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Genetic Diversity of the Afrikaner Cattle Breed

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ABSTRACT: The Afrikaner is one of three indigenous cattle breeds found in South Africa. To assess the level of genetic diversity in the breed, a genetic evaluation was initiated. The objective was to determine the genetic diversity of selected seed stock and commercial herds from the whole Afrikaner population and determine the genetic structure among these herds. Altogether, animals from 28 breeding herds (n=1214) and nine commercial herds (n=166) were genotyped. A total of 11 polymorphic microsatellite markers: BM1824, BM2113, SPS115, ETH3, ETH10, ETH225, INRA23, TGLA53, TGLA122, TGLA126 and TGLA127 were used in the study. Assignment methods (based on STRUCTURE software) revealed a real structure consisting of four genetic populations (K=4). An overall heterozygosity estimate of 0.568 was detected for the Afrikaner breed. Estimates of genetic diversity did not support the hypothesis of significant loss of genetic diversity in the Afrikaner herd.
Keywords: *Bos taurus africanus*; indigenous cattle; heterozygosity

Introduction

The Afrikaner cattle breed (*Bos taurus africanus*) is an indigenous South African breed of the "Sanga" type and is generally found in Southern Africa. Based on the popularity of the Afrikaner cattle breed, it was used as a dam line for crossbreeding purposes to create six other composite breeds. These breeds use the Afrikaner for its adaptive capabilities to extreme environmental conditions and disease resistance. The Afrikaner breed is well adapted to all local cattle producing areas and can be found in various geographical areas in and around Southern Africa.

The current diversity in livestock has been created by the combined forces of both natural and artificial selection. These forces can be described as mutations, adaptations, segregation, selective breeding and genetic drift (Groeneveld et al. (2010)). Genetic diversity in livestock is essential for the adaptive responses needed in ever-changing farming conditions (FAO (1998)) and ultimately to respond to the challenges created by climate change. Diversity also provides a reservoir for genetic variation to ensure that future market demands can be met through selection (FAO (1998)). Little is known about the genetic variation that still resides within the Afrikaner breed. It is important to restrict inbreeding to acceptable levels in breeds and individual herds to avoid deleterious effects on fitness traits, thereby, ensuring viability (Fernández et al. (2005)).

Microsatellite markers are ideal for evaluating the genetic diversity within- and between breeds (Barker (1999)). The markers used for genotyping cattle in South Africa have specifically been designed for European cattle breeds. Problems have been reported where parentage verification could not be established due to some Afrikaner individuals being homozygous at a large number of loci (Marx, personal communication). Therefore, it may be possible that the results generated by these standardized markers, may not be wholly appropriate for indigenous breeds.

The aims of the current study were: (i) to determine the level of genetic diversity within pure Afrikaner cattle seed stock and commercial herds, and thus identify the remaining reservoirs of heterozygosity within the breed; (ii) to determine the genetic structure of the breed and elucidate patterns of differentiation between herds; and (iii) to screen for genetic differences between seed stock and commercial herds.

Differences between seed stock and commercial Afrikaner herds can be ascribed to differences in breeding objectives. Whereas commercial breeders tend to focus on economic traits such as reproduction and production, seed stock breeders tend to also pay attention to breed standards, which are in many cases artificial standards that are not linked to production (Scholtz (2005)).

Materials and Methods

Sample collection. Genotypes for seed stock animals were generated by the Animal Production Institute at the Agricultural Research Council (ARC) and at the Unistel Medical Laboratories (UML). Samples originated from different geographical areas within South Africa, particularly in the Free State, Northwest, Limpopo Provinces and as far afield as Namibia. Altogether 1214 pure seed stock animals from 37 herds were genotyped. The seed stock samples used in the current study were specifically used for parentage verification; therefore all animals within a given herd were most likely to a degree related. Both Laboratories used the same standardized molecular markers to generate genotypes for the cattle, as recommended by the International Society of Animal Genetics (ISAG). This set consists of 11 polymorphic dinucleotide microsatellite markers to be used for parentage testing on cattle as well as tests for genetic diversity (http://www.projects.roslin.ac.uk). Eleven polymorphic microsatellite markers: BM1824, BM2113, SPS115, ETH3, ETH10, ETH225, INRA23, TGLA53, TGLA122, TGLA126 and TGLA127 were used in the study.

In addition, 190 samples were collected from pure commercial Afrikaner animals from nine different geographic areas in South Africa. Plucked hair of the tail from each individual animal was used for DNA analysis. The genetic analysis for the commercial animals was conducted at the Animal Production Institute at the ARC.

Molecular techniques. A direct Polymerase Chain Reaction (PCR) technique was used during this project. The Genetic Analyzer 3130xl (used by the ARC Laboratory) by Life Technologies and Genetic Analyzer 3500 (used by UML), were used for fragment analysis. Results were screened using GeneMapper® Software, version 4.0.A total of three to five hairs per animal were washed with distilled water and air dried for ten minutes. Hair follicles for each individual hair were then cut into 0.2 mL PCR tubes.

Statistical analysis. Genetic diversity within herds, expressed as unbiased heterozygosity (Hz) (Nei (1987)) and the mean number of alleles (A) per locus was calculated with the use of Microsatellite-Toolkit (MSToolkit) for Excel (Park (2001)). In addition, MSToolkit is useful for creating input files for several statistical programs such as ARLEQUIN (Excoffier et al. (2005)), FSTAT (Goudet (2002)), STRUCTURE (Pritchard et al. (2000)) and DISPAN (Ota (1993)). The parameters for STRUCTURE were as follows: all runs consisted of a burn-in period of 100,000 steps that were followed by 200,000 Markov Chain Monte Carlo (MCMC) iterations. Allelic richness (Rs) for each herd was determined as an additional measure of diversity, using FSTAT 2.9.3 software. FSTAT was also used to calculate unbiased F-statistics (Wright (1951)), as the mean within population inbreeding coefficient or FIS and the global inbreeding coefficient or FIT.

Results

Due to differences in genotyping, the loci ETH3 and ETH225 were excluded from further analysis. Consequently, only nine microsatellite loci, BM1824, BM2113, ETH10, INRA23, SPS115, TGLA53, TGLA122, TGLA126 and TGLA227 were used in the remaining statistical analysis.

The Bayesian assignment approach using STRUCTURE and associated Structure-Harvester software showed that the samples from 37 herds had the highest probability of representing only four genetic clusters, with K=4 (from DeltaK values). The geographical distribution of the stud and commercial herds were not a contributing factor to the assignment of populations to specific clusters. A separate cluster analysis was conducted on the commercial sector animals only and the DeltaK illustrated that these herds had no genetic sub-structure since only one cluster could be identified.

A total of 703 herd pair-wise combinations were performed. From these, the number of combinations with significant ($P<0.05$) differentiation after Bonferroni correction (424) outnumbered the combinations that showed no significant differences between herds (242).

The unbiased heterozygosity (Table 1) ranged from a low of 0.456 ± 0.085 to a high of 0.737 ± 0.043. The overall Hz average of the breed across herds was 0.568 ± 0.067 and with an average of 5.18 ± 1.76 alleles per locus. The average observed Hz ± SD for the commercial stock was 0.584 ± 0.034 and 0.594 ± 0.042 for the seed stock. The Average observed Hz for the breed was 0.591 ± 0.041. Within individual populations, the mean number of alleles (A) per locus ranged from 2.67 to 7.78. Allelic richness (Rs) estimates were therefore used for a more accurate view of levels of diversity.

Estimates of FIT and FIS were 0.017 ± 0.005 and -0.024 ± 0.005 respectively. Therefore, the total inbreeding coefficient was 1.7 %. It was assumed that FIS values of -1 were indicative of an excess of heterozygotes presumably indicating outbred populations, whereas values of 1 suggest heterozygote deficiency (Paiva et al. (2011)).

Table 1. Average unbiased heterozygosity (Hz) and average observed heterozygosity estimates for commercial- and seed stock

Status	Average unbiased Hz ± SD	Average observed Hz ± SD
Commercial herds	0.571 ± 0.067	0.584 ± 0.034
Seed stock	0.567 ± 0.071	0.594 ± 0.042

Table 2. Hierarchical distribution of overall genetic diversity in seed and commercial stock (AMOVA)

Source of variation	Variance components	Variation (%)
Among group	0.009	0.335
Among herds within groups	0.105	3.866
Within herds	2.606	95.799

In the AMOVA analysis (Table 2), virtually no variation was detected between the stud and commercial groups, with only 0.34% of variation attributed to differences between stud and commercial herds. By comparison, 3.9% of variation found was due to variation within each of these two groups. The remaining 95.8% of variation was accounted for by differences among individuals within herds.

Discussion and Conclusion

This study represents the first attempt to determine levels of genetic variation in an indigenous South African cattle breed, the Afrikaner.

This extensive use of the Afrikaner breed to develop other composite breeds and in crossbreeding caused a major decline in the numbers of pure bred Afrikaner animals being born each year. A decrease in the number of seeds stock and commercial herds followed and fewer herds meant an increase in inbreeding and a potentially decreased amount of available genetic resources for adaptation purposes.

Factors contributing to heterozygote deficiency in populations are inbreeding, null alleles, population substructure, genetic hitchhiking (Nei (1987)) and restricted gene flow (Frankham et al. (2010)). Higher inbreeding levels were expected in the Afrikaner breed; however the F-statistics (FIT and FIS) calculated for the breed demonstrated low levels of inbreeding with an excess of heterozygous individuals within individual herds as well as in the whole population.

The principle findings of this study are high genetic diversity within but low genetic distances between seed stock- and commercial herds of the Afrikaner cattle breed. The current study showed genetic variability levels within the Afrikaner cattle are higher than expected, with comparatively high heterozygosity values in both the seed stock and commercial herds even though the magnitude of variability is slightly less than literature values reported for other breeds. The study also demonstrated that there is no difference between seed stock- and commercial herds in the breed, which was unexpected. This can be seen as a positive result that can be used in future breeding programs.

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