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Estimation of runs of homozygosity reveals moderate autozygosity in Northern European sheep breeds

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Abstract Runs of homozygosity (ROH) stretches are continuous homozygous fragments of the genome, which are more suitable for calculating the genomic inbreeding, identifying footprints involved in economic traits and understanding the population history in livestock species. In this study, using a dataset of Ovine SNP50 BeadChip genotypes, the distribution of ROH in nine sheep populations (Soay n= 110, Australian Poll Dorset n=108, Australian Suffolk n=109, Finn sheep n= 99, Scottish Texel n= 80, Scottish Blackface n= 56, Galway n= 49, Border Leicester n= 48, German Texel n=46) from Europe were investigated. ROHs were detected by using PLINK v1.09 with the minimal number of SNPs in ROH set at 40; the maximal gap between the adjacent SNPs was set to 1Mb; the minimum SNP density per ROH was set to 1/100 kb and no heterozygote less than 16 Mb was allowed. The detected ROHs were specified to four categories, based on their length: 1–4 Mb, 4–8 Mb, 8–16 Mb and above 16 Mb. A total of 22,204 ROHs were identified, in which ~ 92 – 98 % were less than 16 Mb in length, covering 4.6% to 12.9% of the entire genome. The inbreeding coefficient based on ROH (F_{ROH}) varied among populations (ranging from 0.05 to 0.14). The highest inbreeding rate was found in Border Leicester and Soay breeds. In addition, we detected 90 possible ROH Islands that overlapped with candidate genes associated with different economic traits such as the body weight, meat production, fat deposition, horn-less, and coat color in sheep. Our results suggested that although genetic selection for meat and wool traits in these breeds have been extensively carried out during the last decades, the autozygotic proportion of the genome is still considerably low, and it could lead to an acceptable response to selection in breeding schemes.

Keywords: autozygosity, genomic inbreeding, runs of homozygosity, ROH Islands, sheep

Introduction

Inbreeding is a phenomenon that results from the mating or breeding of closely related organisms. The increase in inbreeding, which may increase the chances of the expression of deleterious recessive alleles, has resulted in several disadvantages such an increase in the widespread distribution of lethal or harmful recessive variants as well as decreases in animal performance and producer's profitability (Bjelland et al., 2013; Gurgul et al., 2016). The conventional method for estimating the inbreeding is based on pedigree information which ignores relatedness among the founders in the base population, and does not take into account the recombination, potential bias resulting from selection, and prevalent errors in sheep and cattle pedigrees (Ferencaković et al., 2013a). Therefore, it is vital to assess the inbreeding level in the populations by using accurate methods. Indeed, incomplete pedigrees with low

generation may underestimate the inbreeding measures (Gurgul et al., 2016). Nowadays, the availability of large number of genetic markers, such as high-density SNPs panels and whole-genome sequence data, have made it possible to estimate the genome autozygosity and calculate the inbreeding coefficients.

Generally, autozygosity occurs when individuals are homozygous at the same DNA segments from common ancestors, which are identical by descent (IBD). This pattern of inheritance gives rise to unusually long continuous homozygous segments, that are defined as runs of homozygosity (ROH) (Carothers et al., 2006). ROH can be characterized as the genetic relatedness among individuals (Howrigan et al., 2011; Zavarez et al., 2016), because it can clarify the autozygotic segment due to IBD comparing to non-autozygotic segments (Howrigan et al., 2011). Previous studies reported a high frequency of short ROHs in the genome, indicating inbreeding by individuals with more ancient common ancestors; however, long ROH presumably indicates that recent inbreeding arose within a pedigree (McQuillan et al., 2008, Kirin et al., 2010). The number of inbred generations and the history of recent selection events can be derived from the extent and frequency of ROH regions (Howrigan et al., 2011; Mastrangelo et al., 2018). In addition, the pattern of ROHs can provide helpful information for populations structure, demography history, and identification of trait-associated ROH in homozygosity mapping studies (Bosse et al., 2012; Herrero-Medrano et al., 2013). The first study on ROH was carried out in the human genome (Carothers et al., 2006; Gibson et al.,2006). In livestock, the first studies on ROH were performed on cattle by Solkner et al. (2010) and Ferencakovic et al. (2011), with further studies reported by others (Purfield et al., 2012; Bjelland et al., 2013; Ferencakovic et al., 2013a; Kim et al., 2013, Asadollahpour Nanaei et al., 2020). In pigs, the first studies on ROH were conducted to highlight the influence of population relationships, demographic history, and the effects of inbreeding on homozygosity (Bosse et al., 2012; Herrero-Medrano et al., 2013).

Sheep and goats were the first livestock animals to be domesticated over ~11,000 years ago around the Fertile Cresent (Chessa et al., 2009; Ruiz-Larrañaga et al., 2020). About 5000 years ago, sheep were transported to Europe. As agriculture spread in Europe during the Neolithic Age, several sheep breeds were developed (Lawson Handley et al., 2007). Today, half of the world's sheep breeds exist in Europe (Peter et al., 2007). Over the past few decades, due to the increasing demands for various animal products, sheep breeders have focused on improving the meat and other economic traits in several breeds with higher genetic potential, which has led to a decrease in the effective population sizes (*Ne*) and exposed them at the risk of extinction (Lawson Handley et al., 2007; Peter et al., 2007). It seems, the herdbook breeding and intensive management, especially in northern and northwestern Europe, have a significant effect on the decrease in genetic diversity and enhanced inbreeding in sheep breeds (Peter et al., 2007; Prieur et al., 2017). Monitoring of inbreeding in these breeds is prerequisite to genetic conservation and protection from extinction, since erosion of diversity can be a problem even in breeds with large population sizes. Few ROH studies based on SNP chip data have been performed on sheep, especially on the studied breeds herein. Moreover, the epigenome comprising different mechanisms e.g., DNA methylation, remodeling, histone tail modifications, chromatin microRNAs, and long non-coding RNAs, interact with environmental factors like nutrition, pathogens, and climate to influence the genes expression profile, and the emergence of specific phenotypes (Barazandeh et al., 2019). Multi-level interactions between the genome, epigenome and environmental factors might occur. Furthermore, numerous lines of evidence suggest the influence of epigenome variation on livestock production (Barazandeh et al., 2019; Moradian et al., 2019). Therefore, the objectives of this work were i) to identify and characterize the ROH patterns and determine the genomic inbreeding inferred from ROH in nine European sheep breeds, ii) to estimate the effective population size for the evaluating selection events, and detect endangered breeds, iii) to investigate the ROH Islands for sheep gene content in the segments shared by most of the populations.

Materials and methods

Breeds, genotyping, and quality control

Nine European sheep populations (n=705) were selected to include the meat, wool and dual-purpose breeds. The Genotyping data of the Illumina OvineSNP50 panel, available by the International Sheep Genomics the web-interfaced next generation database dedicated to genetic diversity exploration (http://widde .toulouse.inra.fr/widde/widde/main.do?module=sheep).Breed names and their abbreviations are listed in Table 1. Only the SNPs on autosomes were included in this analysis; the markers located in the centromere region, and did not map to any chromosome, were removed.

We first filtered out SNPs with minor allele frequency (MAF) < 0.05 from the dataset, because statistical power was deficient for rare SNPs. Moreover, the individuals with missing call rate > 0.05 were also removed. The latest release of the ovine genome sequence assembly, Oar_v4.0, was used to obtain the SNPs genomic coordinates. The data were not pruned based on the linkage disequilibrium (LD); however, to exclude the short ROHs derived from LD, the minimum length of an ROH was set to 1 Mb (Ferencaković et al., 2013a).

ROHs detection and genomic inbreeding coefficients

The ROHs were detected with PLINK v1.09 whole-genome association analysis toolset (Purcell et al., 200-

7). To define the ROHs, the following criteria were used: (i) the minimal number of SNPs in ROH was set to 40; (ii) the maximal gap between the adjacent SNPs was set to 1 Mb; (iii) the minimum SNP density per ROH was set to 1 SNP every 100 kb; (iv) no heterozygote less than 16 Mb was allowed (assuming a genotype error rate of 0.2 %); (v) based on the ROH length, the number of missing genotypes was defined as follows: ROH >1 Mb — 0 missing, >4 Mb — 1 missing, >8 Mb — 2 missing, and ROH > 16 Mb — 3 missing calls; (v) the minimum length that constituted the ROHs was set to 1 Mb. Based on the length, the detected ROHs were specified to four categories: 1–4 Mb, 4–8 Mb, 8–16 Mb and above 16 Mb. All ROHs per animal were summed, and averaged per breed, to calculate the mean sum of ROH per breed. This value was also calculated for each category. The total number, mean number and average length of ROHs were calculated for each breed. In addition, the percentage of the chromosomes covered by ROH was calculated.

Table 1. Sheep breeds and production purpose (Kijas et al., 2012)

Breeds	Abbreviation	Number of animals	Production purpose
Australian Poll Dorset	ADP	108	Meat, wool
Australian Suffolk	ASU	109	Meat, wool
Soay	SOA	110	Wool, meat
Finn sheep	FIN	99	Wool, meat
Scottish Texel	STX	80	Meat, wool
Scottish Blackface	SBF	56	Meat, wool
Galway	GAL	49	Meat, wool
Border Leicester	BRL	48	Meat, wool
German Texel	GTX	46	Meat, wool
Total	9	705	

The identified ROHs were then used to estimate an inbreeding coefficient for each animal (F_{ROH}), using the following formula:

$$F_{ROH} = \frac{\sum_i length\,(ROH_i)}{L}$$

where, i = number of ROHs detected for each animal, and L = total length of the genome covered by SNPs in each breed (Table 2).

Effective population size

The magnitude of *Ne* could be calculated from the molecular data using the measure of LD via r^2 coefficient. The SNeP (v1.1) was used to estimate the effective population size for each breed (Barbato et al., 2015) using the default settings (MAF ≥0.05, ≤ 100,000 SNPs for each chromosome, the minimum distance between SNPs: 10 kb, and maximum distance between SNPs: 20,000 kb). Also, *Ne* was inferred by using the genetic mapping of ROH length (Thompson 2013) whereby the map length of ROH (l) = 100/2g cM, where g is the number of intended generations. This analysis would provide information on F_{ROH} for four different periods; up to <3, 3-6, 6-12, and 12-50 generations ago, which corresponded to $ROH_{>16Mb}$, $ROH_{8\text{-}16Mb}$, $ROH_{4\text{-}8Mb}$, and $ROH_{1\text{-}4Mb}$, respectively.

Identification of the ROH Islands and gene annotation within breeds

Genomic regions most commonly related to ROH, were identified by analyzing the output files generated by the Plink software. To calculate the proportion of SNPs occurrence in ROH, the appearance time of SNPs in ROH were divided by the number of animals in each breed and plotted against the position of the SNP along the chromosomes. The top 1% SNP occurrences was marked as a threshold. The SNP regions with a frequency higher than 1% were specified as possible ROH Islands (Mastrangelo et al., 2019). Genomic coordinates of the identified ROH Islands were used for annotating the genes that were fully or partially located within each selected region using the UCSC Genome Browser (http://genome.ucsc.edu/). Finally, a literature survey was conducted to investigate the biological function of each annotated gene contained in the ROH Islands.

Results

Characterization of ROHs

A total number of 22,204 ROHs were identified (Table 2), which differed clearly between the studied breeds, ranging from 994 (SBF breed) to 5,542 (SOA breed). All animals represented at least one $ROH_{1\text{-}4Mb}$ (705 individuals), and almost one $ROH_{4\text{-}8Mb}$ (697 individuals). The majority of the detected ROHs (92.2 - 98.2%) were less than 16 Mb in length, and the $ROH_{1\text{-}4\ Mb}$ was most frequent in all populations, except in BRL and SOA breeds in which the $ROH_{4\text{-}8\ Mb}$ was prevalent (Figure 1b, supplementary material Table S1). Among all breeds, the Scottish Texel (STX) and German Texel (GTX) breeds exhibited more frequent homozygote segments in 1-4 Mb (42.6%) and inversely, the highest frequency of ROH >16 Mb was observed in Finn sheep (FIN) (7.8%) followed by SBF (6.1%) and Galway (Gal) (5.9%) breeds (Figure 1b).

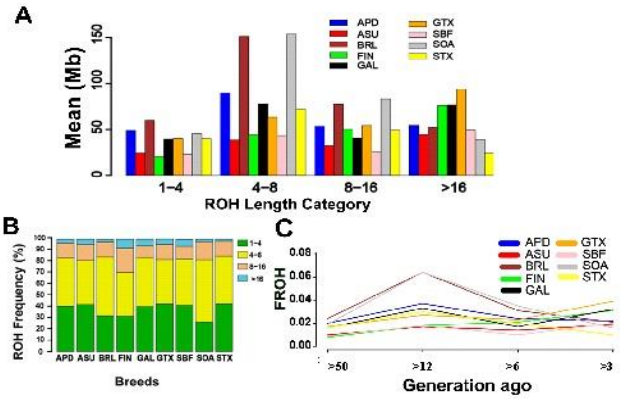

Figure 1. Analysis of ROH for nine sheep breeds. (a) Mean sum of ROHs in each category for all populations. The ROH length in each categories were summed and averaged by individual in each breed (b) the frequency of ROHs in each categories. (c) The trend of F_{ROH} in >50 ($F_{ROH>1}$), < 12 ($F_{ROH>4}$), < 6 ($F_{ROH>8}$), < 3 ($F_{ROH>16}$) generations ago.

The mean sum of ROH lengths (MSRL) was different among the breeds (Figure 2). The Border Leicester (BRL) (12.73%), and Soay (SOA) (12.91%) breeds had the highest MSRL, and most portions of their genomes were located in ROHs (Table 2). Also, in these two breeds, more than half of the homozygote segments were in the $ROH_{4\text{-}8\ Mb}$ category (Figure 1b). In the remaining breeds, MSRL per animal ranged from 110.6Mb (ASU) to 225.7 Mb (APD) (Figure 2, supplementary material Table S3). In each category, MSRL per individual was modified between 20 Mb in $ROH_{1\text{-}4MB}$ for FIN to 153Mb in $ROH_{4\text{-}8MB}$ for the SOA breed (Figure 1a).

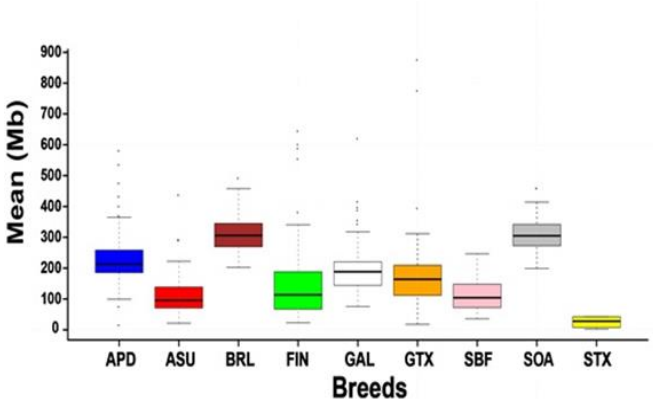

Figure 2. The overall mean sum of ROHs for each breed. The ROH values for animal were summed and averaged per breed (Mb).
Soay (SOA), Australian Poll Dorset (APD), Australian Suffolk (ASU), Finn sheep (FIN), Scottish Texel (STX), Scottish Blackface (SBF), Galway (GAL), Border Leicester (BRL), German Texel (GTX).

The percentage of the autosome covered by ROHs was as low as 1.5% in chr11 for the SOA population to as high as 21.1% in chr23 for the SBF population (Figure 3). The number of ROHs per chromosome was higher in chr1 and chr2 for all breeds, and as chromosome length decreased, chromosome coverage by ROH tended to increase.

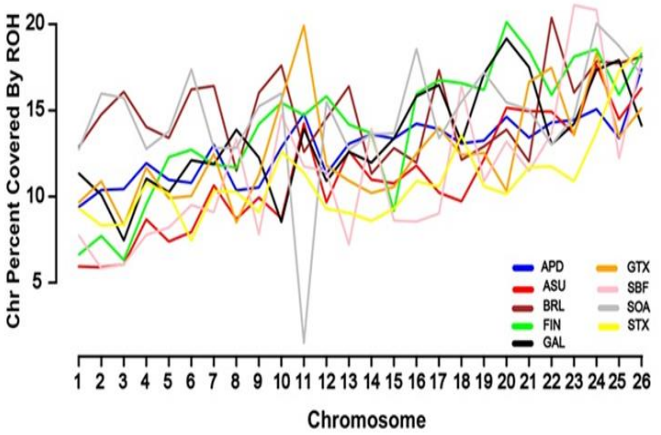

Figure 3. Chromosome percentage covered by ROHs per breed. All breeds had ROH on all chromosomes.
Soay (SOA), Australian Poll Dorset (APD), Australian Suffolk (ASU), Finn sheep (FIN), Scottish Texel (STX), Scottish Blackface (SBF), Galway (GAL), Border Leicester (BRL), German Texel (GTX)

Genomic inbreeding and effective population size

The mean F_{ROH} values varied among breeds and categories (Table 3). The highest value was recorded for SOA (0.134) and BRL (0.14) breeds, and the lowest values for ASU (0.05), SBF (0.05), followed by STX (0.06) (Table 2). In general, F_{ROH} ranged from 0.008 to 0.064 in categories and as the minimum length of ROHs increased, it tended to increase except for BRL, SOA, and STX breeds (Figure1c). The highest value of $F_{ROH1\text{-}4}$ were observed for BRL (0.024) followed by APD breed (0.020), and the largest values of $F_{ROH>16}$ were detected for GTX (0.039) followed by FIN (0.031).

Figure 4 represents the historical trends in *Ne.* For all generations, *Ne* differed between populations and declined during 3520 to 3 previous generation. In three generations ago, STX and GAL approximately had the smallest estimated *Ne*, followed by GTX and BRL, whereas FIN and ASU had the highest value. The other breeds were intermediate between these two groups.

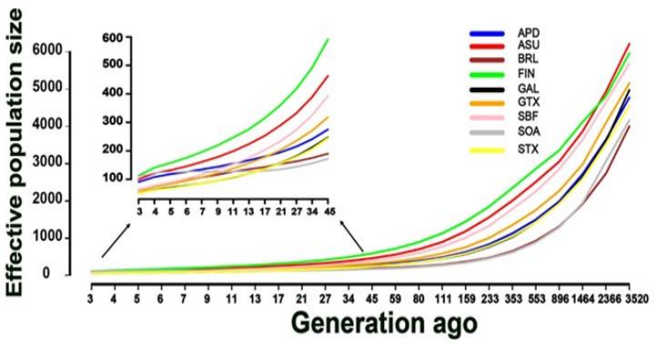

Figure 4. The effective population size in sheep breeds. The decline trend was notably different among breeds Soay (SOA), Australian Poll Dorset (APD), Australian Suffolk (ASU), Finn sheep (FIN), Scottish Texel (STX), Scottish Blackface (SBF), Galway (GAL), Border Leicester (BRL), German Texel (GTX)

Table 2. The statistical characterization of the detected ROHs and F_{ROH} in nine sheep breeds

Breeds	Total No. of ROHs	Average length of ROHs(Mb) (Min-Max)	Average Number of ROHs	Genome coverage by SNPs (Mb)	Genome coverage by ROHs>1 (%)	F_{ROH1-4}	F_{ROH4-8}	$F_{ROH8-16}$	$F_{ROH>16}$	F_{ROH}
APD	4,111	5.92 (1.55-77.76)	38.06	2437.50	9.24	0.020	0.037	0.022	0.022	0.09
ASU	1,924	6.18 (1.71-59.93)	17.65	2304.87	4.80	0.010	0.017	0.014	0.019	0.05
BRL	2,541	5.88 (2.04 - 41)	52.94	2437.20	12.73	0.024	0.064	0.031	0.021	0.0.14
FIN	1,959	7.62 (1.81-76.29)	18.79	2430.80	6.21	0.008	0.018	0.021	0.031	0.062
GAL	1,538	6.56 (2.18-66.51)	31.39	2392.40	8.60	0.016	0.033	0.017	0.032	0.09
GTX	1,346	6.35 (1.96-56.96)	29.26	2376.25	7.81	0.017	0.027	0.023	0.039	0.08
SBF	994	6.42 (1.95-47.75)	17.75	2437.60	4.67	0.009	0.018	0.010	0.02	0.05
SOA	5,542	6.10 (2.19-68.36)	50.38	2159.60	12.91	0.019	0.064	0.035	0.016	0.134
STX	2,249	5.43 (2.08-28.67)	28.11	2437.75	6.34	0.016	0.03	0.02	0.010	0.06

ROH Islands

The frequency of possible ROH Islands varied between breeds and positions of the genome; however, some common islands were observed on chromosomes 1, 2, 3, 4, 5, 6, 7, 10 and, 23 (Figure 5). A total of 90 regions - in different parts of the genome were identified in all breeds. These regions ranged in size from 27.43 kb to 17.65 Mb with the mean length of 1.42 Mb ASU to 5.32 Mb GTX. More detailed statistics on the ROH Islands are presented in Table 3.

Table 3. Characterization of the detected ROH Islands in nine sheep breeds

Breeds	Total No. SNPs	Average SNPs appearance on ROH (%)	Average percentage of top 1% SNPs appearance on ROHs (Min-Max)	ROH Islands No.	Mean length of ROH Islands (Mb)(Min-Max)	Chr with ROH Islands
APD	42,024	9.353	28.008 (24.073-49.074)	12	1.974 (0.186-5.73)	1,2,3,4,8,10,13,23
ASU	44,311	4.529	13.004 (11.927-16.513)	16	1.424 (0.027-4.16)	1,2,3,4,6,7,10,11,19
BRL	36,725	12.833	42.236 (39.583-52.083)	7	4.091 (0.138-6.31)	1,2,3,7,23
FIN	43,187	6.546	14.289 (13.131-16.162)	12	2.114 (0.181-6.14)	1,2,4,5,6,8,13,19
GAL	41,245	8.525	26.789 (24.490-32.653)	10	2.433 (0.441-3.91)	1,2,3,6,7,8,13
GTX	42,830	7.669	37.023 (23.913-50)	5	5.321 (0.497-17.65)	1,2,5,22
SBF	43,255	4.510	19.581 (16.071-26.876)	9	2.626 (0.071-9.13)	1,2,3,6,9,10
SOA	33,909	13.045	40.425 (33.636-56.364)	12	2.644 (0.256-8.86)	2,3,5,6,10,16,25
STX	41,256	6.308	33.196 (26.25-47.5)	6	5.019 (0.041-15.18)	2,5,8,16,26

We identified 85 genes in 37 regions within the 90 possible ROH Islands for all breeds (supplementary material Figure S1 and Table S2). At least five genomic regions were detected throughout the genome for each breed as possible ROH Islands. Two peaks were especially intense; in the highest peak, 56% of the individuals (SOA breed) shared SNP positions in ROH on chromosome 6 (38,545,309-40,423,137). The second intense pattern was observed on chromosome 2 (67,632,592-69,928,905) and 3 (168,193,451-170,984,254) which were detected on 52% of BRL population.

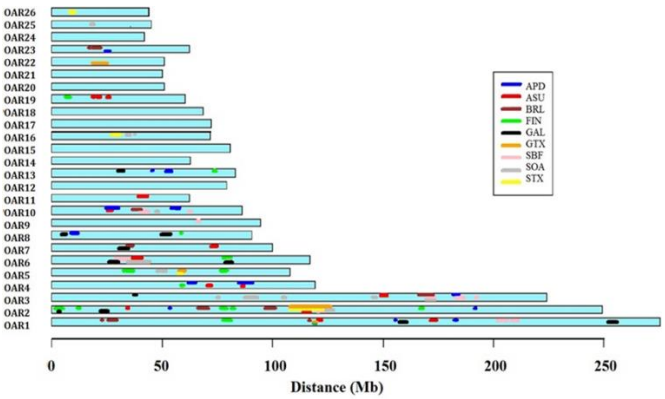

Figure 5. Distribution of ROH Islands throughout genome in sheep breeds. the ROH Islands were not uniformly distributed across all chromosomes.

Soay (SOA), Australian Poll Dorset (APD), Australian Suffolk (ASU), Finn sheep (FIN), Scottish Texel (STX), Scottish Blackface (SBF), Galway (GAL), Border Leicester (BRL), German Texel (GTX).

Discussion

Characterization of ROHs

An ROH greater than 1Mb covered about 4.6% to 12.9% of the genome in all breeds. The BRL and SOA breeds were relatively more inbred based on the high ROH genome coverage (more than 12%). The mean sum of ROH lengths (MSRL) in these two breeds was approximately one to three times higher than other breeds, with most of them related to ROH4-8Mb, which indicated a small population size, closed mating system and/or high artificial selection pressure in these breeds. With regard to their population history, the SOA sheep originated from a small, and isolated population in Soay Island. Long geographical isolation during several years has led to the high level of inbreeding in this breed (McHugo et al., 2019). The BRL breed was also developed from Dishley Leicester rams during the 17th century in England. During breed formation, BRL endured bottlenecks (Al-Mamun et al., 2015). Previously, the largest total number of ROHs (12,561) and mean sum of ROH1-4 (126.06 Mb) for BRL were --

reported compared to Poll Dorset and Merino breeds (Al-Mamun et al., 2015). The highest frequency of ROH >16 was detected in FIN and GTX breeds; in fact, 30-40% of MSRL in these two breeds were related to ROH>16. Regarding the trend of $F_{ROH,}$ Figure 1c illustrates a steady increase in autozygosity up to three previous generations in these breeds. The highest numbers of ROH1-4Mb were detected in STX and GTX, which are originated from the same breed in different geographical regions, confirming the ancient relatedness among them. The ROH1–4 Mb category contained 26-43% of the ROH segments in all breeds; however, it constituted a small proportion of the entire genome. Similar results were reported in other studies (Purfield et al., 2012; Marras et al., 2014; Mastrangelo et al., 2016; Mastrangelo et al., 2019). while some part of this conflict may be related to the fact that the 50k SNP overestimates the ROH1-4 due to low SNP density (Ferencakovic et al., 2013b). The heterozygous SNPs on the denser chip, could not be identified by 50k SNP panel (Ferencakovic et al., 2013b). Therefore, the number and length of ROH estimated from SNP chip may suffer from ascertainment biases (Lencz et al., 2007; Albrechtsen et al., 2010; Kirin et al., 2010; Pemberton et al., 2012; Purfield et al., 2012; Zhang et al., 2015, Asadollahpour Nanaei et al., 2020). To overcome this, we excluded the SNPs with MAF less than 5% to create the consensus panel of SNPs. It seems, in our European sheep breeds which are associated with Ovine 50k BeadChip, the ascertainment bias did not invalidate the trends in observed ROHs level as the mean sum of ROH and also F_{ROH} was higher in breeds with lower numbers of heterozygote loci (supplementary material Figure S2, supplementary material Table S3). In cattle, compared to sheep, the frequency of ROH1-4Mb is equals to only 24% for some local breeds (Zhang et al., 2015). This illustrated that the historical inbreeding in sheep breeds is more relevant compared with the local cattle breeds.

Genomic inbreeding and effective population size

The higher values of F_{ROH} were detected in SOA and BRL breeds which are considered at extinction risk (Food and Agriculture Organization, 2019). This result was compatible with *Ne* size in these two breeds. It is suggested that they need to have suitable breeding strategies to preserve the genetic diversity; however, the declining trend in F_{ROH} from 12 to >3 grenerations ago illustrated that this strategy has been started (Figure 1c). The two breeds with lower values of F_{ROH} were ASU and SBF (Table 2), which had higher effective population size (Figure 1c) In agreement with our results, McHugo et al. (2019) reported the highest and lowest F_{ROH} values for SOA and SBF breeds, respectively. Previous studies reported large variability in F_{ROH} and MSRL in the Suffolk breed (Purfield et al., 2017). We found that F_{ROH} ranged from 0.008 (F_{ROH1-4}) to 0.064 (F_{ROH4-8}), which are in agreement with other reports on sheep (Albrechtsen et al., 2010; Burren et al., 2016; Kim et al., 2016; Purfield et al., 2017; Mastrangelo et al., 2017), and goat (Guangul et al., 2014; Kim et al., 2016), but smaller than the values for cattle (Marras et al., 2014; Gurgul et al., 2016) and pig (Bosse et al., 2012; Herrero-Medrano et al., 2013). It seems that these sheep populations are less inbred and have preserved their genetic diversity compared to the pig and cattle populations.

The effective population size showed a declining trend in all populations (Figure 4). However, initially, the downward slope was steeper in some breeds such as BRL and SOA, which may have resulted from the smaller *Ne* and higher F_{ROH}. In our study, the estimated *Ne* was less than previous reports in the same breeds (Al-Mamun et al., 2015; McHugo et al., 2019), which could be due to differences in the populations origin, software, and procedure to estimate *Ne*. However, the ranking of *Ne* concords with the largest (FIN and ASU), and smallest (BRL, GTX, STX and GAL) in the same breeds. Morever, it has been reported that the estimation of *Ne* is significantly affected by some factors such as the method of estimation. In addition, the amount of *Ne* estimated based on LD, can be underestimated due to the physical linkage between SNPs (Prieur et al., 2017; McHugo et al., 2019). The effective population size in three generation ago for APD, ASU and FIN breeds was higher than the critical threshold (*Ne*>100), which may represent suitable levels of variability in these breeds, that are essential for long-term survival. In remaining breeds, in which *Ne* was less than 100, the F_{ROH} in BRL and STX steadily decreased from 12 to <3 generations ago; however, in GAL and GTX, it decreased to 6 generations ago and then increased (up to <3 generations ago). It seems in BRL and STX, avoiding to close-mating strategy has continued up to 3 generations ago, while in GTX and GAL, mating between close relatives has increased further from 6 generations ago. The GAL, a native breed of Irish sheep, is currently considered at-risk (Food and Agriculture Organization, 2019). McHugo et al. (2019) reported that this breed has an intermediate ROH level, genomic inbreeding, and effective population size compared to conventional and endangered breeds in Europe. Here, we showed that the mean sum of ROH and genome coverage by ROH>16 was slightly higher than intermadiate in this breed. Also, for effective population size, it had the smallest or close to the smallest value in all generations.

In general, it seems, despite of, domestication and breeds formation, improvements in meat and wool traits during several years, and effective population size in these breeds, in comparison to cattle, have kept an acceptable level of genetic diversity, which are important for selection response in the breeding scheme. Other researchs reported adequate level of genetic diversity in sheep (Chessa et al., 2009; McHugo et al., 2019).

ROH Islands and genes characterization

Our findings demonstrated that the 90 possible ROH Islands were not uniformaly distributed in the breeds and

genome. It could be due to the varying recombination rates, LD, and selection across the genome (Al-Mamun et al., 2015; Zhang et al., 2015; Mastrangelo et al., 2019). The positive correlation between the number of ROH in chromosome with the average values of LD, and the negative correlation between the rate of recombination and ROH size were reported (Herrero-Medrano et al., 2013).

We identified 85 genes in 37 regions within the 90 possible ROH Islands for all breeds. It was revealed that several SNPs located in ROHs, occurred in regions with a few or no genes. This may be due to incomplete annotation of the ovine genome (Mastrangelo et al., 2017), ROH Islands located in the non-coding regions, selection performed on uncharacterized regulatory regions, or genetic drift causing fixation of non-coding DNA (Qanbari et al., 2011). Similar results have been reported on Italian sheep breeds (Mastrangelo et al., 2017).

In OAR2 between 108.2 to 125.9 Mb, ROH Islands with different lengths detected in six breeds (ASU, FIN, SBF, GTX, SOA, and STX). This region also found in Australian sheep breeds (Al-Mamun et al., 2019). The HERC2-like gene in this region affects skin pigmentation and hair color in humans (Han et al., 2008). Therefore, this region in OAR2 in sheep may be under selection. We also identified *IGF*-1 (for BRL and SOA breeds) a candidate gene on OAR3 for meat production, which mediates the stimulatory effect of growth hormone, and testosterone on the growth and development of muscle fibers (Meira et al., 2019). The identified candidate genes within the longest ROH Islands, OAR6 (38.5-40.4), were *SLIT2*, *PACRGL* and *KCNIP4*, all of which are involved in fat deposition, body weight and growth rate (Mastrangelo et al., 2016; Mastrangelo et al., 2019). Riggio et al. (2013) detected SNPs (chr 6:33.2-37.7 Mb) associated with body weight at different ages (from 6 to 24 weeks of age) in Scottish Blackface lambs. In Australian sheep, the SNPs between 36.15, and 38.56 Mb on OAR 6 were associated with carcass fatness, lean meat yield, intra-muscular fat and dressing percentage (Daetwyler et al., 2012). In general, detecting some of known genes related to meat production and quality such as *MSTN*, *MYOZ*, *IGF-1* and *CAPN3* (Clop et al., 2006; Fang et al., 2013; Wan et al., 2013; Meira et al., 2019;) and also genes affecting wool production and pigmentation such as *MCR1* and *KRT* (Fontanesi et al., 2009; Fontanesi et al., 2010; Sulayman et al., 2018), confirmed that most of sheep breeds in the current study have been selected for meat and wool production.

Conclusion

In conclusion, our results revealed that the distribution of ROHs in the genome differed among sheep breeds, and ROH>1 covered on the average about 4.6% to 12.9% of the genome in all breeds. The majority of the detected ROHs was less than 16 Mb. The BRL and SOA breeds were relatively more inbred based on F_{ROH}; however; A-SU and SBF had the smallest value for F_{ROH}. The effective population size for APD, ASU and FIN breeds was higher than the critical threshold (Ne>100) in three previous generations. The majority of SNPs located in ROHs, occurred in the regions with a few or no gene; however, most of the detected genes were related to meat production and quality, wool production and pigmentation, the capacity of heat tolerance, immunity response, and polledness. Overall, our findings revealed despite the fact that breeding for meat and wool traits is ongoing in most North European sheep populations, the autozygotic proportion of their genome is still considerably adequate, and this could lead to an acceptable response to selection in breeding schemes.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

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