

## Assessment of genetic variation in Bapedi sheep using microsatellite markers

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### Abstract

This study was conducted to assess genetic variation in Bapedi sheep using 14 microsatellite markers. Blood samples were collected from 174 unrelated Bapedi sheep on six farms in various districts of Limpopo and from the Agricultural Research Council Animal Production Institute (ARC-API) in Gauteng. Genotypes from other South African indigenous sheep, namely Zulu (N = 14), Damara (N = 11), Dorper (N = 8), and Namaqua (N = 11), were included to represent reference populations. The effective number of alleles averaged 5.6 for across the Bapedi flocks and was 4.9 for the reference breeds. Among the Bapedi flocks, the observed heterozygosity ( $H_o$ ) ranged from  $0.56 \pm 0.05$  to  $0.69 \pm 0.03$  and expected heterozygosity ( $H_e$ ) values were between  $0.75 \pm 0.04$  and  $0.88 \pm 0.01$ . Thus, there is considerable genetic diversity within the Bapedi sheep populations. However, the fixation index was high, indicating the possibility of inbreeding becoming a problem for these flocks. A neighbour-joining tree was constructed from the estimates of Nei's genetic distances among flocks. The presence of Bapedi sheep flocks on all of the main branches of the tree along with one of the reference breeds suggests the present-day Bapedi is not an entirely distinct breed and that there are genetic differences between flocks of these South African indigenous sheep. Sustainable breeding and conservation programmes are needed to control inbreeding and to foreclose possible genetic dilution of Bapedi sheep.

**Keywords:** genetic diversity, germplasm conservation, inbreeding, indigenous sheep

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### Introduction

The Bapedi is an indigenous breed of South African sheep that originated from the semi-arid regions of Limpopo. Indigenous sheep are a crucial limited resource for farmers, as over time they have adapted to harsh environmental conditions and have overcome the challenges of drought, lack of good quality feed, and exposure to disease and gastrointestinal nematodes (Kunene *et al.*, 2014). South Africa has the opportunity to better utilize its indigenous small ruminants that are well adapted to harsh environmental conditions and address these challenges. However, most of the indigenous sheep breeds have been neglected as classical 'pure breeds'. Indigenous sheep were perceived to be inferior and were haphazardly mated before their documented characterization. The Food and Agricultural Organization (FAO, 2007) reported that about 30% of indigenous genetic resources are at risk of becoming extinct. Crossbreeding and inbreeding of indigenous sheep have resulted in the decline of locally adapted low-input breeds (Kunene *et al.*, 2010). Johannesburg Zoo and other government institutions are involved in the conservation of Bapedi sheep, with the aim of increasing their numbers. However, the prerequisite for starting any conservation plan is genetic evaluation of the existing population structure and variation within the breed, as well as its differentiation from other breeds (Tariq *et al.*, 2012; Qwabe, *et al.*, 2013). Bapedi sheep are categorized as a Nguni breed type along with the Zulu and Swazi. Surprisingly, Buduram (2004) found Bapedi sheep were genetically distant from

other Nguni breeds, and Hlophe (2011) found that the Bapedi sheep clustered with the Dorper breed. These findings lead to questions of the genetic constitution of Bapedi sheep, and there is limited information from which to address them.

The evolution in genomic technologies has produced many tools, including genetic markers, with which to characterize a breed (Nguluma *et al.*, 2018) and its relationships with other breeds. Microsatellites are amenable to and widely used for population genetic studies (Hanotte & Jianlin 2006; FAO, 2011; Kunene *et al.*, 2014). The goal of this study was to assess genetic variation within Bapedi sheep flocks and to document the degree to which Bapedi sheep constituted a unique genetic resource.

## Materials and Methods

All procedures in the study were performed in accordance with the ethical standards of the Agricultural Research Council (ARC) (reference APIEC17-13). A total of 174 blood samples were collected from unrelated individuals on seven Bapedi sheep farms (15–30 samples/farm). Blood samples from the Zulu and Namaqua sheep were collected from the ARC-API flocks. Blood samples for the Damara and Dorper sheep were collected from the flocks of breeders in Limpopo.

Whole blood was collected from the jugular vein into 6 mL vacutainer tubes that contained ethylenediamine tetraacetic acid (EDTA) using an 18-gauge needle. These samples were stored at 5 °C immediately after collection. Samples were then transported to the laboratory, aliquoted into 2 mL cryotubes, and stored at -20 °C until DNA was isolated. The DNA isolation was done with a High Pure PCR Template Preparation Kit (Roche, Indiana, USA) following the manufacturer's protocol. The isolated DNA was assessed for concentration and quality, using a spectrophotometer (Nano-drop 2000; Thermo Fisher Scientific, Waltham, MA, USA), at 260/280 absorbance ratio and further visualized by electrophoresis on 0.8% agarose gel. The eluted DNA was stored at -20 °C.

The DNA samples were assessed using 14 microsatellites selected from the ISAG–FAO recommended microsatellite markers (FAO, 2011) for diversity studies. Microsatellite markers were selected based on levels of polymorphism, including the number of alleles per locus and genome coverage (Buduram, 2004). The markers used in this study were *HSC*, *OARFCB20*, *OARFCB304*, *CSRD2115*, *THRA*, *MCM527*, *CSRD2111*, *ILSTS39*, *OARFCB128*, *CSRD247*, *BMS1967*, *INRA63*, *MAF65*, and *TCRBV6*.

For PCR amplification, a reaction mixture of 7.5 µL was used, consisting of 2.5 µL of the DNA, 3.225 µL deionized water, 0.75 µL buffer, 0.15 µL of the dNTPs, 0.375 µL MgCl<sub>2</sub>, 0.2 µL Taq and 0.3 µL primers. The amplification was performed with a Perkin Elmer Gene Amp PCR 9700 thermocycler (Perkin Elmer Corp., Midrand, South Africa). The amplification included an initial denaturation step at 95° for 5 minutes, followed by 35 cycles between 94 °C for 45 seconds and 59 °C for one minute, with a final extension step at 72 °C for 10 minutes after the last PCR cycle. An ovine DNA sample was run concurrently with each PCR for accuracy, and to avoid errors, since it is of known size and labelling.

To reduce the possibility of bias, animals with three or more loci with undetermined genotypes were removed from the data and thus not considered further in any of the analyses. The remaining data were analysed using several procedures in the GenAIEx add-in for Excel (Microsoft Corp., Redmond, Washington, USA) software (Peakall & Smouse, 2006, 2012). The neighbour-joining tree was constructed using MEGA7 (Kumar *et al.*, 2016).

## Results and Discussion

All 14 loci that were used in this study were found to be polymorphic, with a mean number of 10.5 alleles per locus when pooled across the 11 populations (Table 1). Sheep from Mara Research Station Bapedi flock had the fewest alleles per locus and the Bapedi sheep from the ARC flock had the greatest number. Among the flocks of Bapedi sheep, the number of effective alleles per locus was also lowest in Mara Research Station flock and highest for the flock at Towoomba Pasture Research Station. The expected frequency of heterozygotes was greater than the observed frequency of heterozygotes in all of the Bapedi sheep populations. Observed heterozygosity was least for Bapedi sheep in the Sekhukhune Communal Farm and greatest in the flock kept at Towoomba Pasture Research Station. Expected heterozygosity was least for the flock at Polokwane Commercial Farm and greatest in Towoomba Pasture Research Station flock. The fixation index reflects as significant excess of homozygotes for all of the Bapedi flocks.

**Table 1** Descriptive statistics for genotypes of seven flocks of Bapedi sheep and four reference populations

Flock	N	NA	Ne	I	Ho	He	F
TWOO	12.2	12.6 ± 0.5	7.1 ± 0.5	2.2 ± 0.06	0.69 ± 0.03	0.88 ± 0.01	0.18 ± 0.03
MOPA	15.5	13.8 ± 0.9	7.5 ± 0.8	2.2 ± 0.09	0.66 ± 0.04	0.87 ± 0.02	0.22 ± 0.05
MADZ	11.3	9.3 ± 0.7	5.9 ± 0.5	1.9 ± 0.09	0.66 ± 0.04	0.85 ± 0.02	0.18 ± 0.04
SEKH	40.5	14.4 ± 1.1	5.0 ± 0.4	2.0 ± 0.06	0.57 ± 0.03	0.80 ± 0.02	0.29 ± 0.03
POLO	13.3	6.9 ± 0.5	4.3 ± 0.5	1.6 ± 0.10	0.56 ± 0.05	0.75 ± 0.04	0.21 ± 0.07
MARA	11.8	7.2 ± 0.4	4.1 ± 0.3	1.6 ± 0.08	0.60 ± 0.05	0.76 ± 0.03	0.19 ± 0.06
ZULU	13.8	13.9 ± 1.0	5.3 ± 0.7	1.8 ± 0.13	0.71 ± 0.06	0.80 ± 0.04	0.06 ± 0.08
ARC	35.0	15.8 ± 1.6	5.6 ± 0.6	2.0 ± 0.13	0.57 ± 0.03	0.80 ± 0.03	0.26 ± 0.05
DAMA	10.4	8.4 ± 0.6	5.0 ± 0.4	1.8 ± 0.08	0.71 ± 0.04	0.82 ± 0.02	0.10 ± 0.05
DORP	7.7	8.6 ± 0.4	6.2 ± 0.4	2.0 ± 0.05	0.65 ± 0.04	0.89 ± 0.01	0.21 ± 0.05
NAMA	10.7	4.4 ± 0.4	2.9 ± 0.2	1.2 ± 0.09	0.61 ± 0.05	0.66 ± 0.03	0.03 ± 0.07

TWOO: Towoomba Pasture Research Station Bapedi; MOPA: Mopani Commercial Farm Bapedi; MADZ: Madzivhandila Agricultural College Bapedi; SEKH: Sekhukhune Communal Farm Bapedi; POLO: Polokwane Commercial Farm Bapedi, MARA: Mara Research Station Bapedi; ZULU: Zulu, ARC: Agricultural Research Council, Animal Production Institute Bapedi, DAMA: Damara, DORP: Dorper, NAMA: Namaqua

N: sample size; NA: mean number of alleles per locus; Ne: effective number of alleles per locus, I: information index; Ho: observed heterozygosity; He; unbiased expected heterozygosity; F: fixation index

The reference breeds had a similar number of effective alleles per locus to the Bapedi sheep, with the exception of the Namaqua, which had fewer. Dorper had a substantially greater level of expected heterozygosity than observed, resulting in a fixation index that was similar to that of the Bapedi sheep flocks. However, the Zulu, Damara and Namaqua sheep had fixation indices that were not greater than zero.

Table 2 shows the analysis of molecular variance for the seven Bapedi sheep populations and four reference breeds in order to partition the genetic variation to among populations, among animals within populations and within animals. These results indicate that 69% of genetic variation was found to occur within the animals, followed by 26% among individuals within populations. Only 4% of the genetic variation arose from differences among the populations.

**Table 2** Analysis of molecular variance for seven flocks of Bapedi sheep and four reference populations

Source of variation	Degrees of freedom	Sums of squares	Means squares	Variance components	Percentage variation
Among populations	10	156.8	15.68	0.250	4.0
Among individuals	180	1317.8	7.32	1.585	26.0
Within individuals	191	793.0	4.15	4.152	69.0
Total	381	2267.6		5.986	100.0

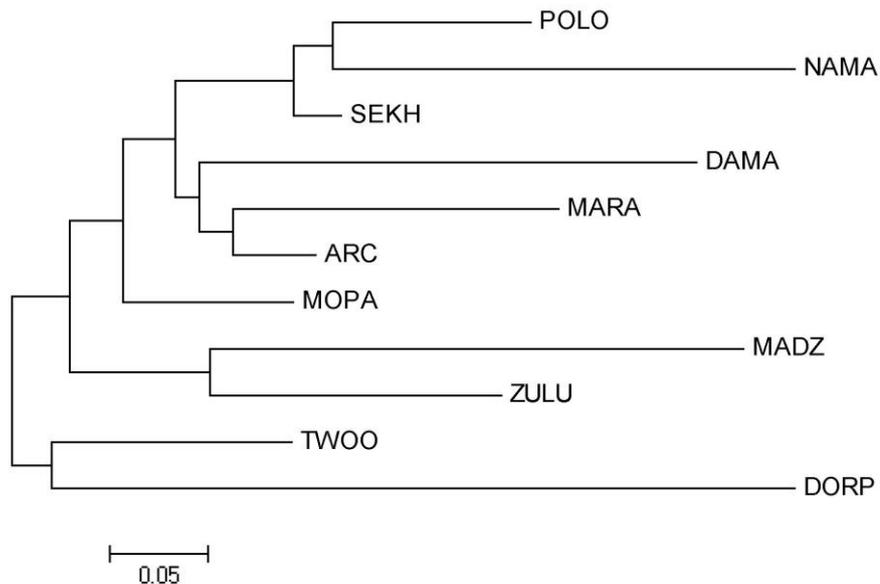
Principal coordinates analysis was used to further examine possible genetic differentiation of the Bapedi sheep and four reference populations. The percentages of variation that were explained by the first three principal coordinates were 36.3, 18.7, and 14.8, respectively. Only the first principal coordinate had an eigenvalue greater than 1.0. The Dorper and Namaqua breeds were at the opposite extremes of the first principal coordinate, whereas the Zulu sheep and Polokwane Commercial Farm flock were at opposite extremes of the second principal coordinate. The Bapedi flocks fell within these extremes.

Table 3 shows the Wright's  $F_{st}$  and Nei's genetic distances between populations. Figure 1 provides a graphic of the neighbour-joining tree illustrating Nei's genetic distances among the Bapedi populations and the reference breeds.

**Table 3** Nei's genetic distances (below diagonal) and Wright's  $F_{st}$  between seven flocks of Bapedi sheep and four reference populations

Flock	TWOO	MOPA	MADZ	SEKH	POLO	MARA	ZULU	ARC	DAMA	DORP	NAMA
TWOO		0.022	0.039	0.029	0.046	0.046	0.046	0.030	0.047	0.036	0.079
MOPA	0.286		0.035	0.021	0.038	0.039	0.041	0.021	0.036	0.037	0.059
MADZ	0.491	0.414		0.045	0.071	0.061	0.044	0.043	0.063	0.051	0.099
SEKH	0.307	0.199	0.458		0.027	0.043	0.037	0.020	0.038	0.050	0.049
POLO	0.369	0.285	0.652	0.168		0.046	0.067	0.034	0.060	0.072	0.065
MARA	0.391	0.307	0.561	0.319	0.292		0.061	0.031	0.054	0.072	0.085
ZULU	0.488	0.412	0.420	0.306	0.516	0.488		0.036	0.053	0.060	0.083
ARC	0.316	0.200	0.453	0.164	0.225	0.208	0.308		0.035	0.047	0.064
DAMA	0.563	0.389	0.737	0.349	0.481	0.436	0.480	0.313		0.052	0.081
DORP	0.501	0.507	0.666	0.588	0.729	0.746	0.682	0.538	0.606		0.105
NAMA	0.583	0.351	0.872	0.256	0.336	0.508	0.512	0.392	0.554	0.965	

TWOO: Towoomba Pasture Research Station Bapedi; MOPA: Mopani Commercial Farm Bapedi; MADZ: Madzivhandila Agricultural College Bapedi; SEKH: Sekhukhune Communal Farm Bapedi; POLO: Polokwane Commercial Farm Bapedi, MARA: Mara Research Station Bapedi; ZULU: Zulu, ARC: Agricultural Research Council, Animal Production Institute, Bapedi, DAMA: Damara, DORP: Dorper, NAMA: Namaqua



**Figure 1** Neighbour-joining tree illustrating Nei's genetic distances among the Bapedi populations and reference breeds

TWOO: Towoomba Pasture Research Station Bapedi; MOPA: Mopani Commercial Farm Bapedi; MADZ: Madzivhandila Agricultural College Bapedi; SEKH: Sekhukhune Communal Farm Bapedi; POLO: Polokwane Commercial Farm Bapedi, MARA: Mara Research Station Bapedi; ZULU: Zulu, ARC: Agricultural Research Council, Animal Production Institute Bapedi, DAMA: Damara, DORP: Dorper, NAMA: Namaqua

The Dorper and Namaqua breeds appeared most distant, as was observed in the analysis of principal coordinates. Towoomba Pasture Research Station Bapedi flock was least distant from the Dorper breed. The ARC and Mara Research Station Bapedi flocks cluster together and were more closely related to the

Damara breed than to the other Bapedi flocks. Likewise, Madzivhandila Agricultural College flock of Bapedi sheep was nearer to the Zulu breed than to other flocks of Bapedi sheep.

South Africa lacks official data from the census of indigenous sheep numbers, and hence strategies and breeding plans for their sustainable conservation. Indigenous sheep such as Bapedi are reported to be endangered (Macaskill, 2018). Thus, it is believed that attention should be given to strategic breeding to reduce the decline of indigenous sheep populations.

In the present study, the mean number of alleles (MNA) was higher than found in the Swazi, Nguni and Pedi indigenous sheep populations of South Africa (Buduram, 2004). Previously, the mean observed number of alleles (MNA) was 5 in the Namaqua breed. In the Zulu breed it was in the range of 3.73 to 6.5 (Kunene *et al.*, 2014; Selepe *et al.*, 2018). Internationally, lower MNA were observed in African fat-tailed sheep and Red Maasia-Mutara (Muigai *et al.*, 2009) and in Najdi sheep of Saudi Arabia (Musthafa *et al.*, 2012). However, the present results are more nearly similar to those for Egyptian (El-Nahas *et al.*, 2008), Spanish (Calvo *et al.*, 2011), and Bhutan sheep breeds (Dorji *et al.*, 2010). Overall, the highly polymorphic markers used in this study produced the high MNA that were observed (Buduram, 2004). However, the effective number of alleles at each locus was consistently less than the census number found in this study. The numbers of effective alleles in the Bapedi sheep populations were similar to or greater than the findings of Sodhi *et al.* (2006) and Ahmed (2014).

No private alleles were observed in any of the populations in the current study. This might be due to gene flow due to migration over the years. The presence of private alleles is an important indicator for the genetic distinctiveness of a population. However, there was no remarkable distinctiveness among the Bapedi populations.

In the current study, high levels of expected heterozygosity (that is, greater than 0.65) were observed for all seven of the Bapedi sheep populations and the four reference breeds, indicating that these populations had high levels of genetic diversity within populations. These estimates are higher than those obtained by Qwabe (2011), who observed heterozygosity ranging from 49% to 55% for Namaqua Afrikaner sheep, and the findings of Buduram (2004) for Pedi (67%), Nguni (69%), Swazi (69%), and Karakul (67%). Soma *et al.* (2012) also reported lower estimates of unbiased heterozygosity for the fat-tailed South African indigenous sheep. However, similar findings have been reported for other African indigenous breeds such as Red Maasai-Matura, Migori, and Transmara (Muigai *et al.*, 2009).

In this study, all of the Bapedi populations and the Dorper reference population manifested levels of expected heterozygosity that were greater than the levels of heterozygosity that were observed. The levels of observed heterozygosity were more similar to their expected values in the Zulu, Namaqua and Damara reference populations. In South Africa, similar frequencies of observed and expected heterozygous loci of Zulu sheep have been found (Kunene *et al.*, 2014). Similarly, Ahmed *et al.* (2014) found good agreement between the level of expected heterozygosity and that which was observed in a study of Kail sheep.

The discrepancies between observed and expected levels of heterozygosity that were observed here led to fixation index values for the Bapedi sheep populations and for the Dorper that were high. The present findings were greater than the fixation indices found in Zulu sheep populations (Kunene *et al.*, 2014) and for previous results from Namaqua and Zulu sheep (Kunene *et al.*, 2010). However, the present findings also were lower than the  $F_{is}$  values obtained for Kail (Ahmed *et al.*, 2014), Hamdani (Al-Barzinji *et al.*, 2011), and Nigerian sheep (Agaviezor *et al.*, 2013) breeds. The observations of reduced heterozygosity relative to expectation and significant positive fixation indices might result from indigenous sheep being most prevalent where breeding is not controlled allowing for the joining of close relatives.

The estimates of Nei's genetic distance (Nei, 1972, 1978) and Wright's  $F_{st}$  (Wright, 1950) and the neighbour-joining tree revealed close relationships of: the Polokwane Commercial Farm and Sekhukhune Communal Farm flocks of Bapedi sheep with the Namaqua breed. The Mara and ARC conservation flocks were closely related to each other and to the Damara breed. The Madzivhandila Agricultural College Bapedi flock was closely related to the Zulu breed; and Towoomba Pasture Research Station Bapedi flock was somewhat closely related to the Dorper breed. These findings are interesting because the Mara and ARC showed a close relationship, although these stations are far geographically distance from each other, and there have been no known exchanges of genetic material between them. The genetic proximity of these flocks with the Damara breed is consistent with the previous reports of this relationship (Muigai, 2002; Buduram, 2004). The Sekhukhune Communal Farm flock of Bapedi sheep was also established with a conservation objective (Snyman, 2014). The observation of the Towoomba flock of Bapedi sheep being at least loosely connected to Dorper can be interpreted as being supportive of the findings of Hlope (2011) and Buduram (2004).

The presence of Bapedi sheep flocks on all of the main branches of the neighbour-joining tree along with one of the reference breeds suggests the present-day Bapedi is not an entirely distinct breed and that there are genetic differences between flocks of these South African indigenous sheep. The conservation of

this breed is deemed important. It is hoped that the findings from this study will support conservation strategies. If Bapedi sheep are to be maintained as a distinct breed, its conservation needs to be undertaken with urgency. Bias in the support for livestock projects that advocate for replacing indigenous stocks with exotic ones and uncontrolled crossbreeding pose threats to conservation of indigenous livestock genetic resources and numbers (Nyamushamba *et al.*, 2017). On the other hand, breeders should evaluate breeds for their potential and incorporate them in well-planned crossbreeding programmes with other breeds for increased production, where such actions should include breeds from within and outside the region. The crossbreeding should not compromise or degrade the numbers and genetic integrity of indigenous sheep.

## Conclusion

Bapedi sheep have considerable genetic diversity. However, inbreeding is at a notable level. Conservation strategies that use genomic information in support of breed identity and in managing inbreeding are recommended. Controlled breeding may be needed to affect the desired results. However, breeders can take advantage of the diversity of Bapedi sheep that was revealed in this study and utilize it sustainably to meet present and future market and food security demands, and as insurance against the challenges posed by climate change.

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## Authors' Contributions

AM, TLN, AKN, HAO and NOM were in charge of project design and writing the manuscript. AM, KSN, RFV, and PJS were in charge of project implementation. All co-authors participated in the results, statistics and interpretation of the study. All authors read and approved the final version of this paper.

## Conflict of Interest Declaration

There are no known conflicts of interest.

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