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ASSESSMENT OF GENETIC DIVERSITY OF EAST AFRICAN GOAT POPULATIONS USING MICROSATELLITE DNA MARKERS

S.W. Chenyambuga[1,3], P.C. Watts[2], J. Hirbo[1], G.C. Kifaro[3], P. H. Petersen[4], P.S. Gwakisa[3], S.J. Kemp[2], O. Hanotte[1] and J.E.O. Rege[5]

1- International Livestock Research Institute, P.O. Box 30709 Nairobi, Kenya, 2-School of Biological Sciences, University of Liverpool, Liverpool, L69 7ZD, UK, 3- Sokoine University of Agriculture, P.O. Box 3004 Morogoro, Tanzania, 4-Department of Animal Science and Health, Royal Veterinary and Agricultural University, Bulowsvej 13DK–1870 Fredenksberg C, Copenhagen, Denmark and 5- International Livestock Research Institute, P.O. Box 5689 Addis Ababa, Ethiopia

SUMMARY

About forty-eight percent of goats of sub-Saharan Africa are found within the East African region. Most of them are indigenous and they are raised under traditional systems, where they provide meat, cash income and secure form of investment. Unplanned crossbreeding with exotic breeds, ravages of drought and civil conflict are some of the factors putting these genetic resources at risk of loss. Characterisation of these indigenous goat types is important to forestall the impact of these factors, but also to enhance their utilization to improve the livelihoods of the local people. In this study, genetic diversity and relationships of 13 indigenous East African goat populations were assessed using 18 microsatellite loci. Within-breed genetic diversity (H_e), between breed genetic differentiation (G_{ST}), genetic distances (using Nei's standard distance, *Ds*) were calculated. Neighbour-joining (NJ) trees and principal component analysis (PCA) were constructed to study the genetic relationships among the populations. Average number of alleles per locus ranged from 5.39 ± 0.69 (Galla) to 6.44 ± 0.73 (Afar). Average heterozygosity was relatively high ranging from 0.55 ± 0.04 (Newala) to 0.66 ± 0.04 (Afar). The overall G_{ST} was 0.12. *Ds* ranged between 0.03 (Ugogo and Masai) to 0.40 (Landim and Tanzanian Coastal). Two major breed groups were identified from neighbour-joining and PCA analyses, the Ethiopian-Kenyan breeds (North East Highland, Afar, Boran, Galla), and the Tanzanian breeds (Ujiji, Sukuma, Masai, Ugogo, Mbeya, Newala, Tanzanian Coastal), the latter being clearly separate from the former group as well as the reference breeds used, the West African Dwarf, the Tswana and the Toggenburg.

Keywords: Genetic diversity, East African goat populations, microsatellite markers

INTRODUCTION

East Africa is home to about forty-eight percent of the sub-Saharan goat populations, ninety-seven percent being indigenous (Okeyo, 1998). They are raised under traditional systems providing meat, cash income and secure form of investment. The region is dominated by the short-eared goats, the most common being the Small East African goats (Mason and Maule, 1960). Genetic relationships between East African goats are unclear. Considerable phenotypic variations are observed among the goat populations, especially with regard to size and coat colour patterns. Unplanned crossbreeding with exotic breeds, ravages of drought and civil conflict are some of the factors putting these genetic resources at risk of loss.

Characterisation of indigenous goat types with the aim of identifying unique populations - to form the foundation on which improvement and conservation programmes can be based - is important not only to forestall the impact of these factors, but also to enhance their utilisation to improve the livelihoods of the people. DNA markers are currently widely used to study genetic polymorphism observed at DNA level. Among the DNA markers, microatellites have proved to be useful polymorphic markers for analysis of closely related populations (MacHugh *et al.*, 1997; Arranz *et al.*, 1998). Studies on genetic differentiation of livestock in Africa using microsatellites have mainly concentrated on cattle (Okomo *et al.*, 1998; Hanotte *et al.*, 2000). No information is yet available on the genetic relationships of East African goats. In this study, genetic diversity and relationships of 13 indigenous East African goat populations were assessed using 18 microsatellite loci. Tswana (from Botswana), West African Dwarf and Toggenburg were used as reference breeds.

MATERIALS AND METHODS

Population samples and DNA extraction

Blood samples were collected from 13 populations of Small East African goats. One European breed and two African breeds from outside the East African region were also sampled. The country of origin and populations sampled were: Tanzania – Ugogo (48 animals analysed), Masai (50), Sukuma (48), Newala (50), Mbeya (48), Ujiji (48) and Tanzanian coastal (48); Kenya – Small East African (41), Boran (40), Galla (20); Ethiopia – Afar (45), North East Highland (46); Mozambique – Landim (36) and Botswana – Tswana (40); Nigeria – West African Dwarf (40), Europe (sampled in Kenya) - Toggenburg (24) for the reference breeds. In each population unrelated animals were randomly sampled from flocks of farmers and approximately equal numbers of females and males were sampled. Blood was collected by jugular vein puncture using 10 ml EDTA vacutainer tubes. DNA was extracted from peripheral blood lymphocytes using a modification of phenol-chloroform procedure (Sambrook *et al.*, 1989).

Microsatellite markers, PCR conditions and fragment analysis

Eighteen microsatellites were chosen for analysis: BM1818, BMC1222, BMS357, BMS1494, ILSTS5, ILSTS17, ILSTS44, ILSTS87, INRA5, INRA63, INRA132, MAF35, MAF65, MAF209, OarAE129, OarFCB304, SRCRSP3 and SRCRSP7. Sixteen markers (BM1818, BMC1222, BMS357, BMS1494, ILSTS17, ILSTS44, ILSTS87, INRA5, INRA63, INRA132, MAF35, MAF209, OarAE129, OarFCB304, SRCRSP3 and SRCRSP7) were analysed using the 4200 LI-COR (MWG-BIOTECH) automatic DNA sequencer and two markers (ILSTS5 and MAF65) were analysed using the 377 ABI (PERKIN-ELMER) automatic DNA sequencer. For all markers PCR amplification was performed in a total volume of 5 µl or 10 µl on either PTC-100™ thermal cycler or GeneAmp® PCR System 9700. Each PCR reaction contained 20 ng template DNA, 0.2 mM of each primer, 0.2 mM of each dNTP, PCR buffer (75 mM Tris-HCl, 2.0 mM $MgCl_2$, 0.01% (v/v) Tween 20, 1.25 units *Taq* DNA polymerase (Promega) or 100 mM Tris-HCl, 500 mM KCl, 2.0 mM $MgCl_2$, 0.01% gelatin, 0.25% Tween 20, 0.25% Nonidet and 0.5 units of *Taq* DNA polymerase (Promega) (ILSTS5 and MAF65 markers). All amplification included an initial denaturing step of 4 min at 95°C, followed by 35 cycles of 45 sec at 94°C, 1 min at the annealing temperature (50 – 60 °C) and 1 min at 72°C. Final extension was for 20 min at 72°C. The PCR products were analysed on a denaturing polyacrylamide gel using 4200 LI-COR and 377 ABI automatic DNA sequencers. Microsatellites were analysed using the Gene ImagIR™ (4200 LI-COR) and Genotyper® (ver 2.0) (377 ABI) softwares.

Statistical analyses

Observed allele frequencies were calculated using the Excel macros (kindly provided by Stephen Park, spark@tcd.ie). Average observed heterozygosity for each population, gene diversity between populations (coefficient of gene differentiation, G_{ST}) and Nei's standard genetic distances (*Ds*) were estimated using the DISPAN programme (Ota, 1993). The neighbour-joining (NJ) methodology was used to construct the phylogenetic tree. Bootstrap resampling (1000 replicates) was performed to test the robustness of the topology of the tree. Principal component analyses were performed using the SAS programme (SAS, 1990).

RESULTS

Within-population genetic variation and population subdivision

Genetic variation within each population was calculated as mean number of alleles per locus and average observed heterozygosity (H_o) (Table 1). Mean number of alleles per locus ranged from 5.11±0.60 in Toggenburg to 6.44±0.67 in Tswana. Among the East African goats, the highest number of alleles was observed in Afar (6.44±0.73) and the lowest in Galla (5.39±0.69). H_o was relatively high in all populations with the highest and lowest values being in Afar (0.660±0.036) and Newala (0.552±0.038), respectively.

The G_{ST} values for each locus across all populations are shown in Table 2. Generally, all loci had low values of G_{ST}, the overall value was 0.118. However, one locus, MAF35, had a very high value (0.698). This was attributed to the locus having non-overlapping alleles in some populations.

Table 1. Mean number of alleles and average observed heterozygosity (H_o) in each population

Population	Mean number of alleles per locus ± S.E.	Observed heterozygosity ± S.E. (H_0)
East African goat populations		
Kenyan Small East African	5.67 ± 0.63	0.605 ± 0.038
Landim	6.06 ± 0.70	0.594 ± 0.052
Boran	5.56 ± 0.82	0.596 ± 0.036
Galla	5.39 ± 0.69	0.604 ± 0.038
North East Highland	6.00 ± 0. 61	0.613 ± 0.040
Afar	6.44 ± 0.73	0.660 ± 0.036
Tanzanian Coastal	6.28 ± 0.50	0.623 ± 0.038
Ugogo	5.89 ± 0.62	0.627 ±0.041
Ujiji	6.11 ± 0.58	0.639 ± 0.034
Masai	5.94 ± 0.53	0.589 ± 0.042
Sukuma	6.17 ± 0.79	0.615 ± 0.046
Newala	5.61 ± 0.63	0.552 ± 0.038
Mbeya	6.22 ± 0.56	0.647 ± 0.029
Reference breeds		
West Africa Dwarf	6.22 ± 0.76	0.618 ± 0.051
Tswana	6.44 ± 0.67	0.641 ± 0.037
Toggenburg	5.11 ± 0.60	0.589 ± 0.060

Genetic distances and breed relationships

Standard genetic distances (*Ds*) between populations are indicated in Table 3. The largest distance was between Landim and Tanzanian Coastal (0.40) while the minimum distance was between Masai and Ugogo (0.03). The NJ tree (Figure 1) indicated two major groups of East African goats. Group one consisted of Ethiopian and Northeast Kenyan goat populations, while group two was made up of the Tanzanian goat populations. The group of Ethiopian and Northeast Kenyan goats could be divided into Ethiopian goats (Afar and North East Highland) and Somali goats (Boran and Galla). The Small East African goats from Kenya were found to be intermediate between the two major groups. The Landim clustered together with the Tswana goats. The plot of the scores for the first three principal components (Figure 2) showed more or less the same clustering as that of the NJ tree. However, for the principal component analysis, Newala goats did not cluster together with the other Tanzanian goats.

Table 2. Coefficient of gene differentiation (G_{ST}) and total number of alleles for each locus

Locus	G_{ST}	Number of alleles
INRA63	0.095	6
ILSTS87	0.081	10
SRCRSP3	0.071	7
SRCRSP7	0.121	5
OarAE129	0.065	13
BMS357	0.094	13
INRA5	0.065	6
BMS1494	0.094	7
BMC1222	0.090	10
INRA132	0.059	7
ILSTS17	0.0754	14
BM1818	0.096	14
MAF209	0.097	10
OarFCB304	0.071	27
MAF35	0.698	6
ILSTS44	0.138	11
ILSTS5	0.115	14
MAF65	0.130	16
All loci	0.118	

Table 3. Nei's standard genetic distances for 13 East African goat populations and 3 breeds of other goats

	Tswana	Small East African	Toggen burg	Landim	Boran	Galla	North East Highland	Afar	Tanzanian Coastal	Ugogo	Ujiji	Masai	Sukuma	Newala	Mbeya	West African Dwarf
Small East African	0.212															
Toggen-burg	0.201	0.241														
Landim	0.101	0.212	0.145													
Boran	0.200	0.057	0.159	0.177												
Galla	0.208	0.077	0.148	0.157	0.037											
North East Highland	0.189	0.058	0.207	0.155	0.076	0.056										
Afar	0.207	0.074	0.189	0.174	0.070	0.078	0.041									
Tanzanian coastal	0.477	0.292	0.478	0.401	0.312	0.326	0.309	0.266								
Ugogo	0.368	0.212	0.400	0.359	0.207	0.216	0.212	0.214	0.119							
Ujiji	0.343	0.196	0.372	0.347	0.185	0.219	0.212	0.209	0.165	0.057						
Masai	0.396	0.210	0.447	0.392	0.210	0.238	0.234	0.251	0.157	0.033	0.069					
Sukuma	0.345	0.210	0.348	0.306	0.173	0.206	0.225	0.217	0.142	0.041	0.075	0.076				
Newala	0.310	0.303	0.469	0.269	0.350	0.323	0.292	0.338	0.250	0.170	0.236	0.196	0.171			
Mbeya	0.313	0.180	0.448	0.329	0.211	0.227	0.211	0.193	0.135	0.040	0.102	0.112	0.080	0.141		
West African Dwarf	0.304	0.214	0.321	0.418	0.189	0.278	0.251	0.191	0.403	0.358	0.334	0.399	0.343	0.561	0.334	

DISCUSSION

The mean number of alleles per locus and the observed heterozygosity were higher in all African populations compared to the single European breed studied. It is possible that East African goat populations have generally larger effective population size than the European ones. However, only twenty-four Toggenburg goats were analysed, all coming from a single population in Kenya. The differences in within-population genetic variation among East African goat populations were small. The coefficients of gene differentiation (G_{ST}) at each locus as well as over all loci were low. Only 11.8% of the diversity was between populations while the within-population diversity was 87.2%. Low G_{ST} values indicate low differentiation between populations at the microsatellite loci analysed (Hedrick, 1999). The genetic distances amongst East African goats were very variable, ranging from 0.03 to 0.40 (Table 3). It is an indication of lack of genetic differentiation between some of the populations following, for example, recent separation from a common ancestral population, recent population admixture and/or lack of selection efforts to create standardised breeds or lines. In some cases, clear genetic differentiation between some of the East African goat populations were also observed. This supports the current separation of these populations into separate breeds. The NJ phylogenetic tree revealed two main clusters of East African goats, a cluster of Ethiopian – Northeast Kenyan goat populations and a cluster of Tanzanian goat populations (Figure 1). This clustering is in agreement with the classification of Rege *et al.* (1996) whereby Ethiopian – Northeast Kenyan goats (North East Highland, Afar, Boran and Galla,) are classified as Intermediate East African goats and the Tanzanian goats as Small East African goats. Within each cluster, geographically adjacent populations were more genetically related. Phylogenetic studies in other species (Bowcock *et al.*, 1994; MacHugh *et al.*, 1998) have shown a similar pattern of population clustering which reflects the geographic origins of the breed. The Landim goats, phenotypically classified as Small East African goats, clustered together with the long lop-eared goats (Tswana). This could be the result of mixing and interbreeding of the Landim with lop-eared goats from adjacent countries in southern Africa. Since 1928 the Landim has been involved in breeding programmes with lop-eared Boer goats of South Africa (Epstein, 1971). The principal component analysis (Figure 2) showed two clusters as those of the NJ tree. However, in contrast to the NJ tree, the Newala goats were separated from other Tanzanian goats. This, probably, indicates little mixing of the Newala goats with other Tanzanian goats as a result of limited movement and trade between the Newala region and other parts of Tanzania.

In conclusion, the 18 microsatellites used in this study were able to differentiate the East African goats according to their geographic origin as shown by clear separation between the cluster of Small East African populations (Tanzanian goats) and that of Intermediate East African populations (Ethiopian and Northeast Kenyan goats). However, the bootstrap values were low indicating lack of clear pattern of differentiation. Also, the study has shown that within-population genetic variability is relatively high in all East African goats, implying that East African goats are well adapted to their local environmental conditions and have high potential for improvement. This supports the need for their conservation and improvement for better utilization to improve the livelihood of the people in East Africa.

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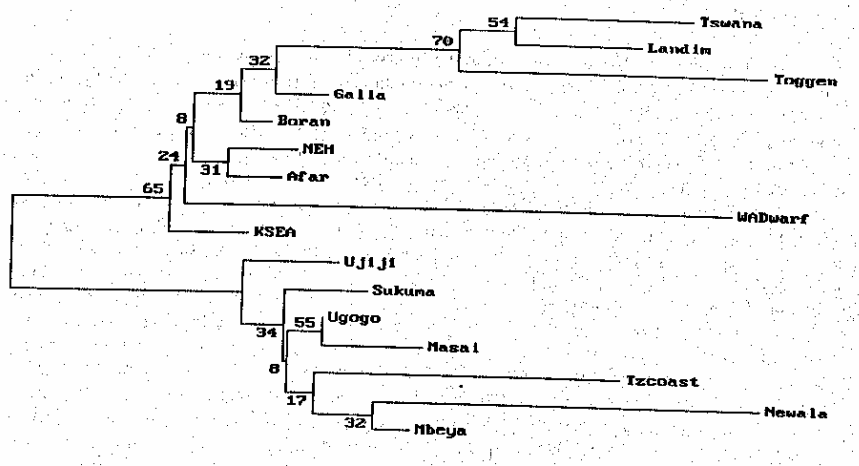

Fig 1: Unrooted neighbour-joining (NJ) tree showing genetic relationships among 13 East African goat populations and 3 reference breeds (Tswana, Toggenburg and West African Dwarf) using the genetic distance *Ds*. Bootstrap values were obtained after resampling of 1000 replicates.

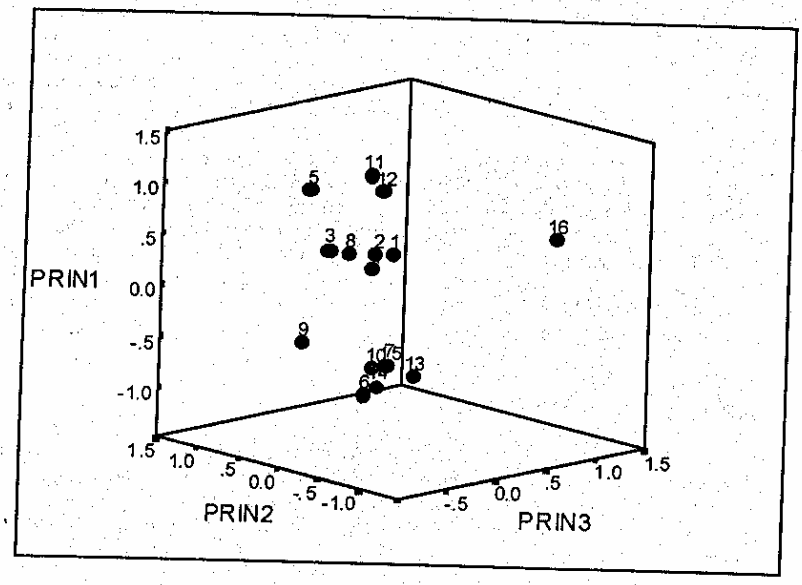

Fig 2. Principal component analysis of allele frequencies from 13 African goat populations and 3 reference breeds. The first three component scores are plotted, with the first, second and third components accounting for 35%, 16% and 10% of the total variation, respectively

PRN1 - Principal component 1 PRN2 - Principal component 2 PRN3 - Principal component 3
1 - Afar 2- Boran 3- Galla 4 - Kenyan Small East African 5 - Landim 6 - Masai 7- Mbeya
8 - North East Highland 9 - Newala 10 - Sukuma 11 - Toggenburg 12 - Tswana 13 - Tanzanian Coastal
14 - Ugogo 15 - Ujiji 16 - West African Dwarf

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