# Genetic diversity and structure in indigenous Africanis dogs from southern Africa

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## 1. Introduction

Boyko et al. (2009) described complex patterns of population structure in the indigenous dogs of Africa (also known as village dogs). These authors showed that Indigenous African dogs show significant genetic distinctiveness but also contain detectable levels of admixture from established Western breeds. The indigenous dog of southern Africa, the Africanis breed, has previously been described by Gallant. Boyko et al. (2009) included Africanis from northern Namibia in their study and described it as distinct from Western breeds. Earlier, Greyling at al. (2004) identified genetic similarities between Africanis and Saluki dogs from the Middle East, that provide further evidence of Africanis as a group distinct from Western breeds. There is thus considerable consensus on the phenotypic and genotypic distinctiveness of Africanis, but the patterns of genetic diversity within this breed across southern Africa remain undescribed. Such data would provide valuable data on patterns migration in southern Africa, and the effect of later introgression from Western breeds.

The aim of this study was to describe patterns of genetic diversity in indigenous Africanis dogs from two regions of southern Africa: the south-eastern regions of South Africa; and areas of Botswana / northern Namibia. These regions may vary in having potentially differing levels of contact and admixture with Western dog breeds. The marker chosen for this analysis was microsatellite DNA regions, since these markers have proved highly informative in previous studies on genetic structure of dogs whereas mtDNA markers are not suited for the timing of events in dogs (Boyko *et al.*, 2009)

## 2. Materials and methods

# 2.1 Populations sampled

A total of 76 dogs, phenotypically classified as Africanis, were genotyped from a bigger group sampled from 10 local populations in South Africa, Botswana and Namibia. The animals were

identified and classified by J. Gallant. The animals consisted of: Nkandla village (8); Byrne area (18); Coffee Bay (7); Kazankulu (9); Caprivi (6); Okavango (10); Rundu /Kunene (5); Western Botswana (8) and Kang village (5). A further 10 dogs, mixed-breed but likely of Western origin, were also sampled in the KwaZulu-Natal Province of South Africa where most of the South African samples originated. Samples collected were either hair or buccal swabs.

The current analyses were restricted to these 86 dogs since these were successfully genotyped at all or the majority of loci studied.

## 2.2 DNA isolation and amplification

Total genomic DNA was extracted using the QIAamp DNA Mini Kit according to the manufacturer's instructions. The quality and quantity of extracted DNA was determined using 1% EtBr stained agarose gels and a Nanodrop spectrophotometer. The Canine Genotyping Kit (© Applied Biosystems), a multiplex panel with 10 microsatellite loci, was used to quantify genetic diversity. The loci included were: PEZ1, PEZ3, PEZ5, PEZ6, PEZ8, PEZ12 and PEZ20; and FHC2010, FHC2054 and FHC2079. All primers were multiplexed for PCR, using 10 μL reaction volumes and standard reaction mixtures and cycling conditions as recommended by ABI© for use with the StockMarks kit.

## 2.3 Statistical analyses

MSToolkit (Park 2001) software was used to organize genotypes, prepare input files and to determine unbiased heterozygosity (Hz – Nei 1987) and the average number of alleles per locus. Genetic diversity within populations was also calculated as allelic richness (Rs), using FSTAT (Goudet, 2001), to compensate for small and un-equal sample sizes.

To determine the true number of genetic populations in the sampled dogs and assign individuals to identified clusters, we used STRUCTURE (Pritchard et al. 2000). A model with assumption of admixture and correlated allele frequencies was used for all STRUCTURE runs, with a burn-in of 50,000 iterations followed by 100,000 MCMC iterations. Runs were repeated five times for each K value of K=1-10. STRUCTURE HARVESTER software was next used to determine the most likely value of K. The analysis was then rerun with 100,000 / 1,000,000 iterations based on the outcome of the initial analyses.

#### 3. Results and discussion

## 3.1 Genetic diversity

Levels of genetic diversity in each population, expressed as heterozygosity (Hz), average number of alleles per locus (A) and allelic richness (Rs), are presented in Table 1 below.

**Table 1.** Unbiased heterozygosity (Hz), average number of alleles per locus (A) and allelic richness (Rs) in individual Africanis populations and a mixed group with Western ancestry.

Population:	Hz:		A:		Rs:	
Nkandla	0.761	±0.030	5.100	±2.183	1.761	±0.095
Byrne	0.795	$\pm 0.037$	8.400	$\pm 3.534$	1.795	±0.116
Coffee Bay	0.699	$\pm 0.055$	4.600	$\pm 1.647$	1.699	$\pm 0.173$
Kazankulu	0.783	$\pm 0.026$	5.300	$\pm 1.494$	1.783	$\pm 0.081$
Caprivi	0.716	$\pm 0.081$	4.300	$\pm 1.337$	1.716	$\pm 0.256$
Okavango	0.801	$\pm 0.036$	6.600	$\pm 2.319$	1.801	±0.115
Rundu	0.800	±0.102	3.000	$\pm 1.054$	1.800	±0.322
Western Botswana	0.788	$\pm 0.047$	6.400	$\pm 1.897$	1.787	$\pm 0.149$
Kang	0.877	±0.024	6.400	$\pm 1.647$	1.887	$\pm 0.078$
Western breeds	0.783	$\pm 0.029$	6.200	$\pm 2.098$	5.320	±1.523

3.1.1 Based on traditional measures of genetic diversity within populations (Hz and A), levels of genetic diversity was closely comparable in all individual Africanis sub-populations. Heterozygosity ranged from 0.699 (Coffee Bay) to 0.877 (Kang), a spread that does not suggest meaningful loss of diversity in any particular population. Values for the hybrids of Western origin was intermediate to these values, with Hz=0.783.

## 3.1.2 Trends from allele diversity (A) mirrored that from Hz.

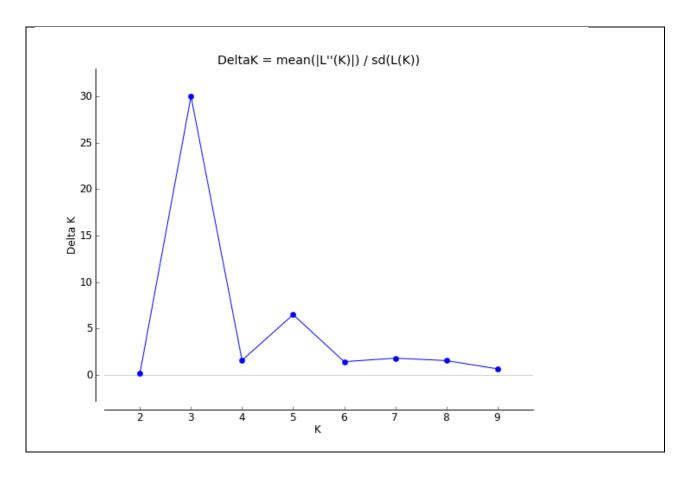
3.1.3 Values from allelic richness, a measure designed to compensate for differing sample sizes, was however much more informative. The Rs values in Africanis ranged from 1.699-1.887, but

with a much higher value of 5.320 in the hybrids of Western origin. This result has two very important implications:

- (i) The high Rs value in the Western hybrids reflects the mixed ancestry (of many domesticated breeds) very clearly, validating the value of the markers used during this study.
- (ii) The fact that none of the Africanis populations shows Rs values that even approaches that of the Western hybrids, may suggest that the populations chosen as representative of Africanis are indeed relatively pure representatives of the type, with no signature of significant introgression from Western breeds.

# 3.2 Genetic structure and uniqueness:

Results from STRUCTURE HARVESTER showed that the most likely real genetic structure of the dogs sampled contain 3 genetic clusters (see Figure 1 below).



**Figure 1.** Analyses of results from STRUCTURE, using STRUCTURE HARVESTER, showing that the 86 animals from 10 populations most likely represent 3 genetic clusters.

Based on the assumption that dogs sampled resort to 3 real genetic groups, an MCMC simulation with 1 million iterations was run, and the output of that used to assign dogs / populations probabilistically to each of the three clusters. The assignment of individuals to each of clusters 1-3 is presented in Table 2.

**Table 2.** Assignment of dog populations to each of 3 genetic clusters. Figures in bold indicates the highest proportion of any population resorting to a particular cluster.

	Cluster 1	Cluster 2	Cluster 3	n
Nkandla	0.376	0.428	0.195	8
Byrne	0.419	0.282	0.299	18
Coffee Bay	0.337	0.500	0.162	7
Kazankulu	0.398	0.455	0.146	9
Caprivi	0.179	0.131	0.690	6
Okavango	0.468	0.330	0.202	10
Rundu / Kunene	0.258	0.145	0.597	5
Western Botswana	0.274	0.307	0.418	8
Kang	0.215	0.210	0.575	5
Hybrids	0.487	0.271	0.242	10

The most notable trends from the STRUCTURE output are as follows:

- 3.2.1 There are 3 identifiable genetic clusters, though with large overlap between clusters. The latter is expected considering that these are all dogs of the same species. Nevertheless, Cluster 1 contains mostly hybrids of Western origin, Cluster 2 Africanis from South Africa and Cluster 3 Africanis of Botswana and Namibian descent.
- 3.2.2 Starting with Cluster 1: most of the hybrid dogs, i.e dogs with a known element of introgression from Western breeds, classify to this cluster, with 48.7% of such dogs falling in this cluster. For most other populations, a minority of the populations is assigned to this cluster (17-39%), except for populations from Byrne and the Okavango (41-46%). The relative scarcity of Africanis dogs in Cluster 1 provides considerable support for the hypothesis of uniqueness in most of the Africanis populations studied (though not all).

- 2.3 Of the South African Africanis dogs, those from Nkandla and Coffee Bay show high uniqueness, with 42.8% and 50% of dogs respectively falling into Cluster "2", a cluster that does not contain a significant component from the Western hybrids (only 27.1%).
- 2.4 For the remaining South African population, Byrne, the biggest single component (41.9%) classify to Cluster 3, dominated by Western breeds. Nevertheless, 58% of Byrne dogs still fall in Clusters 3 & 3, which contains predominantly Africanis dogs. This may however still suggest some introgression of Western breeds at Byrne.
- 2.5 Dogs from Botswana and Namibia show strong identity, with most dogs from these regions resorting to a unique cluster (Cluster 3). This cluster contains 69% of dogs from the Caprivi, 59.7% from Rundu/Kunene, 41.8% of dogs from Western Botswana and 57.5% from the Kang area. Only 17.9-27.4% of dogs from these areas cluster with western hybrids in Cluster 1.
- 2.6 Most dogs from the Kazankulu area (45.5%) cluster with the South African Africanis dogs from Nkandla and Coffee Bay, with another 14.6% in Cluster 3 (dominated by animals from Botswana and Namibia).
- 2.7 The largest component of the dogs sampled from the Okavango area (46.8%) group with western breeds. However, a total of 53.2% of Okavango dogs still cluster in Clusters 2 & 3, dominated by Africanis populations. As with the Byrne population, this result may however show some introgression from Western dogs in the Okavango area.

# 3. Conclusions

Three main conclusions can be made from the current results:

- 3.1 There is evidence of uniqueness in most Africanis populations, based on allelic richness as well as STRUCTURE output.
- 3.2 There appears to be two lineages in the Africanis populations, corresponding to South Africa and Botswana / Namibia. This could be correlated to early human movements into southern Africa, and should be investigated.

3.3 Some nominally Africanis populations show comparatively high levels of introgresion from Western breeds, notably the Byrne and Okavango populations.

## 4. References

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