

Genome-wide characterization analysis reveals potential selection

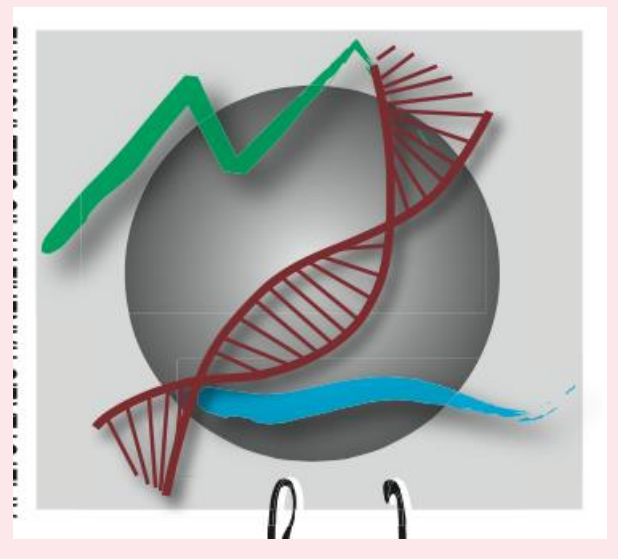
signatures in local Greek sheep.

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INTRODUCTION

Boutsko (BOU), Frizarta (FRZ), Mytilini (MYT) and Pelagonia (PEL) sheep are among the most economically important Greek sheep breeds, which have been adapted to variable environmental conditions and artificially selected for production purposes (milk, meat or both), while reared in different farming conditions [1–4]. However, these genetic processes are largely understudied for Greek livestock species. Runs of homozygosity (ROH) and heterozygosity (ROHet) represent inherited consecutive homozygous and heterozygous regions utilized to estimate inbreeding and characterize population demographic history and their adaptive evolution [5]. The aim of this study was the detection of ROH/ROHet regions throughout the genome of the aforementioned breeds and the identification of ROH and ROHet hotspots potentially related with selection processes.

MATERIALS & METHODS

- Genotyping of 828 Greek sheep (BOU=298, FRZ=363, MYT=107 and PEL=60) - OvineSNP50 Bead Chip
- Quality Control - PLINK v.1.9.
- ROH and ROHet detection & F_{ROH} calculation - R package detectRUNS
- Identification of ROH and ROHet islands by extracting the 99.9 quantile of the frequency distribution of SNPs.
- Gene annotation within distances of ± 250 kb - Ensembl Variant Effect Predictor
- QTL identification in sheep and cattle - Animal QTL database

RESULTS – DISCUSSION

- The majority of ROH were < 2 Mb in length, while most of ROHet ranged from 0.5 to 1 Mb for all Greek breeds (Figure 1).

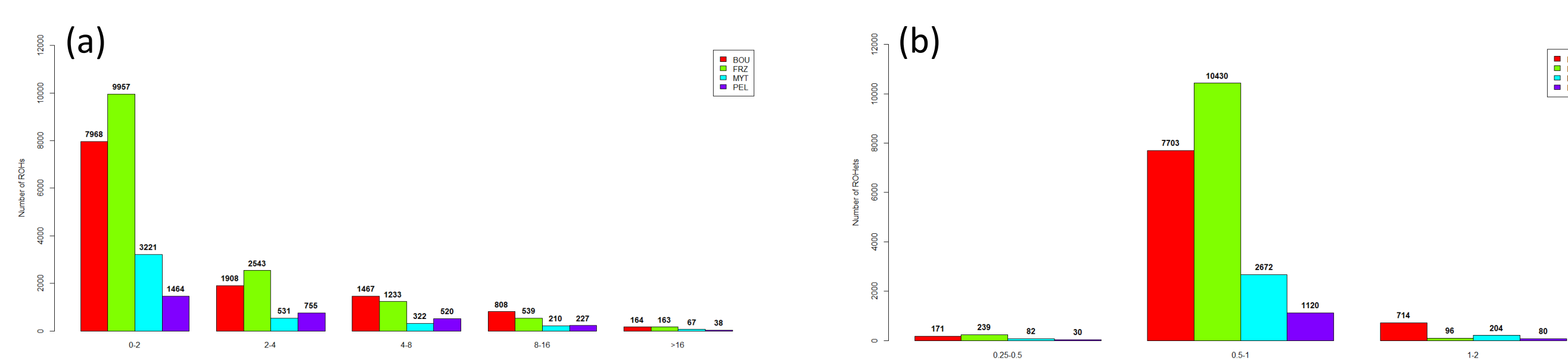


Figure 1. Number of identified ROH (a) and ROHet (b) per breed, classified based on their length.

- The average F_{ROH} for each breed (BOU=0.049, FRZ=0.041, MYT=0.042 and PEL=0.092) suggests that Pelagonia sheep is more inbred than the other three Greek breeds, possibly caused by small population size and crossbreeding practices.
- In total, 13 ROH islands were identified, eight on chromosomes 2 and 10 with overlapping regions among Greek breeds (Table 1; Figure 2a). Moreover, 15 of the 20 detected ROHet islands were distributed on chromosomes 3, 9, 10 and 17 with common heterozygous regions among two or more breeds (Table 2; Figure 2b)
- Analysis on chromosome 10 revealed ROH as well as ROHet islands within the same region for BOU and FRZ sheep (Table 1; Table 2).

Table 1. Runs of homozygosity islands across the four Greek sheep breeds.

	Chrom	From	To	Length (bp)
BOU	10	38216203	39383150	1,166,947
	10	41511331	42126389	615,058
	2	122297721	123491237	1,193,516
FRZ	10	39043416	39403332	359,916
	10	41511331	42358254	846,923
	2	114381876	115466968	1,085,092
MYT	13	48266484	49208171	941,687
	2	52106037	52479757	373,720
	2	114381876	115466968	1,085,092
	2	122092485	123358635	1,266,150
PEL	2	10651915	11743310	1,091,395
	3	130998559	131871923	873,364
	6	78748344	79090228	341,884

Table 2. Runs of heterozygosity islands across the four Greek sheep breeds.

	Chrom	From	To	Length (bp)
BOU	10	41727139	42735479	1,008,340
	17	28915345	29592399	677,054
	2	159902153	160226217	324,064
FRZ	9	77162891	77353198	190,307
	10	38685897	39446610	760,713
	10	41545097	41998194	453,097
	17	28905683	29671064	765,381
MYT	3	109251964	109313746	61,782
	9	77162891	77745675	582,784
	10	41896917	42005547	108,630
	17	29011436	29644074	632,638
	2	110349592	110733324	383,732
PEL	3	71866455	72488674	622,219
	9	77176554	77745675	569,121
	10	41788680	42735479	946,799
	25	18939022	19472002	532,980
	3	72229264	72634273	405,009
	6	12638149	13266900	628,751
	9	63751936	64366158	614,222
	9	77162891	77985259	822,368

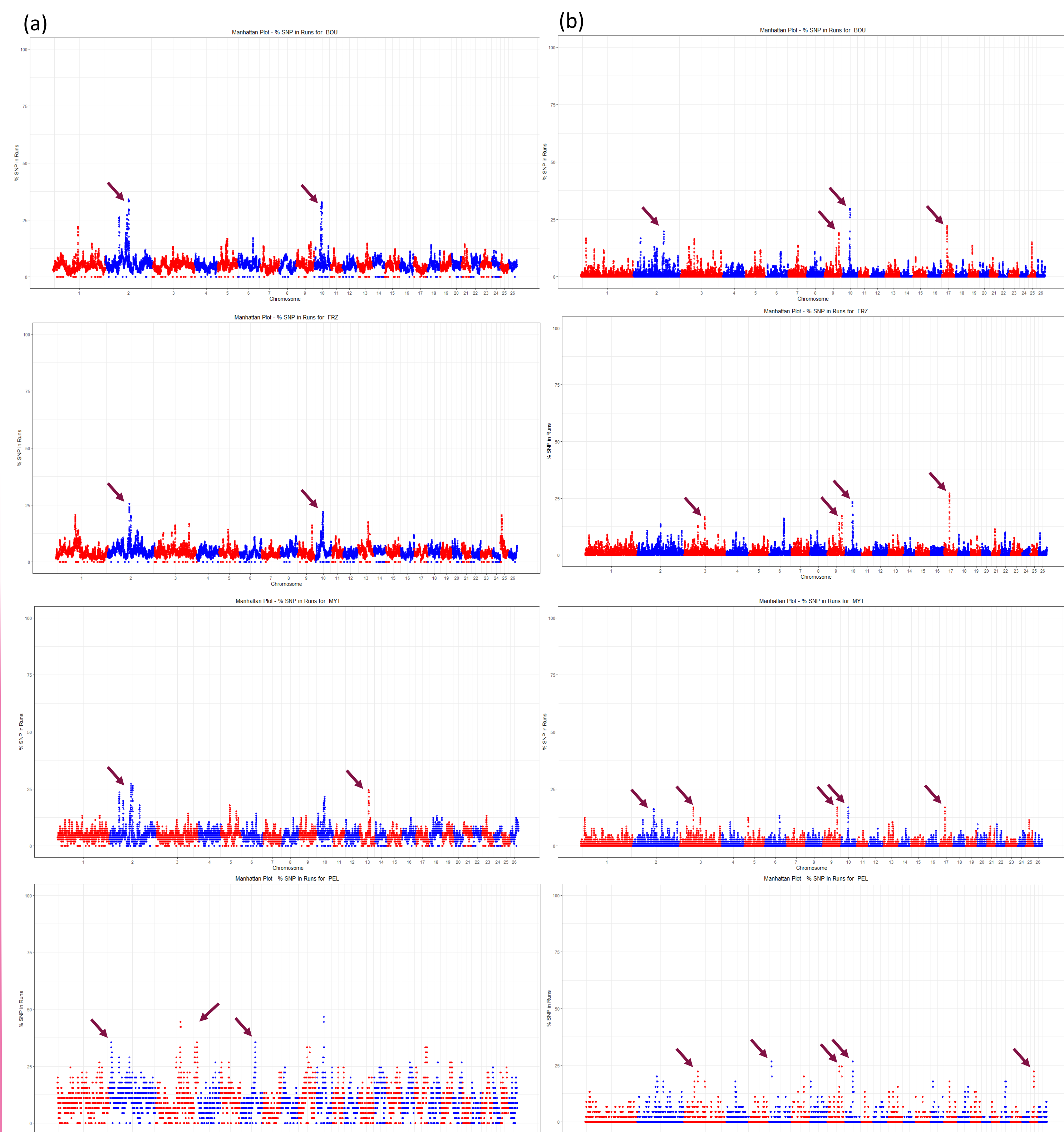


Figure 2. Manhattan plots showing the percentage of times each SNP falls inside homozygous and heterozygous segments in the four Greek sheep breeds. (a) Runs of homozygosity and (b) Runs of heterozygosity

- Functional annotation analysis revealed 117 previously described QTLs and 181 candidate genes within or closely located to the identified ROH and ROHet islands, mostly associated with productivity and environmental adaptation traits (Table 3).
- Our findings contribute to our understanding of the selection impact on Greek sheep genome and to the development of efficient breeding schemes while balancing conservation of genetic resources.

Table 3. Most important genes identified within ROH/ROHet islands.

Trait	Breed	Genes
Milk yield & composition	BOU, FRZ, MYT	VPS13B,, LARP1B, SCLT1, JADE1, ABHD18, MFSD8
	PEL	NCKAP1L
Growth	BOU	MBD5, EPC2
	PEL	NDUFA12, NR2C1, FGD6
Paratuberculosis susceptibility	PEL	GTSF1, ZNF385A, COPZ1, NFE2, CBX5, SMUG1
Parasites resistance	MYT	CLCN3, NEK1, SH3RF1, PALLD
	PEL	FGD6, USP44
Fat deposition & Tail morphology	MYT	HINT2, NPR2, ARHGEF39, HAO1

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