Assessing the occurrence of hybridisation in endangered indigenous sheep

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Summary

Indiscriminate crossbreeding contributes to the loss of unique local genetic resources and the extinction of indigenous livestock species. The Namaqua Afrikaner (NAM), an indigenous fat-tailed sheep, has come under threat of extinction and is now mainly found in conservation flocks in its home country of South Africa. We surveyed three research or resource flocks described as purebred NAM to ascertain whether crossbred individuals were present in these flocks. Samples from the three locations underwent 50K SNP chip genotyping and results were compared with samples of known genetic heritage from ten important commercial and indigenous South African sheep breeds, including NAM. Results from principal component analysis, and F_{ST} and genetic distance calculations indicated individuals from the first two sampling locations were genetically similar to known NAM samples. Samples from the third location did not exhibit a distinct genetic similarity to NAM or any of the other breeds tested. This group may be the result of several generations of crossbreeding between indigenous and commercial breeds, such as the Meatmaster, with largely similar appearance. Continued conservation efforts of the NAM will need to safeguard against indiscriminate crossbreeding and the unintentional introduction of foreign genetics.

Keywords: Namaqua Afrikaner, crossbreeding, SNP chip, conservation

Introduction

Indigenous livestock breeds are increasingly coming under threat of extinction (FAO, 2007). Although indigenous breeds provide genotypes highly suited to local conditions, globalisation, the intensification of agricultural practices and the abandonment of agriculture in marginal regions, have resulted in farmers favouring non-indigenous breeds and utilising crossbreeding (Tabarlet *et al*., 2008). Indiscriminate crossbreeding, without the maintenance of pure lines does, however, pose a major threat to maintaining the unique genepool and adaptive characteristics, such as resistance to local diseases and parasites, of indigenous breeds (Amador *et al*., 2014).

The Namaqua Afrikaner (NAM) is a hardy, fat-tailed sheep indigenous to South Africa. Under challenging environmental conditions the NAM exhibits acceptable levels of production and reproduction rates that are comparable to those of commercial South African breeds (Snyman *et al.,* 1993; Schoeman *et al*., 2010). The NAM stores fat reserves in its tail which results in poor fat distribution throughout the carcass. NAM lambs have been a higher percentage of bone in retail meat cuts, lower carcass meat yield and lower carcass weight in

comparison with commercial South African breeds (Burger, 2015). These characteristics extenuated the decline of the breed and risk of extinction. The South African Department of Agriculture intervened in 1966 and has been maintaining NAM flocks to ensure the survival of the breed. Additional flocks are maintained by producers in smallholding farming systems. The breed is considered endangered with very limited breeding stock remaining (Qwabe *et al*., 2013). The aim of the current study was to investigate whether hybridisation has occurred in research and/or resource flocks that are considered to consist of purebred NAM.

Materials and Methods

Samples were obtained from influential industry or resource flocks for ten important commercial and indigenous South African sheep breeds. Samples from individuals described as purebred NAM were taken from research or resource flocks maintained at three different location (L1-L3) in the Western Cape, South Africa. Blood samples were obtained through jugular venipuncture and stored between -20°C and -80°C. Genotyping was done with the OvineSNP50 beadchip at GeneSeek (Lincoln, NE, USA). PLINK 1.9 (Purcell *et al*., 2007; Chang *et al.,* 2015) software was used to implement the following quality control measures: >0.25 GenCall score; >0.5 GenTrain score; >0.01 MAF; >0.95 call rate, and a sample call rate >0.95. A principal component analysis (PCA) was implemented in adegenet (Jombart & Ahmed, 2011) and visualised with the ggplot2 package (Wickham, 2009) in R (R Core team, 2013). A subset of individuals from indigenous breeds (Damara, NAM, Pedi) or breeds with indigenous ancestry (Dorper, White Dorper, Meatmaster) were selected for further comparisons with the 46 samples of unknown ancestry. Linkage disequilibrium pruning was implemented in PLINK by using the --indep 50 5 2 command. The remaining 164 samples and 15 415 SNPs were used in downstream analyses. Pairwise F_{ST} values were calculated using hierfstat (Goudet, 2005) implemented in adegenet and visualised using a heatmap from gplots (Warnes *et al*., 2009). A neighbourhood-joining unrooted tree based on Nei's genetic distance (Nei, 1972; Saitou & Nei, 1987) was constructed using the ape 3.4 package (Paradis *et al.,* 2004).

Results and Discussion

A total of 522 individuals from ten South African sheep breeds and 46 samples from the three sampling locations met quality control measures (Table S1). The call rate of the remaining 49 180 SNPs was 99.6%. Observed heterozygosity and F_{IS} values were comparable to international studies (Kijas *et al*., 2012; Table S2). Samples from the first and second location exhibited the highest F_{IS} values of 24% and 32%, respectively, across all breeds tested. The principal component analysis indicated ten distinct clusters corresponding to the breeds included in the study (Figure 1). Samples from the first two sampling locations (L1, L2) clustered with the NAM samples, while samples from the third location were isolated from the NAM cluster and loosely clustered near the Meatmaster and Pedi groups. The individual breeds were represented by separate clades within the neighbourhood-joining tree. Samples from L1 and L2 formed a distinctive clade separate from L3 samples. Some individuals from L1 and L2 were grouped within the NAM clade. Due to the apparent similarity between the samples from location 1 and 2 in comparison to location 3, samples from location 1 and 2 were grouped together to calculate F_{ST} values. The pairwise F_{ST} values indicated the least differentiation between the NAM and the combined samples from locations 1 and 2. In contrast, samples from

location 3 exhibited the greatest differentiation from the NAM across the breeds tested.

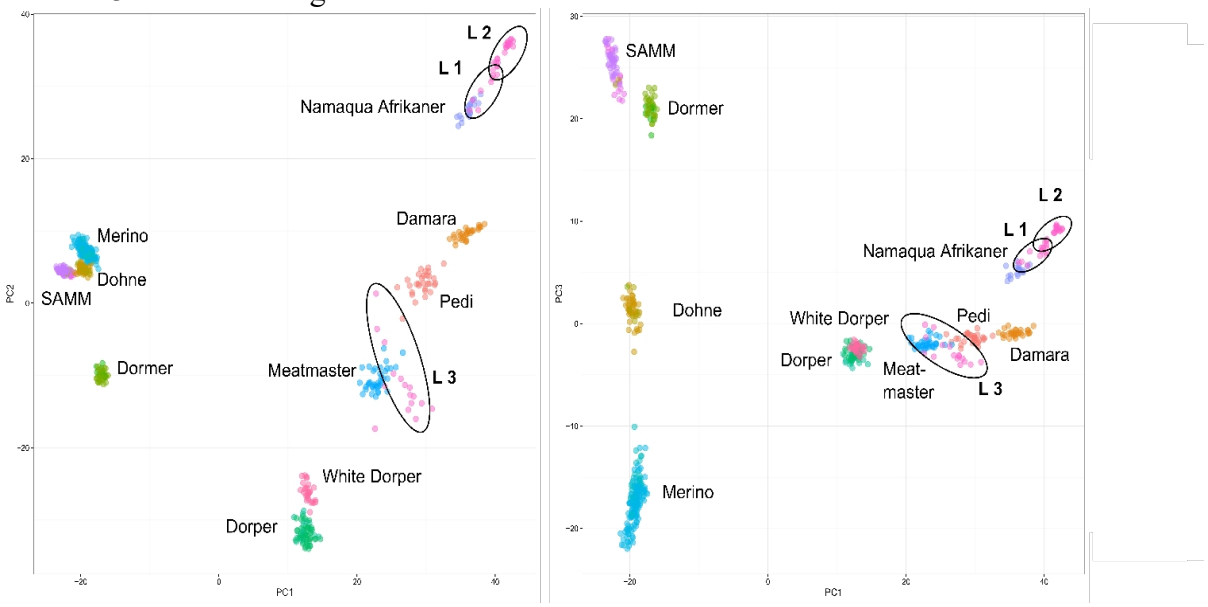

Figure 1. Principal component analysis of 50K SNP chip genotype data of South African sheep breeds and individuals from three sampling locations (L1-L3). The first three principal components explained 12.3% of the variation in the data. SAMM: South African Mutton Merino.

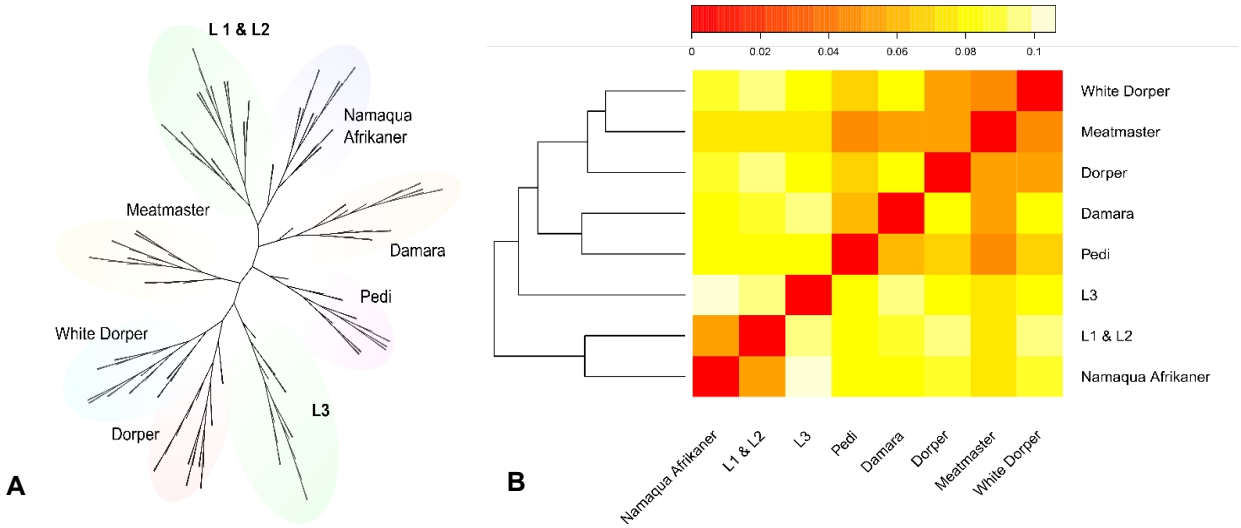

Figure 2. An unrooted neighbourhood-joining tree (A) and pairwise F_{ST} matrix with dendrogram (B) indicating population differentiation between samples of South African sheep breeds and individuals from three sampling locations (L1-L3).

Genetic links have been maintained between samples from L1, L2 and NAM flocks. Careful exchange of genetic material will be necessary to maintain viable levels of genetic diversity within these relatively small flocks. Although samples from L3 were described as NAM, this could not be confirmed by genotype results. Individuals from L3 also did not exhibit a distinct similarity to any of the other breeds tested and therefore most likely represent several generations of hybridisation between several local breeds.

Conclusions

One of the three sampling flocks, reportedly consisting of purebred NAM sheep, were comprised exclusively of individuals genetically distinct from NAMs and most likely representative of hybrids of other local breeds. Continued conservation efforts of the NAM will need to safeguard against indiscriminate crossbreeding and the unintentional introduction of hybrids. The development of a cost effective breed validation diagnostic tools, regulation of breeding practices, and education of sheep breeders will aid future conservation efforts.

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Supplementary information

Table S1. Breeds and sample numbers included in initial analyses.

Breed	Description	n
Damara	Indigenous	30
Dohne Merino	Locally developed, Commercial	48
Dormer	Locally developed, Commercial	40
Dorper	Indigenous influence, Locally developed, Commercial	59
Meatmaster	Indigenous influence, Locally developed, Commercial	38
Namaqua Afrikaner	Indigenous	20
Pedi	Indigenous	30
South African Merino	Locally developed, commercial	156
South African Mutton Merino	Locally developed, commercial	74
Unknown (L1-L3)	-	46
White Dorper	Indigenous influence, Locally developed, Commercial	27
	Total	**568**

Table S2. Sample numbers, observed heterozygosity and inbreeding coefficient (F_{IS}).

Breed	n	Observed heterozygosity(SD)	F_{IS}(SD)
Pedi	18	0.33(0.01)	0.07(0.03)
Damara	20	0.28(0.03)	0.20(0.08)
Dorper	20	0.33(0.01)	0.07(0.03)
Meatmaster	19	0.35(0.01)	0.01(0.02)
Namaqua Afrikaner	20	0.29(0.01)	0.19(0.02)
L1	19	0.27(0.04)	0.24(0.12)
L2	14	0.24(0.03)	0.32(0.08)
L3	13	0.30(0.04)	0.16(0.10)
White Dorper	21	0.33(0.01)	0.07(0.03)

SD: Standard deviation