Genetic diversity and population structure of the endangered Namaqua Afrikaner sheep

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Abstract

The Namaqua Afrikaner is an endangered sheep breed indigenous to South Africa, primarily used in smallholder farming systems. Genetic characterisation is essential for the breed's conservation and utilization. In this study a genetic characterisation was performed on 144 Namaqua Afrikaner sheep kept at the Karakul Experimental Station (KES), Carnarvon Experimental Station (CES) and a private farm Welgeluk (WGK) using 22 microsatellite markers. The mean number of alleles observed was low (3.7 for KES, 3.9 for CES, 4.2 for WGK). Expected Heterozygosity values across loci ranged between 46% for WGK, 48% for KES and 55% for CES, indicating low to moderate genetic variation. The AMOVA analyses revealed that 89.5% of the genetic variation was due to differences within populations. The population structure confirmed the differentiation of three clusters with high relationships between the CES and WGK populations. In the population structure comparison with Pedi and SA Mutton Merino sheep, limited hybridization between the Namaqua Afrikaner sheep and both of these breeds were observed. The results of this study will serve as a reference for genetic management and conservation of Namaqua Afrikaner sheep.

Keywords: Conservation, Genetic characterization, Indigenous breed, Namaqua Afrikaner, Population structure

Introduction

The Namaqua Afrikaner dates back to between 200 and 400 AD when the Khoikhoin tribe entered South Africa with their sheep flocks and is considered the oldest indigenous sheep breed in South Africa (Cloete, 1978; Ramsay *et al*., 2001). The Nama people kept the original Namaqua Afrikaner in the harsh dry areas of the north-west Cape and southern Namibia. It is a fat tailed breed adapted to the environmental conditions of the Karoo in South Africa (Snyman and Herselman, 2005). They have a long life span and their production and reproduction performance recorded under extreme conditions compare favourably with other South African breeds such as Afrinos, Dorpers and SA Mutton Merino sheep (Snyman *et al*., 1993; Snyman *et al*., 2005b). The ewes lamb throughout the year, with an average age at first lambing of 16.5 months (Snyman *et al*. 2005a).

The Namaqua Afrikaner was facing extinction when the Department of Agriculture purchased one of the last purebred flocks from Namies, Springbok in 1966. This flock has been kept at the Carnarvon Experimental Station in the Northern Cape province of South Africa for the past four decades. In 1985, 30 ewes and five rams from the Carnarvon flock were transferred to the Tarka conservation area near Hofmeyer in the Eastern Cape Province. In 1995 these animals were taken to KES to form another breeding flock of Namaqua Afrikaner in the Upington district. At that time there was approximately 2000 Namaqua Afrikaner sheep left in the country (Campbell, 1995). During 1997 a team of researchers at Grootfontein Agricultural Research Institute (GADI) reported that very few famers still owned Namaqua sheep and those who did only had a few sheep (Snyman, 2007). Namaqua Afrikaner sheep are therefore considered to be at risk of becoming endangered as the total number of breeding females is in the range between 100 and 1000 and the total number of breeding males between six and 20 (FAO, 2000). Immediate interventions are required to conserve this breed in order to preserve its genetic diversity.

In order to effectively conserve endangered indigenous breeds it is important that comprehensive knowledge of the breed's characteristics is documented (Groeneveld *et al.,* 2010). These include data on the population size, structure, geographic distribution, and production environments and within and between breed genetic diversity (Groeneveld *et al.,* 2010). This provides essential information on the breed itself and on the amount of existing genetic diversity within the breed (Hanotte and Jianlin, 2005). Several genetic characterisation studies using DNA markers have been reported for indigenous sheep breeds; these include the South African Pedi, Nguni and Damara (Buduram, 2004) and Zulu sheep (Kunene *et al.,* 2009), Kenyan Red Maasai sheep (Muigai *et al*., 2009) and Indian Ganjam sheep (Arora *et al*., 2010). Microsatellite markers have been proved to be a popular and suitable DNA marker, providing useful molecular information for the conservation of rare and endangered breeds (Toro *et al*., 2009; Boettcher *et al*., 2010).

The Namaqua Afrikaner sheep is a unique sheep breed with the potential to be utilized in small holder farming under challenging Southern African conditions. This breed is ideal for breeding intervention as proposed by Rege *et al.* (2011) which depends on the provision of appropriate genotypes to smallholders. The aim of this study was to investigate the genetic variation and population structure of the remaining purebred indigenous Namaqua Afrikaner sheep for conservation as a unique genetic resource using microsatellite markers.

Materials and Methods

Breed sampling and DNA extraction

Three Namaqua Afrikaner flocks kept at the Carnarvon Experimental Station (CES; 30°57' S, 22° 8' E), Karakul Experimental Station (KES; 28°24′S 21°16′E) and Welgeluk farm (WGK; 31°5' S, 21° 8' E), that form part of the Namaqua Afrikaner sheep conservation projects (AP10/1/1, AP10/2/1 and AP 10/3/2) of the Department of Agriculture, Forestry and Fisheries (DAFF) were used in this study. A total of 144 animals from the three flocks were included (48 from each flock, consisting of 10 rams and 38 ewes each). No full pedigrees

were available for any population; therefore to avoid close relationships samples were taken from animals in different mating groups and born in different years at the Carnarvon and Karakul experimental stations. Random sampling, within age groups, was conducted on the animals of the Welgeluk farmer.

DNA was extracted from whole blood samples using the Roche kit (Roche Diagnostic Corporation, Indianapolis, Ind.) according to the Roche protocol at the GADI laboratory. A starting volume of 450 µl was used for all extractions. Extracted DNA was quantified by subjecting it to the electrophoresis system in 1% agarose gel stained with ethidium bromide and visualised using a UV transilluminator. The DNA was stored at minus 40 °C until further analysis.

PCR amplification and genotyping

PCR and genotyping were performed at the Department of Animal and Wildlife Sciences, University of Pretoria. DNA samples were amplified with 22 microsatellite markers selected from the recommended panel for diversity by the International Society of Animal Genetics (ISAG) and under the FAO's MoDAD program (Table 1). Microsatellites markers were selected based on levels of polymorphism, allelic size ranges and amplification success. The following markers were included in the study: BM1824, INRA23, ETH225, TGLA53, MCM527, INRA005, OARCP34, OARCP49, SRCRSP8, SRCRSP9, OARVH72, CSSM47, BM827, INRA63, SRCRSP5, INRABERN192, OARHH35, OARFCB11, OARFCB48, CSRD247, BM7160 and ETH10.

PCR amplification was performed in a total volume of 15 µl, containing10x-Buffer (1.5 µl), 10mM dNTPs (0.3 µl), 25mM $MgCl_2$ (0.75 µl), 5U/µl Taq Gold (0.4 µl), 10pmol/µl primers (0.3 µl each), 5 µl extracted DNA of average 90 µg/µl concentration and deionized water (6.45 µl). The amplification was pwerformed with a Perkin-Elmer GeneAmp PCR System 9700 thermocycler with the following program: 5 minutes at 95 °C, 35 cycles of 45

seconds at 94 °C and 45 seconds at an optimized annealing temperature, 1.5 minutes at 72 °C and a final extension step at 72 °C for 10 minutes. The data was captured using GeneScan 3.1 software and data analysis was carried out using GeneMarker 1.9 (http://www.softgenetics.com/download/genemarker) to determine the fragment sizes in base pairs.

Statistical analysis

Genetic diversity is usually expressed as the frequencies of genotypes (heterozygosity) and alleles, polymorphic information content (PIC), and the observed (Hobs) and expected heterozygosity (Hexp). These estimates were calculated using MSToolkit (Park, 2001). Population subdivision estimates (fixation index F_{ST}) values per populations were obtained from Arlequin 3.1 version (Excoffier *et al*., 2005). Genepop version 4.0 (Raymond and Rousset, 1995) was used for testing deviation from Hardy Weinberg Equilibruim (HWE) at each locus over all populations. An analysis of variance was performed to indicate differentiation within and between populations using Arlequin 3.1 version (Excoffier *et al*., 2005). To detect genetic relationship among three Namaqua Afrikaner populations, the genetic distances was estimated according to Nei's unbiased genetic distance (1978).

Population structure analysis for Namaqua Afrikaner sheep was performed using Structure (Pritchard *et al*., 2000) to infer the true number of genetic populations (clusters or K) in the dataset based on a Bayesian assignment test. The model used for simulation was based on assumption of admixture ancestry and correlated allele frequencies. To estimate the true number of populations the parameter LnPr (X|K) was applied, where $K \leq 5$. Twelve independent runs for each K were used. All runs were carried out with a burn-in period of 20 000 steps followed by 100 000 MCM iterations (Pritchard *et al*., 2000). The probability value for each K was averaged over 12 runs.

Data for comparison

The Namaqua Afrikaner sheep were also compared to the Pedi (indigenous South African sheep breed) and the SA Mutton Merino. To perform a population structure analyses, an extract of genotypic data of SA Mutton Merino and Pedi sheep breeds was obtained with permission from the Agricultural Research Council (ARC), Animal genetics division. Genotypes of 35 samples of SA Mutton Merino and Pedi each were compared using nine microsatellite markers (BM1824, INRA23, ETH225, TGLA53, MCM527, SRCRSP5, INRABERN192, CSRD247 and ETH10) also used in this characterization of the Namaqua Afrikaner. This comparison was performed to assess whether gene introgression has taken place between these breeds and the Namaqua Afrikaner. A population structure analysis (Pritchard *et al*., 2000) was performed as described above using all three Namaqua populations and the SA Mutton Merino and Pedi breeds. To estimate the true number of populations the LnPr(X|K) was applied where $1 > K \leq 10$ and the probability value for each K was averaged over 12 runs. Nei's (1978) genetic distance estimate of the Namaqua Afrikaner sheep population together with the SA Mutton Merino and Pedi sheep breeds were conducted based on the microsatellite markers which corresponded to the study of Buduram (2004).

Results

Genetic diversity within the breed

Twenty of the twenty-two microsatellite markers tested could be used to study genetic diversity in the three populations. Two markers (BM7160 & ETH10) were monomorph in all three populations and were excluded from further statistical analyses. A total of 100 alleles were detected in the 144 individuals. The number of alleles observed varied from one (CSSM47, SRCRSP5) to eight (INRA23, INRA005, CSRD247) over the three populations. The mean number of alleles detected across the populations was low (5.0) over all loci (excluding monomorph markers) (Table 1). The WGK population had the highest mean number of alleles (4.2), followed by CES population (3.8) and KES population (3.6). Ten unique alleles were observed in the WGK population (frequencies between 0.01 and

0.19), five unique alleles in the KES population (frequencies between 0.01 and 0.16) and four in the CES population (0.02 to 0.13).

Table 1 Number of alleles observed (unique alleles), Observed (Hobs) and expected (HExp) heterozygosity values and PIC values

Locus	Nr of Alleles*	Hobs[a]			HExp[b]			PIC		
		CES	KES	WGK	CES	KES	WGK	CES	KES	WGK
OARCP49	6 (0)	0.77	0.73	0.75	0.71	0.66	0.72	0.64	0.60	0.67
SRCRSP08	7 (2)	0.48	0.52	0.10	0.44	0.62	0.10	0.40	0.56	0.10
CSSM47	2 (0)	0.33	0	0.10	0.43	0	0.10	0.34	0	0.09
OARCP34	6 (1)	0.57	0.72	0.48	0.61	0.72	0.46	0.56	0.66	0.43
SRCRSP05	2 (1)	0.16	0	0	0.23	0	0	0.20	0	0
BM827	5 (1)	0.67	0.48	0.52	0.61	0.63	0.45	0.51	0.55	0.36
SRCRSP09	2 (0)	0.47	0.44	0.22	0.40	0.51	0.20	0.32	0.38	0.18
INRABERN192	2 (0)	0.38	0.11	0.60	0.44	0.10	0.48	0.34	0.10	0.36
INRA005	8 (1)	0.75	0.60	0.71	0.78	0.56	0.74	0.75	0.50	0.69
INRA63	7 (2)	0.71	0.67	0.60	0.76	0.67	0.52	0.71	0.60	0.45
OARFCB11	5 (1)	0.51	0.60	0.54	0.47	0.59	0.58	0.37	0.50	0.52
CSRD247	8 (2)	0.71	0.64	0.74	0.70	0.73	0.78	0.63	0.68	0.74
OARVH72	3 (0)	0.17	0.38	0.06	0.16	0.41	0.06	0.15	0.32	0.06
MCM527	4 (1)	0.63	0.70	0.52	0.64	0.66	0.57	0.57	0.58	0.47
OARHH35	5 (1)	0.72	0.71	0.5	0.70	0.72	0.60	0.64	0.66	0.51
OARFCB48	4 (0)	0.67	0.38	0.46	0.62	0.38	0.42	0.54	0.34	0.37
ETH225	5 (2)	0.35	0	0.43	0.56	0.04	0.62	0.49	0.04	0.53
TGLA53	6 (1)	0.45	0.53	0.51	0.48	0.58	0.46	0.43	0.50	0.41
INRA23	7 (2)	0.91	0.53	0.78	0.80	0.56	0.77	0.76	0.51	0.73
BM1824	4 (1)	0.61	0.38	0.60	0.53	0.52	0.62	0.46	0.42	0.54
Average	**5**	**0.55**	**0.46**	**0.46**	**0.56**	**0.48**	**0.46**	**0.49**	**0.43**	**0.41**

*Number of unique alleles indicated in brackets
[a] Observed heterozygosity
[b] Expected heterozygosity

The polymorphic information content (PIC) for the twenty markers are presented in Table 1. Except for CSSM47 and SRCRSP05 which were monomorph in one and two populations respectively, PIC values varied between 0.04 and 0.76. Overall, the average values for PIC in the tested markers were low (0.41-0.49). Microsatellite markers were tested for deviation from HWE over all populations and the results revealed that most of the loci were in Hardy-Weinberg Equilibrium ($P > 0.05$) with only four loci (SRCRSP05, ETH225, TGLA53 and BMI824) not adhering to HWE.

The overall genetic diversity expressed as the heterozygosity in the populations was low to moderate, with the highest H values in CES (55%) compared to KES (48%) and WGK (46%), as indicated in Table 2. With regard to population subdivision, the F_{ST} value for KES (0.112) and WGK (0.113) was marginally higher than the value for CES (0.109), indicating a reduction of heterozygosity in KES and WGK populations, which supported the unbiased heterozygosity estimates (Hartl, 1988).

Table 2 Measures of genetic variation in the populations studied

Population	N	N Loci	Unbiased H[a] ±SD	Hobs ± SD	No Alleles ± SD	F_{ST}
CES	48	20	0.55 ± 0.04	0.55 ± 0.07	3.85 ± 1.42	0.109
KES	48	20	0.48 ± 0.06	0.46 ± 0.02	3.65 ± 1.53	0.112
WGK	48	20	0.46 ± 0.06	0.46 ± 0.02	4.20 ± 1.85	0.113

[a] Heterozygosity

Table 3 AMOVA analyses for the three Namaqua Afrikaner sheep populations

Source of variation	Sum of squares	Variance components	Percentage variation	P-Value
Among Populations	120.01	0.59	10.6	0.001
Within Populations	1356.40	5.00	89.4	0.001
Total	1476.41	5.59		

Measures of population differentiation

The results obtained by AMOVA analyses illustrated that 89.4% of genetic diversity occurred within populations and 10.6% between populations (Table 3).

In Table 4 the proportion of individuals of each of the populations in the three most likely clusters inferred by Structure (Pritchard *et al*., 2000) were presented. The CES population was mainly assigned to cluster 1 (86%), and KES mainly assigned to cluster 2 (89%). The WGK population was divided between a large partitioning in cluster 3 (81%) and a smaller component in cluster 1 (15%). In Figure 1 the bar plot of the proportion of membership of each individual to one or more of the three real clusters were shown. Structure analysis supported the genetic distance estimates where some levels of admixture were observed between CES and WGK populations, indicating a possible gene flow between these populations.

Table 4 Proportion of membership of the analyzed Namaqua Afrikaner sheep populations in each of the three clusters inferred in the structure program

	Inferred clusters			
Predefined populations	**1**	**2**	**3**	**n**
CES	0.86	0.08	0.07	48
KES	0.05	0.89	0.07	48
WGK	0.15	0.04	0.81	48

n = number of individuals

Population structure comparison of Namaqua Afrikaner with SA Merino and Pedi breeds

Structure results in Table 5 indicate that 98% and 95% of SAMM and Pedi populations were assigned to population-specific clusters 4 and 5, respectively. The Namaqua Afrikaner sheep populations were mostly divided between the three remaining clusters (Table 6). Approximately 61% of CES individuals were assigned to cluster 1, 80% KES individuals were assigned to cluster 2 and 68% of WGK individuals were assigned to cluster 3. These

results indicate limited genetic relationships between the Namaqua sheep populations and the SAMM or Pedi sheep.

Table 5 Proportion of membership of the analyzed Namaqua Afrikaner, Mutton Merino and Pedi sheep populations in each of the five clusters inferred in the structure program

Predefined populations	Inferred cluster					
	1	2	3	4	5	n
CES	0.609	0.002	0.326	0.058	0.005	48
KES	0.169	0.798	0.029	0.002	0.003	48
WGK	0.288	0.003	0.677	0.028	0.004	48
SAMM	0.003	0.003	0.003	0.986	0.005	35
Pedi	0.004	0.005	0.041	0.006	0.945	40

n = number of individuals

Discussion

In this study 22 microsatellite markers were used to study the genetic diversity within three Namaqua Afrikaner sheep populations. The low mean number of alleles (5.0) observed was in a similar range as that identified previously in South African indigenous sheep breeds by Buduram (2004) for Blinkhaar Ronderib sheep (4.3) and Namaqua Afrikaner (4.9) sheep using the same microsatellite markers. A higher mean number of alleles have been observed for other South African indigenous sheep breeds including the Swazi (6.5), Nguni (5.4) and Pedi (7.0) (Buduram, 2004). Slightly higher mean number of alleles was also reported by Muigai *et al.* (2009) in Kenyan fat tailed sheep including Red Maasia-Mutara (6.2), Kakamega (6.6) and Transmara (6.4) when compared to markers used in this study. All of these breeds are indigenous to various African countries and were genetically characterized using some of the similar microsatellite markers as used in this study.

The low (46%) to moderate (55%) genetic diversity observed in this study were similar to the values reported by Buduram (2004) in the Namaqua Afrikaner (49%) and Blinkhaar Ronderib Afrikaner (52%) sheep. Compared to other indigenous breeds in South

Africa, the genetic diversity in Namaqua sheep was lower than the values previously reported for Pedi (67%), Nguni (65%), Swazi (69%) and Karakul (67%) sheep breeds by Buduram (2004). These were also lower than the values reported for the other indigenous sheep breeds including Muzzafarnagri sheep (69%) by Arora and Bhati (2004); Red Maasia-Mutara (61%) and Maasia-Olmagogo (58%) by Muigai *et al*. (2009) and Ganjam sheep (68%) by Arora *et al.* (2010). The relatively low level of genetic diversity in the Namaqua Afrikaner populations was expected as they have been kept as a closed population for more than fifteen years.

Genetic differentiation expressed as F_{ST} was moderate for all three populations (Hartl, 1988). More genetic differentiation was observed within the populations (89.5%) than between the populations of Namaqua Afrikaner sheep. The fact that no known selection for or against any specific traits has been carried out in these populations, might contribute to the genetic variation observed within the populations.

The close relationship between CES and WGK, confirmed by the implied gene flow between the CES and WGK population, may be explained by the fact that the owner of WGK farm purchased some of his sheep from CES flock in 1994 and 1995. The KES population had a limited relationship with CES population despite the fact that they share common ancestors. The exchange of breeding animals between the two experimental farms was also limited as far as possible to maintain variation. Overall the population structure analysis of Namaqua sheep suggested a true genetic structure with significant differentiation among all three Namaqua Afrikaner sheep populations. It should be useful to maintain these populations as separate entities, in order to breed replacement animals for each other and increase genetic diversity.

The results of the population structure analysis showed very limited admixture between the Namaqua Afrikaner sheep populations and Pedi or SA Mutton Merino sheep, indicating limited crossbreeding. Pedi sheep was clearly distinct from the Namaqua Afrikaner

even though they are both indigenous fat tail breeds. It is therefore recommended that Pedi sheep is conserved at the small stock biological bank as a separate breed.

The genetic data from this study will form baseline data for the three populations kept at CES, KES and the WGK farm. The current level of genetic diversity within the Namaqua Afrikaner sheep populations should be increased. This could be achieved by the exchange of rams from the different genetic pools among farms that still have the same breed. It is however important that a pure bred Namaqua Afrikaner resource flock will be kept to preserve unique genetic resource (Hoffmann, 2010). Therefore crossbreeding with rams of suspect descendancy should be avoided. Follow-up studies should be performed every five years to ensure that at least the current level of genetic variation is maintained and that inbreeding is kept to a minimum. The current system of cyclic mating should be continued in order to maintain low levels of inbreeding. It is recommended that a development program for Namaqua Afrikaner sheep should be considered with a commercial incentive to increase their numbers for utilization in small holder farming in the Northern Cape province of South Africa. This breed also has the production potential to be used in Southern Africa.

Conclusion

The results of this study showed that the Namaqua Afrikaner sheep breed has a moderate level of genetic variation and that the three populations investigated can be distinguished as separate populations. The genetic information obtained from this study will form the basis for future management of the remaining pure Namaqua Afrikaner sheep. Sheep from the three farms should be sampled and included in the biological reserve bank at GADI.

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