Genome-Wide Association Study to Investigate Shank Skin Colour of Indigenous Chickens from Limpopo and KwaZulu-Natal

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Background

South African indigenous chickens are an important source of protein, income, and play a role in cultural activities. They are highly diverse, hardy and well adapted to their local climates and rural farming environments. The phenotypic as well as genetic characterisation of the indigenous village chicken is crucial for breed definition and for formulating an inventory of agro-ecological ecotypes and informing improvement and conservation efforts.

Shank skin colour is an important adaptive trait and is also largely influenced by the farmer preferences. Generally, it is well established among producers that there is a preference for lighter coloured skin in chicken meat and in shank skin colour. For this reason, it is important to investigate the phenotypic structure as well as genomic regions that are associated with shank skin colour. This can inform improvement programs and breeding strategies for better market uptake of rural free range chickens.

Aim

The aim of this study was to investigate the genomic regions associated with the shank skin colour in South African indigenous chickens from regions of Limpopo and KwaZulu-Natal provinces.

Hypothesis

Shank skin colour is influenced by pigmentation genes environmental factors thus it acts as both a production and an adaptation trait.

Methods

Illumina 60K genotyping data of 115 village-based non-descript chickens from four District Municipalities from Limpopo (Elias Motsoaledi; n=35), Mole Mole =38; Fetakgomo =37) and KwaZulu-Natal in (Umzinyathi (n=5) were selected based on shank colour of either white (n=20), yellow (n=45), pink (n=20), black (n=30). Our approach combined genome-wide association studies (GWAS) and scans for signatures of selection at the regional level.

Figure 1: Blood sample collection from the brachial vein.

Figure 2: After DNA extraction, the DNA is genotyped using the Illumina Chicken 60K Beadchip. Figure 2 depicts the wash step of the genotyping protocol of a Chicken 60K Beadchip.

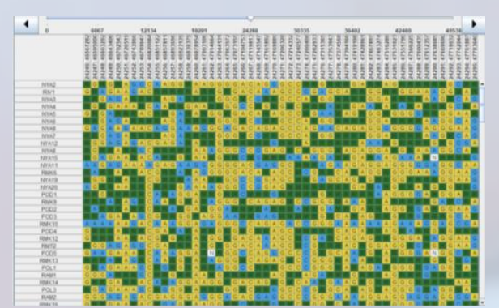
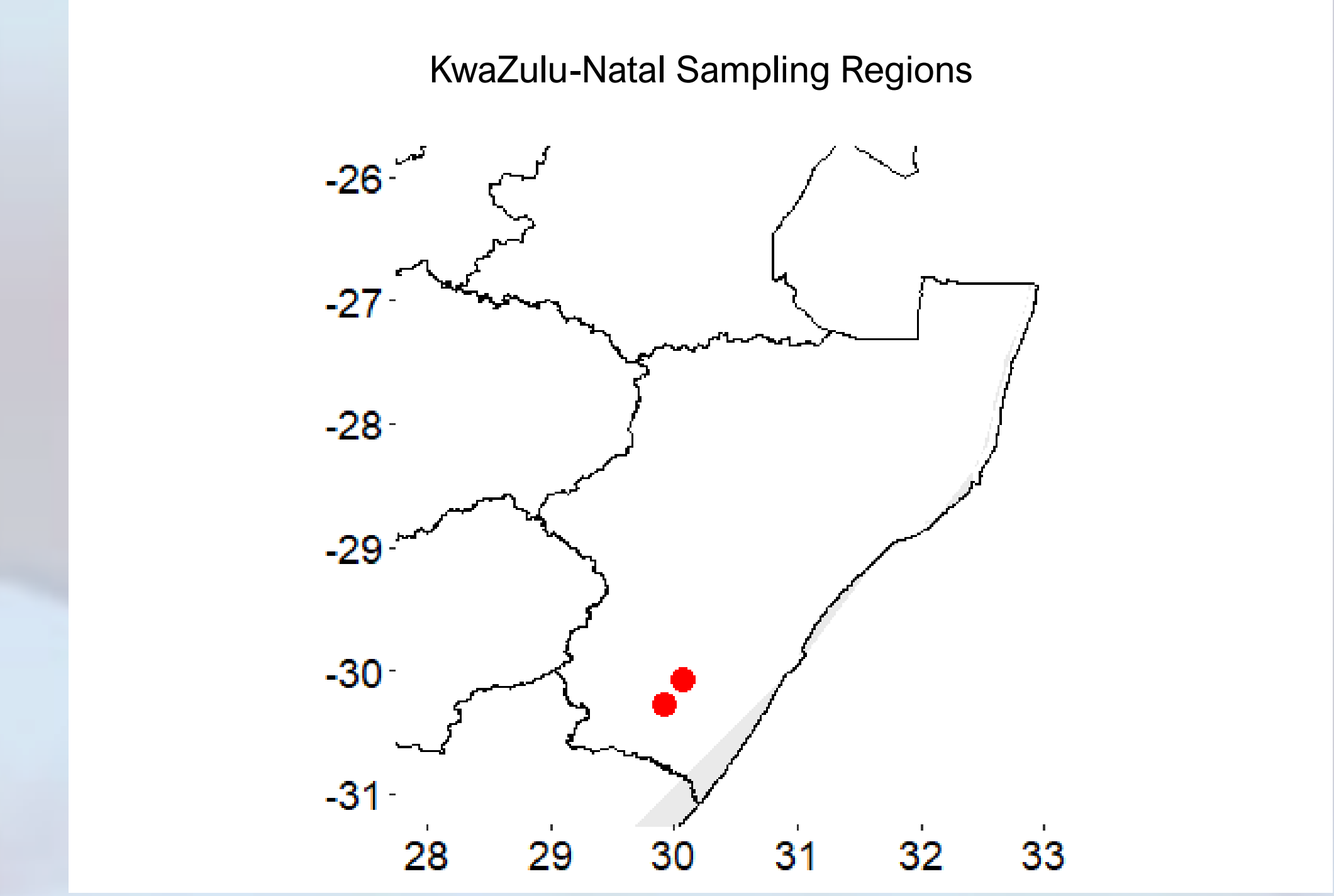
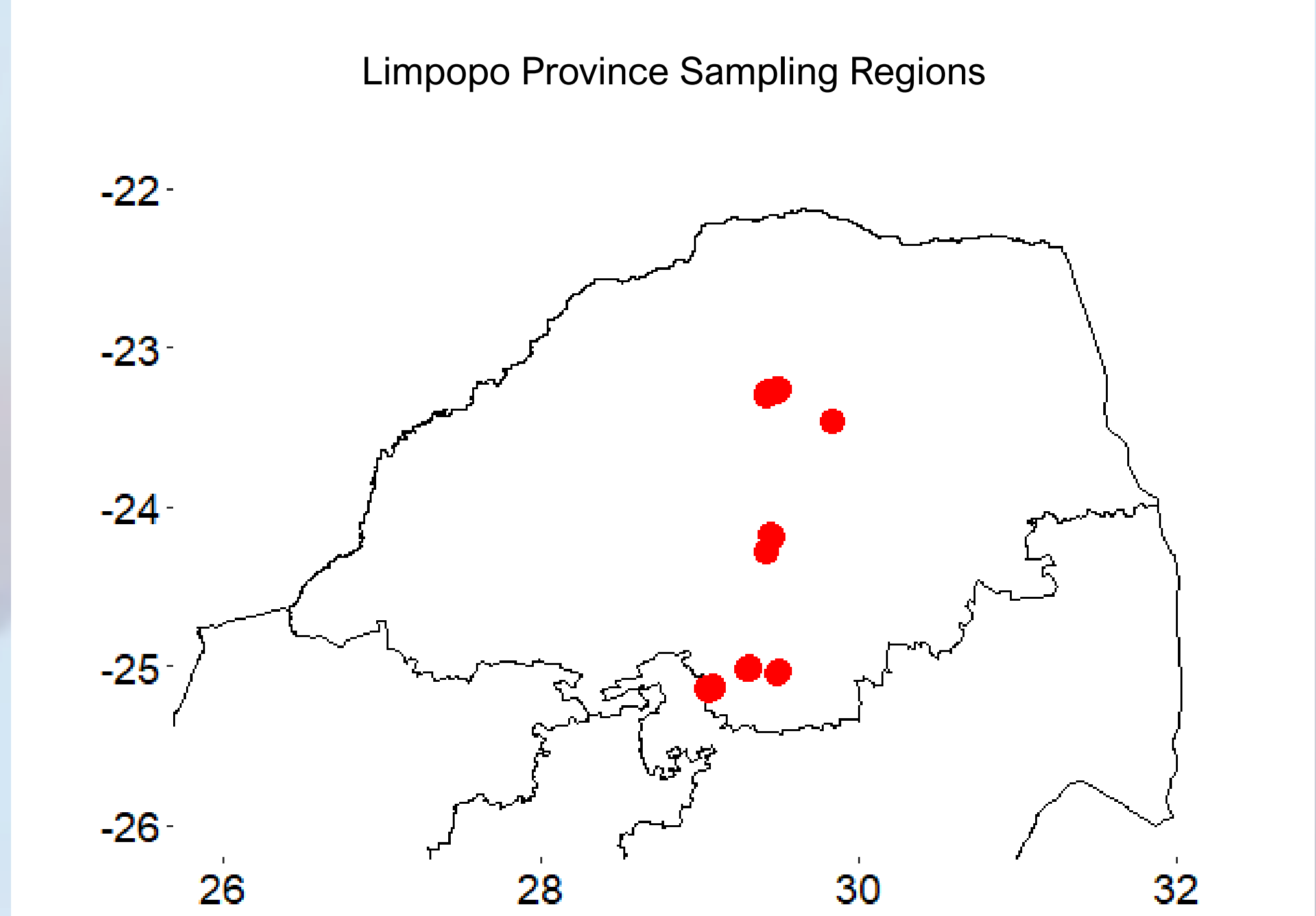


Figure 3: File format conversion and data analysis on the *Tassel 5* platform.



KwaZulu-Natal Sampling Regions

Figure 4 A: The regions in KwaZulu-Natal Province that were sampled for inclusion in the Genome Wide Association Study



Limpopo Province Sampling Regions

Figure 4 B: The regions in the Limpopo Province that were sampled for inclusion in the Genome Wide Association Study

Results

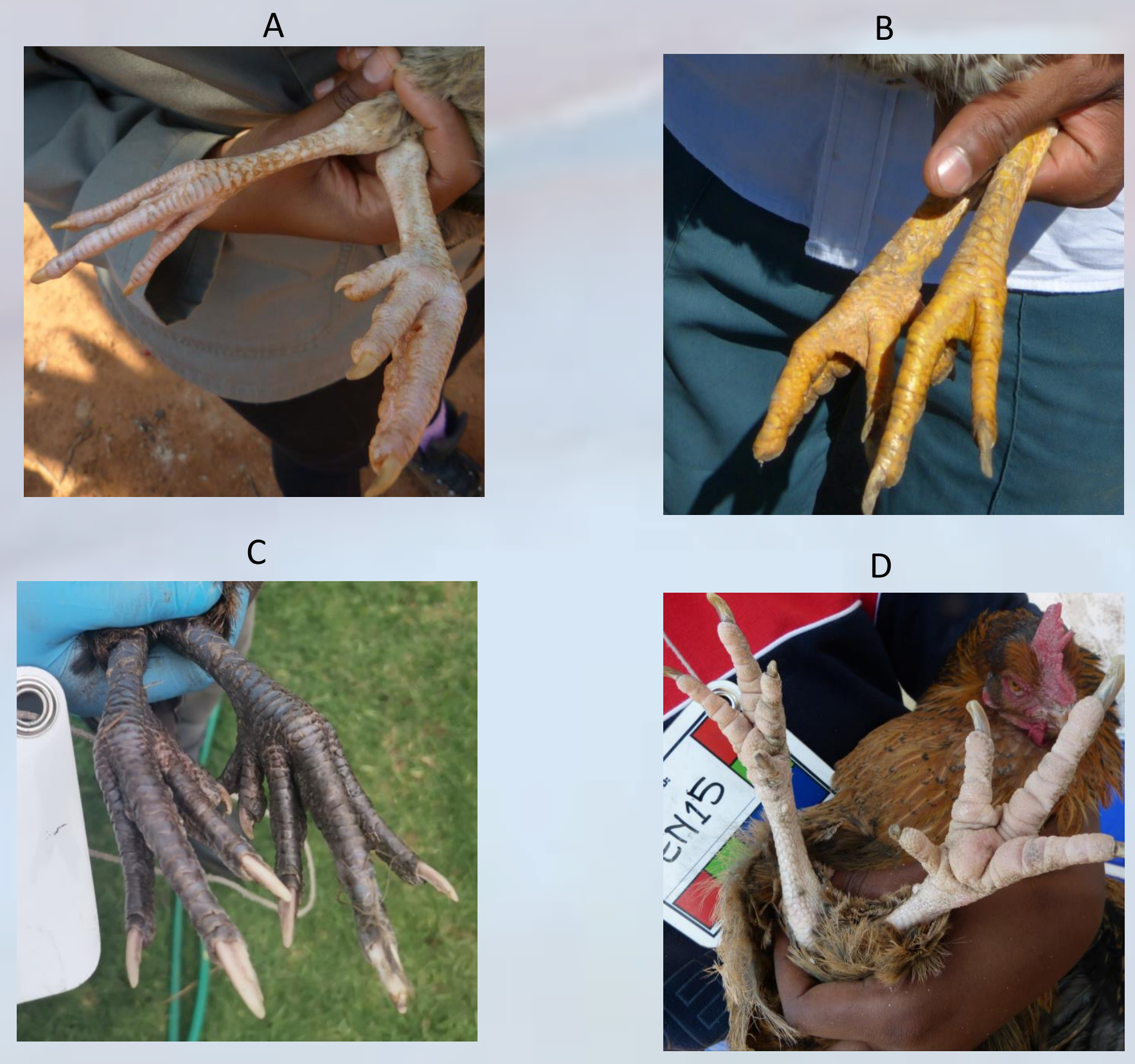


Figure 5: (A) pink shank skin; *(B)* yellow shank skin; *(C)* black shank skin; *(D)* white shank skin.

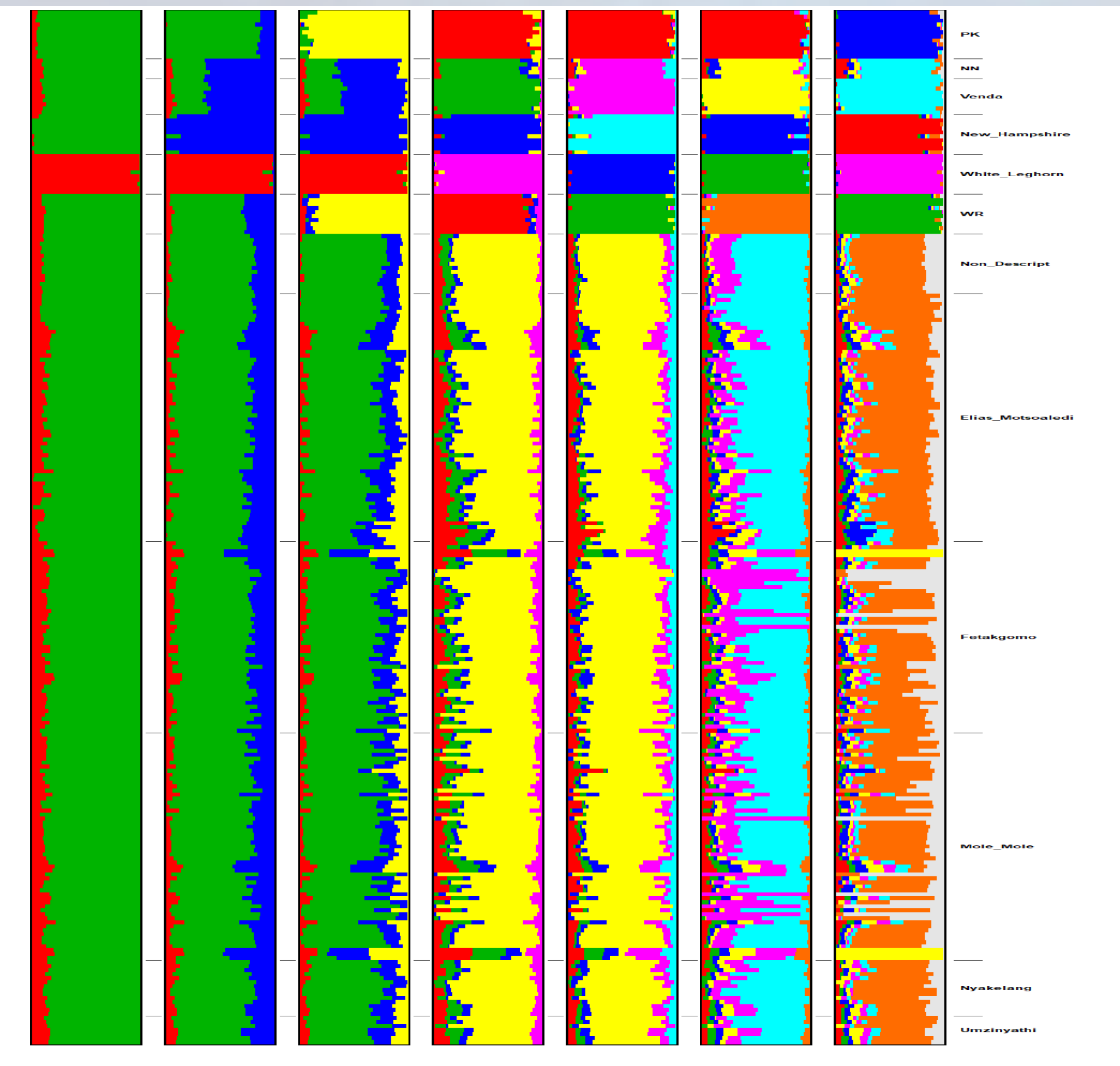


Figure 6: Ancestry proportion of the five populations at District level (Nyakelang District, Mole Mole, Elias Motsoaledi, Fetakgomo and Umzinyathi Districts). Reference population of the Potchefstroom Koekoek, Naked Neck, Venda, New Hampshire, White Leghorn, WR, as well Non-Descript population from the KwaZulu-Natal province are also represented in the diagram.

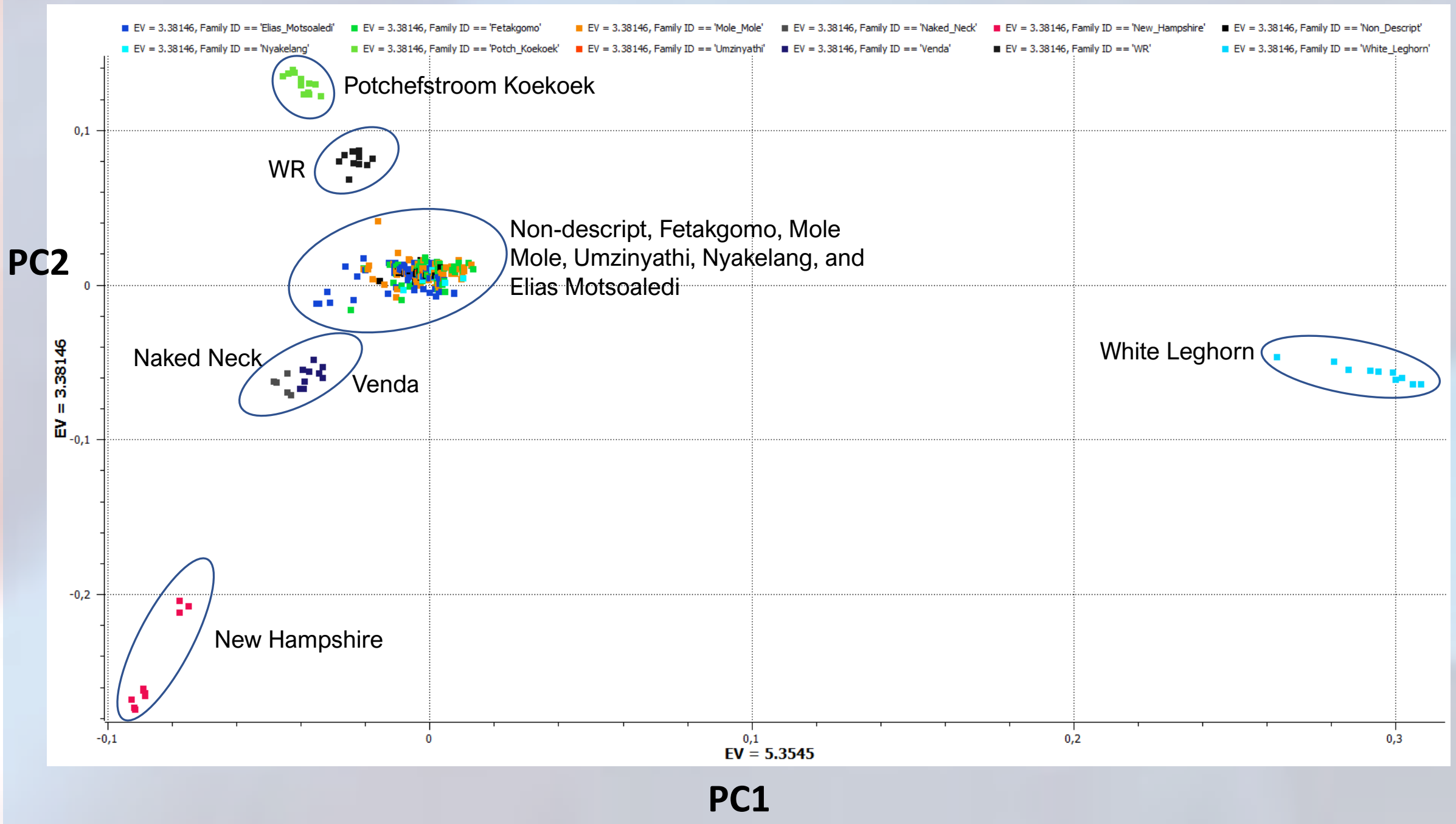
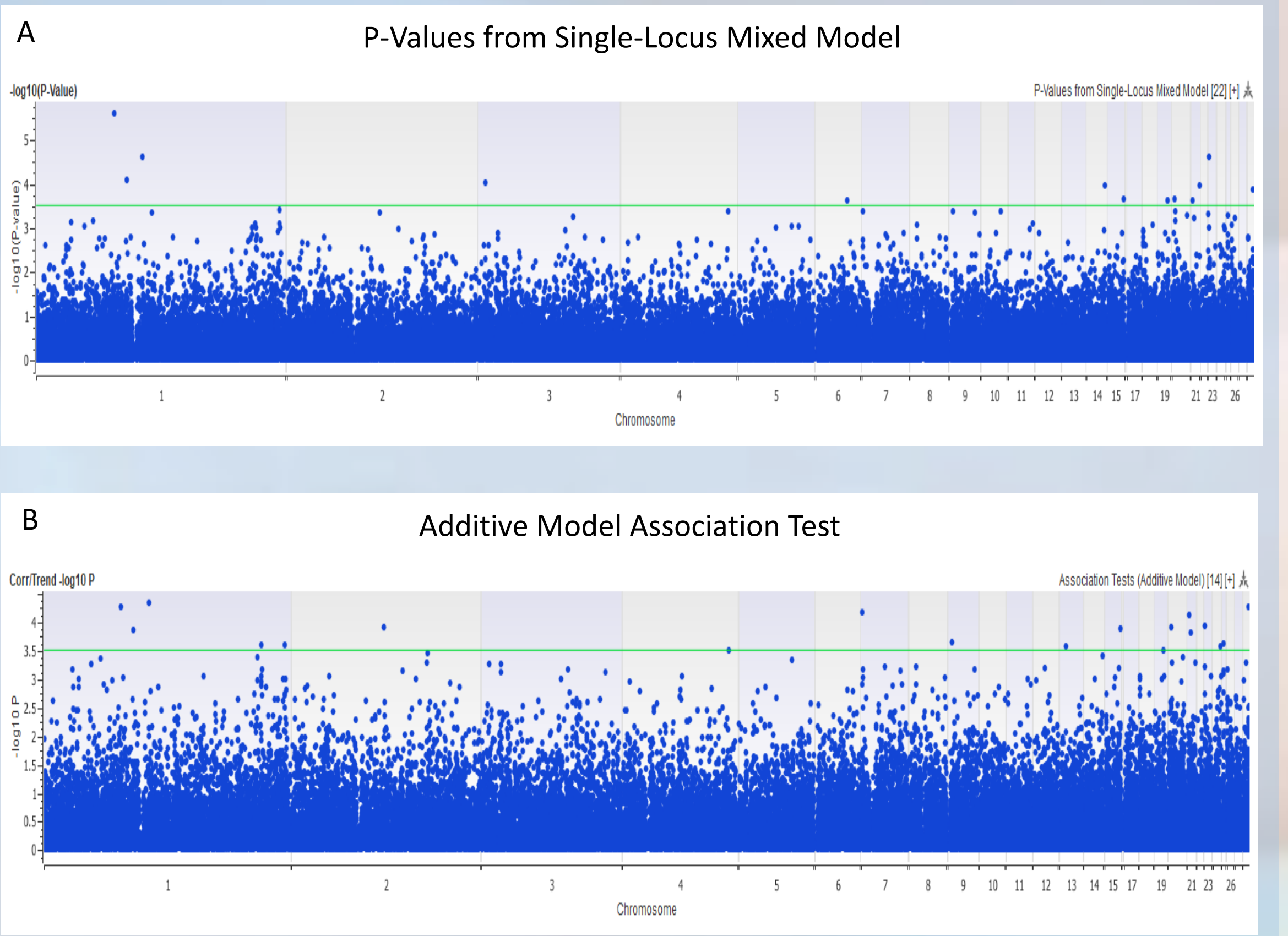


Figure 7: Scatterplot of PC1 and PC2 at District Level with the inclusion of reference populations from conservation flock i.e. Potchefstroom Koekoek, Naked Neck, Venda, New Hampshire, White Leghorn, WR, as well Non-Descript population from the KwaZulu-Natal province . Percentage variance explained by PC1 = 5,3545 whereas percentage variance explained by PC2 = 3,38146

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	13890.491	-80.00552 Va	-0.89
Among populations within groups	3	59584.411	191.21723 Vb	2.14
Within populations	371	3277022.702	8832.94529 Vc	98.76
Total	375	3350497.604	8944.15699	
Fixation Indices				
FST : 0.012	43			

Figure 8: Molecular Variance between within the Districts.

GWAS Results



A

P-Values from Single-Locus Mixed Model

B

Additive Model Association Test

Figure 9: (A) P-Values of Single-Locus Mixed Model shows 15 loci that are under selection; (B) the additive model association test indicates that are 15 candidate regions that are significantly associated with shank skin colour,

Marker	Chromosome	Position	P-Value	-log10(P-Value)
Gga_rs13873864	1	61190085	2,51314E-06	5,599784157
Gga_rs10728329	1	83248503	2,41956E-05	4,616264316
GGaluGA187072	23	237589	2,58742E-05	4,587133549
Gga_rs13883105	1	70983902	8,54513E-05	4,068281456
GGaluGA204924	3	5841372	9,53686E-05	4,020594466
Gga_rs15760220	14	14466819	0,00010719	3,969844744
Gga_rs15186808	21	6673701	0,000111035	3,954538578
Gga_rs14308001	28	4282053	0,000133483	3,874575468
GGaluGA321326	7	37141417	0,00013791	3,860402957
Gga_rs14269662	20	2179663	0,000227449	3,643116094
Gga_rs15787321	15	12468469	0,000228169	3,641743309
Gga_rs14282059	21	1413607	0,000241861	3,616433586
Gga_rs14584500	6	25178685	0,000242707	3,614916947
Gga_rs10728946	19	7320685	0,000242741	3,614856812

Figure 10: A list of the loci that were found to be significantly associated with shank skin color with –log(P-Value) > 3.5. The marker GGA_rs1269662 located at position 2179663 on Chromosome 20 is of particular interest since Cha, J. et al. (2023) also found loci associated with dermal shank colour located on Chromosome 20.

Discussion

- The indigenous chickens exhibited an admixed ancestry, due to uncontrolled mating as well the continuous introduction of exotic cocks into their flocks for improvement.
- It is evident that shank skin colour is a multifactorial trait. Therefore, it is an adaptation as well as a heritable trait. Farmer preferences also have an influence on the phenotype frequencies.
- Fifteen loci were found to be associated with shank skin colour and one of them was on Chromosome 20.
- Additional genotypes, and other production and adaptation traits are necessary to confirm breed composition and increase the differentiation power GWAS.

Conclusion

The results of this study can be used to support community level breeding and conservation strategies in South Africa.

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