Animal xxx (xxxx) xxx

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Genome-wide analyses reveal population structure and identify candidate genes associated with tail fatness in local sheep from a semi-arid area

I. Baazaoui [a], S. Bedhiaf-Romdhani [b,*], S. Mastrangelo [c], E. Ciani [d]

[a] Faculty of Sciences of Bizerte, University of Carthage, 7021 Zarzouna, Bizerte, Tunisia
[b] Institut National de la Recherche Agronomique de Tunisie, Laboratoire des Productions Animales et Fourragères, Université de Carthage, 2049 Ariana, Tunisie.
[c] Department of Agricultural, Food and Forest Sciences, University of Palermo, 90128 Palermo, Italy.
[d] Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, 70121 Bari, Italy

ARTICLE INFO

Article history:
Received 29 June 2020
Received in revised form 20 January 2021
Accepted 25 January 2021
Available online xxxx

Keywords:
Fat tail
Genetic structure
Local sheep
Runs of homozygosity
Selection signature

ABSTRACT

Under a climate change perspective, the genetic make-up of local livestock breeds showing adaptive traits should be explored and preserved as a priority. We used genotype data from the ovine 50 k Illumina BeadChip for assessing breed autozygosity based on runs of homozygosity (***ROH***) and fine-scale genetic structure and for detecting genomic regions under selection in 63 Tunisian sheep samples. The average genomic inbreeding coefficients based on *ROH* were estimated at 0.017, 0.021, and 0.024 for Barbarine (**BAR**, $n = 26$), Noire de Thibar (**NDT**, $n = 23$), and Queue fine de l'Ouest (**QFO**, $n = 14$) breeds, respectively. The genomic relationships among individuals based on identity by state (**IBS**) distance matrix highlighted a recent introgression of QFO into the BAR and a genetic differentiation of NDT samples, possibly explained by past introgression of European gene pools. Genome-wide scan for *ROH* across breeds and within the BAR sample set identified an outstanding signal on chromosome 13 (46.58–49.61 Mbp). These results were confirmed using F_{ST} index, differentiating fat vs. thin-tailed individuals. Candidate genes under selection pressure (*CDS2*, *PROKR1*, and *BMP2*) were associated to lipid storage and probably preferentially selected in fat-tailed BAR animals. Our findings suggest paying more attention to preserve the genetic integrity and adaptive alleles of local sheep breeds.

Implications

Native sheep breeds were considered as a reservoir of genetic variability to produce food under low input production systems. Based on a fine-scale genetic diversity using single-nucleotide polymorphism array, our study highlighted a genetic homogenization between local breeds linked mainly to the absence of coherent breeding strategies and farmers organizations. Genome-wide scan of fat-tailed Barbarine sheep identified signals of selection for fat deposition and adaptive alleles to semi-arid area. Our findings showed that is possible to establish suitable breeding programs as well as coherent crossbreeding activities to enhance native breed's ability to better produce even under harsh conditions.

Introduction

Sheep (*Ovis aries*) were domesticated 11 000 Before Present in the Anatolia region from Asian Mouflon (*Ovis orientalis*). The Northern African sheep is 7 000 years old and contains a remarkable diversity of populations, which have been maintained under traditional farming systems over millennia (Iniguez, 2005). The need to maintain and improve local genetic resources has been recognized as a priority, at the world level (Galal et al., 2000). More than 6.8 million sheep heads are raised in Tunisia, where 80% of them are owned by small farmers with low income and living under harsh conditions in rural areas. About four millions of breeding ewes belong to four different breeds: Barbarine (**BAR**, 58%), Queue fine de l'Ouest (**QFO**, 37%) and Noire de Thibar (**NDT**,2%) (Supplementary Table S1). The remaining sheep populations are represented by the exotic (Moroccan) D'man and the synthetic Sicilo-Sarde dairy breed (Office de l'Elevage et des Pâturages, 2018). The Barbarine breed, the most important native sheep in Tunisia, is the unique fat-tailed breed in Tunisia also exists in Libya and Algeria. It has been introduced into North Africa since the Carthaginian times and was reintroduced until 900 A.D.CE with the Arab invasions (Sarson, 1973). Barbarine breed is at the origin of "Tunis" sheep breed reared in USA (Gammoudi and Bedhiaf, 2015) and, possibly, of the Italian Barbaresca breed (Tolone et al., 2012). The Barbarine breed is characterized by a rich reservoir of genetic ecotypes well adapted to the prevailing agro-ecologies of semi-arid areas (Bedhiaf-Romdhani

* Corresponding author.
E-mail address: romdhani.sonia@iresa.agrinet.tn (S. Bedhiaf-Romdhani).

https://doi.org/10.1016/j.animal.2021.100193

et al., 2008). One of the main threats for this breed is the uncontrolled crossbreeding (i.e., breeding not carried out within the framework of selection plans) with the thin-tailed QFO breed. This crossbreeding is practiced by herders to improve fattening performance of QFO and/or reduce the tail size for easier mating management in crossbreed animals (Bedhiaf-Romdhani et al., 2020). The QFO, originated from the Algerian Ouled Djallel breed, is mainly raised in the west area of Tunisia close to the Algerian frontiers as indicated by its name. The NDT breed is found only in the sub-humid northern region of Tunisia. It was created since 1908, to obtain black-coated animals tolerant to photosensitization caused by *hypericum* weeds and for the production of mutton-fine wool (Baazaoui et al., 2020). Indeed, it is a composite breed, the result of reciprocal crossing between the French Merinos d'Arles breed and local thin-tailed sheep, followed by crossbreeding with the Black Brown Swiss breed (Chalh et al., 2007). Recent studies have underlined the usefulness of single nucleotide polymorphism chip genotyping and Runs of Homozygosity (**ROH**) analyses for monitoring genetic diversity (Ghoreishifar et al., 2019) and genetic relationships in local breeds at national and cross-border level (Meyermans et al., 2020; Rochus et al., 2020). Moreover, analysis of *ROH* classes of different length can help to trace not only the recent but also ancient inbreeding depending from the length of *ROH* fragments (Peripolli et al., 2017). Selection typically leaves signatures in the genome, which are often characterized by high genetic differentiation across breeds and/or a strong reduction of within-breed genetic diversity in regions associated with traits under intense selection pressure. Indeed, *ROH* are commonly used to identify genomic regions under putative selection (Mastrangelo et al., 2017; Almeida et al., 2019; He et al., 2020). In order to follow-up the selection signatures map in fat-tailed sheep initiated by (Moradi et al., 2012), many studies contributed to explore potential candidate genes for the fat tail phenotype using the F_{ST}-outlier method contrasting fat vs. thin-tailed breeds (Moioli et al., 2015; Wei et al., 2015; Yuan et al., 2017; Xu et al., 2017; Zhao et al., 2020) as well as analyzing genomic regions with high *ROH* frequency (Mastrangelo et al., 2019). Tunisian breeds have not been the object so far of such kind of studies. Therefore, this work aimed to (i) understand the genetic relationships among three Tunisian sheep breeds using the Illumina Ovine 50 K SNP BeadChip; (ii) investigate the characteristics of *ROHs* in those breeds, and (iii) identify genomic regions under selection by using a combination of *ROH* hotpots analysis and an F_{ST}-outlier approach.

Material and methods

Sampling, genotyping, and data quality control

Blood sampling was performed on 63 meat sheep samples, belonging to fat-tailed Barbarine ($n = 26$) and thin-tailed animals from the NDT ($n = 23$) and QFO ($n = 14$) breeds (Supplementary Table S1, Fig. 1). To minimize the probability of relatedness among individuals,

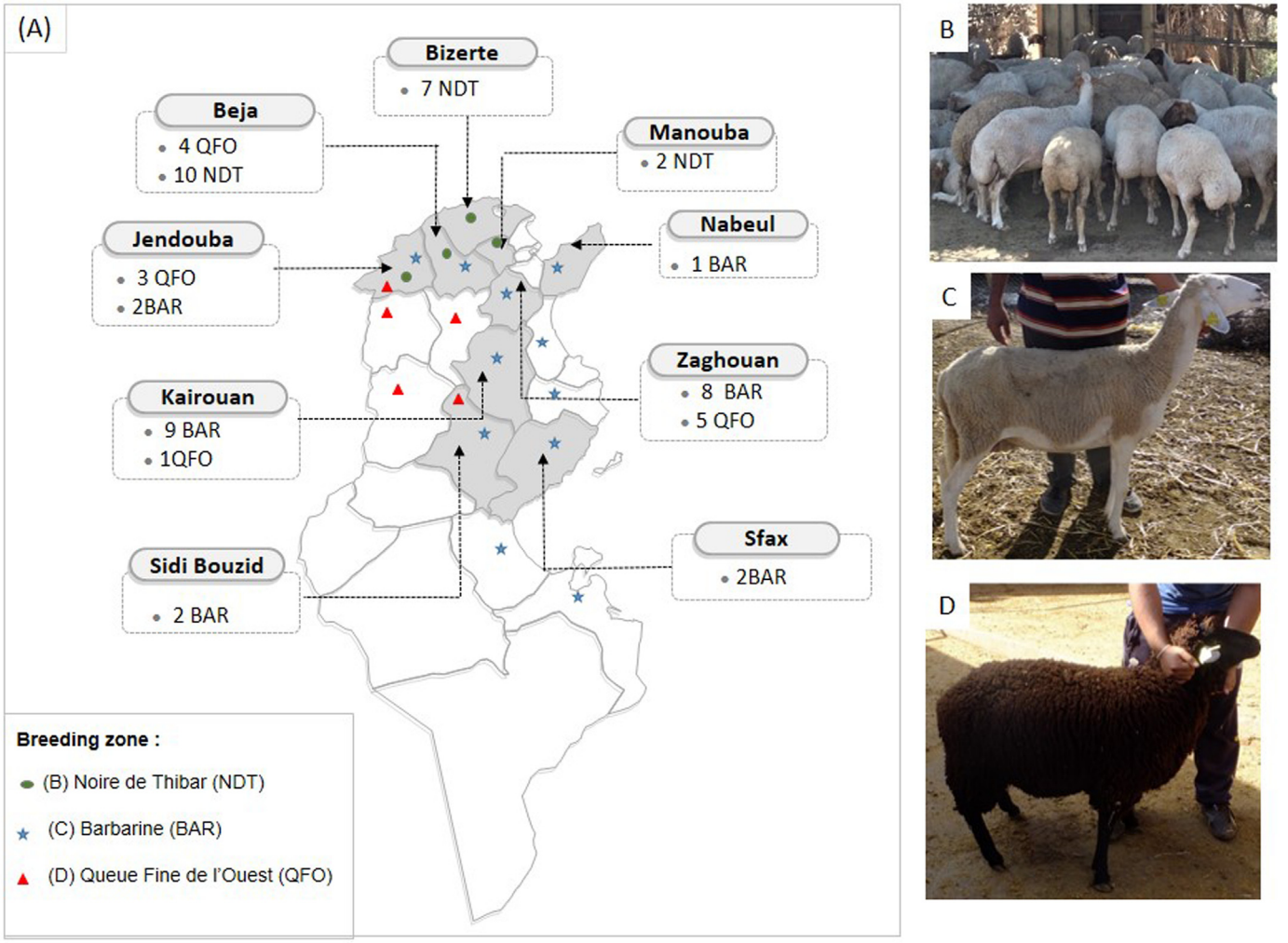

Fig. 1. Sampling sites, breeding zones, and studied sheep breeds in Tunisia.

samples were collected from different farms, including both public farms appointed by the Tunisian Ministry of Agriculture for the breed conservation as well as private farms, located mainly in the northern and central part of the country. The genomic DNA was isolated using the standard phenol-chloroform protocol (Sambrook et al., 1989). The samples were genotyped at 54241 SNP markers using the Illumina Ovine SNP50 BeadChip (San Diego, USA). Data quality control was performed using PLINK v1.07 (Purcell et al., 2007). The SNPs positions were updated to the Ovis_aries_v4.0 version using the information available in the SNPchiMP v3 database (Nicolazzi et al., 2015). Only SNPs located on autosomes were considered in further analyses. The eligible SNPs were then filtered according to the following criteria: (i) SNP call rate > 90%, (ii) minor allele frequency (MAF) > 0.01, (iii) individual call rate > 0.9. Samples and markers that did not satisfy these criteria were excluded.

Population genetic analysis

We computed allele sharing distance (**ASD**) via PLINK 1.07 to perform multidimensional scale (**MDS**) analysis. Genetic admixture was assessed through the model-based clustering algorithm implemented in the software Admixture v. 1.22 (Alexander and Lange, 2011), adopting the default settings. In addition, a network-based approach implemented in NetView v.1.1 software (Neuditschko et al., 2012; Steinig et al., 2016) using identity by state (**IBS**) distance matrix was performed. To better visualize the complex population networks, we used increasing K (i.e. parameter determining the number of mutual nearest neighbors) values up reaching an arbitrarily sufficient level of connectedness among samples.

Runs of homozygosity mapping

The analysis of certain genomic regions often shows reduced genetic diversity and stretches of autozygosity at the individual and population levels, called runs of *ROH* due to identical by descent (**IBD**) chromosomal segments arising from a common ancestor. Runs of homozygosities were computed across autosomes for each individual using PLINK 1.07. The following criteria were used to define the *ROH*: (1) one missing SNP was allowed in the *ROH* and up to one possible heterozygous genotype, (2) the minimum number of SNPs that constituted the *ROH* was set to 30, (3) the minimum SNP density per *ROH* was set to one SNP every 100 kbp (100 000 bp) and (4) maximum gap between consecutive homozygous SNPs of 250 kbp.

Runs of homozygosity distribution, inbreeding coefficient and effective population size

For each breed, the average *ROH* number (MN_{ROH}) was calculated by dividing the total number of *ROHs* by the number of samples. The average total *ROH* length (AL_{ROH}) was calculated as the total *ROH* length divided by the total number of individuals. In addition, each *ROH* was categorized based on its physical length as follows: 1 to < 5 Mbp, 5 to < 10 Mbp, 10 to < 15 Mbp, 15 to < 20 Mbp, and ≥ 20 Mbp. For each of the *ROH* length categories, the mean sum of *ROH* per breed was calculated by summing all *ROHs* (Mbp) per animal in that category and averaging this per breed. The genome-wide inbreeding coefficient, F_{ROH} > 1 Mb, was calculated for each individual using the following method (McQuillan et al., 2008): $F_{ROH} = \frac{L_{ROH}}{L_{AUT}}$ where L is the total length of all *ROHs* per animal while L_{AUT} refers to the length of total autosomal SNP coverage.

The contemporary effective population size (N_e) was estimated using NeESTIMATOR v. 2 (Do et al., 2014) according to the random mating model of the LD (Linkage Disequilibrium) method. We used a method described by Waples and Do (2008) to add a bias correction into the original LD method and a threshold of 0.05 as the lowest allele frequency to derive the least biased results. We reported our estimates with ± 95% confidence intervals.

Identification of putative genes under selection

Detection of runs of homozygosity islands

To detect the genomic regions frequently covered with *ROHs* across and within breeds (BAR, NDT and QFO), the number of times each SNP occurred in *ROH* was calculated separately in each breed. The *ROHs* repeated in more than 10% of the individuals in each breed (less than 1% of the SNPs) were considered as a potential *ROH* islands or hotspots, as suggested in previous studies (Szmatoła et al., 2016; Purfield et al., 2017; Ghoreishifar et al., 2020). Further, the frequency of *ROHs* was plotted against their physical position across individuals and within each breed.

Wright's fixation index

To validate the putatively selected SNPs within the *ROH* hotspots, we used Wright's fixation index F_{ST}-outlier analysis contrasting fat- vs. thin-tailed sheep using the BayeScan 2.1 software (Foll and Gaggiotti, 2008). This package implements an F_{ST}-based hierarchical Bayesian model using a reversible jump MCMC (Monte Carlo Markov Chain) to detect loci that are subject to selection, with F_{ST} being the Wright's fixation index (Wright, 1949). F_{ST} values were ranked, and those falling in the top 0.1% and equivalent to $F_{ST} = 0.5$ were considered as putatively under selection pressure. Genes falling in a window of 100 bp upstream and downstream the most significant SNP position were considered as significant candidate genes. Annotated genes were obtained from the *Ovis aries* reference genome version 4.1 map viewer (http://www.ncbi.nlm.nih.gov/gene/) using the NCBI website. The biological function of the annotated genes was explored via extensive literature review.

Results

Statistics for runs of homozygosity, inbreeding coefficients, and effective population size

Analysis of *ROH* distribution over total samples highlighted 607 *ROHs*, with a mean of 5.58 *ROHs* per individual, out of which 89% are less than 5 Mbp in size. Table 1 presents the descriptive statistics for runs of homozygosity, effective population size (N_e), and the genome-wide inbreeding coefficient, F_{ROH} > 1 Mbp in BAR, NDT and QFO breeds. The average length of *ROH* (AL_{ROH}) showed similar values among the three sheep breeds. The mean number of ROHs per breed ranged from 7.20 (BAR) to 12.60 (NDT). Distribution of runs of homozygosity and inbreeding coefficients (F_{ROH} > 1 Mbp) for each sheep breed are reported in Supplementary Figure S1. BAR breed showed the lowest value of (F_{ROH} > 1 Mbp) (0.017), in agreement with the MN_{ROH} results. However, the QFO revealed high variability in autozygosity levels where F_{ROH} > 1 Mbp values ranged from zero to 0.13. The highest value of

Table 1
Descriptive statistics for effective population size (Ne) runs of homozygosity (ROH) and inbreeding coefficient in the studied sheep breeds.

Breed	Ne[1]	NT_{ROH}[2]	MN_{ROH}[3]	AL_{ROH}[4]	$F_{ROH>1Mb}$[5]
BAR	376.6	180	7.20	5.87	0.017 ± 0.028
NDT	706.5	252	12.60	4.31	0.021 ± 0.009
QFO	174.0	128	9.85	6.58	0.024 ± 0.036

BAR = Barbarine; NDT = Noire de thibar; QFO = Queue fine de l'Ouest.
[1] Contemporary effective population size.
[2] Total number of ROH per breed.
[3] Mean number of ROH per individual.
[4] Average length of ROH in Mb;
[5] Mean ROH-based inbreeding coefficient with standard deviation.

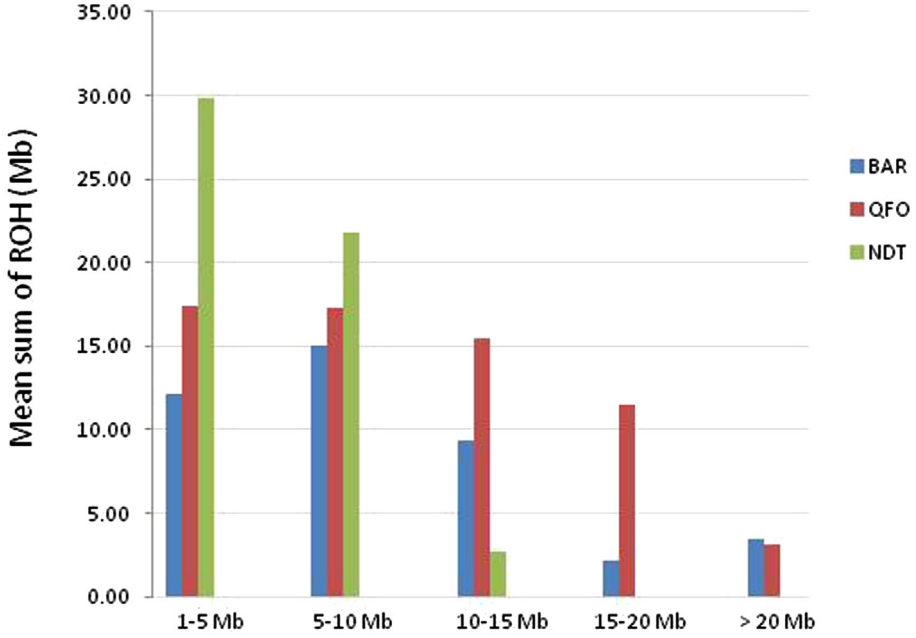

Fig. 2. Classification of runs of homozygosity (*ROH*) in five categories according to size (from 1 to 5 Mbp to more than 20 Mb) (x-axis) and mean sum of *ROH* in Mbp (y-axis) within each *ROH* length category for Barbarine (BAR), Queue fine de l'ouest (QFO), and Noire de thibar (NDT) sheep breeds.

contemporary effective population size was observed in NDT (N_e = 706.5) and the lowest in QFO (N_e = 174). The classification of runs of homozygosity (*ROH*) in five categories according to size from 1 to 5 Mbp to more than 20 Mb for each breed is shown in Fig. 2 and it shows that most *ROH*s in NDT animals are short (1–10 Mbp) while the QFO has long stretches of consecutive homozygous genotypes (10–20 Mbp).

Genomic relationships among breeds

After data filtering, the final number of animals and SNPs retained for further analyses were 58 and 46 232 respectively. The Fig. 3 illustrates the fine-scale genetic structure of BAR, NDT, and QFO sheep breeds. The MDS (Fig. 3a), Admixture (Fig. 3b), and NetView (Fig. 3c) analyses consistently highlighted a differentiation of NDT from BAR and QFO which, instead, showed a marked genetic closeness among them. A notable exception could be highlighted in the clustering of four QFO samples, and this peculiar patterning was consistently observed via MDS, Admixture (at K = 3 and 4), and NetView (at K = 5, 10 and 20) analyses (Fig. 3). These samples originated from a public farm institutionally engaged in QFO genetic conservation. The Supplementary Figure S2 showed that the most appropriate number of ancestral sub-populations inferred through the Admixture cross-validation approach was K = 1. This result confirms the weak genetic structure among Tunisian breeds. Moreover, the topology of the NetView network showed that most of the samples fall in breed-specific clusters, and starting from K = 10, a first link between BAR and QFO appears, mediated by a putatively admixed QFO sample which rather located in the BAR group in the MDS plot.

Genomic regions within runs of homozygosity hotspots

By determining the abundance of SNPs in *ROH*s, several genome regions were identified as potential *ROH* hotspots. Analysis across all individuals (n = 58) showed three genomic regions, on chromosomes 1, 10, and 13, found to contain putative *ROH* hotspots encompassing 84 significant SNPs, with total *ROH* length ranging from 0.47 Mbp in chromosome (OAR) 10 to 3.03 Mbp in OAR 13 (Fig. 4, Table 2). The highest percentage of SNPs found in *ROH* (16.67%) was observed in the chromosome 13, on the genomic region ranging 46.58 to 49.61 Mbp. The abundance of SNPs in *ROH* in BAR, NDT and QFO was nonuniform among chromosomes (Supplementary Figure S3). The chromosome position, number of SNPs, start and end position of *ROH*, and number of annotated genes within the genomic regions of extended homozygosity are reported in Supplementary Table S2. The longest *ROH* island was observed in QFO on OAR 16 (42.16 Mbp), while the shortest one was observed in NDT on OAR 13 (0.15 Mbp). The highest ROH signal was observed in chromosome 13 in BAR samples, with a mean frequency of 0.31. This signal starts at position 46 270 456 and ends at 49 619 573 bp, with a total size of 3.3 Mbp including 28 consecutive homozygous SNPs with frequency of 36%. Surprisingly, the same signal was also observed on OAR 13 when using the F_{ST}-outlier approach, located in the genomic interval 48 193 040–49 070 447 bp with a peak (F_{ST} = 0.869 at genomic position 48 552 093 bp) (Supplementary Figure S4).

Discussion

Genetic diversity and genomic relationships among breeds

The study of the breed genetic diversity based on *ROH* statistics highlighted NDT as the less consanguineous breed and characterized by *ROH* segments higher than 1 Mbp, possibly related to ancient inbreeding and not deriving from linkage disequilibrium (**LD**) patterns (Bosse et al., 2012). On the contrary, QFO looks to have experienced a recent inbreeding since animals showed high inbreeding coefficients and long *ROH*s. This observation could be at the origin of the four QFO inbred samples highlighted in the genetic structure and MDS clustering plots. Moreover, the distribution of F_{ROH} revealed that QFO individuals have high variability in autozygosity levels probably due that different farms may have adopted different strategies in managing the inbreeding problem. These results reflect in general the breed management practices that allow for uncontrolled mating of related individuals especially in smallholder farms where the exchange of rams among flocks is quite unusual. The results of the population structure (MDS, Admixture and Netview analyses) showed two clear genetic patterns of local sheep

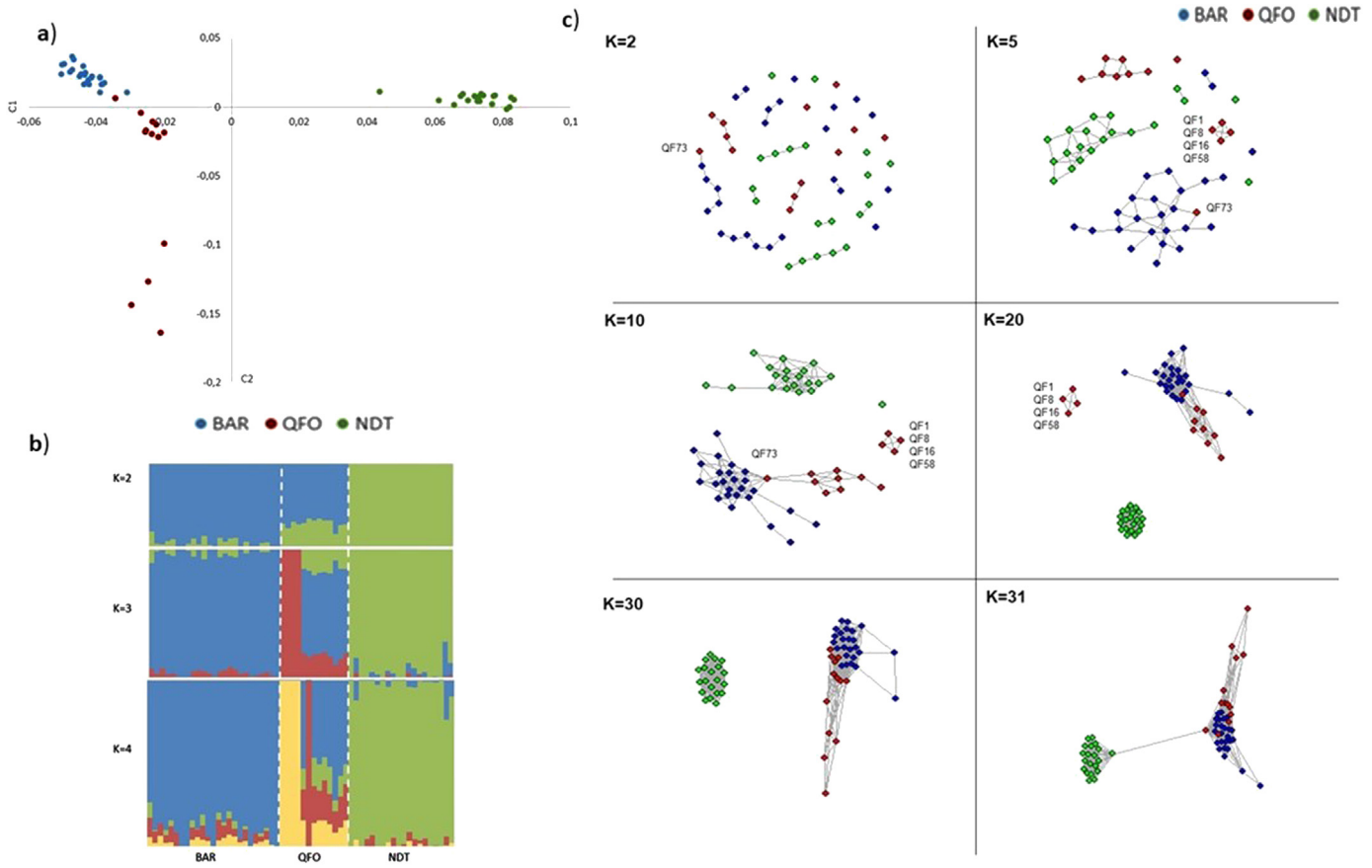

Fig. 3. Fine-scale population structure of Barbarine (BAR), Queue fine de l'ouest (QFO), and Noire de thibar (NDT) sheep breeds. a) Multidimensional scaling (MDS) clustring, b) Admixture ancestry at K = 2, 3, and 4 c) Mutual nearest-neighbor graphs obtained from NetView considering the following k = 2, k = 5, k = 10, k = 20, k = 30 and k = 31. Color shades code for different sheep breeds (BAR = blue, QFO = red and NDT = green). The highlighted QF1, QF8, QF16, QF16, QF58, and QF73 samples represent outliers from the main QFO goup.

breeds in Tunisia: i) a clear differentiation of NDT from the remaining breeds and ii) a high genetic closeness between BAR and QFO breeds; these two points were in agreement with results obtained by Bedhiaf-Romdhani et al. (2020) when using a data set of 40 samples. Indeed, the genetic differentiation of NDT from the other local Tunisian breeds as a consequence of European gene flow (Porter et al., 2016). In addition, the investigation of genomic variation between NDT and its parental breed (QFO), uncover selection signature implicated in tolerance to photosensitization by local toxic weeds (Baazaoui et al., 2020).The genetic closeness between BAR and QFO would not be surprising, if we consider the size reduction observed in the Barbarine population (Sassi-Zaidy et al., 2014) caused by massive cross-breeding between the two breeds. In fact, the shift toward thin-tailed sheep breeds at the expense of the fat tailed Barbarine was mainly due to the butchers' interests. Because of the difficulty in selling the fat of the carcass tail, butchers were reluctant to buy Barbarine animals and farmers admit that butchers' preferences are influencing income because they are paying favorably thin-tailed animals (Bedhiaf-Romdhani et al., 2008). This

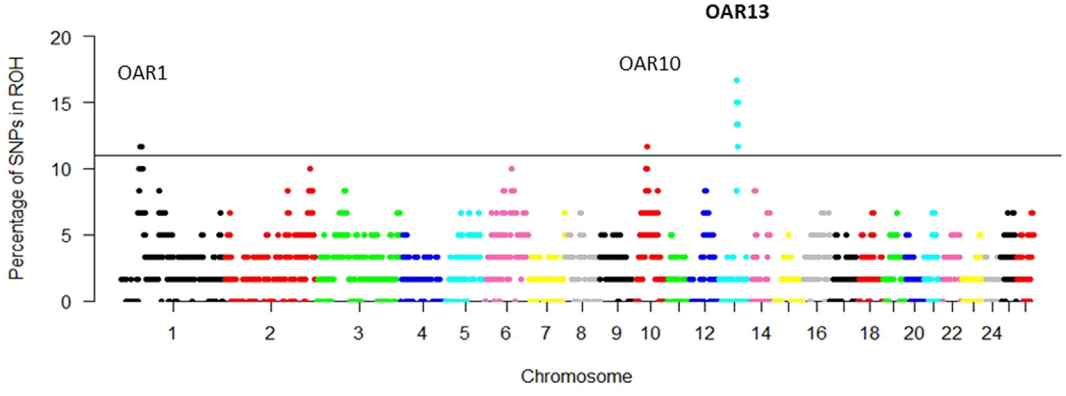

Fig. 4. Manhattan plot of the occurrence of single nucleotide polymorphisms (SNPs) in runs of homozygosity (*ROH)* islands across different chromosomes (OAR) in all sheep individuals ($n = 58$).

Table 2
Details of runs of homozygosity (ROH) hotspots detected across three Tunisian sheep breeds.

Chromosome	Mean percent[1]	SNPs[2]	*ROH* length (kbp)	Start-end positions (bp)	Genes	Genes in *ROH* hotspot
1	11.67	25	1 185	49 985 611–51 170 678	8	*LOC101120030, ERICH3, TRNAW-CCA,CRYZ, TYW3, LHX8, SLC44A5, LOC105611835*
10	11.11	9	473	32 442 135–32 915 074	10	*LOC101113604,LOC101114283, LOC101114533, LNX2, MTIF3, GTF3A, RASL11A, RPL21, LOC105609570, USP21*
13[3]	14.77	50	3 037	46 582 744–49 619 573	20	*CDS2, LOC101109379, PROKR2, GPCPD1, LOC101109635, C13H20orf196, TRNAF-GAA, CHGB, TRMT6, MCM8,LOC105609936, CRLS1, LRRN4, FERMT1, LOC106991507, LOC101117437, BMP2, LOC101117953, LOC101118207, LOC101110166*

[1] Mean percent of significant SNPs ($P > 10\%$) of occurrence in a *ROH* region.
[2] Number of loci identified within *ROH* hotspots.
[3] Chromosome 13 include the highest SNPs frequency in *ROH* region.

shift to thin-tailed breeds, if not controlled, will have a negative impact on the Barbarine breed, which is perfectly adaptated in a variety of production systems. Indeed, crossbreeding among autochthonous Northern African sheep breeds is a current practice (Othman et al., 2016; Harkat et al., 2017) for the purpose to improve production performances and cope with economic pressure. Moreover, these results are consistent with those observed in studies performed on other Maghrebin breeds, which evidenced a clear genetic homogenization among local sheep populations (Belabdi et al., 2019), attesting for similar anthropological and demographic events of Northern African sheep genetic ressources.

Detection of fat tail selection sweeps

Fat deposition in the tail of sheep is an adaptive attribute that allow to cope with periodic feed fluctuations in semi-arid and arid areas (Atti et al., 2004; Bedhiaf-Romdhani and Djemali, 2006). In this study, using both the *ROH* and the F_{ST}-outlier approches, we detected an outstanding signal of selection located on chromosome 13. We focused our attention on this signal because it represents a putatively selected genomic region significantly differentiated the Barbarine fat-tailed breed from the two thin-tailed Tunisian breeds and could harbor genes involved in fat deposition in Tunisian sheep. The genomic region within the *ROH* hotspot spanning 46.5–49.6 Mbp on OAR 13 (Table 2, Supplementary Table S2) encompasses several known genes with specific relevance in lipid metabolic processes (*CDS2*, *PROKR1, and BMP2)*. Among them, the *CDS2* gene, that is involved in the phospholipid biosynthetic process, has been shown to be an important novel regulator of lipid storage (Qi et al., 2016). The prokineticin receptor1 (*PROKR1*) has been shown to control obesity through suppression of preadipocyte proliferation and differentiation in an animal model and in humans (Yuan et al., 2017). The *BMP2* gene has been shown to play an important role in fat tail development in sheep breeds (Lu et al., 2020). It has been detected as a possible candidate gene for the fat tail phenotype in Chinese sheep (Wei et al., 2015; Yuan et al., 2017), in Italian Laticauda breeds and several Mediterranean breeds including the Lybian Barbarine (Mastrangelo et al., 2019). Thus, the observed selection signal is widespread in fat tailed sheep breeds from different geographic origins, suggesting that those breeds may share a common ancestor. Moreover, the *BMP2* gene has been reported to have an influence on regulating body size and muscle development in cattle (Ghoreishifar et al., 2020). A recent study by Pan et al. (2019) suggested that the fixation of fat tail in domestic sheep is caused by a selective sweep near a retro-transposable hotspot on OAR 13 (47 993 040–49 270 447 bp), affecting the expression of the *BMP2* gene. It was, indeed, found to be differentially expressed between fat-tailed and thin-tailed sheep individuals in tail adipose and several other tissue types. Moreover, the same study shows that *LOC101117953* is a novel gene copy derived from a retro-transposable event, originated from protein phosphatase 1 catalytic subunit gamma (*PPP1CC*) gene, located also at ovine chromosome 13. In fact the *PPP1CC* gene was evidenced as determinant of fat deposition and tail-type differences in Chinese sheep (Wei et al., 2015). Genomic structure of three local Tunisian sheep breeds investigated using medium density SNP data revealed a genetic homogenization between the thin-tailed QFO and the fat-tailed BAR breed caused by breeds intermixing and uncontrolled crossbreeding. The adaptation of Barbarine breed to harsh conditions is explained through selection signature on chromosome 13 elucidating the mechanism of tail fat deposition and providing insights into fat tail formation. This study showed that the Tunisian sheep flocks' genetic management and mating programs deserve more attention in order to preserve and valorize adaptive alleles, which are an irreversible heritage.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100193.

Ethics approval

The blood collection was conducted and approved by the Agricultural and Scientific Research Government Committees (CPERA-Tunisia) in accordance with the guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Blood samples from animals were collected by veterinarians during routine blood sampling for medical care or follow-up. All the samples in our study were obtained upon the breeder's and breeding organizations' consent. Those animals were not linked to any experimental trials.

Data and model availability statement

None of the data were deposited in an official repository.

Author ORCIDs

S. Bedhiaf-Romdhani: 0000-0002-8602-9474.
I. Baazaoui: 0000-0002-1035-3841.
S. Mastrangelo: 0000-0001-6511-1981.
E. Ciani: 0000-0001-5673-0975.

Author contributions

I. Baazaoui: Writing, Formal analysis, Investigation, and editing.
S. Bedhiaf-Romdhani: Project administration, Conceptualization, Resources, Supervision, Writing and editing.
S., Mastrangelo: Data analysis, Writing and editing.
E. Ciani: Visualization, Methodology, Writing and editing.

Declaration of interest

The authors declare no financial or commercial conflicts of interest.

Acknowledgements

We would like to thank the following members of AU-IBAR, Simplice Nouala, Mary Mbole-Kariuki and Edward Nengomasha for partial funding through the "Genetics" project. The authors are grateful to OEP members' Mounir Hemdène and Zied Abdennebi who contributed in blood sampling.

Financial support statement

This work was supported by the LABERPAR" Project (code 67), financed by the Apulia Region, Italy. The mobility grant of Imen Baazaoui was supported by the GLOBAL-DOC Project (CUP H96J17000160002) funded by the University of Bari, Italy.

Consent to participate

Both OEP and private, owners of the sheep flocks, gave permission to be included in this study.

References

Alexander, D.H., Lange, K., 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics 12, 246.

Almeida, O.A.C., Moreira, G.C.M., Rezende, F.M., Boschiero, C., de Oliveira, Peixoto J., Ibelli, A.M.G., Ledur, M.C., de Novais, F.J., Coutinho, L.L., 2019. Identification of selection signatures involved in performance traits in a paternal broiler line. BMC Genomics 20, 449.

Atti, N., Bocquier, F., Khaldi, G., 2004. Performance of the fat-tailed Barbarine sheep in its environment: adaptive capacity to alternation of underfeeding and re-feeding periods. A review. Animal Research 53, 165–176.

Baazaoui, I., McEwan, J., Anderson, R., Brauning, R., McCulloch, A., Van Stijn, T., Bedhiaf-Romdhani, S., 2020. GBS data identify pigmentation-specific genes of potential role in skin photosensitization in two Tunisian sheep breeds. Animals 10, 5.

Bedhiaf-Romdhani, S., Djemali, M., 2006. Estimation of sheep carcass traits by ultrasound technology. Livestock Science 101, 294–299.

Bedhiaf-Romdhani, S., Djemali, M., Zaklouta, M., Iniguez, L., 2008. Monitoring crossbreeding trends in native Tunisian sheep breeds. Small Ruminant Research 74, 274–278.

Bedhiaf-Romdhani, S., Baazaoui, I., Ciani, E., Mastrangelo, S., Sassi, M.B., 2020. Genetic structure of Tunisian sheep breeds as inferred from genome-wide SNP markers. Small Ruminant Research 191, 10–192.

Belabdi, I., Ouhrouch, A., Lafri, M., Gaouar, S.B.S., Ciani, E., Benali, A.R., Ouelhadj, H.O., Haddioui, A., Pompanon, F., Blanquet, V., 2019. Genetic homogenization of indigenous sheep breeds in Northwest Africa. Scientific Reports 9, 7920.

Bosse, M., Megens, H.-J., Madsen, O., Paudel, Y., Frantz, L.A., Schook, L.B., Crooijmans, R.P., Groenen, M.A., 2012. Regions of homozygosity in the porcine genome: consequence of demography and the recombination landscape. PLoS Genetics 8, e1003100.

Chalh, A., El Gazzah, M., Djemali, M., Chalbi, N., 2007. Genetic and phenotypic characterization of the Tunisian noire De Thibar lambs on their growth traits. Journal of Biological Sciences 7, 1347–1353.

Do, C., Waples, R.S., Peel, D., Macbeth, G., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (ne) from genetic data. Molecular Ecology Resources 14, 209–214.

Foll, M., Gaggiotti, O., 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics 180, 977–993.

Galal, S., Boyazoglu, J., Hammond, K., 2000. Developing breeding strategies for lower input animal production environments. International Committee for Animal Recording (ICAR), Technical Series 3. Italy, Rome.

Gammoudi, A., Bedhiaf, S., 2015. Molecular characterization and genetic distance evaluation between two sheep breeds: the Tunisian Barbarine and Tunis sheep in USA. Proceedings of the 6th international symposium of animal functional genomics, 27-29 July 2015. Piacenza Università Cattolica del Sacro Cuore, Italy, p. 45.

Ghoreishifar, S.M., Moradi-Shahrbabak, H., Parna, N., Davoudi, P., Khansefid, M., 2019. Linkage disequilibrium and within-breed genetic diversity in Iranian Zandi sheep. Archives Animal Breeding 62, 143.

Ghoreishifar, S.M., Moradi-Shahrbabak, H., Fallahi, M.H., Moradi-Shahrbabak, M., Abdollahi-Arpanahi, R., Khansefid, M., 2020. Genomic measures of inbreeding coefficients and genome-wide scan for runs of homozygosity islands in Iranian river buffalo, Bubalus bubalis. BMC Genetics 21, 16. https://doi.org/10.1186/s12863-020-0824-y.

Harkat, S., Laoun, A., Belabdi, I., Benali, R., Outayeb, D., Payet-Duprat, N., Blanquet, V., Lafri, M., Da Silva, A., 2017. Assessing patterns of genetic admixture between sheep breeds: case study in Algeria. Ecology and Evolution 7, 6404–6412.

He, S., Di, J., Han, B., Chen, L., Liu, M., Li, W., 2020. Genome-wide scan for runs of Homozygosity identifies candidate genes related to economically important traits in Chinese merino. Animals 10, 524.

Iniguez, L., 2005. Characterization of small ruminant breeds in West Asia and North Africa. volume 1. ICARDA, Aleppo, Syria.

Lu, Z., Liu, J., Han, J., Yang, B., 2020. Association between *BMP2* functional polymorphisms and sheep tail type. Animals 10, 739.

Mastrangelo, S., Tolone, M., Sardina, M.T., Sottile, G., Sutera, A.M., Di Gerlando, R., Portolano, B., 2017. Genome-wide scan for runs of homozygosity identifies potential candidate genes associated with local adaptation in Valle del Belice sheep. Genetics, Selection, Evolution 49, 84.

Mastrangelo, S., Bahbahani, H., Moioli, B., Ahbara, A., Al Abri, M., Almathen, F., Da Silva, A., Belabdi, I., Portolano, B., Mwacharo, J.M., 2019. Novel and known signals of selection for fat deposition in domestic sheep breeds from Africa and Eurasia. PLoS One 14, e0209632.

McQuillan, R., Leutenegger, A.-L., Abdel-Rahman, R., Franklin, C.S., Pericic, M., Barac-Lauc, L., Smolej Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., 2008. Runs of homozygosity in European populations. American Journal of Human Genetics 83, 359–372.

Meyermans, R., Gorssen, W., Wijnrocx, K., Lenstra, J., Vellema, P., Buys, N., Janssens, S., 2020. Unraveling the genetic diversity of Belgian Milk sheep using medium-density SNP genotypes. Animal Genetics 51, 258–265.

Moioli, B., Pilla, F., Ciani, E., 2015. Signatures of selection identify loci associated with fat tail in sheep. Journal of Animal Science 93, 4660–4669.

Moradi, M.H., Nejati-Javaremi, A., Moradi-Shahrbabak, M., Dodds, K.G., McEwan, J.C., 2012. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of candidate regions associated with fat deposition. BMC Genetics 13, 10.

Neuditschko, M., Khatkar, M.S., Raadsma, H.W., 2012. NetView: a high-definition network-visualization approach to detect fine-scale population structures from genome-wide patterns of variation. PLoS One 7, e48375.

Nicolazzi, E.L., Caprera, A., Nazzicari, N., Cozzi, P., Strozzi, F., Lawley, C., Pirani, A., Soans, C., Brew, F., Jorjani, H., 2015. SNPchiMp v. 3: integrating and standardizing single nucleotide polymorphism data for livestock species. BMC Genomics 16, 1–6.

Office de l"Elevage et des Pâturages, 2018. Indicateurs du secteur de l'élevage en Tunisie. Ministère de l'Agriculture, des Ressources Hydrauliques et de la Pêche, Tunis, Tunisia.

Othman, O.E.M., Payet-Duprat, N., Harkat, S., Laoun, A., Maftah, A., Lafri, M., Da Silva, A., 2016. Sheep diversity of five Egyptian breeds: genetic proximity revealed between desert breeds: local sheep breeds diversity in Egypt. Small Ruminant Research 144, 346–352.

Pan, Z., Li, S., Liu, Q., Wang, Z., Zhou, Z., Di, R., An, X., Miao, B., Wang, X., Hu, W., 2019. Rapid evolution of a retro-transposable hotspot of ovine genome underlies the alteration of BMP2 expression and development of fat tails. BMC Genomics 20, 261.

Peripolli, E., Munari, D., Silva, M., Lima, A., Irgang, R., Baldi, F., 2017. Runs of homozygosity: current knowledge and applications in livestock. Animal Genetics 48, 255–271.

Porter, V., Alderson, L., Hall, S.J., Sponenberg, D.P., 2016. Mason's world encyclopedia of livestock breeds and breeding, 2 volume pack. CABI Publishing, Wallingford, UK.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics 81, 559–575.

Purfield, D.C., McParland, S., Wall, E., Berry, D.P., 2017. The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. PLoS One 12, e0176780.

Qi, Y., Kapterian, T.S., Du, X., Ma, Q., Fei, W., Zhang, Y., Huang, X., Dawes, I.W., Yang, H., 2016. CDP diacylglycerol synthases regulate the growth of lipid droplets and adipocyte development. Journal of Lipid Research 57, 767–780.

Rochus, C.M., Jonas, E., Johansson, A.M., 2020. Population structure of five native sheep breeds of Sweden estimated with high density SNP genotypes. BMC Genetics 21, 1–9.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Sarson, M., 1973. Ovine in the antiquity after Phonician and Roman traces in Tunisia and Algeria (Les ovins dans l'antiquité d'après les vestiges phéniciens et romains en Tunisie et en Algérie). AGRIS, FAO, Rome, Italy.

Sassi-Zaidy, Y.B., Maretto, F., Zanetti, E., Hajji, G., Charfi-Cheikrouha, F., Cassandro, M., 2014. Genetic structure and variability within and among populations of the fat-tailed Barbarine sheep breed using microsatellites markers. African Journal of Biotechnology 13, 1.

Steinig, E.J., Neuditschko, M., Khatkar, M.S., Raadsma, H.W., Zenger, K.R., 2016. Netview p: a network visualization tool to unravel complex population structure using genome-wide SNPs. Molecular Ecology Resources 16, 216–227.

Szmatoła, T., Gurgul, A., Ropka-Molik, K., Jasielczuk, I., Ząbek, T., Bugno-Poniewierska, M., 2016. Characteristics of runs of homozygosity in selected cattle breeds maintained in Poland. Livestock Science 188, 72–80.

Tolone, M., Mastrangelo, S., Rosa, A., Portolano, B., 2012. Genetic diversity and population structure of Sicilian sheep breeds using microsatellite markers. Small Ruminant Research 102 (18), 25.

Waples, R.S., Do, C., 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8, 753–756.

Wei, C., Wang, H., Liu, G., Wu, M., Cao, J., Liu, Z., Liu, R., Zhao, F., Zhang, L., Lu, J., 2015. Genome-wide analysis reveals population structure and selection in Chinese indigenous sheep breeds. BMC Genomics 16, 194.

Wright, S., 1949. The genetical structure of populations. Annals of Eugenics 15, 323–354.

Xu, S.S., Ren, X., Yang, G.L., Xie, X.L., Zhao, Y.X., Zhang, M., Shen, Z.Q., Ren, Y.L., Gao, L., Shen, M., 2017. Genome-wide association analysis identifies the genetic basis of fat deposition in the tails of sheep (Ovis aries). Animal Genetics 48, 560–569.

Yuan, Z., Liu, E., Liu, Z., Kijas, J., Zhu, C., Hu, S., Ma, X., Zhang, L., Du, L., Wang, H., 2017. Selection signature analysis reveals genes associated with tail type in Chinese indigenous sheep. Animal Genetics 48, 55–66.

Zhao, F., Deng, T., Shi, L., Wang, W., Zhang, Q., Du, L., Wang, L., 2020. Genomic scan for selection signature reveals fat deposition in Chinese indigenous sheep with extreme tail types. Animals 10, 773.