Analysis of Genetic Diversity and Relationships of Tanzanian Local Goat Populations Using Microsatellite DNA Markers

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Abstract

Genetic diversity among seven Tanzanian goat populations (Ujiji, Sukuma, Ugogo, Maasai, Mbeya, Newala and Coastal goats) was investigated by determining polymorphisms at 19 microsatellite DNA loci. West African Dwarf, Tswana, Landim and Toggenburg were included to serve as reference breeds. Among the Tanzanian populations, mean number of alleles per locus was highest (6.26 ± 0.670) in Sukuma and lowest (5.74 ± 0.545) in Newala. Gene diversity ranged from 0.553 ± 0.036 (Newala goats) to 0.646 ± 0.028 (Mbeya goats). The coefficient of gene differentiation (G_{ST}) indicated that 13% of the genetic diversity in all populations was due to differences between the populations. The genetic distance values ranged from 0.068 (between Sukuma and Ugogo goats) to 0.2178 (between Ujiji and Coastal goats). The neighbour-joining dendrogram constructed to show population relationships indicated that the Tanzanian populations were separated from the populations used as reference breeds. The dendrogram revealed three sub-clusters of the Tanzanian populations: Coastal and Maasai goats, Ugogo and Ujiji goats, and Sukuma, Mbeya and Newala goats. The principal component analysis separated the Newala goats from the other Tanzanian goat populations. It is concluded that the level of genetic variation within the goat populations was reasonably high and there was no significant difference between the populations with respect to the number of alleles and the level of heterozygosity.

Key words: genetic differentiation, microsatellites, Tanzanian goats

Introduction

The indigenous goats of Tanzania belong to the Small East African goat type which is the most widespread group in Eastern Africa and some parts of central and southern Africa (Mason and Maule, 1960, Epstein, 1971). Although all the Tanzanian indigenous goats are presumed to belong to one major group, considerable variation in terms of colour, ear type and body size can be observed among the goat populations found in different parts of the country. The different goat populations are named after communities keeping them (e.g. Maasai, Sukuma, and Ugogo) or locations where they are reared (e.g. Ujiji and Newala). It is not known whether this naming reflects distinct genetic entities (breeds or strains) or populations that are genetically similar but

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have been given different names in different places. Earlier attempts to characterise these animals based on morphological characters (Mason and Maule, 1960, Epstein, 1971) did not classify them into distinct breeds or strains due to the considerable variations observed among and within the populations. Furthermore, the history of the different goat populations is not well documented and thus the extent of genetic differentiation among these populations and their evolutionary relationships is not well known. Yet their existence is threatened by replacement or uncontrolled crossbreeding with exotic breeds (especially Toggenburg, Saanen, Anglo-Nubian and Boer goats) as a result of pressures for increased animal production for economic development. The phenotypic traits used in the classification of goat breeds (i.e. ear length and type, horn type, function) (Mason and Maule, 1960, Epstein, 1971) cannot indicate their genetic distinctiveness as well as their evolutionary relationships because they have not been deliberately selected for particular functions and there is no pedigree information. Fortunately, recent advances in the development of nuclear DNA markers, in particular microsatellites, have allowed the determination of the extent of genetic differentiation between closely related populations (Bruford and Wayne, 1993, Saitbekova et al. 1999) and the reconstruction of evolutionary relationships of contemporary populations within a species (MacHugh et al., 1997). The information on population differentiation and evolutionary relationship is used to indicate the genetic uniqueness of breeds within a species. The genetic uniqueness of the breeds can act as an initial guide to objective and rational decision-making in the choice of populations for conservation and sustainable utilisation (Barker, 1994) before other data (e.g. economic values or adaptation to specific environment) become available or can be used in combination with such data.

The objectives of this study were to examine the genetic variation within populations, the extent of population differentiation and the evolutionary relationships of indigenous goat populations in Tanzania by determining polymorphisms at 19 microsatellite loci across the genome.

Materials and Methods

Populations sampled and DNA extraction

Blood samples were collected from seven Tanzanian goat populations. The populations sampled with the number of animals per populations in bracket were as follows: Ugogo (48), Maasai (50), Sukuma (48), Newala (50), Mbeya (48), Ujiji (48) and Coastal goats (48). Three African breeds (West African Dwarf (40), Tswana (40) and Landim (36)) and one European breed (Toggenburg (24)) were also sampled to serve as reference breeds. For each population, approximately equal numbers of females and males were randomly sampled from two districts, and three villages (approximately 15 km apart) per district were selected for sampling. In each village, six to ten animals were sampled from two to four flocks of farmers (approximately 2 km apart). In order to avoid sampling related individuals, farmers were asked about the origins and familial relationships of the animals in their flocks. Blood was collected by jugular vein puncture using 10 mL EDTA tubes. DNA was extracted from peripheral blood lymphocytes using a phenol-chloroform procedure of Sambrook *et al.* (1989).

Microsatellite markers, PCR conditions and fragment analysis

Nineteen (19) microsatellite markers were chosen for analysis; the markers and their characteristics are shown in Table 1. Three markers (*ILSTS005, ILSTS011* and *MAF065*) were typed at International Livestock Research Institute (ILRI), Nairobi, Kenya using the 377 ABI (PERKIN-ELMER) automatic DNA sequencer and the remaining sixteen (16) markers were typed at the University of Liverpool using the 4200 LI-COR (MWG-BIOTECH) automatic DNA sequencer. PCR amplification was done in a total volume of 10 µL on a PTC-100™ thermal cycler (MJ Research, Inc.,). Each PCR reaction contained 20 ng template DNA, 0.2 mM of each primer, 0.2 mM of each dNTP, PCR buffer (100 mM Tris-HCl (pH 8.3), 50 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). 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 the annealing

temperature (shown in Table 1) and 1 min at 72°C. Final extension was for 20 min at 72°C. The PCR products were electrophoresed on a 6% or 4.25% denaturing polyacrylamide gel using 4200 LI-COR (16 markers) and 377 ABI (three markers) automatic DNA sequencers, respectively.

Microsatellite fragments were analysed using the Gene ImagIR™ (for the 16 markers electrophoresed using the 4200 LI-COR) and Genescan analysis™ and Genotyper™ (ver 2.0) softwares (for the 3 markers electrophoresed using the 377 ABI).

Table 1: Microsatelite markers studied and their characteristics

Locus	Chromosome	Primer sequences 5'-3'	Annealing temp.°C	Number of alleles in all populations
BM1818	23	F-AGCTGGAATATAACCAAAGG R-AGTGCTTTCAAGGTCCATGC	55	14
BMC1222	13q12	F-CCAATTTTGCAGATAAGAAAACA R-CCTGAGTGTTCCTCCTGAGT	55	10
BMS357	Unknown	F-TCTGGAGCTTGCAAAAGACC R-AATGGATGACTCCTGGATGG	58	13
BMS1494	Unknown	F-TCTGGAGCTTGCAAAAGACC R-CCAAATAATTGCTGGTCAGG	55	7
ILSTS005	10	F-GGAAGCAATGAAATCTATAGCC R-TGTTCTGTGAGTTTGTAAGC	55	14
ILSTS011	14	F-GCTTGCTACATGGAAAGTGC R-CTAAAATGCAGAGCCCTACC	55	12
ILSTS017	Unknown	F-GTCCCTAAAATCGAAATGCC R-GCATCTCTATAACCTGTTCC	57	14
ILSTS044	Unknown	F-AGCAGACATGATGACTCAGC R-ACATGTTGTATTCCAAGTGC	57	11
ILSTS087	Unknown	F-AGCAGACATGATGACTCAGC R-CTGCCTCTTTTCTTGAGAGC	58	10
INRA005	12	F-CAATCTGCATGAAGTATAAATAT R-CTTCAGGCATACCCTACACC	57	6
INRA063	18q22	F-ATTTGCACAAGCTAAATCTAACC R-AAACCACAGAAATGCTTGGAAG	55	5
INRA132	23	F-AACATTTCAGCTGATGGTGGC R-TTCTGTTTTGAGTGGTAAGCTG	57	7
MAF035	Unknown	F-TCAAGAATTTTGGAGCACAATTCTGG R-AGTTACAATGCAAGCATCATACCTG	55	6
MAF065	15	F-AAAGGCCAGAGTATGCAATTAGGAG R-CCACTCCTCCTGAGAATATAACATG	50	15
MAF209	17	F-GATCACAAAAAGTTGGATACAACCGTGG R-TCATGCACTTAAGTATGTAGGATGCTG	57	9
OarAE129	7	F-AATCCAGTGTGAAAGACTAATCCAG R-GTAGATCAAGATATAGAATATTTTTCAACACC	58	13
OarFCB304	Unknown	F-CCCTAGGAGCTTTCAATAAAGAATCGG R-CGCTGCTGTCAACTGGGTCAGGG	55	23
SRCRSP003	10q36	F-CGGGGATCTGTTCTATGAAC R-TGATTAGCTGGCTGAATGAATGTCC	55	6
SRCRSP007	6	F-TCTCAGCCACCTTAATTGCTC R-GTCAACASTCCAATGGTGAG	57	5

Statistical analyses

Estimations of average observed and expected heterozygosities as well as test for deviations from Hardy-Weinberg equilibrium (HWE) at each locus for each population and for all loci and populations were done by use of GENEPOP package (Raymond and Rousset, 1995a). Unbiased estimate of expected heterozygosity (h) were calculated as $h = 2n(1 - \Sigma x_i^2)/(2n-1)$ (Nei, 1987) where x_i is the population frequency of the i^{th} allele at a locus and n is the number of individuals sampled. Deviations from HWE were tested in two ways: (a) for each locus per population by an exact test using Guo and Thompson's (1992) Markov chain Monte Carlo algorithm and (b) for all loci and populations using Fisher's method in the GENEPOP package (Raymond and Rousset, 1995b). Genetic diversity between populations (coefficient of gene differentiation, G_{ST}) (Nei, 1973) and Nei *et al.'s* (1983) angular genetic distance (D_A) were estimated using a DISPAN programme (Ota, 1993).

The G_{ST} was calculated as $G_{ST} = D_{ST}/H_T$ (Nei, 1987) where D_{ST} is the average gene diversity between subpopulations and H_T is the gene diversity in the total population. The G_{ST} is a drift-based measure of population differentiation and was considered to be more appropriate for this analysis because genetic drift is said to be the main factor in genetic differentiation among closely related populations (Weir, 1990).

The D_A measure of genetic distance was calculated as follows, $D_A = 1/r\Sigma(1-\Sigma\sqrt{x_{ij}y_{ij}})$ (Nei, 1987) where x_{ij} and y_{ij} are the frequencies of the i^{th} allele at the j^{th} locus in populations X and Y, respectively, and r is the number of loci. 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) was used to construct the phylogenetic tree of population relationships from the genetic distance matrix using the DISPAN programme. Bootstrap resampling (1000 replicates) was performed to test the robustness of the topology of the tree. In addition, a principal component analysis (PCA) (Manly, 1986) was carried out using the XLSTAT programme (Fahmy, 2000) to determine population-relationships based directly on the allele frequencies. The PCA is a multivariate technique and involves a linear transformation of the observed allele frequencies into a new set of variables (principal components (PC)), in geometric terms a rotation of the coordinates. The PCs are orthogonal and therefore uncorrelated to each other. The first principal component (PC1) of the observations accounts for more information than PC2 and PC2 more than PC3. The first three PCs are the most informative, and in the present study, they were plotted on a scatter diagram to allow visual inspection of the relationships of all populations.

Results and Discussion

Genetic diversity within the populations

The estimates of population genetic diversity were based on a set of markers with a total of 5 to 23 alleles per locus (Table 1). This level of polymorphism is considered sufficient for genetic characterisation of breeds in order to reduce the standard error of the distance estimates (Barker, 1994). Table 2 shows that, among the Tanzanian populations, Sukuma and Coastal goats had the highest (6.26) mean number of alleles per locus while the Newala goats had the lowest (5.74). The average observed heterozygosity ranged from 0.455 in Newala goats to 0.542 in Ujiji goats. The average expected heterozygosity was lowest in Newala goats (0.553) and highest in Mbeya goats (0.646). The mean numbers of alleles per locus and gene diversities (expected heterozygosities) in the present study indicate a relatively high level of genetic variability within all populations. The values are comparable to those observed in other African goats (Chenyambuga, 2002) and in African cattle (MacHugh *et al.*, 1997, Okomo *et al.*, 1998). The narrow range of the values for both the number of alleles per locus and gene diversity may be due to a recent separation from the ancestral population coupled with continuous mixing and interbreeding among the populations. For the past three decades some communities, especially the Maasai and Sukuma, have been migrating from one place to another in search of grazing land. This has caused mixing and interbreeding among goat populations, thus homogenising the level of the genetic diversity in various populations.

Table 2: Within population genetic variability and test for deviations from HWE at 19 microsatellite loci in indigeous goats of Tanzania and four reference population

Population	Mean number of alleles per locus ± s.e.	Average observed heterozygosity ± s.e.	Average expected heterozygosity ± s.e.	Number of loci Deviating from HWE
Tanzanian Populations				
Coastal	6.26 ± 0.425	0.481 ± 0.042	0.627 ± 0.036	13
Ugogo	5.95 ± 0.527	0.507 ± 0.042	0.630 ± 0.042	14
Ujiji	6.16 ± 0.520	0.542 ± 0.040	0.642 ± 0.032	12
Maasai	5.95 ± 0.449	0.485 ± 0.039	0.595 ± 0.040	12
Sukuma	6.26 ± 0.670	0.524 ± 0.040	0.624 ± 0.044	10
Newala	5.74 ± 0.545	0.455 ± 0.037	0.553 ± 0.036	12
Mbeya	6.21 ± 0.487	0.541 ± 0.039	0.646 ± 0.028	13
Reference breeds				
Tswana	6.53 ± 0.574	0.518 ± 0.044	0.633 ± 0.034	11
Landim	6.21 ± 0.629	0.524 ± 0.045	0.610 ± 0.048	10
WAD	6.32 ± 0.483	0.550 ± 0.053	0.621 ± 0.048	12
Toggenburg	5.11 ± 0.442	0.504 ± 0.063	0.595 ± 0.057	8

For all loci and all populations genotype proportions significantly deviated ($P < 0.001$) from HWE expectations (Table 2). The highest number of loci deviating from HWE was observed in Ugogo goats, 14 loci out of 19 loci deviated from HWE. The significant deviation of genotype proportions from HWE expectations observed in all populations may be due to population admixture effects. The geographical proximity of some of the populations and the movements of some of the pastoral and agro-pastoral people could have favoured the mixing of the different goat populations. However, the deviation from HWE may be due to inbreeding in some of the populations as uncontrolled mating is very common in most traditional flocks. The deviation from HWE also can be caused by presence of experimentally undetected alleles (null alleles). However, it should be remembered that in a genetic survey like this where there are no pedigree information, it is not possible to determine which factors caused the deviations. A non-compliance with HWE observed in this study only means that at least one of the assumptions for HWE (i.e. large population, random mating, with no mutation, selection and migration) did not hold for the populations studied.

Genetic differentiation and relationships between populations

The gene differentiation coefficient (G_{ST}) revealed an overall differentiation of 12.9% between the populations (Table 3). However, by excluding the populations from outside Tanzania, the proportion of genetic diversity due to between population differences dropped to 7.2% of the total genetic variation, the remaining 92.8% corresponded to differences among individuals within the populations, indicating that the extent of differentiation among the populations is low. The low level of genetic differentiation is probably due to intermixing and interbreeding among the goat populations as communities keeping them intermingle.

Table 3: Estimates of genetic differentiation at each locus

Microsatelite Locus	G_{ST} (All populations)	G_{ST} (Tanzanian populations)
BM1818	0.114	0.11
BMC1222	0.11	0.087
BMS1494	0.107	0.026
BMS357	0.099	0.081
ILSTS005	0.124	0.049
ILSTS011	0.16	0.135
ILSTS017	0.079	0.038
ILSTS044	0.169	0.039
ILSTS087	0.068	0.082
INRA005	0.071	0.058
INRA063	0.077	0.063
INRA132	0.052	0.024
MAF035	0.736	0.028
MAF209	0.111	0.034
MAF65	0.15	0.133
OarAE129	0.069	0.041
OarFCB304	0.087	0.034
SRCRSP003	0.094	0.084
SRCRSP007	0.136	0.138
All loci	0.129	0.072

G_{ST} = coefficient of gene differentiation

Among the Tanzanian populations, the smallest genetic distance, D_A, was found between the Sukuma and Ugogo goats (0.068) and the highest distance was observed between Ujiji and Coastal goats (0.2178) (Table 4). The neighbour-joining (NJ) tree constructed from the genetic distances to represent therelationships among the populations (Figure 1) indicated that the Tanzanian populations were separated from the populations used as reference breeds. Among the Tanzanian populations, three sub-clusters could be identified from the tree. The first sub-cluster consisted of the Coastal and the Maasai goats. The second sub-cluster was made up of the Ugogo and Ujiji goats. The third sub-cluster had the Sukuma, Mbeya and Newala goats, with Mbeya and Newala goats being more closely related. The bootstrap values (number of times a node was observed in 1000 replicates of resampled loci) for the tree ranged from 20% to 69%.

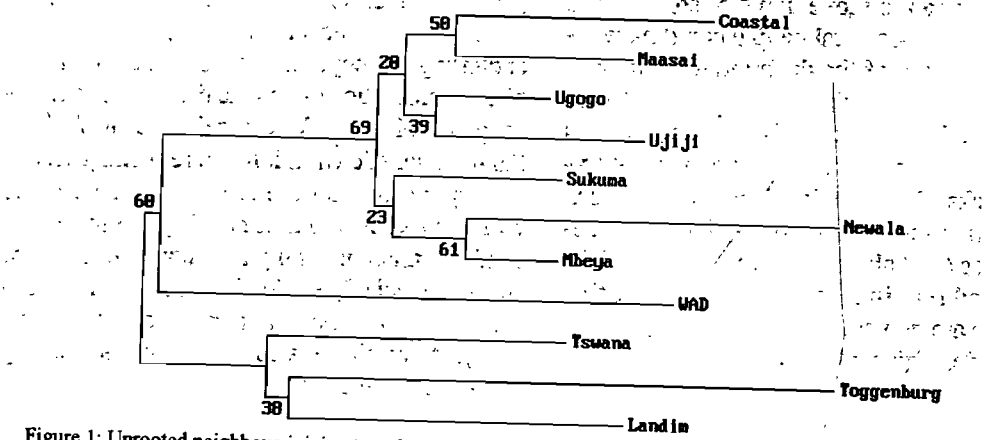

Figure 1: Unrooted neighbour-joining tree showing genetic relationships of seven Tanzanian goat populations and three other African goat populations and one European population (numbers at the nodes are percentage boostrap values obtained after 1000 replications of resampled loci).

Table 4: Nei's DA genetic distance matrix for seven Tanzanian populations and four reference populations

	Tsw	Tog	Lad	Cos	Ugo	Uji	Mas	Suk	New	Mby
Tsw										
Tog	0.195									
Lad	0.133	0.189								
Cos	0.221	0.265	0.207							
Ugo	0.204	0.254	0.217	0.098						
Uji	0.198	0.236	0.226	0.218	0.070					
Mas	0.221	0.279	0.213	0.094	0.075	0.097				
Suk	0.192	0.227	0.196	0.120	0.068	0.095	0.094			
New	0.199	0.302	0.180	0.165	0.141	0.081	0.146	0.125		
Mby	0.161	0.261	0.180	0.099	0.074	0.107	0.100	0.079	0.100	
WAD	0.194	0.222	0.284	0.215	0.192	0.185	0.216	0.196	0.296	0.198

Tsw - Tswana, Tog - Toggenburg, Lad-Landim, Cos - Coastal, Ugo - Ugogo, Uji- Ujiji, Mas - Maasai, Suk- Sukuma, New- Newala, Mby - Mbeya, WAD - West African Dwarf.

Figure 2 shows the PCA plot constructed using the first three principal components. In the PCA plot, the Newala goats were separated from the other Tanzanian populations while the Ugogo, Maasai, Coastal and Mbeya goats were grouped together and the Ujiji and Sukuma goats were more closely related. Unlike the NJ phylogenetic tree, the PCA plot separated the European breed (Toggenburg) from the African breeds. Among the African breeds, the WAD was well separated from the others and the Tswana and Landim were slightly separated from each other and from the Tanzanian populations. The first, second and third principal components accounted for 19, 17 and 13% of the total variations, and in total 10 principal components were required to account for all the variations.

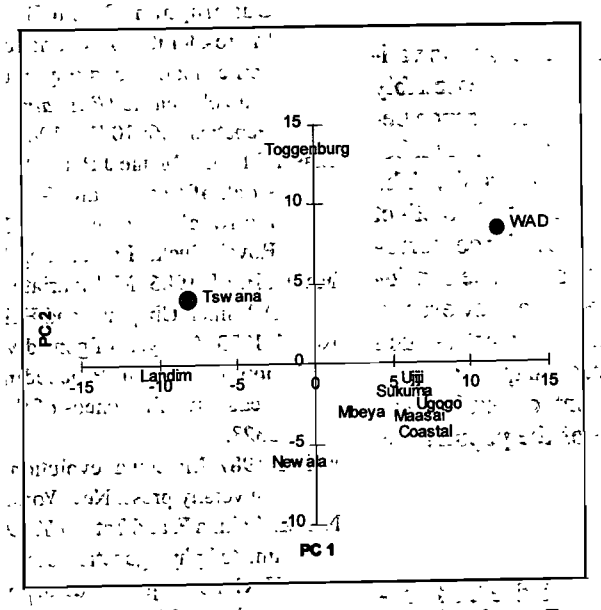

Figure 2: Three-dimensional PCA plot showing the genetic relationships of seven Tanzanian populations and three other African populations and one European population. The PC 1, PC2, and PC3, accounted for 19,17 and 13% respectively, of the total variation in the allele frequency data.

The two methods of graphically representing population relationships, the neighbour-joining dendrogram and PCA, indicate that the PCA plot revealed better the genetic relationships of the populations than the neighbour-joining dendrogram. This is in agreement with the finding of MacHugh *et al.* (1997) who reported the PCA, using microsatellite allele frequency data, to be a more powerful tool than the phylogenetic tree for revealing the underlying evolutionary relationships of cattle populations from Africa, Europe and Asia. In the present study the pattern of population grouping was according to expectations in the PCA. The European breed (Toggenburg) was separated from the African breeds, the Landim from Mozabique was close to Tanzanian populations and among the Tanzanian populations the Newala goats were separated from the other Tanzanian goats. The close relationships observed among the Tanzanian populations (Sukuma, Ujiji, Ugogo, Maasai, Mbeya and Coastal goats) are due to admixture of these populations resulting from the movements of animals for trade or in search of grazing land. The PCA indicates that the Newala goats are distinct from the other Tanzanian goats. This is, probably, because of little mixing of the Newala goats with other Tanzanian goats as a result of limited movement and trade between the Newala district and other parts of Tanzania.

Conclusions

The present study has shown that genetic variation within the goat populations was reasonably high but there was no significant difference between the populations with respect to the number of alleles and the level of heterozygosity. The Newala goats appeared to be genetically distinct from the other populations. Hence, for conservation purposes the Newala goats should be considered as an important genetic resource. However, in order to have rational decision, other factors such as traits of economic importance, unique traits, adaptation to specific environment, cultural values and degree of endangerment of the populations should be established.

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