

## **RUNS OF HOMOZYGOSITY IN SWAKARA PELT PRODUCING SHEEP: IMPLICATIONS ON SUB-VITAL PERFORMANCE**

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### **ABSTRACT**

Sub-vital performance is a phenotype in which some pure white Swakara sheep die within 48 hours of birth. Postmortem of sub-vital lambs have shown underdeveloped digestive organs. It is hypothesized that due to high levels of inbreeding, Swakara sheep carry a recessive mutation that affects some of the white fleece colour subpopulation resulting in the sub-vital production performance. The genetic basis of the sub-vital effect is however unknown. The aim of this paper was to use the Ovine SNP50K data to investigate inbreeding levels and occurrence of ROH in the Swakara sheep genome in order to uncover the genetic basis of sub-vital performance. Runs of homozygosity (ROH) are long stretches of contiguous DNA fragments that are homozygous and occur due to parents transmitting similar haplotypes to their offspring through shared ancestry. Long stretches of ROH observed in SNP markers indicate probable underlying stretches of DNA inherited identical by descent from recent common ancestors. The aim of the study was to screen for extended ROH shared across animals, estimate levels of inbreeding using ROH and infer association with sub-vital performance which could indicate regions associated with the sub-vital mutation. Ninety-four Swakara individuals that belonged to the grey (n =22), black (n=15) and white vital (n = 41) and white sub-vital (n=16) were genotyped using the OvineSNP50 beadchip. Four hundred and thirty six unique ROH regions that spanned between 1001 to 6594 Kb were observed on the 25 chromosomes in 94 individuals of the four colour subpopulations. Three consensus ROH (cROH) were more prevalent in sub-vital Swakara sheep. Results suggested alternative genetic mechanisms to sub-vital performance other than was initially hypothesised that sub-vital performance was due to recessive mutations prevalent in inbred white Swakara sheep.

### **INTRODUCTION**

Swakara is a fat-tailed sheep breed that was improved through generations of selective breeding from the Karakul breed which originates from the Middle East (Campbell 2007; Soma *et al.* 2012). White pelts are preferred to other colours (brown, black and grey) on the market (Campbell 2007) because they can be dyed to any desired colour to make coats and other fashion products. Production of white pelt is however hampered by a sub-vital factor that affects some of the pure white Swakara sheep that die within 48 hours of birth. Postmortem of affected lambs have shown underdeveloped digestive organs. The pure white animals require special care that includes specialized diets and most die before reproductive maturity, even under special care. As a practice the breeders mate A-white (sub-vital pure white fleece) rams that are raised under specialized care with black ewes to produce 100% B-white and C-white progeny, which are predominantly white with black patches (and are vital). Mating of B-white X B-white (C-white) produces 25% black; 50 % B/C-white and 25% A-white (sub-vital).

It is hypothesized from the products of the different matings that the appearance of the sub-vital factor in pure white Swakara could have resulted from intensive selection leading to the accumulation of the homozygous recessive allele associated with sub-vital performance. However, there are other white Swakara sheep that do not experience sub-vital performance implying some level of independent segregation of the sub-vital loci from the coat colour loci in some

populations. An understanding of the genomic structure and diversity of the white vital, white sub-vital and the two other Swakara colour variants (black and grey) is important in determining the genetics of sub-vital performance and to aid breed improvement programmes.

Runs of homozygosity (ROH) are defined as long and continuous stretches of homozygous genotypes (Ku *et al.* 2011) that result from parents transmitting identical haplotypes to their offspring. ROH have been used as an estimate of inbreeding (Zhang *et al.* 2013). Recent studies have found associations between regions of extended homozygosity with both complex and simple gene disorders (Kijas *et al.* 2012; Suarez-Vega *et al.* 2013). Due to the small population sizes of Swakara breeding flocks and the intense selection, high levels of inbreeding and longer extended ROH could be occurring at a high frequency and also contributing to the expression of the sub-vital factor. In this study, we investigated inbreeding levels and screened the genome of Swakara sheep for the occurrence of ROH using the Ovine SNP50K data. The genes covered by ROH were investigated and inference made on associations with sub-vital performance.

## MATERIALS AND METHODS

**Animal genotyping and quality control.** Blood was collected from 96 Swakara sheep sampled from two research stations and 3 farms in Namibia (n=60), Carnavon Research Station in South Africa (n=30) and consisting of four colour sub-populations i.e. black (n=16), grey (n=22), white vital (n=35) and white sub-vital (n=17). The white-vital referred to a sub-population of white Swakara that did not experience sub-vital performance whilst the white sub-vital would die prematurely. The ovineSNP50 beadchip was used for genotyping. Quality control was done to remove SNPs where the genotype call rate was < 0.05, missing genotypes were >0.10, the minor allele frequency was < 0.05 or there was a deviation from Hardy Weinberg equilibrium ( $P < 0.001$ ). At least 80.35 % of the SNPs were left for further analysis after quality control. Two individuals, one white sub-vital and one black, were excluded from downstream analysis due to missing genotypes.

**Inbreeding and runs of homozygosity.** Inbreeding coefficients ( $F_{IS}$ ) and runs of homozygosity (ROH) were estimated from SNP genotypes using Plink v.1.7 (Purcell *et al.* 2007). In this analysis, the ROH were defined as homozygous stretches along a 1000kb sliding window with a minimum of 20 SNPs allowed in the tract, with allowance of at most 2 missing SNPs and no heterozygous SNPs. The threshold for SNP density was set at 50kb/SNP. When two SNPs within a segment were too far apart (>500 kb), the segment was split in two using the *--homozygous gap* function. Pools of overlapping segments were estimated using PLINK. The size of the consensus region was determined as well as the distribution of the consensus region in the sub-vital and normal Swakara sheep.

**Identifying potential trait association of SNPs in ROH segments.** The Golden Helix GenomeBrowse® was used to identify genes within the consensus regions of ROHs using the sheep reference genome *Ovis Aries OAR v.3.1* and *Ensembl* ([www.ensembl.org/sheep](http://www.ensembl.org/sheep)). The genomic base location of the first and last SNP of a ROH was used to denote the region of interest.

## RESULTS AND DISCUSSION

**Inbreeding levels and ROH patterns in Swakara subpopulations.** The inbreeding levels of the four Swakara subpopulations are shown in Table 1. The Grey subpopulation was the least inbred with an  $F_{IS}$  value of 0.01 whilst the black Swakara had the highest  $F_{IS}$  of 0.09. The low inbreeding levels observed in the grey subpopulation were expected since homozygous grey karakul also experience a lethal factor documented by Groenewald (1993). The black sub-population is known not to experience any sub-vitality or lethal factors and is currently used in crossbreeding with pure white Swakara as a way of managing sub-vital

performance. Four hundred and thirty-six (436) ROH were observed in 94 Swakara sheep. All sheep had at least one reported ROH (Table 1), and there were an average of about 5 ROH events reported per animal in each subpopulation.

**Chromosomal coverage of ROH in Swakara population.** The ROH length ranged from 1001Kb to 6594 Kb and covered at least 13.96% of the Swakara sheep genome. There were no ROHs observed on chromosome 23. The highest chromosomal coverage was observed on chromosome 9 that had 35 unique ROHs covering 44% of the chromosome.

**Table 1. Inbreeding ( $F_{IS}$ ) levels and the characteristics and number of ROH Swakara sheep.**

Subpopulation	$F_{IS}$	No. ind. with ROH	No. ROH	Mean $\pm$ SD ROH length (KB)	Mean $\pm$ SD NSNPs
White-vital	0.089 $\pm$ 0.063	41	214	1661.13 $\pm$ 788.92	35.01 $\pm$ 16.07
White subvital	0.078 $\pm$ 0.13	16	84	1610.18 $\pm$ 804.56	34.19 $\pm$ 16.76
Black	0.094 $\pm$ 0.075	15	72	1567.67 $\pm$ 776.72	33.28 $\pm$ 15.32
Grey	0.011 $\pm$ 0.069	22	109	1749.98 $\pm$ 922.79	37.05 $\pm$ 18.58
Overall	0.089 $\pm$ 0.063				

**Consensus (overlapping) ROH and putative function.** The 436 unique ROH grouped into 131 consensual ROH (cROH). Of these only three on chromosomes 3, 4 and 25 had a distribution biased towards sub-vital Swakara sheep (Table 2). The consensus ROH (cROH) on chromosome 4 was observed in 10 sheep six of which were from the sub-vital group. Those cROH on chromosomes 3 and 25 were observed in 2 individuals each both of which were of sub-vital performance.

**Table 2. The consensus ROH common in white sub-vital sheep and the associated genes.**

cROH	Chr	No. of individuals	Length of cROH (Kb)	No. Sub-vital sheep	Genes within region
cROH1	3	2	1201.90	2	C2orf74
cROH2	4	10	2768.36	6	DPP6;HTR5A-ASI;SHH
cROH3	25	2	1018.51	2	LRRTM3

Consensus regions cROH1 and cROH3 on chromosome 3 and 25, respectively, were observed in only in 2 white sub-vital Swakara sheep whereas cROH2 on chromosome 4 was observed in 6. Using the GenomeBrowse® tool we found that cROH3 is within a genomic region carrying the leucine-rich repeat transmembrane 3 (LRRTM3) gene. This gene family is associated with late onset of Alzheimer's disease in mice (Laakso *et al.*, 2012). The consensus region cROH2 on chromosome 4 is a 2.8MB region on which lies the DPP6, HTR5A-ASI and SHH genes. No information was found on the putative functions of HTR5A-ASI, while studies on DPP6 suggest it functions on the development of the brain and skeletal system in livestock (Buzanskas *et al.*, 2014). The mammalian SHH has been reported to encode a signaling molecule that is vital for the developmental patterning especially of the nervous system and the skeletal system (Dorus *et al.*, 2006). The trend in the observed cROH in white sub-vital Swakara sheep observed in this study suggests some candidate genes impacting on the nervous function and skeletal and brain development. The cROH2 carrying these genes was however also observed in a few (40%) of vital Swakara sheep which makes it a less likely candidate for sub-vital performance under the assumption that the sub-vital performance was a recessive mutation only experienced in white Swakara sheep. These results suggest alternative genetic mechanisms to sub-vital performance

other than was initially hypothesised that sub-vital performance was due to recessive mutations prevalent in inbred white Swakara sheep. Small sample sizes of both the vital and sub-vital sheep were a major limitation of this study. Alternative analyses such as genome-wide association studies and population stratification based analysis could be used to further investigate potential genes and the genetic mechanisms for sub-vital performance in white Swakara sheep.

## CONCLUSION

Apart from the grey subpopulation, the Swakara sheep are moderately inbred with an average inbreeding co-efficient ( $F_{IS}$ ) of about 10% percent. Our results do not support the presence of a recent recessive-lethal mutation causing the sub-vital phenotype in white Swakara sheep.

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