (He) ranged from 0.104 (ILSTS 005) to 0.883 (SRCSP 9). The Shannon index (I) ranged from 0.236 to 2.429 and in most cases were higher than 1.0. The inbreeding index (F_{is}) was negative at six (6) loci (ILSTS029, ILSTS087, SPS113, SRYM18, OarFCB34 and MAF209) displaying some level of out-breeding at such loci and was positive (inbred) for the remaining loci ranging from 0.033 (OarFCB48) to 0.854 (INRABERN172).

The number of effective alleles (Ne), observed (Ho) and expected heterozygosity (He), Shannon (I) and fixation index (F_{is}) using the 25 microsatellite markers for the non-descript goat population are revealed in Table 3. The number of available alleles ranged from 2-14. The highest value obtained for the effective allele was 8.989 (ILSTS 087) while the least was 1.133 (ILSTS 005). The observed heterozygosity (Ho) ranged from 0.125 (ILST 005) to 1.000 (SPS 113 and SRYM 18), while the expected heterozygosity (He) ranged from 0.117 (ILSTS 005) to 0.889 (ILST 087 and MAF 65). Most of the primers/markers used for this population were informative and displayed sufficient polymorphism in markers with the exception of ILSTS 005 and INRABERN 185 which had allele numbers of 2 and 3, respectively.

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Table 2: Allelic Frequencies (Na, Ne), Shannon Index (I) Heterozygosity (Ho, He) and F-statistics (F_{is}) in West African dwarf Goats

Locus	N	Na	Ne	I	Ho	He	F _{is}
OarFCB20	32	7.000	4.188	1.657	0.594	0.761	0.220
BM6444	60	19.000	8.036	2.429	0.783	0.876	0.105
CSRD247	56	8.000	5.733	1.876	0.732	0.826	0.113
ETH10	60	15.000	4.417	1.948	0.533	0.774	0.311
ILSTS005	55	3.000	1.116	0.236	0.073	0.104	0.300
ILSTS11	43	12.000	3.365	1.705	0.651	0.703	0.073
ILSTS029	60	11.000	2.601	1.380	0.683	0.616	-0.110
ILSTS087	60	14.000	8.072	2.227	0.900	0.876	-0.027
INRA023	41	10.000	5.086	1.837	0.634	0.803	0.211
INRABERN185	36	5.000	1.468	0.701	0.250	0.319	0.215
INRABERN172	25	9.000	5.580	1.950	0.120	0.821	0.854
MAF65	37	4.000	1.214	0.403	0.027	0.176	0.847
MAF70	59	9.000	4.284	1.711	0.746	0.767	0.027
MCM527	60	5.000	2.374	1.171	0.267	0.579	0.539
OarFCB48	60	11.000	3.411	1.598	0.683	0.707	0.033
OarFCB304	59	13.000	3.920	1.756	0.644	0.745	0.135
SPS113	60	10.000	2.684	1.264	1.000	0.627	-0.594
SRCRSP3	60	5.000	3.216	1.294	0.550	0.689	0.202
SRCSP9	58	10.000	8.571	2.207	0.707	0.883	0.200
SRYM18	60	16.000	4.672	2.021	0.967	0.786	-0.230
TCRVB6	44	9.000	2.076	1.235	0.386	0.518	0.255
OarPC34	48	9.000	5.075	1.841	0.813	0.803	-0.012
SRCSP5	59	12.000	6.574	2.046	0.678	0.848	0.200
MAF209	60	6.000	4.162	1.480	0.767	0.760	-0.009
INRA063	58	6.000	2.530	1.176	0.414	0.605	0.316
Mean	52.400	9.520	4.177	1.568	0.584	0.679	0.167
SE	2.149	0.796	0.417	0.110	0.054	0.041	0.059

N=sample size, Na= no. of alleles, Ne= no.of effective alleles, I= Shannon index, Ho = observed heterozygosity, He= expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

The Shannon index (I) for this population ranged from 0.234 to 2.395. The ILSTS005 marker had low Shannon index (0.234) and number of alleles, whereas the inbreeding index (F_{is}) ranged between (-0.012 to 0.444) and were negative at eleven loci for this population (BM6444, ILSTS005, ILSTS11, ILSTS029, ILSTS087, INRABERN185, OarFCB304, SPS113, SRYM18, SRCSP9, TCRVB6).

In both of the goat populations, the marker ILSTS005 was observed to have low polymorphism (allelic numbers below 4).

Some measure of out-breeding was observed in both populations as recorded by the negative F_{is} values. These homozygous deficiencies may have arisen from population sub-division owing to genetic drift, null alleles and selection against inbreeding. These negative values have also been reported in other studies on goats (Barker *et al.*, 1997, Luikart *et al.*, 1999, Agha *et al.*, 2008, Rout *et al.*, 2008, Dixit *et al.*, 2009)

Table 4 revealed the average observed and expected heterozygosities of the two goat

Table 3: Allelic Frequencies (Na, Ne), Shannon Index (I), Heterozygosity (Ho, He) and F-statistics (F_{is}) in a Non-descript goat population

Locus	N	Na	Ne	I	Ho	He	F _{is}
OarFCB20	14	5.000	4.170	1.509	0.429	0.760	0.436
BM6444	20	8.000	2.676	1.331	0.750	0.626	-0.198
CSRD247	19	9.000	5.870	1.933	0.789	0.830	0.048
ETH10	20	13.000	7.143	2.235	0.650	0.860	0.244
ILSTS005	16	2.000	1.133	0.234	0.125	0.117	-0.067
ILSTS11	18	8.000	2.521	1.307	0.722	0.603	-0.197
ILSTS029	20	4.000	1.891	0.899	0.500	0.471	-0.061
ILSTS087	20	14.000	8.989	2.394	0.950	0.889	-0.069
INRA023	16	8.000	4.876	1.793	0.750	0.795	0.057
INRABERNI185	13	3.000	1.807	0.733	0.615	0.447	-0.377
INRABERN172	17	11.000	6.494	2.104	0.471	0.846	0.444
MAF65	8	9.000	8.000	2.133	0.750	0.889	0.143
MAF70	18	6.000	3.447	1.448	0.611	0.710	0.143
MCM527	20	4.000	2.432	1.074	0.500	0.589	0.151
OarFCB48	20	9.000	6.612	2.005	0.650	0.849	0.234
OarFCB304	20	10.000	6.250	2.048	0.850	0.840	-0.012
SPS113	20	8.000	3.774	1.586	1.000	0.735	-0.361
SRCRSP3	17	5.000	3.524	1.352	0.647	0.716	0.097
SRCSP9	20	7.000	4.348	1.622	0.800	0.770	-0.039
SRYM18	20	12.000	7.767	2.220	1.000	0.871	-0.148
TCRVB6	12	7.000	5.143	1.772	0.833	0.806	-0.039
OarPC34	14	7.000	4.840	1.729	0.643	0.793	0.190
SRCSP5	19	10.000	6.067	1.972	0.737	0.835	0.118
MAF209	20	6.000	3.941	1.530	0.600	0.746	0.196
INRA063	17	5.000	2.861	1.209	0.588	0.651	0.096
Mean	17.520	7.600	4.663	1.607	0.678	0.721	0.041
SE	0.638	0.611	0.425	0.104	0.038	0.035	0.041

Heterozygosity, Na = No. of alleles, Ne = effective number of alleles, I = Shannon index, Ho = observed heterozygosity, He = expected heterozygosity, and F_{is} = fixation index inbreeding coefficient

populations as well as their Hardy-Weinberg equilibrium values. The average expected heterozygosity was lower in WAD goats (0.679) as compared with the Non-descript goats (0.721). Also, the Non-descript goats had higher observed

heterozygosity (0.678) than the WAD goat (0.568). In both cases, the average observed heterozygosities were lower than the expected heterozygosities and both populations showed significant deviations ($P < 0.01$) from Hardy-Weinberg expectations.

Table 4: Hardy -Weinberg Equilibrium for the Two Nigerian goat populations

Populations	Ho	He	HWE	
Non-descript goat population	0.678	0.721	0.0026	**
West African Dwarf	0.568	0.679	0.0133	**

Ho = observed heterozygosity, He = expected heterozygosity, HWE = Hardy-Weinberg Equilibrium ** = highly significant at ($P < 0.01$)

The significant deviations from the Hardy-Weinberg Equilibrium for the two goat populations, implies that there was a deficit of heterozygosity resulting from the non-random union of gametes in the population i.e. the mating among individuals in the population which are more related than the average relationship. Similarly, migration, mutation and selection might have caused these deviations. Deviations from Hardy-Weinberg equilibrium at microsatellite level have been reported in various studies (Barker *et al.*, 2001; Hassan *et al.*, 2003; Laval *et al.*, 2000; Luikart *et al.*, 1999).

Conclusion

This study has shown that there are significant phenotypic differences between the West African dwarf goat and the non-descript goat from Zaria. Also, most of the primers/markers used for this population were highly informative and displayed sufficient polymorphism. Significant deviations from the Hardy -Weinberg Equilibrium were observed in the two goat populations.

Recommendations

Improved conservation and management practices, which could ensure uniqueness of the individual goat populations and avoid inbreeding, should be implemented.

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