GENOME-WIDE ANALYSES ELUCIDATE THE POPULATION STRUCTURE OF NOTICIANA SHEEP BREED IN THE MEDITERRANEAN CONTEXT

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Introduction

Sheep species plays an important role in genetic resources in the Mediterranean area, thanks to its capability of adapting to harsh environments. Italy has a long history of sheep breeding and still counts several local populations (more than 60) that may represent a unique source of genetic diversity. Noticiana is a Sicilian sheep breed, reared in a restricted area under a semi-extensive system, which can be considered a dual-purpose breed.

The Herd Book was established in 2002, and today Noticiana is listed by FAO as an endangered breed with a total of about 400 individuals. Its breeding has been favoured mainly by its low nutritional requirements and good adaptation to harsh conditions, such as high summer temperatures. Few small flocks with a restricted number of males make appropriate breeding programs necessary.

Aim of the research

The availability of high-density single nucleotide polymorphism (SNP) panels is a suitable method for analysing genome structure and estimating genetic diversity. The results could help to establish conservation programs of the Noticiana breed, in order to avoid an increase in inbreeding, and therefore the consequent deterioration of fitness and survival-related traits.

Material and Methods

DNA was extracted from blood samples of 48 individuals of the Noticiana sheep breed and genotyped using the Illumina Ovine SNP600K BeadChip array (Illumina, San Diego, California, USA). The assembly was updated to the latest OAR4.0 version and only SNPs in autosomes were maintained. Noticiana (NOT) was investigated for genetic diversity indices and Run of Homozygosity (ROH) analysis. Furthermore, the genomic SNP data were merged to a Worldwide (WOR), Italian (ITA) and Sicilian (SIC) sheep datasets to characterize the genetic relationships and population structure (Table 1).

	NOT		WOR		ITA		SIC	
Original SNPs in autosomes	576,582	SNPs	35,834	SNPs	46,827	SNPs	46,827	SNPs
	48	IDs	2,943	IDs	548	IDs	377	IDs
	1	breed	155	breeds	22	breeds	5	breeds
Merging to NOT			35,834	SNPs	41,382	SNPs	41,382	SNPs
			2,991	IDs	596	IDs	425	IDs
			156	breeds	23	breeds	6	breeds
Quality Control	501,662	SNPs *	35,560	SNPs **	39,644	SNPs **	39,315	SNPs **
*maf 0.01; geno 0.05; mind 0.10	48	IDs	2,991	IDs	592	IDs	425	IDs
**maf 0.01; geno 0.05; mind 0.05	1	breed	156	breeds	23	breeds	6	breeds
Pruning 50 10 0.2			33,557	SNPs	33,184	SNPs	31,167	SNPs
			2,991	IDs	592	IDs	425	IDs
			156	breeds	23	breeds	6	breeds
Analyses	• Genetic diversity indices • Runs of homozygosity		• Multidimensional scaling analysis		• Multidimensional scaling analysis • Bayesian clustering • Pairwise distances • Bayescan: NOT vs COM			

Table 1. Dataset composition and management

Results

The genetic diversity indices in Table 2 suggest an isolate-breaking effect due to gene flow ($H_O > H_E$) and show relative low level of inbreeding. Moreover, if 100 is considered as the minimum acceptable Ne to conserve a population, the estimate for Noticiana breed is below this critical value.

The high-density SNP panel allowed us to discover a total of 4,618 ROHs, mostly on OAR2. Almost all segments of homozygosity were less than 4 Mb (93%), suggesting old inbreeding events and a reduced ROH coverage of the whole genome (Table 2 and Figure 1).

The analysis identified 10 ROH islands on six chromosomes (OAR2, OAR3, OAR6, OAR9, OAR12, and OAR15), harboring genes and QTLs (quantitative trait loci) associated with productive traits (e.g., milk protein percentage, meat fatty acid content and meat and carcass traits). In particular, ROH islands in OAR3 and OAR9 highlighted correlations with traits and genes responsible for local adaptation and fitness.

H_O	H_E	F_{IS}	F_{ROH}	MAF	Ne	N_{ROH}	NC_{ROH}	L_{ROH}	LC_{ROH}
0.336	0.324	-0.035	0.083	0.242	76	96.21	117.62	2.05	2.11

Table 2. Genetic diversity indices and results of ROH analysis for Noticiana. Observed (H_O) and expected (H_E) heterozygosity; inbreeding coefficients on heterozygosity (F_{IS}) and ROH (F_{ROH}); minor allele frequency (MAF); effective population size (Ne); mean number of ROH per individual (N_{ROH}); mean number of ROH per chromosome (NC_{ROH}); average length of ROH in Mb per individual (L_{ROH}); average length of ROH in Mb per chromosome (LC_{ROH}).

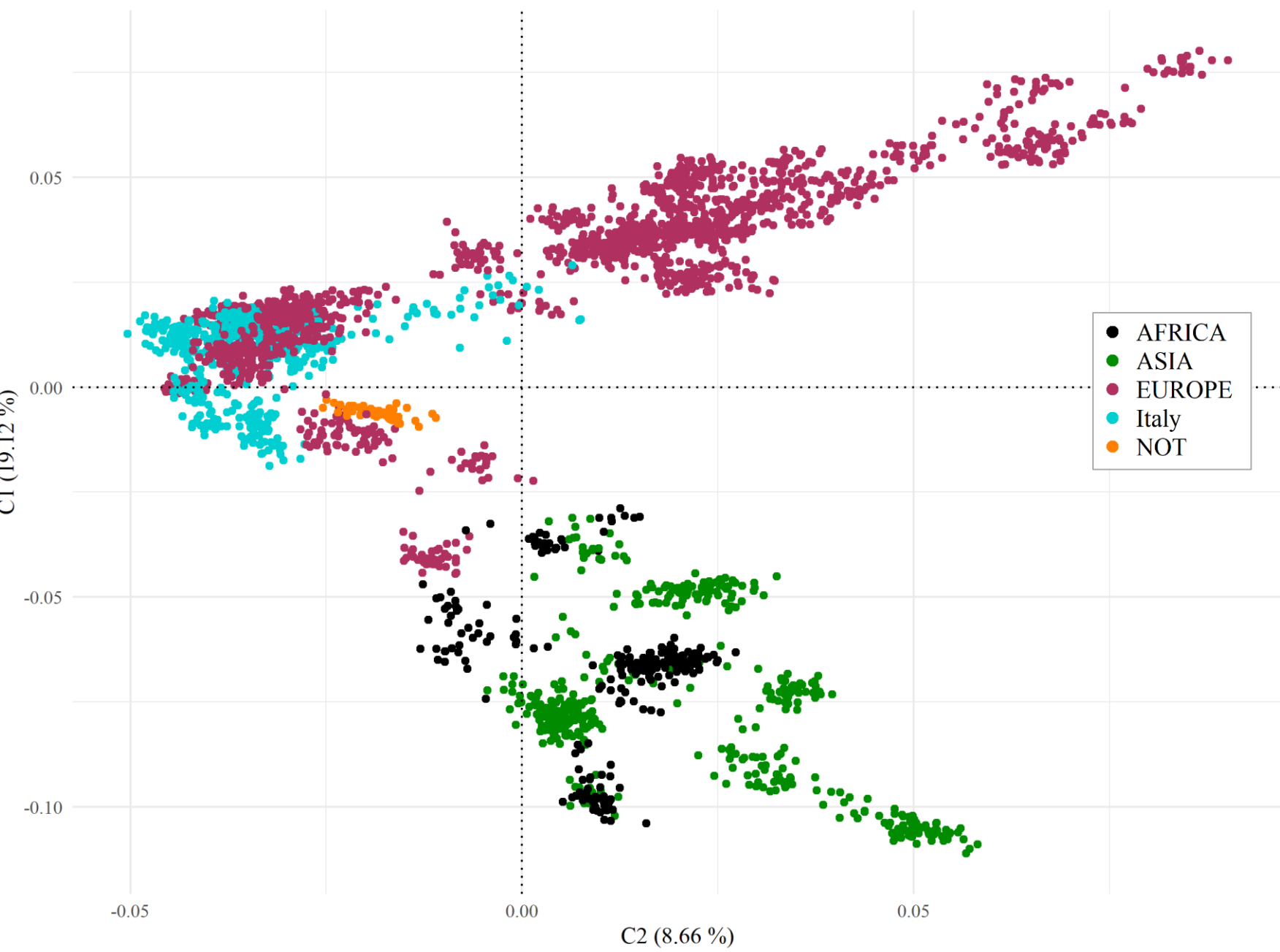Figure 1. ROH count per chromosome and ROH length in different colors.

Figure 2. MDS plot of the WOR dataset comprehensive of 156 breeds.

Population structure analyses revealed a clear separation of breeds according to their geographical origin and historical admixtures. Noticiana grouped within the European cluster, close to South-Italian, Albanian and Spanish breeds (Figure 2).
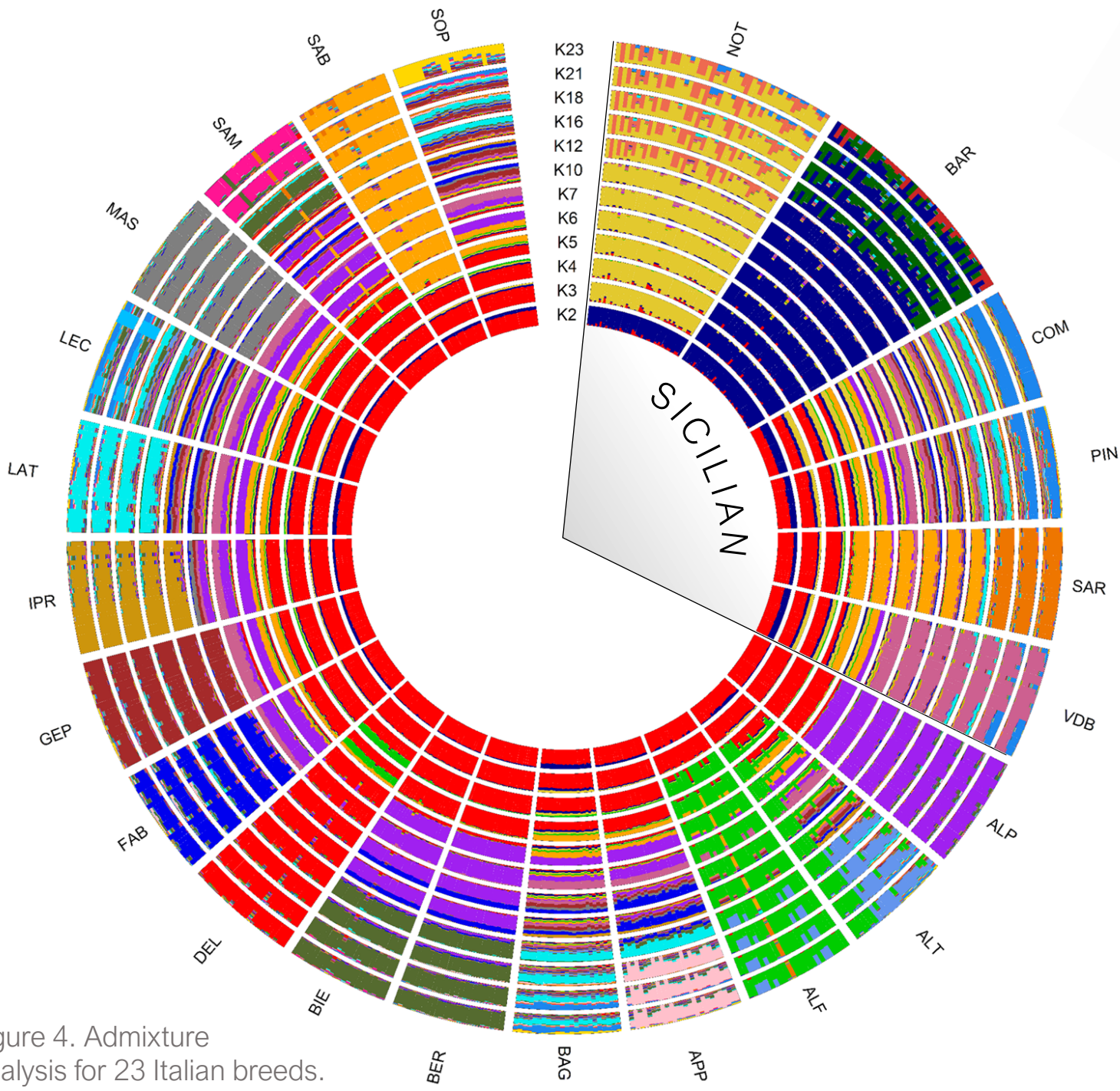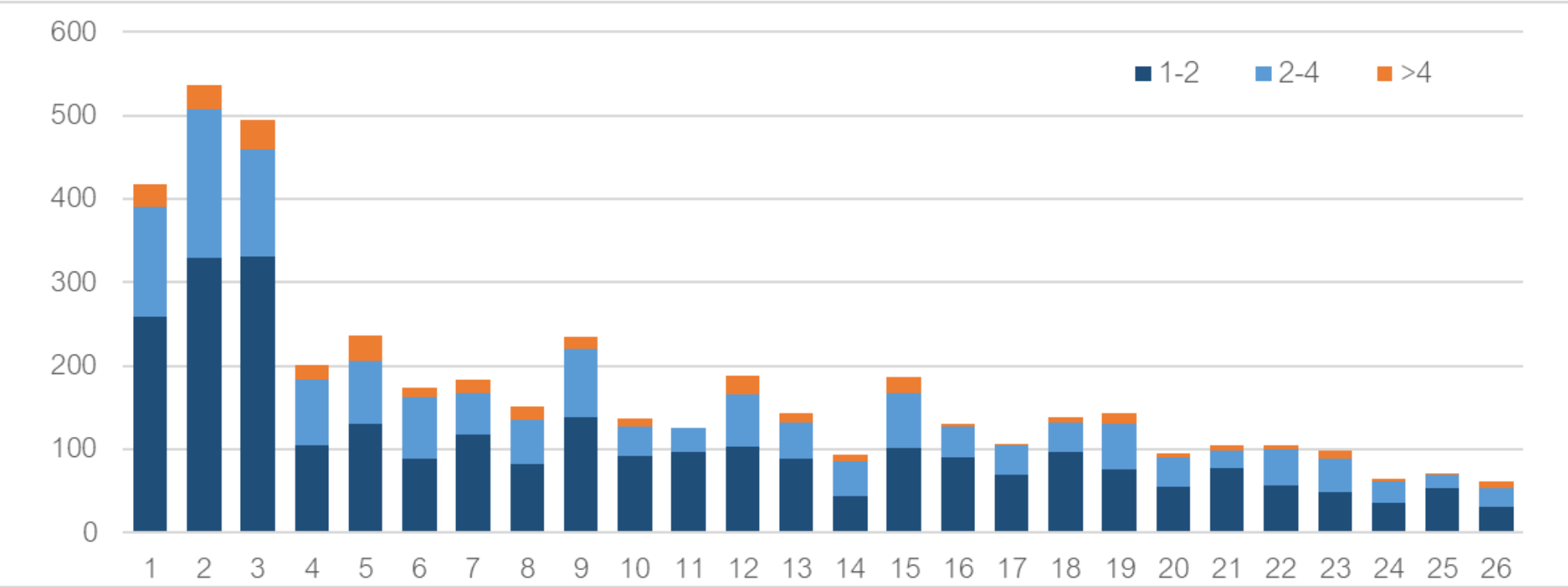

Figure 4. Admixture analysis for 23 Italian breeds.

The Bayesian clustering analysis reported the lowest CV error for K = 23 in ITAPOP, corresponding to the number of investigated breeds (Figure 4). Breeds were progressively assigned to different clusters, revealing genetic admixture among Sicilian breeds, especially for Comisana and Pinzirita. Noticiana showed a separate pattern with an evident internal substructure.
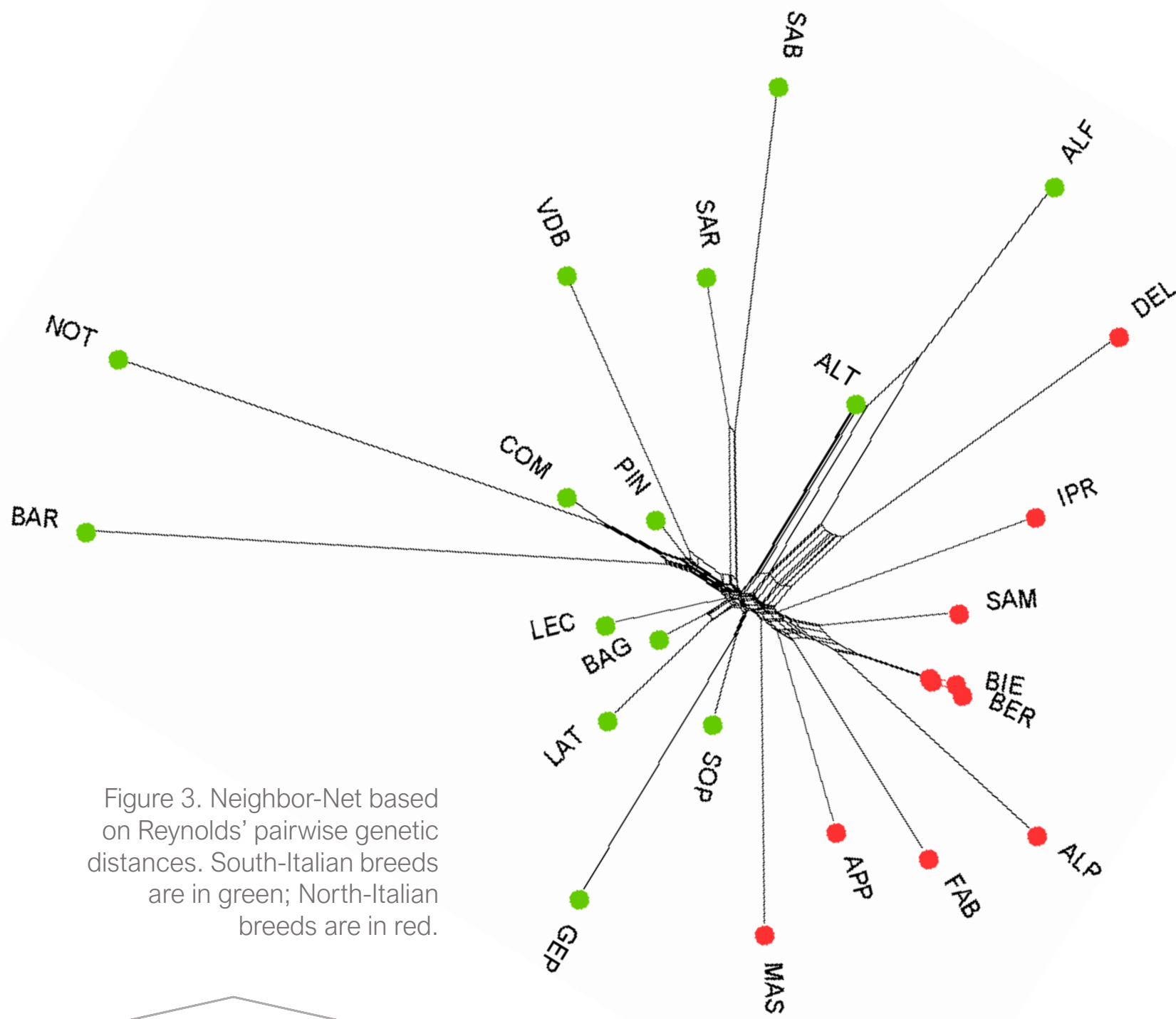

Figure 3. Neighbor-Net based on Reynolds' pairwise genetic distances. South-Italian breeds are in green; North-Italian breeds are in red.

Neighbor-Net separated distinctly the North-Italian breeds from the South ones. Moreover, Noticiana showed a common root with Comisana (Figure 3). The F_{ST}-outliers analysis identified a total of 20 outlier SNPs as a putative signal of differentiation between Noticiana vs Comisana. In particular, the main genetic differences lay in productive and adaptability traits.

References

Ciani et al., 2014; Mastrangelo et al., 2017; Persichilli et al., 2021; Bigi & Zanon, 2008; FAO, 2022; Tolone et al., 2022; McQuillan et al., 2008; Rochus et al., 2020; Edea et al., 2019; Wei et al., 2016; De Léon et al., 2021; Jia et al., 2019; Kijas et al., 2012; Lv et al., 2022; Mastrangelo et al., 2014.

Conclusion

This study provided for the first time the genome-wide assessment of the genetic diversity and population structure of the Noticiana sheep breed, using a high-density SNP panel and providing a thorough analysis of the breed's structure, fine mapping of ROH islands, and identification of potential selection signatures. The results demonstrated its clear distinction from the rest of the breeds, revealing a moderately low level of inbreeding and its shared ancestry components with Comisana. ROH analysis identified several genes and QTLs positively involved in milk and meat production traits, as well as adaptation to harsh environments. The information generated in this study is of significant importance because it will help to design and implement conservation strategies in order to recover the Noticiana breed. However, additional analyses and a wider sampling would contribute refining and validating these results.

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