Genetic variation from 12 microsatellite makers in an indigenous Tswana goat flock in South-eastern Botswana

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Abstract

The objective of this study was to preliminary characterize genetic variability from 12 microsatellite markers in an indigenous Tswana goat flock in South-eastern Botswana. The genomic DNA from 87 partially pedigreed indigenous Tswana goats from Botswana College of Agriculture farm was amplified via PCR with a panel of 12 microsatellite markers.

The program POPGENE Version 1.31 was used to determine the polymorphism information content (PIC), allele frequencies, number of alleles at each locus, effective number of alleles, average heterozygosity (H) and the inbreeding coefficient (F_{IS}) in the population. The flock exhibited low levels of allelic diversity (1.83±0.83) and heterozygosity (0.16±0.20). The number of alleles ranged from 1 to 3 with allele sizes ranging from 99 to 260 bp. The inbreeding coefficient (F_{IS}) across all 12 markers was 0.12±0.16 for this indigenous flock. Five markers namely BM1329, CSRD247, ILST002, RM004 and INRA63 were fixed indicating no genetic variation, three markers i.e. SRCRSP8, OARFCB20 and MAF65 were in Hardy-Weinberg equilibrium ($P > 0.05$) indicating random mating at those marker loci and four markers namely SRCRSP5, MCM527, ILST087 and INRA006 differed ($P < 0.05$) from Hardy-Weinberg equilibrium. Further studies are required using more microsatellite markers to compare this flock with country-wide flock and to generate information on effectiveness of directional selection for traits of economic importance and general productivity.

Key words: genetic characterization, goats, heterozygosis, microsatellite markers, polymorphism

Introduction

Indigenous goats in Botswana are generally known as Tswana but the distinction of different breeds, strains or ecotypes according to the ecological regions is not known (FAO 2001, Nsoso et al 2004). A country-wide preliminary study based on phenotypic characterization indicated the presence of different strains/breeds of the indigenous Tswana goats (Nsoso et al 2004). Definitive existence of strains/breeds can be established through molecular taxonomic characterization, which can in turn serve as a guide in decisions relating to their conservation and improvement (Hall 1996). The local goats which are more numeruous than other breeds constitute a valuable genetic resource because of their adaptation to harsh climatic conditions, their ability to better utilize the limited and poor quality feed resources and resistance/tolerance to parasites and diseases found in their habitats (Animal Production and Range Research Unit 1970-1990). The indigenous Tswana goat breed is kept by traditional farmers (Nsoso et al 2004) hence they can help in poverty reduction and provision of food self-sufficiency. The aim of this research was to assess preliminary genetic variation using 12 microsatellite markers in the indigenous Tswana goat flock kept by the Botswana College of Agriculture farm in South-eastern Botswana.

Materials and Methods

Animals

A base population of 100 mixed age (3+ years) Tswana does was established at Botswana College of Agriculture (BCA) in late 1996 from goats purchased from all over Southern Botswana (Katongole et al 1996). The goats were classified as Tswana using phenotypic appearance (Nsoso et al 2001). These does were used in an experiment to select for parasite resistance/tolerance coupled with increased productivity (Nsoso et al 2001) after grading up for 10 years using pure Tswana bucks from the Department of Agricultural Research flock in Botswana, which had been closed for over 30 years.

Each year in April –May the does were group-mated to 3-5 bucks of Tswana goat breed from the Department of Agricultural Research in Botswana. All male progeny were castrated at 3-4 months of age while all female progeny were kept as replacements. Does were culled for old age (more than 7 years) and poor reproductive performance. The BCA Tswana goat flock was specifically selected for the study because it represents a unique genetic material that has deliberately undergone selection for parasite resistance/tolerance coupled with increased productivity for slightly over 16 years.

Blood sampling and DNA extraction

Whole blood samples were collected from all the 87 available does of indigenous Tswana goats at BCA farm. Blood was collected from each animal via jugular venipuncture into EDTA coated Vaccutainer™ tubes and stored at -20ºC until DNA extraction. Total genomic DNA was extracted using Quick-gDNA Mini Prep kit (Zymo Research Corporation, CA, USA) following the manufacturer's protocol.

Microsatellite Markers

The primer pairs used for selective amplification of different microsatellite markers were synthesized by Inqaba Biotechnical Industries (Pty) Ltd (Hartfield, Pretoria, South Africa) (Table 1).

Table 1: Characteristics of the 12 microsatellite loci used to characterize 87 indigenous Tswana goats of Botswana College of Agriculture in South-eastern Botswana

Primer	Chromosome Number	Size Range	Sequence (forward + reverse)
SRCRSP5	21	166 - 180	F 5'-GGA CTC TAC CAA CTG AGC TAC AAG-3' R 5'-TGA AAT GAA GCT AAA GCA ATG C-3'
SRCRSP8	Unknown	210 - 260	F 5'-TGC GGT CTG GTT CTG ATT TCA C-3' R 5'-CCT GCA TGA GAA AGT CGA TGC TTA G-3'
MCM527	5	155 - 173	F 5'-GTC CAT TGC CTC AAA TCA ATT C-3' R 5'-AAA CCA CTT GAC TAC TCC CCA A-3'
BM1329	6 (sheep)	168 - 182	F 5'-TTG TTT AGG CAA GTC CAA AGT C-3' R 5'-AAC ACC GCA GCT TCA TCC-3'
OARFCB20	2	99 - 125	F 5'-AAA TGT GTT TAA GAT TCC ATA CAG TG-3' R 5'-GGA AAA CCC CCA TAT ATA CCT ATA C-3'
CSRD247	14	236 - 244	F 5'-GGA CTT GCC AGA ACT CTG CAA T-3' R 5'-CAC TGT GGT TTG TAT TCA GG-3'
ILST087	28	145 - 165	F 5'-AGC AGA CAT GAT GAC TCA GC-3' R 5'-CTG CCT CTT TTC TTG AGA GC-3'
ILST002	14	118 - 127	F 5'-TCT ATA CAC ATG TGC TGT GC-3' R 5'-CTT AGG GGT GAA GTG ACA CG-3'
RM004	15	138 - 146	F 5'-CAG CAA AAT ATC AGC AAA CCT-3' R 5'-CCA CCT GGG AAG GCC TTT A-3'
INRA63	18	174 - 190	F 5'-ATT TGC ACA AGC TAA ATC TAA CC-3' R 5'-AAA CCA CAG AAA TGC TTG GAA G-3'
INRA006	3	109 - 123	F 5'-AGG AAT ATC TGT ATC AAC CTC AGT C-3' R 5'-CTG AGC TGG GGT GGG AGC TAT AAA TA-3'
MAF65	15	117 - 127	F 5'-AAA GGC CAG AGT ATG CAA TTA GGA G-3' R 5'-CCA CTC CTC TGA GAA TAT AAC ATG-3'

Microsatellite loci amplification and genotyping

Selective amplification of different microsatellite markers was performed using the thermocycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). PCR reagents were synthesized by Fermentas Life Sciences (Opelstrasse, Germany). The PCR mixture contained 50 ng genomic DNA, 0.6 µM of each primer, 0.2 mM dNTPs mixture, 3 mM Magnesium chloride and 1.0 unit of Taq DNA polymerase in a final reaction volume of 50 µl. PCRs were performed in a programmable thermalcycler with the following protocol: 94°C for 5 minutes; followed by 33 cycles of 94°C for 30 seconds, desired primer annealing temperatures for 45 seconds and 72°C for 1 minute; with a final extension step of 72°C for 10 minutes. Amplicons of the different microsatellite markers was confirmed by running the PCR products on 1% agarose gel and visualizing under UV rays using gel documentation system (Major Science CCD Image System, Saratoga, CA95070, USA).

Statistical Analysis

The data were collected by Genescan™ analysis software (Version 3.1, Applied Biosystems). The allele sizes were determined with the Genotyper™ software (Version 2.0, Applied Biosystems).

The program POPGENE (Version 1.31) was used to determine the polymorphism information content (PIC), the allele frequencies, number of alleles at each locus, effective number of alleles reflecting the interrelationship among different alleles, the population average heterozygosity (H) and the inbreeding coefficient (F_{IS}).

Results and discussion

A total of 22 alleles were observed over all the 12 loci across the 87 individuals in the flock (Table 2). The mean number of alleles per locus was 1.83±0.83 and varied from 1 for BM1329, CSRD247, ILST002, RM004 and INRA63 to 3 for SRCRSP5, OARFCB20 and ILST087 with the rest being intermediate. Out of 12 markers, 7 had 2 or more alleles while the rest had only one allele (Figure 1). These results are consistent with those of Garrine et al (2010) who reported 9 alleles for Maputo population of Mozambican goats from the following 6 markers: SRCRSP8, BM1329, CSRD247, RM004, INRA006 and MAF65 out of 17 markers used. The mean PIC value of the Tswana goats across the population was 58.3% which was comparable with 65% PIC value observed in Mehsana goats (Aggarwal et al 2007). In contrast, a lower PIC value of 35% was reported by Kim et al (2002) for Korean goats. Since the genetic markers exhibited a PIC value more than 50% in this indigenous Tswana flock, this is considered as informative (Botstein et al 1980) in genetic population analysis.

The mean effective number of alleles in Tswana goats at BCA farm was 1.28±0.38 across all the 12 microsatellite loci (Table 2). This was low compared to the 7.7 alleles for the Kalahari Red goat breed from South Africa (Kotze et al 2004), the 5.58 alleles for Mozambique goat breed of Tete and 6.94 alleles for the Pafuri breed (Garrine et al 2010). Generally, the mean number of alleles is highly dependent on the sample size and the number of sires used in a breeding program because of the presence of unique alleles in populations which occur at very low frequencies. The lower observed and effective number of alleles in Tswana goats at BCA farm was as a result of using very few sires i.e. 3-5 per year for 16 years of the almost closed breeding program and also directional selection for parasite resistance/tolerance coupled with increased productivity (Nsoso et al 2001) hence possibly accumulated inbreeding. This is consistent with Pandey et al (2006) who reported that inbreeding in a population is likely to be a manifestation of diminished population size and confinement to a small breeding territory, coupled with insufficient number of breeding males in the breeding region.

The overall inbreeding coefficient for the whole goat population at BCA farm was 0.12±0.16 which represented an average increase in the number of homozygous loci or conversely an average decrease in the proportion of heterozygous loci of 12% in BCA goat population (Table 2). The overall F_{IS} values per locus ranged from -0.2340 (INRA006) indicating low levels of inbreeding at that marker locus to 0.8772 (MCM527) depicting high levels of inbreeding ($F_{IS} > 0$) with respect to that marker locus with others being intermediate (Table 2). This inbreeding coefficient is comparable to that of 10% reported for 45 rare breeds of 15 European and Middle Eastern countries (Cañon et al 2006) and 7.6 to 10.5% reported by Hoda et al (2011) in six local Albanian goat breeds. The 12% inbreeding coefficient reported for the BCA goat population could be due to the small population size, closed breeding system and limited number of breeding males used, which is consistent with the findings of Pandey et al (2006) who reported similar phenomena. Significant heterozygote deficiency has also been reported in Marwari goats (F_{IS} = 0.264) by Kumar et al (2005) and in Mehsana goats (F_{IS} = 0.156) by Aggarwal et al (2007) who attributed this phenomenon to the few bucks that were used for breeding purposes in closed populations in these studies.

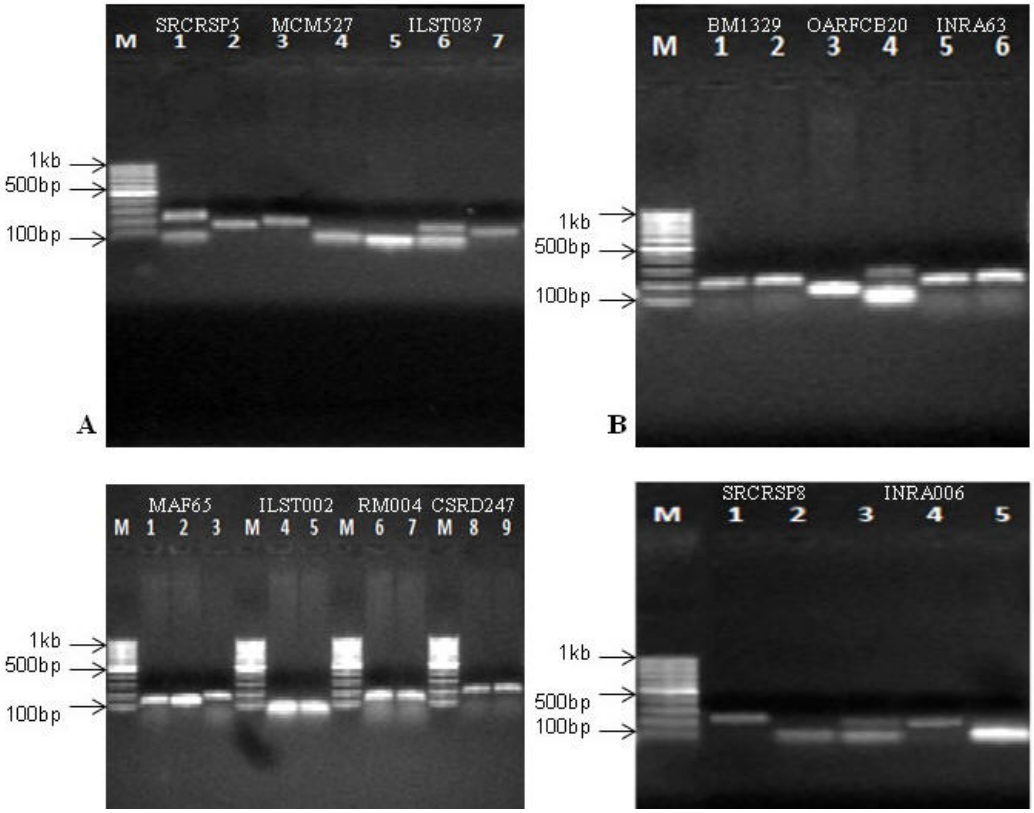

Figure 1: An example of PCR genomic DNA products from 12 microsatellite loci amplified from one of the 87 indigenous Tswana goat of Botswana College of Agriculture in South-eastern Botswana. **A** - Lanes 1 and 2 (SRCRSP5); Lanes 3 and 4 (MCM527) and Lanes 5 – 7 (ILST087). **B** Lanes 1 and 2 (BM1329); Lanes 3 and 4 (OARFCB20) and Lanes 5 and 6 (INRA63). **C** – Lanes 1 – 3 (MAF65); Lanes 4 and 5 (ILST002); Lanes 6 and 7 (RM004) and Lanes 8 and 9 (CSRD247). **D** - Lanes 1 and 2 (SRCRSP8) and Lanes 3 - 5 (INRA006). Lane M (O'GeneRuler™ 100 bp DNA ladder - Fermentas).

Four out of the 12 loci i.e. SRCRSP5, MCM527, ILST087 and INRA006 differed significantly from the Hardy-Weinberg equilibrium (HWE) indicating that these loci have been subjected to systematic selection and dispersive forces such as genetic drift and inbreeding as there were some significant increase and decrease between both observed and expected number of genotypes at those marker loci (Table 2). Five markers namely; BM1329, CSRD247, ILST002, RM004 and INRA63 were fixed indicating no genetic variation and three markers i.e. SRCRSP8, OARFCB20 and MAF65 were in Hardy-Weinberg equilibrium (P > 0.05) indicating random mating at the three marker loci. These trends are generally consistent with the findings of Thilagam et al (2006) on Kanniadu goats of India that revealed a deviation of 80% of the loci (16 out of 20) from the Hardy-Weinberg equilibrium. The possible reasons for the deviations given by Thilagam et al (2006) were existence of "null" alleles, high mutation rate and size homoplasy of microsatellite loci, besides the small study population. These reasons can also apply to the flock under study because some of the marker loci observed in the current study could be due to small and almost a closed breeding population of goats at BCA farm hence random genetic drift, incidences of inbreeding and selective breeding for parasite resistance/tolerance and increased productivity over the years (Nsoso et al 2001). According to Aminafshar et al (2008), deviations from Hardy-Weinberg equilibrium could also be due to a variety of causes

including: excess of heterozygote individuals than homozygote individuals, migration, high mutation rate at microsatellite loci and artificial selection.

Across all loci, the mean observed and expected heterozygosity values were 0.12±0.16 and 0.16±0.20, respectvely (Table 2). The observed average heterozygosity was significantly lower than expected hence being consistent with the effects of inbreeding and selective breeding in small and closed population, indicating low genetic variation among the goat population at BCA farm. In Namibia, Els et al (2004) reported average heterozygosity of 71% in the Kavango goat population and average heterozygosity of 57% in the Caprivi goat population. The BCA goat population also had very low levels of heterozygosity when compared to Asian goat populations whose heterozygosity values ranged from 31 to 48% using a panel of 25 microsatellite markers (Barker et al 2001). Visser et al (2004) in South Africa observed heterozygosity values of 63 to 69% in Boer goats population. Average heterozygosity is an appropriate measure of genetic variability within a population because genetic diversity can be measured as the amount of actual or potential heterozygosity (Ramamoorthi et al 2009). The mean allele number per locus and the average heterozygosity are normally used as estimators to evaluate genetic characteristics and diversity. The indigenous Tswana goats at BCA farm exhibited consistently low levels of allelic diversity (1.83±0.83) and heterozygosity (0.16±0.20) which is attributed to its almost closed breeding history and selection for improved parasitic resistance/tolerance and increased productivity (Nsoso et al 2001) hence that strategy led to the similarities between the animals.

Table 2: Number of alleles, heterozygosity and inbreeding estimate (*F*is) from 12 microsatellite loci and mean estimate of different parameters for 87 indigenous Tswana goat of Botswana College of Agriculture, South-eastern Botswana

Locus	Number of alleles		Heterozygosity		Nei**	Shannon index	F_{IS}	*P* value
	Observed (n_a)	Effective (n_e)	Observed (H_o)	Expected (H_e)				
SRCRSP5	3	1.889	0.402	0.473	0.470	0.733	0.145	0.000001*
SRCRSP8	2	1.084	0.081	0.078	0.077	0.169	-0.042	0.718128
MCM527	2	1.880	0.058	0.471	0.468	0.661	0.877	0.000000*
BM1329	1	1.000	0.000	0.000	0.000	0.000	****	****
OARFCB20	3	1.084	0.081	0.078	0.078	0.185	-0.037	0.987969
CSRD247	1	1.000	0.000	0.000	0.000	0.000	****	****
ILST087	3	1.850	0.379	0.462	0.460	0.803	0.175	0.000467*
ILST002	1	1.000	0.000	0.000	0.000	0.000	****	****
RM004	1	1.000	0.000	0.000	0.000	0.000	****	****
INRA63	1	1.000	0.000	0.000	0.000	0.000	****	****
INRA006	2	1.444	0.379	0.309	0.307	0.486	-0.234	0.031961*
MAF65	2	1.071	0.069	0.067	0.067	0.150	-0.036	0.761701
Mean	1.833	1.275	0.121	0.162	0.161	0.266		
St. Dev.	0.835	0.381	0.164	0.204	0.203	0.315		

**Indicate highly significant deviation from Hardy-Weinberg Equilibrium (P<0.05)*
*** Nei's (1973) expected heterozygosity*
***** No value for F_{IS} because of fixed alleles (Monomorphic locus)*

The goat population in this study has been subjected to pressures of selection for parasite resistance/tolerance and increased productivity for a period of 16 years (Nsoso et al 2001) which could have eliminated some alleles at some marker loci and fixed the alleles observed at the monomorphic marker loci. These monomorphic markers could be linked to genes responsible for parasitic resistance as revealed in the study by Beh et al (2002) where the genome scans have revealed quantitative trait loci (QTLs) for parasite resistance on the same chromosomes where the monomorphic microsatellite markers observed in this study are located. This implies that the monomorphic microsatellite markers might be in linkage disequilibrium with favorable alleles for parasite resistance. Beh et al (2002) reported that a QTL for parasite resistance and genes with significant influence on faecal egg count (FEC) have been mapped to chromosome 3 where the monomorphic INRA 006 marker used in this study has also been mapped. QTLs for Nematodirus have also been mapped to chromosome 6 according to Beh et al (2002) where the monomorphic BM1329 marker used in this study has also been mapped. A QTL for Nematodirus has also been mapped to chromosome 14 where the monomorphic CSRD247 and ILST002 markers used in this study were mapped. Marshall et al (2005) reported several significant QTL for *H. contortus* FEC in sheep from the Golden Ram flock within which a major gene for parasitic resistance is believed to be segregating.

Conclusions

- This is the first molecular evaluation study on the indigenous Tswana goats.
- The indigenous Tswana goat population exhibited some degree of inbreeding which manifested as a decrease in the total number of heterozygous loci and a concomitant increase in the number of homozygous loci.
- The decrease in the total number of heterozygous loci and some degree of inbreeding may be a result of directional selection for parasite resistance/tolerance and increased productivity, which the goat flock was subjected to for the past 16 years.
- Further studies should be carried out using more microsatellite markers and covering the whole country to fully characterize and identify useful genes and genotypes within the indigenous Tswana goat breed if it is to better benefit the resource poor farmers who are farming with it.

Acknowledgements

The authors wish to thank the Department of Animal Science and Production, Botswana College of Agriculture for their financial support for this study. The authors are grateful to the staff at the Botswana College of Agriculture Farm for their excellent assistance in sampling the flock.

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Received 24 November 2012; Accepted 15 January 2013; Published 5 February 2013

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