

## A comparison of genetic diversity between South African conserved and field chicken populations using microsatellite markers

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### Abstract

The objective of the study was to determine genetic diversity within South African indigenous chicken populations and the effectiveness of the current conservation flocks in capturing the available diversity in the founder populations. Two chicken populations, Venda (VD\_C) and Ovambo (OV\_C) conservation flocks (n = 56) from the Animal Production Institute in Irene and two founder population from which these conservation flocks were sampled; Venda (VD\_F) and Ovambo (OV\_F) field populations (n = 72) were genotyped for 29 autosomal microsatellite markers. All microsatellites typed were found to be polymorphic. A total of 213 alleles were observed for all four populations. The mean number of alleles per population ranged from  $3.52 \pm 1.09$  (VD\_C) to  $6.62 \pm 3.38$  (OV\_F). Mean observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity in the conservation flocks were 0.55 and 0.57 respectively. The corresponding values for the founder population were 0.62 and 0.68. The observed within population diversity measures indicated that field populations are more diverse than conservation flocks. The Reynolds genetic distance ( $D_{\text{Reynolds}}$ ) between conservation flocks and field population observed was 0.22 between VD\_C and VD\_F and 0.09 between OV\_C and OV\_F. STRUCTURE was used to cluster individuals to  $2 \leq K \leq 5$ . The most probable clustering was found in  $K = 3$ , in which the populations were grouped into three clusters. VD\_C and OV\_C conservation flocks separated as independent clusters, while VD\_F and OV\_F field populations formed one cluster for any  $K$  value. Clustering analysis indicated a clear subdivision of the conservation flocks and field population into genetically distinct populations. The present study suggests that conservation flocks are less diverse compared to field populations.

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**Keywords:** SA chickens, genetic diversity, microsatellites, conservation strategies

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### Introduction

The South African Ovambo and Venda chicken breeds are kept at the Animal Production Institute in Irene as conservation flocks. These chickens are still reared by different communities involved in low input agriculture in isolated villages in different farming regions of South Africa. Native chickens provide food, income, and employment in the rural communities of South Africa. Numerous animal species have gone extinct or are critically threatened due to various evolutionary forces (Diamond, 1989; Pimm *et al.*, 1995; Hilton-Taylor, 2000; Pimm & Raven, 2000). The loss of native chicken species may represent a social or economic loss for human populations, especially in developing countries. For long-term conservation and development purposes, it is therefore necessary to assess the genetic diversity of South African conserved and field native chicken species to maintain both species survival and within-species genetic diversity. To date no studies have systematically determined the effectiveness of the current conservation flocks in capturing the available diversity of the founder populations. Thus, the objective of this study was to determine genetic diversity within indigenous South African chicken populations and the effectiveness of the current conservation flocks in capturing the available diversity in the founder populations.

### Materials and Methods

Two chicken populations, Venda (VD\_C) and Ovambo (OV\_C) conservation flocks (n = 56) from the Animal Production Institute in Irene and two founder population from which these conservation flocks were sampled; Venda (VD\_F) and Ovambo (OV\_F) field populations (n = 72) were used in this study. Field

populations were sampled in several villages of Limpopo Province (VD\_F chickens) and Northern Cape Province along the border post of Namibia (OV\_F chickens). Blood samples were collected from the wing vein onto FTA@Micro Card (Whatman Bio Science, UK). DNA isolation was done using a Phenol/Chloroform extraction method (Sambrook & Russell, 2001). Population genetic structure was determined using 29 autosomal microsatellite markers. These markers have been used in several chicken biodiversity studies (Cuc *et al.*, 2006; Muchadeyi *et al.*, 2007; Granevitze *et al.*, 2007; Bodzsar *et al.*, 2009). Of the 29 markers, 28 were established and used in the AVIANDIV (1998-2000) project ([http://aviandiv.tzv.fal.de/primer\\_table.html](http://aviandiv.tzv.fal.de/primer_table.html)). The marker loci correspond to the revised set of microsatellites, which were suggested by FAO-ISAG (2004) for the MoDAD project (<http://dad.fao.org/en/refer/library/guidelin/marker.pdf>). PCR was used to amplify the specific DNA fragments containing microsatellites. PCR products were generated using primers fluorescently labelled with IRD700 and IRD800 and visualized on 8% polyacrylamide gel with LICOR semi-automated DNA analyser (LICOR Biotechnology Division). Allele ladder for each locus were used to adjust allele scoring and allele size scoring was performed using RFLPscan software (Scanalytics).

Genetic variation within individual populations were estimated and compared. The observed number of alleles at each locus and the respective allele frequency were calculated using the FSTAT software, version 2.9.3 (Goudet, 2001). Mean observed and expected frequency of heterozygotes for each population, and overall, as well as  $F_{IS}$  and  $F_{IT}$  values for each locus were calculated with Microsatellite-Toolkit (Park 2001). Genetic differentiation among conservation and field populations were determined by multi-locus estimator of  $F_{ST}$  between all pairs of populations using Weir & Cockerham's (1984) estimations of Wright's (1951) fixation indices. Reynolds standard genetic distances ( $D_{Reynolds}$ ) (Reynolds *et al.*, 1983) were calculated using the PHYLIP 3.66 software package (Felsenstein, 2005). The STRUCTURE software was applied for clustering of individuals from multilocus genotypes (Pritchard *et al.*, 2000). The analysis involved an admixture model with correlated allele frequencies and no linkage. Burn-in phase for 20 000 iterations followed by 50 000 iterations for each of a user defined number of clusters ranging from  $2 \leq K \leq 5$  was performed. For each K-value, 100 repeated runs were compared, by calculating similarity coefficients (Rosenberg *et al.*, 2002), in which solutions with similarity coefficients over 95% were considered as identical. The most frequent solution of each K-value was considered the most probable clustering and was visualized using the DISTRUCT software (Rosenberg, 2004).

## Results and Discussion

All microsatellites typed were found to be polymorphic. A total of 213 alleles were observed for all four populations. The mean number of alleles (MNA) per locus, expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity and inbreeding coefficient ( $F_{IS}$ ) per population are presented in Table 1. The mean number of alleles per population ranged from  $3.52 \pm 1.09$  for VD\_C to  $6.62 \pm 3.38$  for OV\_F. Conservation flocks has less alleles per locus compared to field populations. A similar mean number of alleles ranging from 2.3 to 4.3 in South African conservation chicken lines was reported by Van Marle-Köster & Nel (2000), while the field populations displayed an allele range similar to that of other free-ranging chickens reported by Muchadeyi *et al.* (2007) in Zimbabwean, Malawian and Sudanese populations. Mean observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity for VD\_C and OV\_C in the conservation flocks were 0.55 and 0.57, while the corresponding values for the founder populations were 0.62 and 0.68. The observed within population diversity measures indicated that field populations are more diverse than conservation flocks.

**Table 1** Mean number of alleles (MNA) per locus, expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity and inbreeding coefficient ( $F_{IS}$ ) per population

Population	Sample size	$H_E \pm SD$	$H_O \pm SD$	Alleles $\pm SD$	$F_{IS}$
OV_F	42	$0.68 \pm 0.02$	$0.63 \pm 0.01$	$6.62 \pm 3.38$	0.075
VD_F	30	$0.67 \pm 0.02$	$0.61 \pm 0.02$	$5.97 \pm 3.30$	0.085
OV_C	26	$0.62 \pm 0.03$	$0.59 \pm 0.02$	$4.66 \pm 1.82$	0.041
VD_C	30	$0.51 \pm 0.03$	$0.51 \pm 0.02$	$3.52 \pm 1.09$	0.002

The mean  $F_{IT}$ ,  $F_{ST}$  and  $F_{IS}$  inbreeding coefficients and their standard errors (SE) for conservation flocks and field populations are given in Table 2. High heterozygote deficiency ( $F_{IT} \pm SE = 0.179 \pm 0.028$ ) and high level of variation ( $F_{ST} \pm SE = 0.161 \pm 0.026$ ) was observed in conservation flocks compared to field populations, with  $F_{IT} = 0.088 \pm 0.016$  and  $F_{ST} = 0.010 \pm 0.003$  observed in the field populations. This may be attributed to population structuring of closed conservation flocks caused by controlled mating and limited gene flow between flocks. Presumably the field populations are managed extensively with continuous gene flow between neighbouring flocks. Similarly, greatest heterozygote deficiency was also reported by Muchadeyi *et al.* (2007) on purebred commercial lines in comparison to the local Zimbabwe ecotypes. Granevitze *et al.* (2007) showed that genetic variation is highly dependent on population management. The  $F_{IS}$  estimate for conservation flocks was lower ( $0.021 \pm 0.020$ ) compared to the field populations ( $0.079 \pm 0.016$ ), which suggests that conservation flocks retain considerable genetic variability and low level of inbreeding. In contrast, Van Marle-Köster *et al.* (2008) reported highest level of potential inbreeding for conservation flocks.

**Table 2** Overall populations ( $F_{IT}$ ), between populations ( $F_{ST}$ ) and within population ( $F_{IS}$ ) inbreeding coefficients and their standard errors (SE)

Population	$F_{IT} \pm SE$	$F_{ST} \pm SE$	$F_{IS} \pm SE$
Field	$0.088 \pm 0.016$	$0.010 \pm 0.003$	$0.079 \pm 0.016$
Conservation	$0.179 \pm 0.028$	$0.161 \pm 0.026$	$0.021 \pm 0.020$
Overall	$0.147 \pm 0.014$	$0.096 \pm 0.012$	$0.057 \pm 0.012$

Reynolds standard genetic distances ( $D_{Reynolds}$ ) between population pairs are presented in Table 3. Genetic distance between conservation flocks and field population observed was 0.22 between VD\_C and VD\_F and 0.09 between OV\_C and OV\_F. The smallest genetic distance of 0.05 was observed between VD\_F and OV\_F field population and the largest distance of 0.23 was found between VD\_C conservation and OV\_F field. Muchadeyi *et al.* (2007) using same set of 29 markers with the present study, observed small genetic distances for the local Zimbabwe ecotypes similar to those observed between South African field populations and larger genetic distances for the purebred lines. The conservation flocks on the other hand exhibit high genetic distances similar to what was observed between purebred commercial flocks reported by Muchadeyi *et al.* (2007).

**Table 3** Reynolds standard genetic distances ( $D_{Reynolds}$ ) between populations

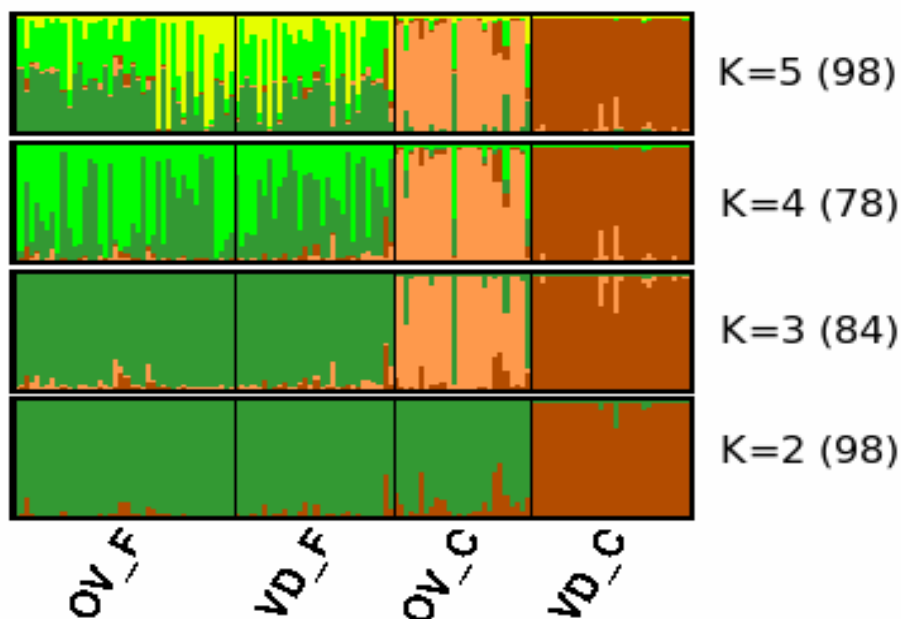
Population	OV_F	VD_F	OV_C	VD_C
OV_F	-			
VD_F	0.046	-		
OV_C	0.086	0.095	-	
VD_C	0.232	0.224	0.221	-

STRUCTURE software was applied for clustering individuals to  $2 \leq K \leq 5$  (Figure1). At the lowest  $K$ -value ( $K = 2$ ), the VD\_C conservation split from others to form its own cluster and remained separated until the highest  $K$ -value ( $K = 5$ ). The most probable clustering was found in  $K = 3$  ( $n = 84$ ), in which the populations were grouped into three clusters. VD\_C and OV\_C conservation flocks separated as independent clusters, while the VD\_F and OV\_F field populations formed one cluster for any  $K$  value. Cluster analysis

indicated a clear subdivision of the conservation flocks that are genetically distinct even from the field populations.

### Conclusion

The present study suggests that conservation flocks are less diverse compared to field populations. The STRUCTURE analysis indicated a clear subdivision of the conservation flocks and field population into genetically distinct populations. For effective management and utilization of South African indigenous chicken populations, effort should be made to conserve the unique genetic resources in the field populations.



**Figure 1** STRUCTURE clustering of four South Africa indigenous chicken populations. Numbers in parentheses indicate the number of identical solutions at 95% threshold. Keys: Conservation flock: OV\_C = Ovambo; VD\_C = Venda; Field population: OV\_F = Ovambo; VD\_F = Venda.

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