Genetic Characterization of Indigenous Goats of Sub-saharan Africa Using Microsatellite DNA Markers*

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ABSTRACT : Genetic diversity of sub-Saharan African goats was assessed using 19 microsatellite markers. Breeds were sampled from eastern Africa (Maasai, Kigezi, Mubende, North West Highland, Arsi-Bale), southern Africa (Ndebele, Pafuri) and West Africa (West African Dwarf, Maure, Djallonke). European breeds (Grisons Striped, Toggenburg), Asian breeds (Mongolian Cashmere, Bandipur) and a Middle East breed (Arab) were also included. The mean number of alleles per locus and average gene diversity ranged from 5.26 ± 0.464 (Djallonke) to 7.05 ± 0.516 (Mubende) and from 0.542 ± 0.036 (Pafuri) to 0.672 ± 0.031 (Ndebele), respectively. The between breeds variation evaluated using G_{ST} and θ were found to account for 14.6% (θ) and 15.7% (G_{ST}) of the total genetic variation. The D_A measure of genetic distance between pairs of breeds indicated that the largest genetic distance was between Pafuri and Djallonke while the lowest genetic distance was between Arsi-Bale and North West Highland. A neighbour-joining tree of breed relationships revealed that the breeds were grouped according to their geographic origins. Principal component analysis supported the grouping of the breeds according to their geographic origins. It was concluded that the relationships of sub-Saharan African goat breeds were according to their geographical locations implying that the goats of eastern Africa, West Africa and southern Africa are genetically distinct. Within each sub-region, goat populations could be differentiated according to morphological characteristics. *(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 4 : 445-452)*

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INTRODUCTION

The goats of sub-Saharan Africa can be divided into five major types (Mason and Maule. 1960; Rege et al.. 1996): (i) The Long lop-eared goats of North-east Africa which includes the Sudanese Nubian and Sudanese Desert found mainly in northern Sudan, the Shukria of Eritrea and Ethiopia and the Benadir of Somalia; (ii) the Small short-eared goats which extend from Ethiopia and Somalia through East Africa up to Zimbabwe and some parts of South Africa; (iii) the Dwarf short-eared goats which are found along the coast of West and Central Africa; (iv) the Sahelian and Intermediate goats found in the Savannah belt and (v) the Southern lop-eared goats found in southern Africa. In between these major types there are many intermediate and less distinguishable subtypes. The genetic relationships among the major types as well as the subtypes is unclear because their history (how they were established and if later on intercrossing occurred between the types) is not well documented. The considerable variation observed among the goat populations. in terms of size and coat colours has led to some inconsistencies in the classification of the various local populations into breeds/strains. Often the subtypes are named after locations or communities keeping them (Rege et al.. 1996), for instances. goats kept by Somali and Maasai people of East Africa are called Somali and Maasai goats, respectively. This naming system may not reflect the genetic differentiation of the breeds. However. given the wide geographic area and the diversified climate and topography of the region. local populations in different places might have been isolated over a long period while also being subjected to varying selection pressures and genetic drift. thus have become genetically divergent. However. the extent to which various populations are different from each other is not clear.

In recent years. a range of innovations in molecular genetics have been developed for the study of genetic variation and evolution of populations using DNA marker genotype information. One of the recent DNA markers are microsatellites. The analysis of microsatellites is currently

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Table 1. Microsatellite markers used and their annealing temperature and size ranges in the goat breeds studied

Locus	Chromosome	Primer sequences 5'-3'	Annealing temp, °C	Observed size ranges (bp)
BM1818	23	F-AGCTGGGAATATAACCAAAGG R-AGTGCTTTCAAGGTCCATGC	55	250-300
BMC1222	13q12	F-CCAATTTTGCAGATAAGAAAACA R-CCTGAGTGTTCCTCCTGAGT	55	273-297
BMS357	Unknown	F-TCCAAACAAGTCTTCTCTATTTACC R-CCAAATAATTGCTGGTCAGG	58	102-138
BMS1494	Unknown	F-TCTGGAGCTTGCAAAAGACC R-AATGGATGACTCCTGGATGG	55	252-284
ILSTS005	10	F-GGAAGCAATGAAATCTATAGCC R-TGTTCTGTGAGTTTGTAAGC	55	164-194
ILSTS11	14	F-GCTTGCTACATGGAAAGTGC R-CTAAAATGCAGAGCCCTACC	55	250-316
ILSTS17	Unknown	F-GTCCCTAAAATCGAAATGCC R-GCATCTCTATAACCTGTTCC	57	122-142
ILSTS44	Unknown	F-AGTCACCCAAAAGTAACTGG R-ACATGTTGTATTCCAAGTGC	57	135-175
ILSTS87	Unknown	F-AGCAGACATGATGACTCAGC R-CTGCCTCTTTTCTTGAGAGC	58	139-179
INRA5	12	F-CAATCTGCATGAAGTATAAATAT R-CTTCAGGCATACCCTACACC	57	133-167
INRA63	18q22	F-ATTTGCACAAGCTAAATCTAACC R-AAACCACAGAAATGCTTGGAAG	55	141-179
INRA132	23	F-AACATTTCAGCTGATGGTGGC R-TTCTGTTTTGAGTGGTAAGCTG	57	139-159
MAF35	Unknown	F-TCAAGAATTTTGGAGCACAATTCTGG R-AGTTACAAATGCAAGCATCATACCTG	55	99-119
MAF65	15	F-AAAGGCCAGAGTATGCAATTAGGAG R-CCACTCCTCCTGAGAATATAACATG	50	104-138
MAF209	Unknown	F-GATCACAAAAAGTTGGATACAACCGTGG R-TCATGCACTTAAGTATGTAGGATGCTG	57	101-131
OarAE129	7	F-AATCCAGTGTGTGAAAGACTAATCCAG R-GTAGATCAAGATATAGAATATTTTTCAACACC	58	130-176
OarFCB304	Unknown	F-CCCTAGGAGCTTTCAATAAAGAATCGG R-CGCTGCTGTCAACTGGGTCAGGG	55	128-180
SRCRSP3	10q36	F-CGGGGATCTGTTCTATGAAC R-TGATTAGCTGGCTGAATGTCC	55	107-127
SRCRSP7	6	F-TCTCAGCACCTTAATTGCTCT R-GTCAACACTCCAATGGTGAG	57	115-131

regarded as the most useful technique in the study of genetic diversity of closely related populations. The usefulness of microsatellites in the study of genetic variation within and among livestock breeds has been documented by numerous studies (Buchanan et al., 1994; MacHugh et al., 1997; Okomo et al., 1998; Arranz et al., 1998; Pandey et al., 2002; Dorji et al., 2003). Unfortunately, for the sub-Saharan African goats, no work has been done to determine genetic variation among breeds/populations using microsatellite polymorphism information. Therefore, the objectives of this study were to quantify genetic diversity within and among selected goat populations in sub-Saharan Africa and to clarify the genetic relationships among the major goat types found in the region.

MATERIALS AND METHODS

Breeds and sampling strategy

A total of 10 breeds representing the major types of goats in sub-Saharan Africa were sampled from Ethiopia (Arsi-Bale and North West Highland (NWH)), Uganda (Mubende and Kigezi), Tanzania (Maasai), Mozabique (Pafuri), Zimbabwe (Ndebele), Nigeria (West African Dwarf (WAD)), Mali (Maure) and Senegal (Djallonke). The Ethiopian breeds are classified as Short-eared Intermediate East African goats while the Ugandan and Tanzanian breeds are classified as Short-eared Small East African goats. The Pafuri and Ndebele goats belong to the Southern Long Lop-eared goat type. The West African Dwarf (WAD) and Djallonke belong to the Short-eared Dwarf African goat

Table 2. Mean number of alleles per locus and average heterozygosity values obtained in sub-Saharan African goat breeds and breeds from outside Africa

Breed	Mean number of alleles±SE	Average observed heterozygosity±SE	Average expected heterozygosity±SE
African breeds			
Maasai (48)	5.84±0.327	0.420±0.040	0.588±0.037
Kigezi (45)	6.00±0.452	0.376±0.032	0.649±0.032
Mubende (47)	7.05±0.516	0.356±0.034	0.656±0.035
Ndebele (40)	6.84±0.318	0.342±0.038	0.672±0.031
WAD (40)	6.11±0.483	0.366±0.051	0.637±0.036
NWH (42)	5.79±0.570	0.406±0.041	0.597±0.040
Pafuri (40)	5.74±0.523	0.376±0.048	0.542±0.036
Maure (40)	6.05±0.521	0.421±0.053	0.609±0.041
Arsi-Bale (40)	5.95±0.623	0.475±0.038	0.623±0.034
Djallonke (40)	5.26 ± 0.464	0.423±0.056	0.569±0.045
Reference breeds			
Arab (33)	5.53±0.564	0.391±0.044	0.587±0.050
Grisons Striped (40)	5.58±0.608	0.509±0.060	0.559±0.056
Toggenburg (20)	4.05±0.442	0.473 ±0.068	0.553±0.060
Mongolian Cashmere (40)	6.53±0.707	0.547±0.048	0.634±0.044
Bandipur (40)	7.47±0.646	0.498±0.055	0.645±0.049

The numbers in brackets are numbers of animals genotyped for each breed. WAD: West African Dwarf. NWH: North west highland.

type and the Maure belong to the Short-eared twisted horn intermediate or Intermediate West African goat type. In each breed 40 to 48 (Table 2) animals with approximately equal number of females and males were sampled from two districts. In each district five villages/communes were selected and one to five unrelated animals per flock were sampled. In order to avoid sampling related individuals, farmers were asked about the origins and familial relationships of individual animals. In Addition, breeds from Europe (Toggenburg and Grisons Striped from Switzerland), Asia (Mongolian Cashmere from Mongolia and Bandipur from Nepal) and Middle East (the Arab breed from the United Arab Emirates) were sampled to serve as reference breeds. Samples collected were either blood or hair roots. Blood was collected by jugular vein puncture using 10 ml EDTA vacutainer tubes. Hair samples were collected by plucking 50 to 100 hairs from each animal, making sure that the hairs had hair roots.

DNA extraction

DNA was extracted from either peripheral blood lymphocytes (PBL) or hair roots. DNA from PBL was extracted using a modification of phenol-chloroform extraction procedure and ethanol precipitation (Sambrook et al., 1989). To extract DNA from hair roots, 15 hairs were cut at about 5 mm from the hair root base and put into an eppendorf tube. One hundred (100) µl of lysis buffer (10 mM Tris (pH 8.3), 50 mM KCl and 0.5% Tween 20) were added into the tube. Then 20 µl of 20 µg/µl solution of Proteinase K in 10 mM Tris-HCl (pH 7.5) were added. The contents were vortexed for one minute, centrifuged at 14,000 rpm for one minute, incubated overnight in a water-

bath set at 56°C, incubated for 10 minutes in a water-bath set at 94°C, cooled down to room temperature and then centrifuged at 14,000 rpm for one minute. The resultant sample was used as PCR template.

Microsatellite markers, PCR conditions and genotyping

Nineteen (19) microsatellite markers were chosen across the genome as shown in Table 1. The PCR protocol applied was as follows: All PCR amplifications were performed in a total volume of 10 µl on a GeneAMP®PCR System 9700 (PE Applied Biosystems). Each PCR reaction contained 20 ng template DNA, 200 mM each primer, 2.5 mM each dNTP (i.e. dATP, dCTP, dGTP, dTTP). PCR buffer (2.0 mM MgCl$_2$, 100 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin and 0.25% Tween 20) and 0.5 units Taq DNA polymerase (Promega). All amplifications included an initial denaturing step of 4 min at 95°C, followed by 35 cycles of 45 sec at 94°C, 1 min at an appropriate annealing temperature (Table 1) and 1 min at 72°C. Final extension was for 20 min at 72°C. The PCR products were analysed by electrophoresis on a 4.25% denaturing polyacrylamide gel using a 377 ABI automatic DNA sequencer (PERKIN-ELMER). Sizes of amplified fragments were analysed using the 672 GENESCAN™ analysis software (version 2.0) and the GENOTYPER™ software (version 2.0).

Statistical analyses

Determination of genetic variation within populations : Genetic diversity within each population was determined as the mean number of alleles per locus and average observed and expected heterozygosities. Number of alleles per locus

Table 3. Number of alleles, gene diversity and estimates of population differentiation (G_{ST} and θ) for the nineteen (19) loci analysed

Locus	Number of alleles	Mean gene diversity±SE	G_{ST}	θ
BM1818	13	0.759±0.020	0.111	0.096
BMC1222	13	0.685±0.029	0.125	0.108
BMS357	18	0.734±0.033	0.168	0.159
BMS1494	15	0.695±0.019	0.104	0.097
ILSTS5	12	0.480±0.049	0.109	0.094
ILSTS11	19	0.705±0.024	0.151	0.136
ILSTS17	11	0.658±0.039	0.099	0.082
ILSTS44	17	0.493±0.062	0.242	0.225
ILSTS87	19	0.599±0.037	0.205	0.207
INRA5	9	0.569±0.032	0.102	0.092
INRA63	10	0.624±0.031	0.095	0.076
INRA132	11	0.512±0.045	0.227	0.201
MAF35	8	0.321±0.044	0.481	0.504
MAF65	14	0.796±0.019	0.125	0.115
MAF209	9	0.443±0.063	0.148	0.333
OarAE129	22	0.713±0.034	0.164	0.157
OarFCB304	23	0.651±0.044	0.167	0.154
SRCRSP3	11	0.585±0.045	0.099	0.082
SRCRSP7	9	0.582±0.025	0.110	0.097
All loci			0.157	0.146

per population was obtained by direct counting. Average observed and expected heterozygosities were calculated using the GENEPOP package version 3.2 (an update version of GENEPOP (V. 1.2) described in Raymond and Rousset, 1995a).

Measurement of population differentiation : The correlation of genes between individuals in the same population (θ) (Weir and Cockerham, 1984) and the coefficient of gene differentiation (G_{ST}) (Nei, 1973) were used to measure the genetic differentiation among the populations. The parameters θ and G_{ST} were estimated using the FSTAT programme (version 2.9.1), an update of FSTAT version 1.2 (Goudet, 1995). These drift-based measures of population differentiation were considered more appropriate for this analysis because genetic drift is said to be the main factor for genetic differentiation among closely related populations or for short-term evolution (Weir, 1990). The significance of breed differences for all pairs of populations was tested using an exact test of population differentiation (Raymond and Rousset, 1995b) implemented in the GENEPOP package version 3.2. The null hypothesis (H_o) tested was that "the allelic distribution was identical across populations".

Determination of genetic variation and relationships among populations : Genetic variation between pairs of populations was measured using Nei et al.'s (1983) angular genetic distance (D_A). This genetic distance was computed using the DISPAN program (Ota, 1993). The D_A measure of

genetic distance was selected due to its superior performance in phylogeny reconstruction when using microsatellite data (Takezaki and Nei, 1996). The Neighbour-joining (NJ) methodology (Saitou and Nei, 1987) implemented in the DISPAN program was used to construct the phylogenetic tree of breed relationships. The reliability of the tree topology was examined by performing 1,000 bootstrap resampling. A principal component analysis (PCA) (Manly, 1986) was carried out on the allele frequencies to determine breed relationships based directly on the allele frequencies. PCA was performed using the XLSTAT program (version 4.3) (Fahmy, 2000). PCA is a multivariate technique, which condenses the information from a large number of alleles and loci into a few synthetic variables (principal components) (MacHugh et al. 1997). It involves a linear transformation of the observed allele frequencies into a new set of variables, which are uncorrelated with each other. The coefficients defining the linear transformations are chosen so as to maximize the variation of the transformed data measured along each new coordinate-axis (principal component). The first three principal components are the most informative and, in the present study, these were plotted on a three-dimensional scatter diagram to allow visual inspection of the relationships of all populations.

RESULTS

Genetic variation within breeds

Genetic variability within breeds as indicated by the mean number of alleles per locus and average heterozygosity (both observed and expected) is shown in Table 2. Among the sub-Saharan African breeds, the mean number of alleles ranged from 5.26±0.464 to 7.05±0.516 in Djallonke and Mubende, respectively. For all breeds the lowest mean number of alleles (4.05±0.442) was observed in Toggenburg while the highest (7.47±0.646) was found in Bandipur. Observed average heterozygosity ranged from 0.342±0.038 in Ndebele to 0.547±0.048 in Mongolian Cashmere. The expected heterozygosity was lowest (0.542±0.036) in Pafuri and highest in Ndebele (0.672± 0.031). In all cases, average observed heterozygosities were lower than the heterozygosities expected under Hardy-Weinberg Equilibrium (HWE) and all populations showed significant deviation ($p \leq 0.001$) from HWE expectations.

Measures of population differentiation

The two measures of population differentiation, the G_{ST} and θ shown in Table 3 were more or less the same. The overall values were 0.157 and 0.146 for G_{ST} and θ, respectively. The exact test for population differentiation for all pairs of breeds across all loci showed that all breeds were significantly ($p \leq 0.001$) different from each other.

Figure 1. Unrooted neighbour-joining tree showing genetic relationships of 10 breeds from sub-Saharan Africa and 5 breeds from outside Africa based on D_A genetic distances. Numbers at the nodes are bootstrapping values from 1,000 replicates.

Genetic distances and phylogenetic relationships among breeds

The D_A genetic distances between pairs of breeds are shown in Table 4. The lowest distance (0.087) was observed between Arsi-Bale and North West Highland (i.e. between Ethiopian breeds), followed by that between Pafuri and Ndebele (0.125). Among the sub-Saharan African goat breeds, the largest distance was found between Pafuri and Djallonke (0.273), followed by that between WAD and Pafuri (0.256). For all the 15 breeds analysed, the largest distance were found between Mongolian Cashmere and Djallonke (0.382) and between Toggenburg and Arab (0.382).

The neighbour-joining (NJ) tree (Figure 1) revealed six clusters. The first cluster consisted of East African breeds (Maasai, Kigezi, Mubende), the second cluster consisted of West African breeds (Maure, Djallonke and West African Dwarf) and the third cluster was made up of the Ethiopian

breeds (North West Highland and Arsi-Bale). The fourth cluster was made up of breeds from Southern Africa (Pafuri and Ndebele) and the fifth cluster consisted of one breed, the Arab from the Middle East. Finally, the sixth cluster consisted of the European-Asian breeds. Within the sixth cluster, two sub-clusters could be identified, one for the European breeds (Toggenburg and Grisons Striped) and the other for the Asian breeds (Mongolian Cashmere and Bandipur). The bootstrap values ranged from 38 to 97%.

Principal component analysis

The first principal component accounted for 16.1% of the total variability and separated the European and Asian breeds from the African breeds. The East African breeds were also separated from the other African breeds by this principal component. The second principal component accounted for 12.4% of the total variability and separated the European breeds from the Asian breeds. The Arab breed was also separated from all other breeds by this principal component. The third principal component accounted for 9.1% of the total variation and separated the West African breeds from the other African breeds. The first ten principal components accounted for 86.1% of the variation. The plot for PCA (Figure 2) obtained by combining the first three principal components indicated three separate groupings of the breeds of sub-Saharan Africa, the first group being for the West African breeds (WAD, Djallonke and Maure), the second for the eastern African breeds (Maasai, Kigezi, NWH and Arsi-Bale) and the third for the breeds of southern Africa (Ndebele and Pafuri). However, the Mubende goats were well separated from all other African breeds. When all the breeds were considered, the principal component analysis revealed that the European breeds were well separated from the Asian breeds.

DISCUSSION

The main focus of this study was to examine the genetic

Table 4. Nei's D_A genetic distances matrix for 10 breeds of sub-Saharan Africa and 5 breeds from outside Africa

	Mas	Kig	Mub	Ara	Ndeb	WAD	NWH	Paf	Mau	Asib	Djal	Gris	Tog	Mong
Kig	0.135													
Mub	0.137	0.149												
Ara	0.284	0.300	0.267											
Ndeb	0.181	0.193	0.176	0.279										
WAD	0.195	0.239	0.217	0.333	0.243									
NWH	0.130	0.168	0.150	0.231	0.183	0.218								
Paf	0.170	0.177	0.170	0.257	0.125	0.256	0.145							
Mau	0.169	0.199	0.194	0.303	0.214	0.129	0.164	0.208						
Asib	0.136	0.173	0.137	0.254	0.157	0.217	0.087	0.163	0.171					
Djal	0.199	0.236	0.202	0.349	0.261	0.167	0.204	0.273	0.160	0.217				
Gris	0.292	0.289	0.295	0.357	0.238	0.294	0.229	0.197	0.194	0.249	0.315			
Tog	0.362	0.363	0.355	0.382	0.284	0.339	0.301	0.266	0.253	0.296	0.351	0.157		
Mong	0.338	0.343	0.310	0.310	0.251	0.328	0.293	0.261	0.256	0.272	0.382	0.204	0.272	
Band	0.314	0.353	0.319	0.295	0.217	0.296	0.271	0.244	0.253	0.264	0.351	0.240	0.291	0.167

Mas-maasai, Kig-kigezi, Mub-mubende, Ara-arab, Ndeb-ndebele, WAD-west African dwarf, NWH-north west highland, Paf-pafuri, Mau-maure, Asib-Arsi-bale, Djal-djallonke, Gris-grisons striped, Tog-toggenburg, Mong-mongolian Cashmere, Band-bandipur.
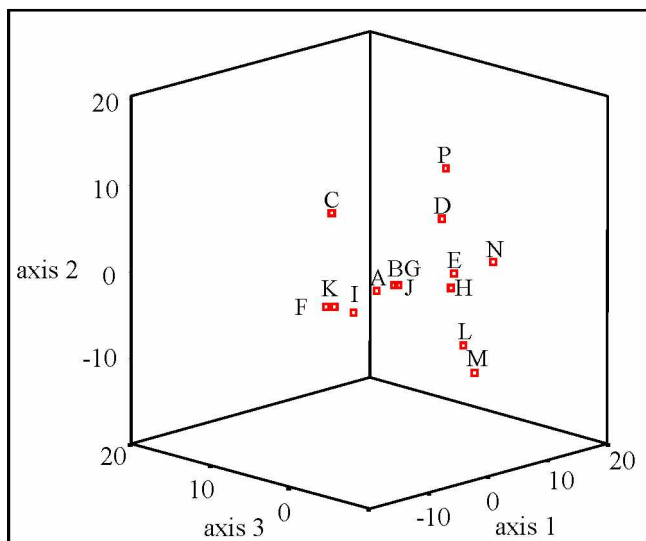
Figure 2. Principal component analysis of allele frequencies from 10 African breeds and 5 reference breeds from outside Africa. The first three principal components were plotted with the first, second and third principal components represented by the axes 1, 2 and 3, respectively. The letters stand for the breeds: A-Maasai, B-Kigezi, C-Mubende, D-Arab, E-Ndebele, F-west African dwarf, G-north west highland, H-paturi, I-Maure, J-Arsi-Bale, K-Djallonke, L-Grisons Striped, M-Toggenburg, N-mongolian cashmere and P-Bandipur.

diversity within- and between-populations of goats from sub-Saharan Africa using allele frequencies data obtained from microsatellite analysis. The total number of alleles per locus ranged from 8 (MAF35) to 23 (OarFCB304). This observed number of alleles for each locus demonstrates that all the microsatellite loci were sufficiently polymorphic in the goat populations studied. Thus, the markers used were appropriate because the number of alleles for each marker was higher than the minimum number of alleles (at least four alleles) recommended for microsatellite markers to be used in the estimation of genetic distance (Barker, 1994).

Genetic variation within breeds

Mean number of alleles observed over a range of loci in different populations is considered to be a reasonable indicator of genetic variation within the populations (MacHugh et al., 1997). Generally, all populations studied revealed high level of allelic diversity. When the means for the number of alleles per locus are compared among the goat breeds studied, it is evident that the African and Asian populations had slightly higher values than the European breeds. This phenomenon, of lower level of allelic diversity in European breeds, has also been observed in cattle (MacHugh et al., 1997) and has been attributed to the recent evolutionary history of European populations.

A more appropriate measure of genetic variation within a population is gene diversity (average expected

heterozygosity) (Nei. 1987). Gene diversity for each breed ranged from 54.2% in Pafuri to 67.2% in Ndebele. This level of genetic diversity is similar to the values reported in Swiss goat breeds (51 to 58%) for 20 microsatellite loci (Saitbekova et al., 1999), but is slightly lower than those reported in Chinese goat breeds (77.7 to 82.3%) for 6 microsatellite loci (Yang et al., 1999). In general, the levels of gene diversity observed in the goats of sub-Saharan Africa were comparable to the values reported in other African livestock species such as cattle (MacHugh et al., 1997; Okomo et al., 1998) and chicken (Wimmers et al., 2000).

Population differentiation

The two estimators of population subdivision indicated that the levels of population differentiation were considerable as all values for G_{ST} and θ differed from zero and the exact test of population differentiation indicated significant differentiation between pair-wise comparisons of breeds. Reproductive isolation due to geographic barriers had caused the genetic differentiation observed between the breeds. Since there have been no deliberate efforts undertaken to create distinct goat breeds in sub-Saharan Africa, founder effects and genetic drift may have played a major role in the differentiation of the African breeds. The multi-locus G_{ST} and θ values indicated that between 14.6% and 15.7% of the total genetic variation was due to population differences, the remaining 85.4 (for G_{ST}) and 84.3% (for θ) corresponded to differences among individuals within the populations. These values for the between-breed variation are close to that reported in Swiss goats (17%) (Saitbekova et al., 1999) using microsatellites and higher than the amount of variation observed among goat breeds (10.7%) from Africa, Middle East, Asia and Europe using mitochondrial DNA analysis (Luikart et al., 2001).

Genetic relationships

A close examination of the genetic distances data revealed that the smallest distance values were found between eastern African breeds and southern African breeds while the largest distance values were those between West African breeds and the breeds from southern Africa. This indicates that the breeds of southern Africa were more closely related to the eastern African breeds than to the breeds of West Africa. The phylogeny constructed from the genetic distance data indicated that the breeds were grouped according to their geographic locations of origin and that the sub-Saharan African breeds were clearly separated from the breeds from outside Africa. A similar observation of populations clustering according to their geographic origins has been reported in humans (Bowcock et al., 1994), cattle (MacHugh et al., 1997) and chickens (Wimmers et al.,

2000). This implies that geographically adjacent populations are more genetically related, probably because of founder effects and interbreeding, especially around bordering areas. Bootstrap values (percentage of occurrence of a node in 1,000 bootstrap resampling of loci) ranged from 38 to 97%, with only two nodes with values below 50%. This indicates that the topology of the phylogeny constructed from D_A distances was more reliable in recovering the evolutionary relationships of the populations studied.

The principal component analysis (PCA) supported the grouping of the African populations according to their geographic locations. The eastern African breeds were found in between the southern African breeds and the West African breeds. Unlike the results of the phylogeny, the plot for PCA revealed the separation between the European and Asian breeds more clearly. Hence, it is conceivable that the close relationships between European and Asian breeds evident in the phylogenetic tree may be a result of the limitation of phylogenetic analysis procedure to show the relationships of distantly related breeds when using microsatellite data. This is in agreement with the finding of MacHugh et al. (1997) who reported the PCA, using microsatellite allele frequencies, to be a powerful tool for revealing the underlying evolutionary history among distantly related populations of cattle from Africa, Europe and Asia.

The classification of African goat breeds is based on body size, ear shape and length, horn types and functions. It is assumed that the differences in such traits reflect distinct origins or genetic identity. Investigation of the phylogeny and the plot for PCA indicated that the African breeds were grouped according to their geographic locations of origin. It seems that there is a clear association between particular goat types and sub-regions of sub-Saharan Africa. Within a sub-region, populations could be grouped according to phenotypic characteristics. For instance, among the West African breeds, the Maure goats, classified as "Twisted horn intermediate" breed type, were separated from the Dwarf small horn breeds, the WAD and Djallonke, indicating that the WAD and the Djallonke are more close to each other than to the Maure. However, it should be noted that morphological differences between populations are not taken into account by neutral molecular markers such as microsatellite (Hartl and Clark, 1997). It is genetic drift process that causes the genetic differentiation between populations detected by the use of neutral molecular markers. Hence, breed relationships were more related to geographical locations of the breeds than to the morphological differences between the breeds.

CONCLUSSIONS

This study has shown that microsatellite analysis can be used to classify the goat populations of sub-Saharan into distinct genetic groups or breeds. The phylogenetic tree and the plot for principal component analysis grouped the goats of sub-Saharan Africa according to their geographic locations of origin indicating that the goats of eastern Africa, West Africa and southern Africa are substantially differentiated from each other. This means that the goat populations from different sub-regions of sub-Saharan Africa have remained relatively genetically distinct, despite the fact that no strict breeding rules has been applied to create standard breeds. Within each sub-region, goat populations could be differentiated according to morphological characteristics.

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REFERENCES

Arranz, J. J., Y. Bayón and F. San Primitivo. 1998. Genetic relationships among Spanish sheep using microsatellites. Anim. Genet. 29:435-440.

Barker, J. S. F. 1994. A global protocol for determining genetic distances among domestic livestock breeds. In: Proceedings of the 5th World Congress of Genetics Applied to Livestock Production. Vol. 21:501-508.

Bowcock, A. M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J. R. Kidd and L. L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455-457.

Buchanan, F. C., L. J. Adams, R. P. Littlejohn, J. F. Maddox and A. M. Crawford. 1994. Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics 22:397-403.

Dorji, T., O. Hanotte, M. Arbenz, J. E. O. Rege and W. Roder. 2003. Genetic Diversity of indigeous cattle populations in Bhutan: Implications for conservation. Asian-Aust. J. Anim. Sci. 16:946-951.

Fahmy, T. 2000. XLSTAT program version 4.3. http://www.xlstat.com

Guodet, J. 1995. FSTAT version 1.2: a program to calculate F-statistics. J. Hered. 86:485-486.

Hartl, D. L. and A. G. Clark. 1997. Principles of population genetics, 3rd ed. Sinauer Associates. Sunderland, Massachusetts.

Luikart, G., L. Gielly, L. Excoffier, J. Vigne, J. Bouvet and P. Taberlet. 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. In: Proceedings of the National Academy of Science of the USA. Vol. 98:5927-5932.

MacHugh, D. E., M. D. Shriver, R. T. Loftus, P. Cunningham and D. G. Bradley. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146:1071-1086.

Manly, B. F. J. 1986. Multivariate statistical methods: A Primer. Chapman and Hall. London.

Mason, I. L. and J. P. Maule. 1960. The indigenous livestock of Eastern and Southern Africa. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. In: Proceedings of the National Academy of Science of the USA. Vol. 70:3321-3323.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University press, New York.

Nei, M., F. Tajima and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. J. Mol. Evol. 19:153-170.

Okomo, M. A., J. E. O. Rege, A. Teale and O. Hanotte. 1998. Genetic characterisation of indigenous East African cattle breeds using microsatellite DNA markers. In: Proceedings of the 6th World Congress of Genetics Applied to Livestock Production. Vol. 27:243-246.

Ota, T. 1993. DISPAN: Genetic Distance and Phylogenetic Analysis. Pennsylvania State University, University Park PA, USA.

Pandey, A. K., M. S. Tantia, D. Kumar, B. Mishra, P. Chaudhary and R. K. Vijh. 2002. Microsatellite analysis of three poultry breeds of India. Asian-Aust. J. Anim. Sci. 15:1536-1542.

Raymond, M. and F. Rousset. 1995a. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.

Raymond, M. and F. Rousset. 1995b. An exact test for population differentiation. Evolution 49:1280-1283.

Rege, J. E. O., C. V. Yapi-Gnaoré and C. L. Tawah. 1996. The indigenous domestic ruminant genetic resources of Africa. In: Proceedings of the second All Africa Conference on Animal Agriculture, 1-4, April 1996, Pretoria, South Africa, pp. 57-75.

Saitbekova, N., C. Gaillard, G. Obexer-Ruff and G. Dolf. 1999. Genetic diversity in Swiss goat breeds based on microsatellite analysis. Anim. Genet. 30:36-41.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning-A laboratory manual. Vol. 2, 2nd edition. Cold Spring Harbour, Laboratory Press, USA.

Takezaki, N. and M. Nei. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389-399.

Weir, B. S. 1990. Genetic data analysis: Methods for discrete population genetic data. Sinauer Associates, Inc. publishers. Sunderland, Massachusetts.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wimmers, K., S. Ponsuksili, T. Hardge, A. Valle-Zarate, P. K. Mathur and P. Horst. 2000. Genetic distinctness of African, Asian and South American local chickens. Anim. Genet. 31:159-165.

Yang, L., S. H. Zhao, K. Li, Z. Z. Peng and G. W. Montgomery. 1999. Determination of genetic relationships among five indigenous Chinese goat breeds with six microsatellite markers. Anim. Genet. 30:452-455.