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VARIOUS APPROACHES FOR THE GENOMIC ANALYSIS OF THE GENETIC STRUCTURE OF DIFFERENT CATTLE POPULATIONS

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CHAPTER 1

General introduction

Introduction

The *Bos* species can be distinguished in two subspecies: *Bos taurus taurus* (B. taurus; Figure 1), which is humpless and farmed in Europe, Middle East, North and West Africa, and *Bos taurus indicus* (B. indicus; Figure 2), humped and present in arid regions such as India, and eastern Africa (Bruford et al., 2003).

Figure 1. *Bos taurus taurus* (https://www.ncbi.nlm.nih.gov).

Figure 2. *Bos taurus indicus* (https://www.ncbi.nlm.nih.gov).

Modern cattle descend from the extinct wild aurochs, the *Bos primigenius*, which was endemic over most of Asia, Europe, and North Africa (Felius et al., 2014), and which was firstly domesticated about 8,500 years BC. There are two main hypotheses about cattle domestication. The first theory was proposed by Epstein and Mason (1984) and it asserted that taurine cattle had been directly domesticated from the aurochs; all the modern cattle breeds, both humped and humpless, derived from this domestication as the result of breeding and selection occurred in different places according to the human migrations from the Near East. The second theory suggested an independent domestication of the *B. indicus* in the actual Pakistan (Meadow, 1993). This was confirmed by Loftus et al. (1994a), who compared *B. taurus* from Europe and Africa with *B. indicus* from India and Africa: the Indian zebu showed very different mtDNA restriction fragment length polymorphism profiles compared to both *B. taurus* and other zebu populations; these differences date back much earlier than the cattle domestication. Thus, the most accredited hypothesis is that the differences between *B. taurus* and *B. indicus* are due to different subspecies of *B. primigenius* domesticated in different regions. In fact, three subspecies of *Bos primigenius* existed in three different regions: the *Bos primigenius namadicus* in Asia, the *Bos primigenius opisthonomus* in North Africa, and the *Bos primigenius primigenius* in Europe (Loftus et al., 1994b). The presence of different subspecies located in different regions leads to the theory of independent domestication events: one in the actual Pakistan and another in the Fertile Crescent (Helmer et al., 2005); moreover, a third independent domestication may have happened in Africa (Bradley et al., 1996), where the *B. taurus* has been domesticated before the arrival of *B. indicus* (Bradley et al., 1998; Hanotte et al., 2002; Pérez-Pardal et al., 2010).

In any case, after the domestication, cattle followed the human migrations, reaching Europe and Asia, and then Africa, where they probably intermixed with the respective local wild animals (Beja-Pereira et al., 2006; Ajmone-Marsan et al., 2010). During the domestication, cattle became smaller in size (Tchernov and Horwitz, 1991), and, after the migrations, adapted to the different environments.

At the beginning of the animal breeding, farmers managed the reproduction favoring individuals with better phenotypes leading to animals that better met the farmers needs and that were adapted to the local environment (Taberlet et al., 2011). This first selection shaped the cattle genome, giving birth to the breeds (Purfield et al., 2012). The breeds were then improved, starting from the 18th century, by the genetic isolation of phenotypically homogenous groups (Felius et al., 2014) to standardize their morphology and performances (Taberlet et al., 2011). Moreover, from the 19th century, some breeds, such as Holstein-Friesian, were exported out of their region of origin, and this led to the development of regional varieties of the same cosmopolitan breed (Felius et al., 2015).

Domestication, natural and artificial selection, migrations, adaptation, and crossbreeding during the breeds history left the so called "selection signatures" or "selective sweeps". In fact, these different events or processes drafted the genome through an increase of specific mutations frequencies, which probably gave to the animals the ability to respond to various challenges, such as human demands or adaptation (Fan et al., 2010).

Breeds can be considered as genetic reservoirs, because each one contributes to the diversity of the specie since they contain a portion of the livestock variation (Felius et al., 2015). At the same time, the intra-breed selection led to a decrease in the genetic diversity and, thus, to an increase in homozygosity (Felius et al., 2014). Moreover, in the last half century, the within-breed homozygosity increased because of the wide use of the artificial insemination, for which only a few males have been involved in the reproduction schemes. Another side effect of this process could be identified in the reduction of the effective population sizes (Taberlet et al., 2011).

The specialized breeds, more productive and thus more profitable, have gradually replaced the traditional local breeds, resulting in a loss of genetic variability (Groeneveld et al., 2010; Medugorac et al., 2009). As a consequence of this replacement, the cattle industry lost its plasticity needed to respond to changes in the market demands, environment or other future challenges (Ajmone-Marsan et al., 2010). For this reason, the autochthonous breeds represent a reservoir of genes that should be maintained to ensure a proper variability within the specie (Giovambattista et al., 2001). Indeed, if locally adapted populations are conserved, also the genes responsible of this adaptation are conserved (McKay and Latta, 2002). Moreover, the local breeds are well adapted to extensive systems based on natural grazing, which can be not suitable for specialized breeds; for this reason, the products of the local autochthonous breeds are economically important in environments where genetically improved breeds may not fit (Felius et al., 2014). To maintain the abovementioned "reservoir of genes", a special effort should be put on conserving genetic diversity, i.e., the genetic variation among breeds (Lenstra et al., 2012). In fact, local breeds are usually small populations and, thus, it is important to maintain their genetic diversity through accurate management and breeding strategies (Mastrangelo et al., 2016).

In Italy, a project to maintain and preserve dual-purpose cattle breeds ("Dual Breeding Project", "Le razze bovine a duplice attitudine: un modello alternativo di zootecnia eco-sostenibile", http://www.dualbreeding.com/it/) has been financed by the European Agricultural fund for Rural Development (EAFRD) through the National Rural Development Program (Programma di Sviluppo Rurale Nazionale, PSRN, 2014/2020;

Sub-measure 10.2 “Support for conservation, use and sustainable development of genetic resources in agriculture – Activities for the characterization of the livestock genetic resources and for the protection of biodiversity”). The aims of this project were: i) to maintain the genetic biodiversity of the dual-purpose cattle breeds farmed in Italy, ii) to preserve the autochthonous breeds with limited diffusion, iii) to increase the production efficiency by improving the disease resistance and the animal welfare. A total of 16 dual-purpose cattle breeds were involved in the project. In order to meet the aim of the project, various phenotypic and genetic analyses were expected. In particular, animals belonging to all breeds have been genotyped with SNP BeadChip to highlight the genetic diversity among them. The following paragraphs will present details about these tools.

Single Nucleotide Polymorphisms

Single Nucleotide Polymorphisms (SNPs) are bi-allelic genetic markers widely distributed throughout the genome (Fan et al., 2010) that identify a variation of a single nucleotide. In 2009, the Bovine Genome Sequencing and Analysis Consortium produced the first reference sequence of taurine genome; during the sequencing process, a total of about 2.2 million SNPs have been identified. Thanks to this sequencing, commercial BeadChips (Figure 3), that allow to genotype an animal with up to hundreds of thousand SNPs, were developed.

Figure 3. BovineSNP50 v3 DNA Analysis BeadChip (www.illumina.com).

BeadChip allows to automatically identify SNPs in the DNA extracted from blood, saliva, or semen. Most of the BeadChips developed for cattle are reported in Table 1. Among the Illumina SNP chips, the overlapping rate ranged from 74% (between Bovine 3k and the LD v1.1 or v2) to 99%; 2,084 SNPs were found in all the considered Illumina SNP chips. The average overlapping rate among the GeneSeek chips was 86±15%, and it ranged from 53% (between the LDv2 and the HD) to 98%. As far as the HD SNP chips were considered, the GPP HD shared 97% of the markers with the Illumina BovineHD BeadChip, whereas the GGPHD v2 the 96%. Lower overlapping rates were observed for the only one HD Affymetrix chip considered in Table 1, i.e. 17% with Illumina BovineHD BeadChip and from 5% to 8% with the two GGP HD SNP chips. A total of 1,863 SNPs was found in common among all the SNP chips.

The availability of genotyped animals with large amount of SNPs provided the opportunity to clarify the genetic relationship among breeds, to investigate the history of the breed and to identify genomic regions and gene associated with particular traits of interest (Fan et al., 2010). Moreover, studies on population genetics, other than investigating the historical events that made the formation of the specific breed, can assist in preserving the genetic diversity within breeds (Fan et al., 2010).

The development of these dense SNP arrays made more accurate and reliable the investigation on both homozygosity and heterozygosity, and the interpretation of their signals.

Table 1. List of the most common Beadchips developed for cattle genotyping.

Producer	BeadChip name	Number of SNPs
Illumina	Bovine 3k	2,900
	BovineLD	6,999
	BovineLD v1.1	6,912
	BovineLD v2	7,931
	BovineSNP50v1	54,001
	BovineSNP50v2	54,609
	BovineHD	777,962
GeneSeek	Dairy Ultra LD v2	7,049
	GGP LD v1	8,610
	GGP LD v2	19,721
	GGP LD v3	26,151
	GGP HD	76,879
	GGP HD v2	139,480
Affymetrix	Axiom BovMDv3	65,003
	Axiom Bovine	648,875

Runs of Homozygosity

Runs of homozygosity (ROH) are continuous stretches of homozygous genotypes (Gibson et al., 2006) without heterozygosity in the diploid state. These homozygous regions were firstly introduced by Broman and Weber (1999) in human populations, and they were reported to be related to autozygosity, i.e., the result of the mating of related individuals which pass shared chromosomal segments (identical by descent, IBD) to their progeny. The ROH occurrence has been reported to have negative implications on human health. This latter hypothesis was confirmed by Lencz et al. (2007), who found an association between ROH and the genetic risk for Schizophrenia; in the same study, the name "Runs of Homozygosity" has been proposed for the first time. These findings about an association between ROH and health were confirmed even in livestock (e.g., Biscarini et al., 2014; Mészáros et al., 2015), because autozygosity is related to an increase of homozygosity and to a loss of genetic variation, and thus to an higher inbreeding level. Inbreeding is known to increase the risk of many disorders (Howrigan et al., 2011) because of the possible presence of deleterious recessive alleles inside a ROH (Mc Parland et al., 2009; Ferenčaković et al., 2013a; Peripolli et al., 2017). In addition, inbreeding could be associated with a loss in performance and vitality of the offspring, resulting in the so-called "inbreeding depression" (McParland et al., 2007).

Gibson et al. (2006) deepened the ROH investigation introducing the statistics about these regions, studying the implications of their number, length, and distribution: the results showed that i) ROH were more common in regions with high linkage disequilibrium (LD) and low recombination rate, ii) ROH mapped in similar genomic regions across different populations, and iii) the length of the homozygosity regions reflected the relatedness of the ancestry. Indeed, the length of ROH is a temporal indicator of the inbreeding occurrence: long ROH are due to recent inbreeding (VanRaden et al., 2011), whereas shorter ROH are the results of the breaking of the original long segment due to meiotic recombination, which involves a decrease of the length during the progression of generations (Forutan et al., 2018). Consequently, a long ROH can be divided in a larger number of shorter ROH and, thus, the average number of these regions per individual tend to increase over the generations.

Since ROH are correlated with autozygosity, a ROH-based inbreeding coefficients (F_{ROH}) was proposed by McQuillan et al. (2008) to represent the proportion of genome that is autozygous. F_{ROH} is calculated as the ratio between the total genome length covered by ROH and the total length covered by SNPs. As reported by Forutan et al. (2018), F_{ROH} is the inbreeding coefficient closest to the true inbreeding, with a correlation close to 1. In fact, since ROH technique is sensitive even to short regions (according to the parameters used), the F_{ROH} can detect both recent and old inbreeding (Ferenčaković et al., 2013a; Marras et al., 2015). For this reason, it is more comprehensive compared to the traditional pedigree-based inbreeding coefficient that usually consider only few generations. Moreover, pedigree files can contain errors and unknown parenthood, or they are not even recorded, whereas the computation of inbreeding using genomic data, e.g., F_{ROH}, can be done also for animals without known relatives.

Since ROH can be due to mating of related individuals, they are more frequent after demographic events that decrease the effective population size, such as bottlenecks and genetic drift (Purfield et al., 2012).

ROH are associated to selection pressure (Gaspa et al., 2014): they are more common in population under artificial selection because the individuals with high genetic merit usually have a large contribution in the population; this obviously increases the rate of inbreeding (Forutan et al., 2018). Additionally, the number of ROH per individual differ between genomic and traditional breeding schemes (Forutan et al., 2018): the latter is usually associated to a lower (+ 0.57±0.01 per year) increase in the ROH occurrence compared to the genomic selection (+ 2.1±0.05 per year).

Under natural or artificial selection, ROH can also arise from the fixation of favorable alleles that tend to increase their frequency and, thus, to be fixed in the population; this variation of allele frequency may extend to the neighboring loci due to linkage disequilibrium, resulting in a ROH (Cesarani et al., 2021). In fact, short ROH may represent an ancestral region, known as LD hotspots, which persist in the population due to the lack of recombination (Marras et al., 2015).

The ROH seem to be not randomly distributed across the genome, and, thus, an association between ROH and selection signatures can be observed. For example, Kim et al. (2013) reported that two-third of selection signatures found in a Holstein population overlapped with ROH highlighted in another population of the same cattle breed. Moreover, it is interesting to analyze which genes are mapped in ROH that are shared among individuals of the same breed or among different breeds to trace events at population level (Gorssen et al., 2021).

For all these reasons, ROH features can be used to investigate the history of the investigated population (Kirin et al., 2010; Peripolli et al., 2017) or the differences between breeds with different management: e.g., autochthonous breeds generally show very different ROH patterns (i.e., number, length, and distribution) compared to commercial cattle breeds (Szmatola et al., 2016). Moreover, ROH can be an important tool to account for inbreeding when managing reproduction in small populations in which genetic diversity needs to be maintained, especially because the pedigree is usually not available in those populations (Peripolli et al., 2017).

ROH can be detected using different methods and software, such as PLINK, Golden Helix, GERMLINE SNPandVariation suite (SVS), BEAGLE, and R (Horwigan et al., 2011; Peripolli et al., 2017). Two approaches have been mostly adopted: the sliding window or the consecutive runs. The former works with a window of fixed size whereas the latter scans the genome SNP by SNP. Both approaches can be used choosing different parameters to define a ROH (e.g., minimum number of SNPs, minimum length, number of heterozygous or missing SNPs allowed within the region). The ROH detection is strongly influenced by the BeadChip density (Purfield et al., 2012). The HD SNP panel (~800k SNPs) seems to be the one that allow the more accurate detection in cattle: as the SNP density increases, the number of detected ROH increases and the length decreases, because among the new added SNPs there are probably heterozygous or missing SNPs that broke the continuity (Hillestad et al., 2018). This means that the detection is more accurate, avoiding falsely long ROH and allowing the detection of shorter ROH. In any case, at low density (~50k SNPs) almost all the ROH longer than 5 Mb are recognized, but the detection tends to be less accurate for the shorter ROH (Purfield et al., 2012).

Finally, ROH detection is influenced by the adopted parameters, for which, up to now, there is not a consensus. The differences in the parameters used to define a ROH make difficult to compare results among studies, and this represents a particular problem when ROH are used to estimate inbreeding (Hillestad et al., 2018). In general, when heterozygote SNPs are allowed inside a ROH, the detection of false ROH increases (Ferenčaković et al., 2013b), especially when using low SNP density (Marras et al., 2015). On the contrary, the number of missing SNP does not strongly affect the detection because generally the data are filtered before, and the adoption of a call rate > 95% tends to avoid the problem (Hillestad et al., 2018).

Runs of Heterozygosity

Runs of Heterozygosity (ROHet) were firstly introduced by Williams et al. (2016) in a study on the Chillingham cattle. ROHet are defined as consecutive stretches of heterozygous SNP genotypes or as heterozygosity-rich regions (Marras et al., 2018). The Chillingham is a rare cattle breed of the northern England that is highly inbred (i.e., 95% of the genome was covered by ROH) because it remained a close herd for at least 350 years. Williams et al. (2016) observed that the heterozygous loci were not homogeneously distributed across the genome, but clustered in specific regions, probably under balancing selection. Indeed, they suggested that those clustered heterozygous regions could contain genes with a major effect on fitness and resistant to genetic drift. Balancing selection is a form of natural selection due to the persistence of multiple variants of a given trait within populations (Llaurens et al., 2017) via selective advantage of heterozygous genotypes (Fijarczyk and Babik, 2015). Indeed, heterozygosity, and therefore ROHet, can contribute to avoid deleterious homozygotes which can increase the environmental adaptation, fitness (Santos et al., 2021) and disease protection (Samuels et al., 2016).

After the first pilot study, Ferenčaković et al. (2016) performed a ROHet analysis on austrian Pinzgauer cattle, in which, taking into consideration the top 0.1% SNPs in runs, 11 regions were investigated. The genes mapped in those heterozygosity-rich regions confirmed the hypothesis that ROHet can harbor genes important for biological processes (e.g., immune and inflammatory response) and under balancing selection.

In the last years, more studies focused on ROHet in cattle. Biscarini et al. (2020) investigated the genome of the Maremmana cattle breed, and they found out that the results were not comparable with the two abovementioned studies because of the differences in methodologies and SNP arrays. However, the common finding was that ROHet were for sure rarer and shorter than ROH. This is because heterozygotes represent only a small portion of the genome and, thus, the ROHet are usually fewer than ROH (Ablondi et al., 2022).

The study by Biscarini et al. (2020) represents the first sensitivity analysis on the parameters for the ROHet detection, performed varying the missing and homozygous SNPs allowed inside the region. Because of the nature of this type of region, the number of both missing and opposite SNPs was found to affect the detection much more than what is reported for ROH. In addition, Biscarini et al. (2020) firstly identified the ROHet islands by selecting SNPs that were inside a ROHet in more than 25% of the individuals. Using this criterion, only three islands were identified, located on BTA6, BTA14, and BTA21; the genes mapped in these regions were related to immune resistance, reproduction traits, and evolutionary response. This pointed out again how these regions can harbor loci involved in fitness and resistance traits, and how a high level of genetic diversity can be important to face different challenges.

ROHet islands were also analyzed by Hidalgo et al. (2021), who defined them by selecting the top 0.1% SNP of the distribution. The three ROHet islands detected

(BTA1, BTA8, and BTA13) mapped genes related with reproduction traits, other than with the mammary gland functionality and milk production.

A second sensitivity analysis on ROHet detection was performed by Mulim et al. (2022) by changing the gap between consecutive SNPs, and the number of homozygous and missing markers allowed within the region. They found out that the parameter with the greatest influence on ROHet detection was the number of homozygous SNPs allowed, whereas the number of missing SNPs resulted significant for only one breed. On the contrary, the gap between consecutive SNPs had no effect on the number of ROHet detected. Moreover, they compared the power of detection using two different genotyping densities (i.e., 35K and 50K) and they concluded that the use of at least the 50K is suggested. This is because, as far as for ROH, the lower density panels can underestimate the number of regions due to the larger distance between consecutive markers distributed along the genome.

After these first studies on this quite new technique, only other two studies have been published on cattle (Lashmar et al., 2022; Kenny et al., 2022), but few studies have been conducted in the other livestock species. Among the studies about ROHet in pigs (Chen et al., 2022; Bordonaro et al., 2023), Ruan et al. (2022) analyzed the genome of two different populations of Duroc pig and open new insights on ROHet shared between different populations, which might be related with survival rate and fitness. Indeed, the ROHet shared between the two populations included genes related to fitness, immunity, and reproduction traits. Moreover, the same study adapted the F_{ROH}, providing a heterozygosity-based coefficient to evaluate the proportion of the genome covered by runs of heterozygosity.

ROHet in goats have been investigated only by Li et al. (2022), who focused on the ROHet shared between two chinese indigenous breed with the aim to search for balancing selection, validating it using the Tajima’s D test.

As far as the ROHet studies in sheep are concerned, Selli et al. (2021) performed a sensitivity analysis about the parameters used for the ROHet detection in sheep, confirming that the homozygous SNPs allowed in a ROHet affect the number of detected regions and their length.

Finally, three (Santos et al., 2021 and 2023; Ablondi et al., 2023) and one (Marras et al., 2018) studies investigated the ROHet features in horse and turkey, respectively.

Objective of the Thesis

This work is structured into a general introduction, three chapters of experimental contributions and general conclusions.

The first Chapter contains the general introduction, aiming to provide a synopsis about some techniques used to decipher the genetic diversity between and within cattle breeds.

The first research contribution (Chapter 2) concerned the investigation of several cattle breeds raised worldwide to highlight the genomic regions associated to the environmental adaptation.

The last two research contributions (Chapters 3 and 4) focused on the Runs of homozygosity and heterozygosity of Italian cattle breeds. Different approaches were tested in order to identify the best method to detect selection signatures in the genome of cattle.

Finally, the general conclusions reported in Chapter 5 offered a short summary of the main results obtained from the research contributions reported in the PhD thesis.

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CHAPTER 2

Analysis of runs of homozygosity of cattle living in different climate zones

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Abstract

Aim of this study was to analyze the distribution and characteristics of runs of homozygosity in *Bos taurus taurus* and *Bos taurus indicus* breeds, as well as their crosses, farmed all around the word. With this aim in view, we used SNP genotypes for 3,263 cattle belonging to 204 different breeds. After quality control, 23,311 SNPs were retained for the analysis. Animals were divided in seven different groups: 1) Continental taurus, 2) Temperate taurus, 3) Temperate indicus, 4) Temperate composite, 5) Tropical taurus, 6) Tropical indicus, and 7) Tropical composite. The climatic zones were created according to the latitude of their country of origin: i) Continental, latitude ≥ 45°; ii) Temperate, 45°< Latitude >23.26°; iii) Tropics, Latitude ≤ 23.26°. Runs of homozygosity were computed as 15 SNPs spanning in at least 2 Mb; number of ROH per animal (nROH), average ROH length (meanMb), and ROH-based inbreeding coefficients (FROH) were also computed. Temperate Indicus showed the largest nROH, whereas Temperate Taurus the lowest value. Moreover, the largest meanMb was observed for Temperate Taurus, whereas the lowest value for Tropics Indicus. Temperate Indicus breeds showed the largest F_{ROH} values. Genes mapped in the identified ROH were reported to be associated with the environmental adaptation, disease resistance, coat color determinism, and production traits. Results of the present study confirmed that runs of homozygosity could be used to identify genomic signatures due to both artificial and natural selection.

Keywords

Runs of Homozygosity, Taurus, Indicus, Hybrid, cattle breeds, environmental adaptation

Introduction

Modern cattle breeds arise from the extinct wild auroch (*Bos Primigenius*), that has been domesticated about 8.500 years ago in the Fertile Crescent, during the early Neolithic development of pastoralism (Diamond, 2002; Magee and Bradley, 2006). There are two main hypotheses about the origins of *Bos taurus taurus* and *Bos taurus indicus*. The first is that *Bos taurus taurus* derived from *Bos primigenius*, and then *Bos taurus indicus* would have been derived from *Bos taurus taurus* because of artificial selection during pastoral movements to India, in 4000 BC approximately (Magee and Bradley, 2006). The second hypothesis, which is largely accepted, is that modern cattle arose from two different domestications of aurochs, and *B. taurus indicus* would derive from a southern Asian subspecies of aurochs, the *B. primigenius namadicus* (Bradley et al., 1998).

In any case, the modern breeds are the result of a complex interaction between natural and artificial selection, and adaptation to environment (Gautier et al., 2010; Taberlet et al., 2011). *Bos taurus indicus* is characterized by a shoulder hump and is diffused in Eurasia and eastern Africa, whereas *Bos taurus taurus* has no hump and it is diffused mainly in Europe, North and West Africa, and Middle East (Bruford et al., 2003). Indicine cattle usually present larger ears and excess skin across the entire ventral midline, especially around the neck and chest (Utsunomiya et al., 2019). The long exposure to harsh environments shaped their genome, with lower nutritional demands and more adapted to digest low-quality forages (Hennessy et al., 2000; Obeidat et al., 2002).

After indicine and taurine post-Neolithic diffusion across Eurasia and Africa, they have been crossed to obtain hybrids with intermediate characteristics. Because of different evolution histories, the genomes of animals belonging to the three different types (taurus, indicus, and composite) living under distinct environmental conditions, may harbor different selection signatures (Senczuk et al., 2021). Among the techniques used to investigate these signals, Runs of Homozygosity (ROH) are getting an increasing popularity. ROH are continuous homozygous chromosomal segments identical by descendent (IDB) (Gibson et al., 2006). The length of the ROH is a temporal indicator of the inbreeding occurrence: recent inbreeding results in longer ROH, whereas old inbreeding is associated with shorter ROH because recombination events tend to break the segments. ROHs can be also due to either artificial or natural selection because homozygous genotypes arise from the fixation of favorable alleles at selected loci. Different studies have reported relationships between ROH and local adaptation or production and reproduction traits (e.g., Mastrangelo et al., 2017; Doekes et al., 2019; Cesarani et al., 2021; Antonios et al., 2021).

As already mentioned, also the environmental adaptation left traces in the genome of the species. In the literature there are increasing examples of ROH associated to environmental adaptation in different species. Àlvarez et al. (2020) found significant regions in the Djallonkè sheep, a West African local breed, harboring some genes associated to immunity, resistance to infections and to parasites, and response to stress, which are important functions for the adaptation to the environment of the hot-humid and trypanosome challenged area in which this breed is farmed. Peripolli et al. (2020), analyzing the ROH hotspots in indicine and taurine Brazilian cattle breeds, found two genes involved in the temperament and body size traits, which are associated with stress responsiveness and climate condition tolerance, respectively. Other candidate genes were associated with parasite vector resistance, reproduction traits, coat color, and morphology traits. All these traits are related with the local environmental adaptation and could have helped these breeds to overcome the many stressing factors typical of several Brazil environments. Another example of environmental selection signatures in the genome of livestock is the study of Cesarani et al. (2022) about the Sarda dairy sheep breed. These authors analyzed the genome of ewes farmed at different altitude levels (plain, hill, and mountain) and they reported differences in ROH lengths and frequencies among the three groups, suggesting the role of the environment in the evolution of the analyzed breed and the possible existence of different subpopulations.

Given the multiplicity of environments where taurine and indicine breeds (and their crosses) are farmed, it could be of interest to test if relationships among ROH and adaptation reported by analyses carried out at local level can be confirmed worldwide. With this aim in view, we investigated the distribution of ROH in the genomes of several indicine and taurine cattle (and their hybrids) farmed all around the world to characterize the ROH patterns and to detect ROH islands that contain the candidate genes related to specific traits.

Materials and methods

Animals and genotypic data

In this study, SNP genotypes for a total of 3,263 cattle belonging to 204 different breeds were available. Genotypes were retrieved from previously published studies (Decker et al., 2014; François et al., 2017; Upadhyay et al., 2017; Ben Jemaa et al., 2018; Ramljak et al., 2018; Mastrangelo et al., 2018; Yurchenko et al., 2018) or online public repository and were merged in a previous study (Mastrangelo et al., 2020). All individuals were genotyped with the Bovine SNP50K BeadChip (v1 and v2). Several of these available datasets contained filtered SNPs; therefore, they did not report all the markers present in the original file (about 54K). In fact, the number of common overlapping markers among the datasets that remained after the merger was about 25K. Breeds with fewer than 3 samples were removed. To reduce the bias from over-represented breeds, data were restricted to a maximum of 30 animals per breed. After these edits and quality control (Mastrangelo et al., 2020), 23,311 SNPs mapping on the 29 autosomes were retained for the analysis (with no imputation). The positions of all retained markers were updated on ARS-UCD 1.3 assembly. Number of animals per breed ranged from 3 to 30, with an average value of 16±7. Breeds originated from all around the world: Asia, Africa, Americas, Australia, and Europe. For each breed, latitude and longitude were retrieved using the centroid of the origin area. Animals were divided in seven groups: 1) Continental taurus, 2) Temperate taurus, 3) Temperate indicus, 4) Temperate composite, 5) Tropical taurus, 6) Tropical indicus and 7) Tropical composite. The three geographic zones were created according to the latitude of the Countries of origin: i) Continental, latitude ≥ 45°; ii) Temperate, 45°<

Latitude >23.26°; iii) Tropics, Latitude ≤ 23.26°. Details about the involved dataset are reported in Supplementary Table 1.

Principal component analysis

The genetic structure of the cattle populations was analyzed using a principal component analysis (PCA), which is a multivariate technique aimed to reduce the total variance of a system is a smaller number of new variables (Morrison, 1976). Individual PCA scores (i.e., new values associated to the animals) are often used to highlight clusters or trends in the investigated dataset. A dispersion plot of the scores of the first two PCs extracted from the genomic relationship matrix (GRM), built according to VanRaden (2008), was used to display the genetic stratification among zones and types. Scores of the first two PCs were compared using an ANOVA test and means were declared significant for $P < 0.05$ (Tukey HSD test).

Runs of homozygosity

Consecutive ROH were computed using the "*detectruns*" R package (Biscarini et al., 2018). The following parameters were adopted to define a ROH: at least 15 homozygote SNPs in a row covering a minimum of 2 millions base pairs; no heterozygote or missing allowed. These strict parameters were adopted to avoid false ROH calling. Number of ROH per animal (nROH) and average (meanMb) ROH length were computed. A ROH that started and finished at the same positions within chromosome was defined as unique. These unique ROH could be identified in more than one animal; regions found in more than one sample were identified as repeated (ROHREP; Cesarani et al., 2018; Macciotta et al., 2021).

According to McQuillan et al. (2008), the ROH-based inbreeding coefficient (F_{ROH}) was calculated for each animal as:

$$F_{ROH} = \frac{L_{ROH}}{L_{GEN}}$$

where L_{ROH} was the sum of all ROH lengths per animal and L_{GEN} was the total genome length covered by SNP (2.48 Gbp).

For each SNP, the ratio between number of animals with a given SNP inside a ROH and total number of animals was defined as SNP_{ROH}. These values were compared among the seven identified groups using an ANOVA test and means were declared significantly different for $P < 0.05$ (Tukey HSD test). SNP were flagged as significant if their SNP_{ROH} exceeded the 99th percentile of the chromosomal distributions (Macciotta et al., 2021). These values led to the use of different thresholds for each chromosome within each group. According to the SNP_{ROH} values, ROH islands were defined as genomic region with adjacent significant SNPs ±250kb downstream and upstream (Manca et al., 2020).

Gene discovery was carried out in the significant regions highlighted with the ROH_{REP} and SNP_{ROH} analysis. Annotated genes were retrieved from the UCSC Genome Browser Gateway (http://genome.ucsc.edu/) and the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm. nih.gov) databases.

Statistical analysis

The nROH parameter was analyzed using the following generalized linear model (GLM) with a lognormal distribution:

$$nROH = \mu + GROUP + bPCs + e \qquad [1]$$

where: μ is the overall mean; GROUP is the fixed effect of the considered group (7 levels; i.e., Continental taurus, Temperate taurus, Temperate indicus, Temperate composite, Tropical taurus, Tropical indicus, and Tropical composite); bPCs are the covariates of the principal components extracted from the GRM associated to variance greater than 1%; e was the residual term.

Differences in the ROH mean length were investigated using the following mixed linear model with a lognormal distribution:

$$\text{meanMb} = \mu + \text{GROUP} + \text{CHROM} + \text{ANIM} + \text{bPCs} + e \qquad [2]$$

where: μ is the overall mean; GROUP is the fixed effect of the considered group (7 levels; i.e., Continental taurus, Temperate taurus, Temperate indicus, Temperate composite, Tropical taurus, Tropical indicus, and Tropical composite); CHROM is the fixed effect of chromosome (29 levels); ANIM is the random effect of animal (3,177 levels considering animals with at least 5 ROH); bPCs are the covariates of the principal component extracted from the GRM associated to variance greater than 1%; and e is the random residual term. The two random effects were assumed to be normally distributed with parameters $N(0, I\sigma_a^2)$ and $N(0, I\sigma_e^2)$, where I is an identity matrix and σ_a^2 and σ_e^2 the variances associated with the animal and the residual random effects, respectively. Mixed models were computed using SAS PROC GLMMIX (SAS Inc. 2012). The effect of PCs was included in all models to account for the population stratification.

Results and Discussion

Principal component analysis

Figure 1 shows the plot of the first two principal components extracted from the GRM matrix.

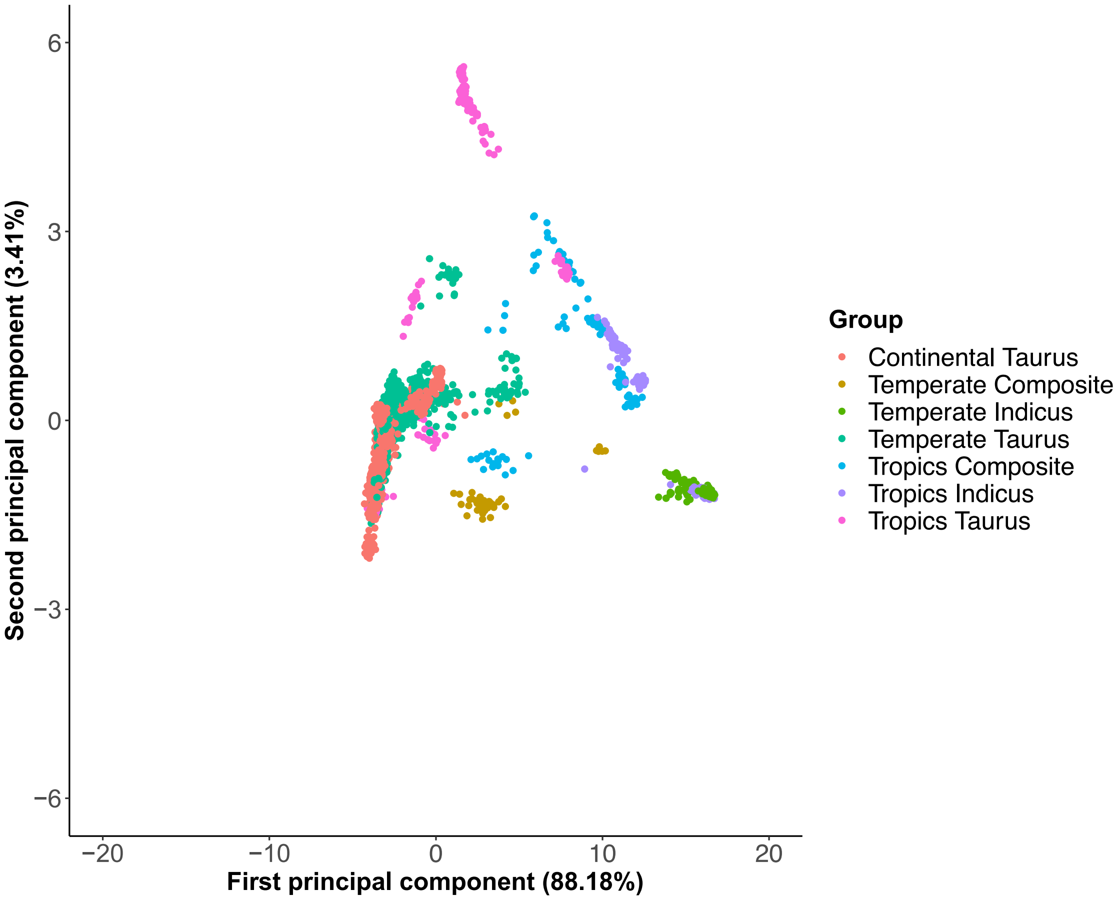

Figure 1. Plot of the first two principal components extracted from the genomic relationship matrix (GRM).

The PC1, which explained 88.18% of the total variance, showed that all the taurus had negative or strictly positive values, whereas indicus were at positive values. As expected, composite showed intermediate values. The PC2 explained about 3.41% of the total variance and it showed all the animals living at Tropics with positive values, or only a little negative in the case of the tropics composite. On the contrary, animals living in the Continental or Temperate zones showed values around 0 or negative. Both PC1 and PC2 scores were significantly different (P<0.0001) among the seven considered groups. The plot could be seen as bidimensional triangle and it is similar to the ones reported in comparable studies (e.g., Decker et al., 2014; Mastrangelo et al., 2020; Magnier et al., 2022). In fact, PCA may decipher the geography of the subjects, as it can reflect migrations, isolation by distance, and exchanges among neighboring populations (Reich et al., 2008).

Analysis of runs of homozygosity

A total of 136,922 ROHs were found in 3,177 animals (considering the animals with at least 5 ROH). As far as the distribution among the groups is concerned, Continental taurus showed the largest number (47,445 ROH, 35% of the total), whereas Temperate composite the lowest number (2,036 ROH, 1% of the total). The same proportions were found also considering a minimum ROH length of 4Mb instead of 2Mb (data not shown).

Table 1 shows the results of the mixed model analysis for both nROH and meanMb. All the three fitted PCs were significant for both analyzed traits. GROUP was highly significant for both nROH and meanMb (P<0.0001). Temperate indicus showed the highest number of ROH per animal (124.62±17.70), whereas Temperate taurus showed the lowest nROH (27.52±18.44). As far as the mean ROH length is concerned, Temperate taurus showed the largest value (5.90±0.16), whereas Tropics indicus the lowest value (3.90±0.32). The Temperate and Tropics composite showed intermediate values both for the average number of ROH (41.55±11.96 and 48.73±21.16, respectively) and for the mean length (4.45±0.31 and 4.07±0.25, respectively).

Nothangel et al. (2010) demonstrated that the number and the length of ROHs in human genomes are correlated with the latitude of their sample origin, although not with the longitude, maybe because the major migrations in Europe have followed a South to North gradient. In goat, Bertolini et al. (2018) reported significant differences for ROH patterns (ROH number and coverage) between the sub-geographical groups with a broad geographic distribution. A study on the pattern of ROH in world-wide sheep populations showed clear differences in the number of ROHs among breeds and regions, ranging from 50 in Southwest European breeds to 5,542 in North European breeds (Nosrati et al., 2021). In the present study, the nROH was moderately and negatively correlated with the latitude of origin (-0.42, $P<0.0001$); thus, nROH increased as the latitude decreased. In goat, the differences in climate between the Northern and Souther groups have resulted in different ROH lengths and numbers (Cortellari et al., 2021). The relationship between ROH features and environment was also recently observed in sheep (Cesarani et al., 2022) where animals living in hot environments exhibited a higher number of ROH compared to animals living in cold environments. The findings in humans, goat and sheep were confirmed in the present study where the Tropics, i.e., zone with the hotter climate and lower values of Latitude, showed the largest number of ROH per animal.

Table 1. Basic statistics and results of the statistical analyses.

	Animals		ROH			Model		
	N	%	N	%		nROH[1]	meanMb[1]	SNPROH
					PC1	($P<0.001$)	($P<0.001$)	–
					PC2	($P<0.001$)	($P<0.05$)	–
					PC3	($P<0.001$)	($P<0.001$)	–
					CHROM	–	($P<0.001$)	–
GROUP[2]						($P<0.001$)	($P<0.001$)	($P<0.001$)
Continental Taurus	1,314	41%	47,445	35%		36.11 ± 21.64^{F}	4.9 ± 0.18^{BC}	8.25 ± 1.87^{D}
Temperate Composite	49	2%	2,036	1%		41.55 ± 11.96^{E}	4.45 ± 0.31^{BC}	7.82 ± 5.24^{E}
Temperate Indicus	174	5%	21,684	16%		124.62 ± 17.70^{A}	4.28 ± 0.37^{BC}	18.80 ± 13.66^{A}
Temperate Taurus	1,205	38%	33,159	24%		27.52 ± 18.44^{G}	5.90 ± 0.16^{A}	7.85 ± 1.44^{E}
Tropics Composite	109	3%	5,312	4%		48.73 ± 21.16^{D}	4.07 ± 0.25^{B}	6.94 ± 5.47^{F}
Tropics Indicus	156	5%	18,625	14%		119.39 ± 39.66^{B}	3.90 ± 0.32^{BC}	15.78 ± 11.09^{B}
Tropics Taurus	170	5%	8,661	6%		50.95 ± 24.81^{C}	5.26 ± 0.25^{AC}	9.22 ± 3.74^{C}

[1] nROH = number of ROH per animal; meanMb = mean of ROHs length per animal; SNPROH = ratio between number of animals with a given SNP inside a ROH and total number of animals.

[2] Continental = Latitude ≥ 45°; Temperate = 45°< Latitude >23.26°; Tropics = Latitude ≤ 23.26°.

These results could have been influenced by the ascertainment bias, which effect is larger in indicus since the Bovine SNP50 BeadChip was originally derived from the sequencing of taurus breeds (e.g., O'Brien et al., 2014; Utsunomiya et al., 2014). The utility of a specific SNP genotyping platform is influenced by its development and the application of such platforms can adversely impact on population-specific SNP parameters estimated for breeds that were not represented in its design. Breeds with unclear, but presumably diverse ancestry, may therefore display variation in these parameters due to the origin of the investigated SNP. This might have resulted in ascertainment bias into autozygosity estimates for indicus populations. However, studies on sequence data revealed a larger genetic variability of the indicine breeds (Murray et al., 2010). Moreover, the traditional breed formation is largely a European phenomenon, and it is absent in the African cattle. Purfield et al. (2012) highlighted that African *Bos taurus taurus* breeds, humped *Bos taurus indicus* breeds and indicine/taurine hybrids, tended toward low levels of ROH per genome, reflecting traditional management practices in Africa, characterized by less controlled mating. On the contrary, indicus breeds, mostly raised at the tropics, showed the highest number of ROH but with the lowest average ROH length. This is probably because the natural selection due to environmental adaptation acted on these breeds long time ago, and these ROH have been broken by recombination events due to progression of the generations. Indeed, short ROH are older and probably derive from environmental adaptation, whereas long ROH are recent and probably due to artificial selection (Macciotta et al., 2021), or inbreeding due to non-optimal management or small population size. Therefore, the lowest average ROH length (meanMb) in indicus breeds may be due to the involved SNP chip (ascertainment bias), but it may also be due to an ancient inbreeding. Saravanan et al. (2021), in a study on taurus and indicus breeds, showed the variations in the frequency of different ROH-length classes. In the smaller length category (< 5 Mb), the Ongole breed (indicus) showed the highest number of ROH, whereas in the longer length category (>40 Mb), the largest number of ROH was observed for the Jersey breed (taurus). Several indicus breeds showed zero number of ROH in the longer length category (>40 Mb). A study performed on whole-genome sequencing data from European and African taurines, in addition to four indicine populations, reported that genome ROHs did not vary greatly among the investigated breeds (Upadhyay et al 2019). Mastrangelo et al. (2020) confirmed these findings, as their results showed that African populations (both taurine and zebuine cattle) had ROH patterns comparable with those of most European taurine breeds. Moreover, the length of ROH represents an important source of information on demographic and genetic processes; therefore, the results in indicus breeds could be also due to admixed events or different selection pressure.

Individual ROH-based inbreeding coefficients (Table 2) were computed considering four different minimum ROH length (F_{ROH} > 2Mb, F_{ROH} > 4Mb, F_{ROH} > 8Mb and F_{ROH} > 16Mb). As expected, F_{ROH} values decreased as the minimum ROH length increased (Table 2). The largest F_{ROH} values were computed always for Temperate indicus, whereas the lowest were observed for Tropics composite in the first two classes (F_{ROH} < 8 Mb) and for Tropics indicus in the last two classes (F_{ROH} > 8 Mb). Also in this case, the different F_{ROH} values computed in the identified groups using various minimum ROH length could be ascribed to the above-mentioned reasons (ascertainment bias, admixture events, selection pressure).

Table 2. Inbreeding coefficients (mean±SD) estimated according to different minimum ROH length in the seven considered groups.

Group[1]	F_{ROH} > 2 Mb		F_{ROH} > 4 Mb		F_{ROH} > 8 Mb		F_{ROH} > 16 Mb	
	Mean	n	Mean	n	Mean	n	Mean	n
Continental Taurus	0.08±0.06^{C}	1,314	0.06±0.05^{B}	1,292	0.04±0.04^{B}	1,182	0.03±0.03^{B}	805
Temperate Composite	0.08±0.03^{C}	49	0.05±0.02^{BC}	48	0.03±0.02^{B}	44	0.02±0.01^{B}	24
Temperate Indicus	0.19±0.08^{A}	174	0.08±0.08^{A}	174	0.06±0.08^{A}	130	0.06±0.07^{A}	86
Temperate Taurus	0.08±0.07^{C}	1,205	0.07±0.07^{AB}	1,122	0.06±0.06^{A}	944	0.05±0.05^{A}	686
Tropics Composite	0.07±0.05^{C}	109	0.03±0.04^{C}	106	0.03±0.04^{B}	49	0.03±0.03^{B}	29
Tropics Indicus	0.16±0.06^{B}	156	0.05±0.04^{BC}	156	0.02±0.03^{B}	112	0.02±0.02^{B}	48
Tropics Taurus	0.09±0.05^{C}	170	0.05±0.05^{B}	170	0.04±0.05^{AB}	133	0.04±0.04^{AB}	77

[1] Continental = Latitude ≥ 45°; Temperate = 45°< Latitude >23.26°; Tropics = Latitude ≤ 23.26°.

In sheep, the frequencies of ROH class lengths of 8–16Mb and >16Mb were lowest for European breeds and highest for Southwest Asian breeds followed by African and Asian populations (Nosrati et al., 2021). The largest average inbreeding coefficients for the first two classes ($F_{ROH} > 2$Mb and $F_{ROH} > 4$Mb) were observed for indicus, as also reported in Mastrangelo et al. (2020); moreover, indicus showed the same value (i.e., not significantly different) of taurus from 8 Mb.

Figure 2 shows the Manhattan plot of SNP_{ROH} computed for all markers within each of the seven identified groups. Although the distribution of the ROH among group was relatively balanced and the signals were moderate in height, we found several outstanding peaks with a high percentage of SNPs in ROH, especially in Temperate and Tropics indicus. On average, Temperate indicus showed the largest SNP_{ROH} (18.80%), whereas Tropics composite the lowest (6.94%) value (Table 1). A total of 887 SNP was flagged as significant; however, no SNP exceeded the threshold of 99% in all the seven groups.

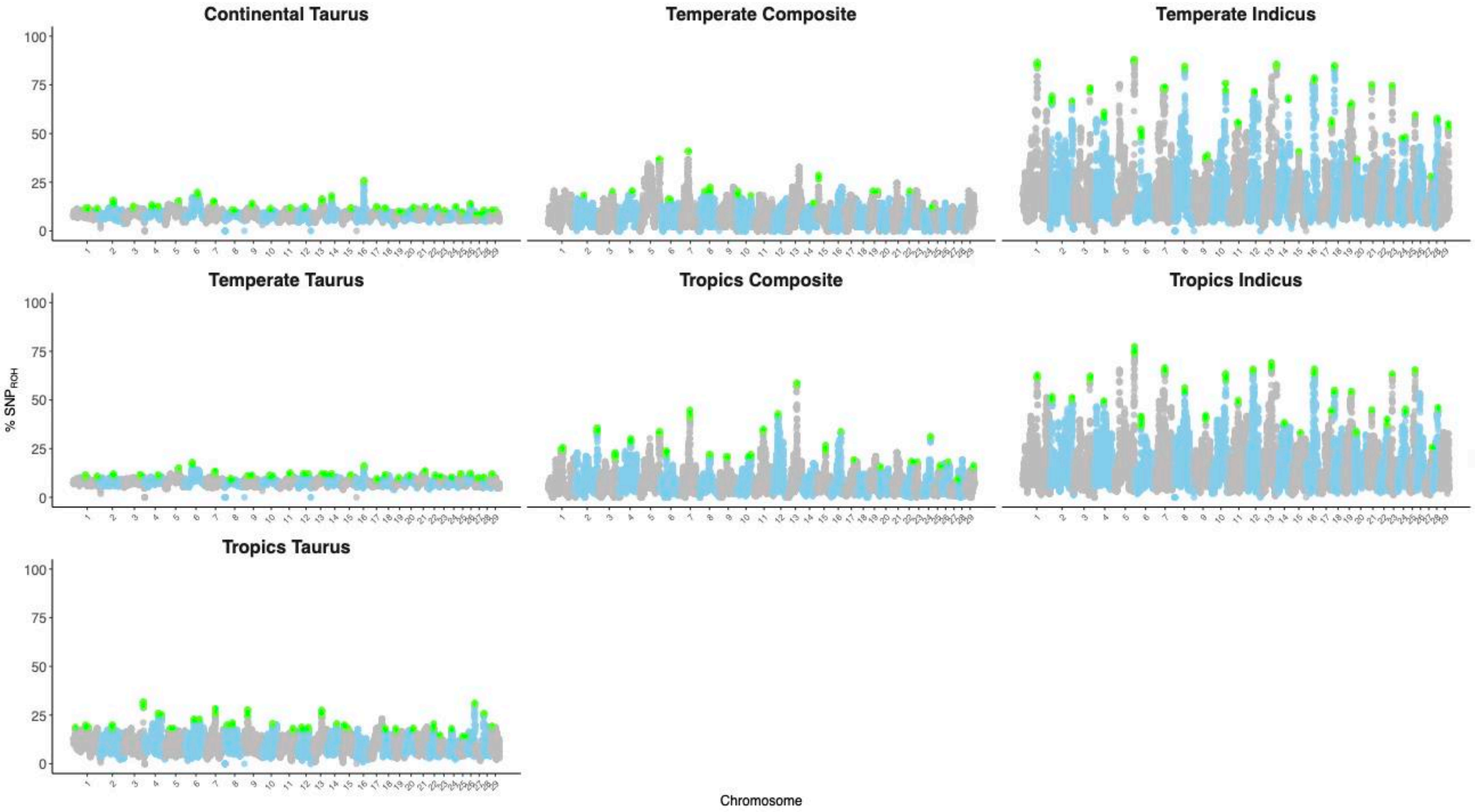

Figure 2 Manhattan plot of SNP$_{ROH}$ computed for all markers within Type and Zone. The green dots represent SNP exceeding the 99th percentile of the SNP$_{ROH}$ distribution.

Analysis of the shared ROH

Removing the duplicated regions, 94,925 unique ROH were found. As reported in Table 3, the top shared ROH were mainly found in the European breeds, which was expected because of their larger sample size. The first region on BTA12 was found in 94 animals, of which the 84% were from European taurine breeds; while the remaining were found in other 7 taurus breeds, 1 African composite breed, and 5 indicus breeds from India. Interestingly, Martikainen et al. (2020) found a ROH on BTA12 (65.13-66.37 Mb), which is included in the larger region highlighted in the present study, negatively associated with the interval from calving to the first insemination in Finnish Ayrshire cattle. The second region (BTA16) was found in European breeds the 77% of the times; the other animals were of taurine (11), composite (1) and indicine (8) breeds. The breeds which shared the third region (BTA27) were mostly worldwide (13%) and European (77%) Taurus; the same ROH was also found in 1 composite and 4 indicus breeds. Finally, the fourth region (located on BTA15) was shared by European breeds for 69%. It was shared also by other 7 taurus, 3 composite, and 5 indicus breeds.

The ROH shared by the largest number of animals (94), across TYPE and ZONE, was found on BTA12. Two genes that map in this region, *GPC5* and *GPC6*, were found to be associated with reproduction traits in cattle (Dubon et al., 2021; Purfield et al., 2019; Purfield et al., 2020). Others two genes located in this region, *GPR180* and *SOX21*, are involved immune functions (Minozzi et al., 2010) The *GPR180* gene was associated also with clinical ketosis (Soares et al., 2021). Finally, the *DCT* gene was associated to coat color (Jung et al., 2020), a trait related to environmental adaptation (e.g., Gaughan et al., 2019; Laible et al., 2021).

Table 3. TOP shared runs of homozygosity.

Region			Continental	Temperate	Temperate	Temperate	Tropics	Tropics	Tropics
BTA	Mb	Animals	Taurus	Composite	Indicus	Taurus	Composite	Indicus	Taurus
12	63.40-69.68	94	38	–	4	47	2	2	1
16	1.03-4.77	91	32	1	5	46	–	5	2
27	0.43-6.04	86	34	1	2	42	–	2	5
15	1.03-6.30	77	32	3	8	29	3	1	1

[1] Continental = Latitude $\geq 45°$; Temperate = $45° <$ Latitude $> 23.26°$; Tropics = Latitude $\leq 23.26°$.

The second most frequent ROH, spanning about 3.7 Mb, was located on BTA16, and it was shared by 91 animals (Table 3). This region was particularly rich of annotated genes. A first group are those associated with feed efficiency: *FMOD*, *GOLT1A*, (Chen et al., 2011), *ATP2B4* (Yang et al., 2021), *SNRPE* (Connor et al., 2010), *MDM4* (Rathert et al., 2020). Another group of genes annotated in this region are involved in disease resistance and immune functions: *RBBP5* (Swanson et al., 2009), *KLHDC8A* (McConnel et al., 2020), *RAB7B* (Ibeagha-Awemu et al., 2021), *ZC3H11A* (Rossetti et al., 2011), *SLC26A9* (Rinaldi et al., 2010). A cluster of 12 genes that map in this region was reported to be associated to clinical ketosis in Holstein cattle (Soares et al., 2021): *IKBKE*, *RASSF5*, *EIF2D*, *DYRK3*, *MAPKAPK2*, *IL10*, *IL19*, *IL20*, *IL24*, *FCMR*, *PIGR* and *FCAMR*. Three of them (*IKBKE*, *RASSF5*, and *FCMR*) have been also associated to female reproduction in cattle (Guarini et al., 2019; May et al., 2022). Other genes involved in reproduction that map in this ROH island are: *EIF2D* (Singh et al., 2018), *PPP1R15B* (Melo et al., 2018), *KISS1* (Singh et al., 2020), *LRRN2* (Gaddis et al., 2016), *ETNK2* (Hummitzsch et al., 2014), *PIK3C2B* (Mota et al., 2022), *SRGAP2* (Forde et al., 2012), and *PRELP* (Rodríguez-Alonso et al., 2019). We also identified several genes associated to disease resistance and immune response: *IL20* (Moré et al., 2019), *IL10* (Fonseca et al., 2009), and *IL19* (Saravanan et al., 2021). Two genes in the cluster were of particular interest, being associated to thermotolerance: *DYRK3* (Dikmen et al., 2015), and *FCAMR* (Dado-Senn et al., 2018). Another gene related to environmental adaptation within this region is the *SOX13* (Wiener et al., 2021). This ROH located on BTA 16 harbors also genes related to production traits. A first group are those related to beef traits, such as: *BTG2* (Sasaki et al., 2006), *AVPR1B* (Srikanth et al., 2020), *ELK4* (de las Heras-Saldana et al., 2020), and *CDK18* gene (Pan et al., 2021). Another gene, *REN*, was found to be associated with body traits (Cole et al., 2011). A second group of genes consisted of those related to dairy traits. The *NUCKS1* (Yuan et al., 2019) and the *LAX1* (Iso-Touru et al., 2016) genes were found to be associated with milk yield. A cluster of 5 genes was associated with lactose yield (Costa et al., 2019): *CNTN2*, *TMEM81*, *RBBP5*, *DSTYK* and *TMCC2*. The *PM20D1* was found associated with protein yield (Xu et al., 2019). Other genes were related to milk fatty acid composition (Iung et al., 2019): *MFSD4A*, *SLC45A3* and *SLC41A1*.

The third most frequent ROH mapped on the BTA27 and included 12 genes. Some of them (*ERICH1*, *DLGAP2*, *KBTBD11* and *ARHGEF10*) were not found previously highlighted. Two other genes, *CLN8* and *MYOM2*, were reported to be associated with environmental adaptation in buffaloes (Mokhber et al., 2018). *CSMD1* (Gonzalez et al., 2020) and *ANGPT2* (Tetens et al., 2013) were found to be related to dairy traits. In this ROH island are located also genes involved in beef traits, as *MCPH1* (Lim et al., 2013), *AGPAT5*, and *XKR5* (Edea et al., 2020). Finally, the *TAP* gene was reported to be involved in resistance to bovine respiratory disease (Berghuis et al., 2014).

The fourth ROH, mapped on the BTA15, was shared by 77 animals and it included 23 genes. Among these, 5 were not reported previously in study on cattle: *IZUMO1R*, *ANKRD49*, *GPR83*, *KBTBD3* and *DDI1*. Some of them were found related with immune functions: *CASP4* (Kabara et al., 2010; Sharifi et al., 2018), *CASP1* (Shen et al., 2019), *PDGFD* (Welderufael et al., 2018), MMP12 (Evans et al., 2014), and *MMP7* (Kosciuczuk et al., 2017). The *TMEM123* was reported to be downregulated in feed restriction-induced ketosis in Holstein (Loor et al., 2007). Some of the genes located in this region are related to environmental adaptation: *MSANTD4* and *GRIA4*, reported to be related to cold-stress resistance (Igoshin et al., 2019), and *MMP3*, associated to adaptation to hypoxic environment (Chen et al., 2018). Several genes were reported to be associated with reproduction: *DCUN1D5*, *MMP13*, *MMP20*, *MMP27*, *MRE11*, *MMP1*, *MMP3*, *MMP8* and *MMP13* (Guarini et al., 2019; Walker and Biase, 2020; Wathes et al., 2011). *MMP8* was identified also as candidate gene for the identification of Mycobacterium avium subsp. Paratuberculosis infection (Blanco Vázquez et al., 2020). Finally, we identified two genes associated to dairy traits: *AASDHPPT* (Saravanan et al., 2021), and *DYNC2H1* (Strillacci et al., 2014).

In the most shared ROH located on BTA12, we found genes mainly associated with reproductive and immune traits; the second region, on BTA16, included genes related to meat traits, body conformation, and feed efficiency, but also to milk traits; in the third shared ROH located on BTA27, some interesting genes associated with environmental adaptation in buffaloes and other associated with meat production were retrieved; finally, the fourth shared ROH (BTA15) included genes mainly associated with immune function and reproductive disorders.

In general, meat traits included fat deposition, growth, body size, backfat thickness and carcass quality traits, but also residual feed intake and feed intake. Milk traits, instead, included CLA milk content, milk yield, lactose yield, protein yield and somatic cell score. The immune functions regarded ketosis, antibody response to Mycobacterium avium subsp. Paratubercolosis, response and recoverability from mastitis, resistance to tick infestation, response to inflammation and susceptibility to brucellosis. The reproductive function covered quality of bull semen, pregnancy, conception rate, calving difficulty and oocyte developmental competence, but also reproductive disorders. Finally, the genes associated with environmental adaptation were found related both to cold-stress and heat-stress resistance. More details about the genes mapped in these four shared ROHs are reported in the Supplementary Table 2.

Conclusions

Results of the present study confirmed that runs of homozygosity could be used to identify genomic signatures due to both artificial and natural selection. Temperate indicus showed larger numbers of ROH per individual which resulted in larger inbreeding coefficients. However, Temperate taurus had numerical higher mean ROH length and Composite, both Tropics and Temperate, showed always intermediate values. Despite the limitation of the ascertainment bias, limited number of genotyped animals for indicus and reduced genomic coverage of the SNP dataset used, differences in ROH and an interesting list of potential genes have been identified, which will be the foundation for future investigations.

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Appendix Chapter 2

Analysis of runs of homozygosity of cattle living in different climate zones

Supplementary Table 1. Description and details of the investigated breeds.

Supplementary Table 2. List of the genes mapped in the top shared runs of homozygosity.

Supplementary Table 1. Description and details of the investigated breeds.

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
GUEL	Africa	Guelmoise	Algeria	35.8	7.4	24	Temperate Taurus
BORG	Africa	Borgou	Benin	9.6	2.7	20	Tropics Composite
ZFU	Africa	Zebu Fulani	Benin	-9.5	2.27	20	Tropics Indicus
LAG	Africa	Lagune	Benin	6.3	2.4	20	Tropics Taurus
TULI	Africa	Tuli	Botswana	-22.4	26.7	4	Tropics Composite
BAO	Africa	Baoule	Burkina Faso	10.4	-4	20	Tropics Taurus
ZBO	Africa	Zebu Bororo	Chad	15	14.5	20	Tropics Indicus
KUR	Africa	Kuri	Chad	15	14.5	20	Tropics Taurus
SHK	Africa	Sheko	Ethiopia	6.6	35.8	17	Tropics Composite
NDAM	Africa	N'Dama	Ivory Coast, Africa Burkina Faso	12.6	-8	20	Tropics Taurus
ZEB	Africa	East African Shorthorn Zebu	Kenya	1	35	20	Tropics Composite
ZMA	Africa	Zebu from Madagascar	Madagascar	-24.75	45.4	20	Tropics Indicus
OUL	Africa	Oulmès-Zaer	Morocco	22.5	-14.3	19	Tropics Taurus
ANKW	Africa	Ankole-Watusi	Ruanda	-2	29.3	5	Tropics Composite
AFR	Africa	Africander	South Africa	-34	18.5	4	Tropics Composite
BOR	Africa	Boran	Southern Ethiopia	11.75	37.05	20	Tropics Composite
SOM	Africa	Somba	Togo	7.4	0.7	20	Tropics Taurus
CANC	Americas	Canchim	Brazil	-22	-47.8	20	Tropics Composite
NEL	Americas	Nelore	Brazil	-12.9	-38.4	20	Tropics Indicus
ROMO	Americas	Romosinuano	Columbia	8.7	-75.9	8	Tropics Taurus
CRK	Americas	Florida Cracker	Florida, United States	30.3	-81.6	9	Temperate Taurus
BR	Americas	Brahman	Gulf Coast, United States	34	-81	20	Temperate Indicus
CORR	Americas	Corriente	Sonora, Mexico	28.5	-105.9	5	Temperate Taurus
BEFM	Americas	Beefmaster	Texas, United States	27.4	-98.5	20	Temperate Composite
SGT	Americas	Santa Gertrudis	Texas, United States	27.5	-97.85	20	Temperate Composite
TXLH	Americas	Texas Longhorn	Texas, United States	33.8	-98.5	20	Temperate Taurus

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
BSW	Americas	Brown Swiss	United States	42.4	-71.2	19	Temperate Taurus
ANR	Americas	Red Angus	United States	38.3	-98.7	19	Temperate Taurus
SENP	Americas	Senepol	United States Virgin Islands	17.7	-64.7	19	Tropics Taurus
ONG	Asia	Ongole Grade	Andhra Pradesh, India	15.5	80	20	Tropics Indicus
LX	Asia	Luxi	China	36.1	103.7	5	Temperate Composite
QC	Asia	Qinchuan	China	35.2	109.6	4	Temperate Composite
CHO	Asia	Cholistani	Cholistan Desert, Punjab, Pakistan	28.5	71.5	11	Temperate Indicus
GIR	Asia	Gir	Gujerat, India	21.1	70.8	20	Tropics Indicus
GUZ	Asia	Guzerat	Guzarat, India	21.4	69.9	3	Tropics Indicus
HN	Asia	Hainan	Hainan Province, China	19.75	110	4	Tropics Indicus
HAR	Asia	Hariana	Haryana plains, India	28.9	76.6	10	Temperate Indicus
BRE	Asia	Brebes	Indonesia	-6.85	109.5	9	Tropics Indicus
PES	Asia	Pesisir	Indonesia	-1.6	100.7	6	Tropics Indicus
WAGY	Asia	Wagyu	Japan	35.5	137.5	12	Temperate Taurus
BAG	Asia	Bhagnari	Kaochi, Kalat, and Baluchistan, Pakistan	28.9	67.8	10	Temperate Indicus
GBI	Asia	Gabrali	Khyber Pakhtun Khwa, Pakistan	35.5	72.4	10	Temperate Indicus
ACH	Asia	Achai	Khyber Pakhtun Khwa, Pakistan	34.9	72.3	12	Temperate Indicus
HANW	Asia	Hanwoo	Korea	37.6	127	8	Temperate Taurus
MAD	Asia	Madura	Madura Island, Indonesia	-7.1	113.4	7	Tropics Indicus
MG	Asia	Mongolian	Mongolia	45.5	96.5	5	Continental Taurus
KAN	Asia	Kankraj	North Gujerat, India	23.6	70.6	10	Temperate Indicus
LOH	Asia	Lohani	Northwest Pakistan	30.4	68.6	10	Temperate Indicus
SAHW	Asia	Sahiwal	Punjab, Pakistan	31	72.5	17	Temperate Indicus
HIS	Asia	Hissar	Punjab, Pakistan	29.15	75.7	10	Temperate Indicus
DHA	Asia	Dhanni	Punjab, Pakistan	33.8	72.4	12	Temperate Indicus
DAJ	Asia	Dajal	Punjab, Pakistan	29.55	70.4	10	Temperate Indicus
ROJ	Asia	Rojhan	Punjab, Pakistan	30.5	70.15	10	Temperate Indicus
RSIN	Asia	Red Sindhi	Sindh, Pakistan	25.8	68.7	10	Temperate Indicus

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
THA	Asia	Tharparkar	Southeast Sindh, Pakistan	24.9	70.2	12	Temperate Indicus
ACE	Asia	Aceh	Sumatra, Indonesia	4.7	96.7	12	Tropics Indicus
MUGR	Australia	Murray Grey	Australia	-36	147.4	4	Tropics Taurus
YKT	Eurasian	Yakut	Arctic Circlle, Republic of¬†Sakha	62	129.7	25	Continental Taurus
GVR	Eurasian	Gorbatov Red	Gorbatov, Russia	56.3	44	11	Continental Taurus
ALA	Eurasian	Ala-Tau	Kazakhstan	43.35	77.15	14	Temperate Taurus
KAW	Eurasian	Kazakh Whiteheaded	Kazakhstan, Russia	48.7	44.5	20	Continental Taurus
KHO	Eurasian	Kholmogory	Kholmogory	64.2	41.65	20	Continental Taurus
IST	Eurasian	Istoben	Kirov	58.6	49.6	5	Continental Taurus
KAL	Eurasian	Kalmyk	Mongolia and northwestern China	45.8	44.6	23	Continental Taurus
YAR	Eurasian	Yaroslavl	Russia	57.8	39.7	19	Continental Taurus
BURY	Eurasian	Buryat	Russia	53.8	37.8	24	Continental Taurus
KOS	Eurasian	Kostroma	Russia	57.8	40.9	18	Continental Taurus
BLP	Eurasian	Black Pied	Russia	57.8	41	24	Continental Taurus
RUHE	Eurasian	Russian Hereford	Russia	57.8	41	9	Continental Taurus
TAG	Eurasian	Tagil	Russia	59.1	59.1	19	Continental Taurus
UKG	Eurasian	Ukrainian Grey	Ukraine	47.5	29.9	20	Continental Taurus
UKW	Eurasian	Ukrainian Whiteheaded	Ukraine	51.1	25.2	7	Continental Taurus
YUR	Eurasian	Yurino	Ukraine	49.4	38.9	3	Continental Taurus
BST	Eurasian	Bestuzhev	Uzbekistan	42.4	62.5	19	Temperate Taurus
TGV	Europe	Tirolean Grey cattle	Austria	47.3	11.4	20	Continental Taurus
HRI	Europe	Istrian cattle Boskarin	Balkans Croatia	45.3	13.9	20	Continental Taurus
HRP	Europe	Croatian Podolian cattle	Balkans Croatia	48.6	27.5	24	Continental Taurus
DBB	Europe	Dibra cattle Busa	Balkans, Albania	44	16.7	25	Temperate Taurus
IMB	Europe	Middle Albanian Busa	Balkans, Albania	44	16.7	20	Temperate Taurus
SKB	Europe	Skodra cattle Busa	Balkans, Albania	44	16.7	14	Temperate Taurus
PRE	Europe	Prespa cattle Busa	Balkans, Albania	40.8	20.9	20	Temperate Taurus
LKB	Europe	Lekbibaj cattle Busa	Balkans, Albania	42.4	20.2	27	Temperate Taurus

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
RHS	Europe	Rhodope Shorton Busa	Balkans, Bulgaria	44	16.7	24	Temperate Taurus
HRB	Europe	Croatian Busa	Balkans, Croatia	44	16.7	28	Temperate Taurus
BHB	Europe	B&H Busa	Balkans, Herzegovina	44	16.7	18	Temperate Taurus
RMB	Europe	Red Metohian Busa	Balkans, Kosovo	44	16.7	26	Temperate Taurus
SHB	Europe	Sharri Busa	Balkans, Kosovo	44	16.7	21	Temperate Taurus
DGB	Europe	Dukagjini Busha cattle	Balkans, Kosovo	42.4	20.4	21	Temperate Taurus
MKB	Europe	Macedonia Busa	Balkans, Macedonia	44	16.7	24	Temperate Taurus
MNB	Europe	Montenegro Busa	Balkans, Montenegro	44	16.7	20	Temperate Taurus
RMNG	Europe	Romenian Grey	Balkans, Romania	46.2	25.2	4	Continental Taurus
SRB	Europe	Serbian Busa	Balkans, Serbia	44	16.7	20	Temperate Taurus
TG	Europe	Turkish Grey	Balkans, Turkey	43.2	23.7	8	Temperate Taurus
AB	Europe	Anatolian Black	Balkans, Turkey	37.1	31.1	8	Temperate Taurus
ABB	Europe	Anatolian cattle	Balkans, Turkey	37.1	31.1	20	Temperate Taurus
ASY	Europe	Anatolian Southern Yellow	Balkans, Turkey	37.1	31.1	8	Temperate Taurus
EAR	Europe	East Anatolian Red	Balkans, Turkey	37.1	31.1	8	Temperate Taurus
SANR	Europe	South Anatolian Red	Balkans, Turkey	37.1	31.1	8	Temperate Taurus
ZVT	Europe	Zavot	Balkans, Turkey	43.6	45.05	5	Temperate Taurus
FLR	Europe	Flemish Red	Belgium	51.2	3.2	27	Continental Taurus
BBB	Europe	Blanc Bleu Belge	Belgium	50.8	4.42	20	Continental Taurus
CORS	Europe	Corsa	Corsica	42.05	9.05	20	Temperate Taurus
MSH	Europe	Milking Shorthorn	England	54.5	-1.6	9	Continental Taurus
REDP	Europe	Red Poll	England	52.2	0.7	5	Continental Taurus
LINC	Europe	Lincoln Red	England	53	-0.3	8	Continental Taurus
SDEV	Europe	South Devon	England	50.4	-4.2	3	Continental Taurus
DEV	Europe	Devon	England	50.7	3.6	4	Continental Taurus
SH	Europe	Beef Shorthorn	England	55	-2.9	17	Continental Taurus
LH	Europe	Longhorn	England	54.1	-2.2	3	Continental Taurus
SUSS	Europe	Sussex	England	51.1	0	4	Continental Taurus

Code	**Continent**	**Breed**	**Origin**	**Latitude**	**Longitude**	**Samples**	**Group**
DAN	Europe	Deutsche Angus	England	51.4	10.4	20	Continental Taurus
RHF	Europe	Red Holstein	Europe	53	5.8	20	Continental Taurus
HOLST	Europe	Holstein	Europe	47.5	9.6	20	Continental Taurus
ABO	Europe	Abondance	France	46.2	7	20	Continental Taurus
LIM	Europe	Limousin	France	45.1	2.4	20	Continental Taurus
MAAN	Europe	Maine-Anjou Rouge de Pres	France	47.5	-0.5	15	Continental Taurus
MONT	Europe	Montbeliard	France	47.4	6.1	20	Continental Taurus
SAL	Europe	Salers	France	45.1	2.7	20	Continental Taurus
MARA	Europe	Maraichine (Parthenaise)	France	47	-0.95	19	Continental Taurus
TARE	Europe	Tarentaise	France	45.6	6.6	20	Continental Taurus
VOS	Europe	Vosgienne	France	48	7	20	Continental Taurus
BPN	Europe	Bretonne Black Pied	France	48.2	-2.9	18	Continental Taurus
RPLAIN	Europe	Pie Rouge de Plaines	France	48.2	-2.9	20	Continental Taurus
PRP	Europe	French Red Pied Lowland	France	48.2	-2.9	20	Continental Taurus
NORM	Europe	Normande	France	49.5	0.1	20	Continental Taurus
CHAR	Europe	Charolais	France	46.5	4.3	20	Continental Taurus
TAR	Europe	Tarine	France	45	5	18	Continental Taurus
AUB	Europe	Aubrac	France	44.6	3.1	20	Temperate Taurus
GAS	Europe	Gascon	France	43.7	0.2	20	Temperate Taurus
BAQ	Europe	Blonde d'Aquitaine	France	44.8	-0.6	30	Temperate Taurus
DFV	Europe	Fleckvieh	Germany	49.4	11.1	20	Continental Taurus
MWF	Europe	Marnau Werdenfelser	Germany	47.5	11.1	20	Continental Taurus
GEL	Europe	Gelbvieh	Germany	49.9	10.9	20	Continental Taurus
GNS	Europe	Guernsey	Guernsey Island	49.45	-2.6	20	Continental Taurus
DEX	Europe	Dexter	Ireland	52.5	-8.5	4	Continental Taurus
KERR	Europe	Kerry	Ireland	52.5	-8.5	3	Continental Taurus
PROropa	Europe	Pezzata Rossa d'Oropa	Italy	45.55	8.05	23	Continental Taurus
ITSIMM	Europe	Italian Simmental	Italy	46.1	13.2	20	Continental Taurus

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
PUST	Europe	Pustertaler	Italy	46.75	12.3	24	Continental Taurus
BARPUR	Europe	Barà Pustertaler	Italy	45.15	7.2	24	Continental Taurus
POD	Europe	Podolica	Italy	47.4	29.5	24	Continental Taurus
GRIALP	Europe	Grigia Alpina	Italy	46.5	11.4	23	Continental Taurus
ITBROWN	Europe	Italian Brown	Italy	47	8.6	20	Continental Taurus
BUR	Europe	Burlina	Italy	45.9	11.5	24	Continental Taurus
REND	Europe	Rendena	Italy	46.1	10.75	24	Continental Taurus
MRM	Europe	Maremmana	Italy	42.55	11.25	24	Temperate Taurus
PMT	Europe	Piedmontese	Italy	44.9	8.2	21	Temperate Taurus
ITHOLST	Europe	Italian Holstein	Italy	41.9	12.2	20	Temperate Taurus
MPIS	Europe	Mucca Pisana	Italy	43.7	10.4	23	Temperate Taurus
CABA	Europe	Cabannina	Italy	44.6	9.4	22	Temperate Taurus
ROSSIC	Europe	Rossa Siciliana	Italy	37.9	14.7	24	Temperate Taurus
VAROTT	Europe	Varzese-Ottonese	Italy	44.4	9.7	20	Temperate Taurus
PONTR	Europe	Pontremolese	Italy	43.85	10.5	24	Temperate Taurus
GARF	Europe	Garfagnina	Italy	44.1	10.4	23	Temperate Taurus
SAM	Europe	Sardo-Modicana	Italy	39.65	9	28	Temperate Taurus
CHIAN	Europe	Chianina	Italy	43.2	11.8	23	Temperate Taurus
AGER	Europe	Agerolese	Italy	40.65	14.5	22	Temperate Taurus
CALV	Europe	Calvana	Italy	43.9	11.3	24	Temperate Taurus
SAR	Europe	Sarda	Italy	40	9	30	Temperate Taurus
MOD	Europe	Modicana	Italy	36.85	14.75	29	Temperate Taurus
CINI	Europe	Cinisara	Italy	38.2	13.1	30	Temperate Taurus
REGG	Europe	Reggiana	Italy	44.7	10.6	25	Temperate Taurus
SABR	Europe	Sardo-Bruna	Italy	40.6	9	10	Temperate Taurus
MDN	Europe	Modenese	Italy	44.8	10.9	23	Temperate Taurus
RMG	Europe	Romagnola	Italy	44.2	12.1	21	Temperate Taurus
MARCH	Europe	Marchigiana	Italy	43.3	13.3	22	Temperate Taurus

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
PINZ	Europe	Pinzgauer	Italy-Austria	47.8	13	20	Continental Taurus
JER	Europe	Jersey	Jersey Island, Channel Island	49.2	-2.15	20	Continental Taurus
LWB	Europe	Lithuanian White Backed	Lithuania	54.4	23.9	3	Continental Taurus
MALT	Europe	Maltese	Malta	35.9	14.4	4	Temperate Taurus
LKV	Europe	Lakenvelder	Netherlands	52.4	4.9	21	Continental Taurus
DERE	Europe	Deep Red	Netherlands	51.4	5.4	20	Continental Taurus
MRY	Europe	Meuse Rhine Issel	Netherlands	5.5	5.5	20	Tropics Taurus
NRC	Europe	Norwegian Red	Norway	59.9	10.7	20	Continental Taurus
MAR	Europe	Maronesa	Portugal	41.4	-7.5	14	Temperate Taurus
BAR	Europe	Barrosa	Portugal	41.95	-8.45	14	Temperate Taurus
MOR	Europe	Morucha	Salamanca	41	-5.7	5	Temperate Taurus
AN	Europe	Angus	Scotland	56.75	-3	20	Continental Taurus
BGAL	Europe	Belted Galloway	Scotland	55.1	-4.5	4	Continental Taurus
GLW	Europe	Galloway	Scotland	54.95	-4.5	20	Continental Taurus
AYR	Europe	Finnish Ayrshire	Scotland/Finland	55.5	-4.6	18	Continental Taurus
HGL	Europe	Highland cattle	Scottland	57.3	-57.3	19	Continental Taurus
SIC	Europe	Cika	Slovenia	46.3	13.9	26	Continental Taurus
TL	Europe	Toro de Lidia	Spain	42.7	-1.7	19	Temperate Taurus
TER	Europe	Terrana	Spain	43.1	-2.9	5	Temperate Taurus
CAR	Europe	Cardena Andaluza	Spain	37.9	-4.8	5	Temperate Taurus
AVIL	Europe	Avilena	Spain	40.7	-4.7	6	Temperate Taurus
NGA	Europe	Negra Andaluza	Spain	37.4	-4.5	5	Temperate Taurus
PASIEG	Europe	Pasiega	Spain	43.2	-4.05	5	Temperate Taurus
TUD	Europe	Tudinca	Spain	43.2	-4.05	6	Temperate Taurus
ASTU	Europe	Asturiana	Spain	43.4	-5.9	5	Temperate Taurus
MOST	Europe	Mostrenca	Spain	37.05	-6.45	5	Temperate Taurus
MARES	Europe	Maresmana	Spain	37.05	-6.45	30	Temperate Taurus
PIR	Europe	Pirenaica	Spain	42.6	0.5	5	Temperate Taurus

Code	Continent	Breed	Origin	Latitude	Longitude	Samples	Group
BC	Europe	Berrenda en Colorado	Spain	37.9	-6.1	5	Temperate Taurus
BN	Europe	Berrenda en Negro	Spain	37.9	-6.1	5	Temperate Taurus
RET	Europe	Retinta	Spain	37.4	-6	10	Temperate Taurus
MEN	Europe	Menorquina	Spain	40	4.1	3	Temperate Taurus
SIM	Europe	Simmental	Switzerland	46.85	7.85	20	Continental Taurus
SMR	Europe	Simmentaler	Switzerland	46.9	8.2	4	Continental Taurus
OBV	Europe	Original Braunvieh	Switzerland	47	8.6	20	Continental Taurus
BRHV	Europe	Braunvieh	Switzerland	47	8.65	20	Continental Taurus
WHPK	Europe	White Park	Wales	51.88	-4.01	5	Continental Taurus
HFD	Europe	Hereford	Wales-England	52.1	-2.7	20	Continental Taurus

Supplementary Table 2. List of the genes mapped in the top shared runs of homozygosity.

BTA	Position (Mb)	Gene acronym	Associated traits	Reference
12	65.75-67.33	*GPC5*	Gestation length in beef cattle	Purfield et al., 2019
12	67.70-68.21	*GPC6*	Age at first calving in Nellore Perinatal mortality, maternal calving difficulty and direct calving difficulty	Dubon et al., 2021 Purfield et al., 2020
12	68.96-69.00	*DCT*	Melanogenesis	Jung et al., 2020
12	69.07-69.09	*TGDS*		
12	69.09-69.15	*GPR180*	Clinical ketosis in Canadian Holstein *Mycobacterium avium* subsp. *Paratubercolosis* antibody response in Holstein cows	Soares et al., 2021 Minozzi et al., 2010
12	69.200-69.204	*SOX21*	*Mycobacterium avium* subsp. *Paratubercolosis* antibody response in Holstein cows	Minozzi et al., 2010
15	1.300-1.303	*IZUMO1R*		
15	1.39-1.40	*GPR83*		
15	1.43-1.50	*MRE11*	Oocyte developmental competence	Walker and Biase, 2020
15	1.50-1.52	*ANKRD49*		
15	1.56-1.59	*AASDHPPT*	Milk production traits in Jersey cattle	Saravanan et al., 2021
15	1.59-1.67	*KBTBD3*		
15	1.76-1.77	*MSANTD4*	Cold-stress resistance	Igoshin et al., 2019
15	1.81-2.49	*GRIA4*	Cold-stress resistance	Igoshin et al., 2019
15	3.23-3.24	*CASP1*	Ketosis	Shen et al., 2019
15	3.25-3.26	*CASP4*	Response to *Escherichia Coli* induced mastitis	Sharifi et al., 2018
15	4.25-4.53	*PDGFD*	Recoverability from mastitis in Danish Holstein cows	Welderufael et al., 2018
15	4.390-4.392	*DDI1*		
15	5.05-5.45	*DYNC2H1*	CLA milk content in Italian Brown Swiss dairy cattle	Strillacci et al., 2014
15	5.47-5.50	*DCUN1D5*	Reproductive disorders, in particular cystic ovaries, in Canadian Holstein dairy cows	Guarini et al., 2019

BTA	Position (Mb)	Gene acronym	Associated traits	Reference
15	5.67-5.68	*MMP13*	Reproductive disorders, in particular cystic ovaries, in Canadian Holstein dairy cows Negative energy balance in postpartum dairy cows	Guarini et al., 2019 Wathes et al., 2011
15	5.75- 5.76	*MMP12*	Inflammation, in particular in the case of Bovine digital dermatitis treponemes	Evans et al., 2014
15	5.77-5.78	*MMP3*	Adaptation to hypoxic environment in Tibetan cattle Negative energy balance in postpartum dairy cows	Chen et al., 2018 Whates et al., 2011
15	5.81-5.82	*MMP1*	Negative energy balance in postpartum dairy cows	Whates et al., 2011
15	5.93-5.94	*MMP8*	Negative energy balance in postpartum dairy cows *Mycobacterium avium* subsp. *Paratuberculosis* infection	Whates et al., 2011 Blanco Vázquez et al., 2020
15	5.95-5.96	*MMP27*	Reproductive disorders, in particular cystic ovaries, in Canadian Holstein dairy cows	Guarini et al., 2019
15	6.03-6.08	*MMP20*	Reproductive disorders, in particular cystic ovaries, in Canadian Holstein dairy cows	Guarini et al., 2019
15	6.14-6.15	*MMP7*	Response to inflammation	Kosciuczuk et al., 2017
15	6.22-6.30	*TMEM123*	Feed-restriction induced ketosis	Loor et al., 2007
16	1.00-1.03	*CHIT1*		
16	1.08-1.09	*BTG2*	Intramuscular fat deposition	Sasaki et al., 2006
16	1.12-1.32	*FMOD*	Feed intake in Angus bulls	Chen et al., 2011
16	1.23-1.25	*PRELP*		
16	1.25-1.26	*OPTC*		
16	1.39-1.50	*ATP2B4*	Residual feed intake in Chinese beef bulls	Yang et al., 2021
16	1.50-1.51	*LAX1*	Milk yield in Nordic Red Cattle	Iso-Touru et al., 2019
16	1.58-1.62	*ZC3H11A*	Susceptibility to brucellosis	Rossetti et al., 2011
16	1.63-1.64	*SNRPE*	Feed efficiency in Angus	Connor et al., 2010
16	1.86-1.91	*SOX13*	Environmental adaptation in Ethiopian sheep populations	Wiener et al., 2021
16	1.91-1.93	*ETNK2*	Health of follicles	Hummitzsch et al., 2014

BTA	Position (Mb)	Gene acronym	Associated traits	Reference
16	1.93-1.95	*REN*	Body size traits in Holstein cows, and in particular stature, strength, body depth, rump width and udder attachment	Cole et al., 2011
16	1.95-1.96	*KISS1*	Reproductive performance in Indian cattle	Singh et al., 2020
16	1.96-1.97	*GOLT1A*	Feed intake in Angus bulls	Chen et al., 2011
16	1.98-2.02	*PLEKHA6*		
16	2.14-2.15	*PPP1R15B*	Reproductive functions in Brahman and Nellore cattle	Melo et al., 2018
16	2.16-2.24	*PIK3C2B*	Heifers early calving	Mota et al., 2022
16	2.25-2.30	*MDM4*	Residual feed intake in Hereford x Angus steers	Rathert et al., 2020
16	2.35-2.36	*LRRN2*	Heifers conception rate	Gaddis et al., 2016
16	2.60-2.80	*NFASC*	Stromal cells of the endometrium	Pereira et al., 2022
16	2.82-2.85	*CNTN2*	Lactose yield in Fleckvieh cattle	Costa et al., 2019
16	2.861-2.864	*TMEM81*	Lactose yield in Fleckvieh cattle	Costa et al., 2019
16	2.86-2.90	*RBBP5*	Lactose yield in Fleckvieh cattle Mastitis	Costa et al., 2019 Swanson et al., 2009
16	2.93-2.98	*DSTYK*	Lactose yield in Fleckvieh cattle	Costa et al., 2019
16	3.00-3.04	*TMCC2*	Lactose yield in Fleckvieh cattle	Costa et al., 2019
16	3.06-3.08	*NUAK2*		
16	3.100-3.105	*KLHDC8A*	Bovine metritis and early postpartum disease	McConnel et al., 2020
16	3.14-3.17	*LEMD1*		
16	3.24-3.27	*CDK18*	Adipogenic differentiation	Pan et al., 2021
16	3.33-3.36	*MFSD4A*	C18:0 in Brazilian Holstein	Iung et al., 2019
16	3.37-3.38	*ELK4*	Fat deposition and growth in Hanwoo steers	de las Heras-Saldana et al., 2020
16	3.40-3.46	*SLC45A3*	C18:0 in Brazilian Holstein	Iung et al., 2019
16	3.46-3.49	*NUCKS1*	Milk synthesis	Yuan et al., 2019

BTA	Position (Mb)	Gene acronym	Associated traits	Reference
16	3.50-3.51	*RAB29*		
16	3.53-3.55	*SLC41A1*	C18:0 in Brazilian Holstein	Iung et al., 2019
16	3.60-3.62	*PM20D1*	Protein yield in milk in Chinese Holstein cows	Xu et al., 2019
16	3.64-3.67	*SLC26A9*	*Escherichia Coli* infection	Rinaldi et al., 2010
16	3.72-3.75	*RAB7B*	Immune response to *Mycobacterium avium* subsp *paratuberculosis*	Ibeagha-Awemu et al., 2021
16	3.82-3.85	*RHEX*		
16	3.87-3.88	*AVPR1B*	Backfat thickness in Hanwoo steers	Srikanth et al., 2020
16	3.98-3.99	*FAM72A*		
16	3.99-4.25	*SRGAP2*	Pregnancy	Forde et al., 2012
16	4.25-4.28	*IKBKE*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Endometritis in Holstein	May et al., 2022
16	4.93-4.36	*RASSF5*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Endometritis in Holstein	May et al., 2022
16	4.36-4.39	*EIF2D*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Quality of bull semen	Singh et al., 2018
16	4.42-4.43	*DYRK3*	Respiration rate and thermotolerance	Dikmen et al., 2015
			Clinical ketosis in Holstein cattle	Soares et al., 2021
16	4.46-4.51	*MAPKAPK2*	Clinical ketosis in Holstein cattle	Soares et al., 2021
16	4.551-4.555	*IL10*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Mastitis	Fonseca et al., 2009
16	4.62-4.63	*IL19*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Innate immune response	Saravanan et al., 2021
16	4.653-4.656	*IL20*	Clinical ketosis in Holstein cattle	Soares et al., 2021

BTA	Position (Mb)	Gene acronym	Associated traits	Reference
			Resistance to tick infestation in Bradford cattle skin	Moré et al., 2019
16	4.682-4.687	*IL24*	Clinical ketosis in Holstein cattle	Soares et al., 2021
16	4.68-4.70	*FCMR*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Metritis in Canadian Holstein cows	Guarini et al., 2019
16	4.71-4.73	*PIGR*	Clinical ketosis in Holstein cattle	Soares et al., 2021
16	4.73-4.75	*FCAMR*	Clinical ketosis in Holstein cattle	Soares et al., 2021
			Heat-stress in Holstein cows	Dado-Senn et al., 2018
27	0.49-0.53	*ERICH1*		
27	0.58-1.18	*DLGAP2*		
27	1.19-1.25	*CLN8*	Environmental adaptation in buffaloes	Mokhber et al., 2018
27	1.34-1.40	*ARHGEF10*		
27	1.41-1.43	*KBTBD11*		
27	1.45-1.51	*MYOM2*	Environmental adaptation in buffaloes	Mokhber et al., 2018
27	2.14-4.21	*CSMD1*	Udder conformation in Holstein	Gonzalez et al., 2020
27	5.44-5.68	*MCPH1*	Marbling in Hanwoo cattle	Lim et al., 2013
27	5.55-5.61	*ANGPT2*	Somatic cell score in Holstein cattle	Tetens et al., 2013
27	5.81-5.85	*AGPAT5*	Beef production and carcass quality traits in Chisko and Hanwoo cattle	Edea et al., 2020
27	5.89-5.91	*XKR5*	Beef production and carcass quality traits in Chisko and Hanwoo cattle	Edea et al., 2020
27	6.013-6.015	*TAP*	Microbial activity against bacterial pathogens causing bovine respiratory disease	Berghuis et al., 2014

CHAPTER 3

Runs of heterozygosity and homozygosity in Italian dual-purpose cattle breeds

Abstract

Dual purpose cattle have been progressively replaced by specialized breeds; this led to an increase of homozygosity and to a loss of biodiversity and fitness traits. Differences between the genomes of dual-purpose and specialized breeds can be investigated through Runs of Homozygosity (ROHom) and Runs of Heterozygosity (ROHet), continuous stretches of homozygous or heterozygous SNP genotypes, respectively. Three examples of Italian dual-purpose cattle (Italian Simmental, Modicana, and Cinisara) were considered to test four different approaches in ROHet detection.

The Italian Simmental showed the largest number of ROHom, coupled with the lowest average length. Among the considered ROHet approaches, two provided results more consistent with the literature, whereas the other two probably overestimated the number of detected regions. The statistics of the first two approaches were consistent with the assumption that ROHet are fewer and shorted than ROHom.

Being ROHom and ROHet considered opposite, the correlation between the respective statistics were computed, but only the correlation between number of ROHom and ROHet in Italian Simmental was negative and significant, whereas the results for the other two breeds were not so clear. Moreover, only for the Italian Simmental the correlation between the ROHom-based and ROHet-based coefficients was coherent (i.e., negative value) with the expectation.

The genes mapped in ROHom and ROHet islands as well as those found in the regions shared among breeds were investigated. Interestingly, a region on BTA23 was identified by both ROHom and ROHet analyses. The *KH RNA Binding Domain Containing, Signal Transduction Associated 2* (*KHDRBS2*) gene mapped in this region and it was found to be related with different traits of interest. Results of the present study established the basis for future studies to better understand the relationship between homozygosity and heterozygosity in cattle.

Keywords

Dual-purpose cattle breeds, genetic diversity, detection parameters.

Introduction

In the last decades, dual purpose and autochthonous cattle populations have been progressively substituted with cosmopolite specialized breeds, genetically improved for production traits (Medugorac et al., 2009). This process led to a biodiversity loss and to a general increase in homozygosity (Baes et al., 2019). The specialized breeds show larger production levels compare to the dual-purpose ones, that, conversely, are characterized by hardiness, longevity, disease resistance, and adaptability (Mäki-Tanila et al., 2010). Moreover, the dual-purpose breeds can be considered as a valid resource in terms of biodiversity and heterozygosity (Giovambattista et al., 2001).

Italian Simmental (IS), Modicana (MOD), and Cinisara (CIN) are some examples of Italian dual-purpose cattle breeds. The IS breed is farmed mostly in small herds located in the mountainous areas of the Northeastern Italy (Cesarani et al., 2020), and it is the third largest Italian cattle breed (www.vetinfo.it, Sistema Informativo Veterinario 2022). The MOD and CIN breeds are mostly farmed in Sicily (Italy), since they are well adapted to the harshness of Sicilian marginal mountain areas (Mastrangelo et al., 2016). The differences between dual-purpose and specialized cattle breeds are probably due also to differences in their genome. Among the various techniques, Runs of Homozygosity (ROHom) and Runs of Heterozygosity (ROHet) allow to investigate the genetic structural variation of livestock population. In particular, ROHom are continuous homozygous chromosomal segments (Gibson et al., 2006) used to investigate inbreeding and selection signatures, since homozygous genotypes arise from the fixation of favorable alleles at selected loci (Macciotta et al., 2021). On the contrary, ROHet are regions rich of heterozygosity (Marras et al., 2018), defined as consecutive stretches of heterozygous SNP genotypes (Williams et al., 2016) in which haplotype diversity could be advantageous for fitness or survival traits (Mc Parland et al., 2009). For these reasons, ROHet can be used to investigate the maintenance of heterozygosity and, eventually, the balancing selection (Mulim et al., 2022), which is a type of natural selection that maintains genetic diversity via heterozygote advantage (Fijarczyk and Babik, 2015).

The first study about ROHom in cattle dates to 2010 (Sölkner et al., 2010). Several subsequent studies investigated the differences in the detection of ROHom when different parameters and software are used. Nevertheless, there is still a lack of consensus on the ROHom detection methodology that leads to difficulties in comparing the results among different studies (Howrigan et al., 2011). On the contrary, there is a lack of literature about ROHet, since this is a quite new technique. ROHet were firstly analyzed in cattle by Williams et al. (2016), who recognized in the genome of the Chillingham, a rare and highly inbred cattle breed, a particular clusterization of the heterozygous SNPs at specific chromosomal locations. After this pilot study, ROHet have been investigated in cattle by other authors, all using a wide plethora of parameters and methods to detect them, making the results difficult to compare. Regarding the detection method, some studies (i.e., Mulim et al., 2022; Lashmar et al., 2022; Biscarini et al., 2020) considered the consecutive windows method, whereas other studies used the sliding windows one (Williams et al., 2016; Kenny et al., 2022). Between the two methods, the consecutive one seemed to be preferred and more sensitive to ROHet (Mulim et al., 2022). In addition to these, other two studies investigated ROHet in cattle: Hidalgo et al. (2021) used the same parameters proposed by Biscarini et al. (2020), whereas Ferenčaković et al. (2016) used a different approach and software (SNP&Variation Suite) to detect ROHet.

The aim of this study was to investigate the ROHom and ROHet patterns in three Italian dual-purpose cattle breeds. Moreover, we investigated the consensus among different parameters to detect the ROHet and we analyzed the relationship between homozygosity and heterozygosity regions.

Materials and methods

Animals and genotypic data

The dataset consisted of genotypes for a total of 1,019 cattle belonging to three different breeds: 391 Italian Simmental, 304 Modicana, and 324 Cinisara. Within the Italian projects DUALBREEDING and DUALBREEDING-2, all animals were genotyped with the GGP Bovine 150k BeadChip, and the SNPs were filtered to exclude unmapped markers or those assigned to sex chromosomes according to the ARS-UCD 1.2 release. Moreover, SNPs with call rate less than 0.95 or with minor allele frequency lower than 0.01 were removed. After quality control, all animals and 119,375 SNPs were retained for further analysis.

Runs of homozygosity

Consecutive ROHom were computed using the "*detectRUNS*" R package (Biscarini et al., 2019). The minimum number of SNP that constituted a ROHom was calculated using the following formula from Purfield et al. (2012):

$$l = \frac{\log_e \frac{\alpha}{n_s . n_i}}{\log_e(1 - hom)} \qquad [1]$$

where n_s is the number of SNPs per individual, n_i is the number of individuals, α is the percentage of false positive (0.05), and *het* is the average heterozygosity. The same formula was adapted to compute the number of missing SNP allowed in a ROHom by using the call-rate in the denominator instead of the heterozygosity:

$$missing = \frac{\log_e \frac{\alpha}{n_s . n_i}}{\log_e(1 - AvgCallRate)} \qquad [2]$$

To calculate the number of opposite SNP (i.e., heterozygous) allowed, we considered the ratio between the minimum number of SNP in a ROHom and the minimum number of SNP in a ROHet. The minimum ROHom length was fixed at 1 million base pairs. Table 1 lists the parameters adopted to define a ROHom.

The number of ROHom per animal (nROHom) and average ROHom length ($\mathrm{meanMb}_{\mathrm{hom}}$) were computed using the R software.

The number of times a SNP fall into a ROHom was computed as $\mathrm{SNP}_{\mathrm{ROHom}}$, and the top 0.1% of these SNPs were considered as part of ROHom islands.

The ROHom-based inbreeding coefficient (F_{ROHom}) was calculated for each animal as:

$$F_{ROHom} = \frac{L_{ROHom}}{L_{GEN}} \qquad [3]$$

where L_{ROHom} was the sum of all ROHom lengths per animal and L_{GEN} was the total genome length covered by SNP (McQuillan et al., 2008).

Runs of heterozygosity

Consecutive ROHet were computed using the "*detectRUNS*" R package (Biscarini et al., 2019). To detect the ROHet, four different approaches were used based on the existing literature: ROHet1 (Purfield et al., 2012), ROHet2 (Mulim et al., 2022), ROHet3 (Biscarini et al., 2020), and ROHet4 (Lashmar et al., 2022). The latter three used the same method (i.e., consecutiveRUNS) applied in this study, whereas Purfield et al. (2012) involved a different computation strategy.

For the first approach (ROHet1), the same formula used to compute the minimum number of SNP, the missing SNP and the opposite SNPs allowed in a ROHom was applied (Purfield et al., 2012), using homozygosity instead of heterozygosity. In Table 1, the parameters used to call ROHet in the different approaches were reported.

Table 1. Parameters adopted to define ROHom and ROHet.

Metric	Breed	SNPs[1]	Opposite[2]	Missing	Minimum length
ROHom					
	IS	48	–	6	1Mb
	MOD	51	–	5	
	CIN	46	–	5	
ROHet1					
	IS	20	2	6	500kb
	MOD	18	3	5	
	CIN	20	2	5	
ROHet2		20	2	1	500kb
ROHet3		15	3	2	250kb
ROHet4		10	0	2	10kb

[1]SNPs = minimum consecutive homozygote markers for ROHom or heterozygotes for ROHet.

[2]Opposite = heterozygote markers for ROHom and homozygote markers for ROHet.

Number of ROHet per animal (nROHet) and average ROHet length (meanMb$_{het}$) were computed. As abovementioned, ROHet shared by at least 2 animals for each breed and ROHet islands were identified.

The ROHet-based heterozygosity coefficient (F_{ROHet}), already proposed by Ruan et al. (2022), was calculated for each animal as:

$$F_{ROHet} = \frac{L_{ROHet}}{L_{GEN}} \qquad [4]$$

where L_{ROHet} was the sum of all ROHet lengths per animal and L_{GEN} was the total genome length covered by SNP.

In order to evaluate the differences among the four considered methods, the number of ROHet and their average length were analyzed using the following generalized linear model (GLM) with a lognormal distribution:

$$y = \mu + \mathrm{METHOD} + e \qquad [5]$$

where: y is the number of ROHet or their average length; μ is the overall mean; METHOD is the fixed effect of the investigated method (4 levels, i.e., Purfield et al., 2012; Mulim et al., 2022; Biscarini et al., 2020; Lashmar et al., 2022); e was the random residual term.

Gene annotation

The genes mapped in the regions shared among the three breeds or within the ROHom and ROHet islands were retrieved using online databases (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov).

Results and Discussion

Analysis of Runs of Homozygosity and Runs of Heterozygosity

The descriptive statistics of ROHom are shown in Table 2. The IS showed the highest nROHom, whereas CIN the lowest. As far as the mean length was concerned, CIN and IS showed the highest and the lowest mean length, respectively. As already pointed out, ROHom results vary according to the adopted parameters and, thus, results of the present study differ from reports found in literature. The required number of minimum SNP to define a ROHom (Table 2), adopted in the present study was generally larger compared to other studies, i.e., 15 SNP (e.g., Cesarani et al., 2021; Hidalgo et al., 2021; Szmatola et al., 2016; Ferenčaković et al., 2011; Marras et al., 2015) or 30 (Bjelland et al., 2013; Mészáros et al., 2015; Punturiero et al., 2023). Indeed, in literature different nROHom values could be found: 82.1±20.9 in European IS (Cesarani et al., 2021), 53.97±17.15 in Romosinuano breed (Hidalgo et al., 2021), from 54±7.2 to 94.6±11.6 in Piemontese and Italian Brown cattle breeds (Marras et al., 2015), and from 106.8 to 125.1 in Italian Holstein cattle breed (Punturiero et al., 2023). As far as the mean length was concerned, the values found in the present study were more similar to those already reported in literature: e.g., 2.45 Mb for European Simmental (Cesarani et al., 2021), 3.3 Mb for Romosinuano (Hidalgo et al., 2021), from 1.9 to 3.9 Mb in Italian cattle (Marras et al., 2015), and 2.6 Mb in Holstein (Punturiero et al., 2023) breeds, respectively. Therefore, in general, a larger nROHom together with a slightly lower average ROHom length can be found in literature.

Table 2. Basic statistics for ROHom and ROHet computed using different approaches.

		Breed		
Approach	Parameters	IS	MOD	CIN
ROHom	ROHom (animals)	21,871 (390)	8,958 (277)	6,538 (293)
	nROHom[1]	56.08±19.20	32.34±23.40	22.31±15.64
	meanMb$_{hom}$[2]	2.80±2.63	4.11±4.84	4.37±5.66
ROHet1[3]	ROHet (animals)	3,118 (391)	2,826 (281)	1,523 (287)
	nROHet	7.97±6.34^{C}	10.06±6.73^{C}	5.31±3.02^{C}
	meanMb$_{het}$	0.63±0.14^{A}	0.63±0.18^{B}	0.68±0.22^{A}
ROHet2	ROHet (animals)	2,192 (387)	678 (196)	1,461 (285)
	nROHet	5.66±2.36^{C}	3.46±1.98^{C}	5.13±2.56^{C}
	meanMb$_{het}$	0.62±0.13^{B}	0.68±0.24^{A}	0.68±0.22^{A}
ROHet3	ROHet (animals)	98,857 (391)	40,729 (299)	63,713 (319)
	nROHet	252.83±37.50^{A}	136.22±68.52^{A}	199.73±60.02^{A}
	meanMb$_{het}$	0.37±0.11^{C}	0.38±0.12^{C}	0.38±0.12^{B}
ROHet4	ROHet (animals)	40,056 (391)	14,700 (299)	24,299 (319)
	nROHet	102.45±23.97^{B}	49.16±29.98^{B}	76.17±27.33^{B}
	meanMb$_{het}$	0.23±0.09^{D}	0.23±0.10^{D}	0.23±0.10^{C}

Different superscript letters within a column within a breed indicate significant difference among the approaches tested for ROHet detection ($P<0.05$).

[1] nROHom/nROHet = number of ROHom/ROHet per animal.

[2] meanMb$_{hom}$/meanMb$_{het}$ = mean of ROHom/ROHet length per animal.

[3] Parameters for ROHet1: PRI = minSNP 20, minlenght 500.000, maxOpp 2, maxMiss 6; MOD = minSNP 18, minlenght 500.000, maxOpp 3, maxMiss 5; CIN = minSNP 20, minlenght 500.000, maxOpp 2, maxMiss 5.

Parameters ROHet2: minSNP 20, minlenght 500.000, maxOpp 2, maxMiss 1.

Parameters ROHet3: minSNP 15, minlenght 250.000, maxOpp 3, maxMiss 2.

Parameters ROHet4: minSNP 10, minlenght 10.000, maxOpp 0, maxMiss 2.

The four approaches used to detect the ROHet led to very different results (Table 2). As far as the differences among the approaches were considered, the nROHet computed using the first two approaches (i.e., Purfield et al., 2012; Mulim et al., 2022) were not significantly different (Table 2). When the average ROHet length was considered, the differences were always significant among the four different approaches, with the only exception of the CIN breed.

When ROHet1 approach was considered, MOD had the highest nROHet (10.06±6.73) and CIN the lowest (5.31±3.02), respectively, whereas different results were obtained using the other three approaches. In the ROHet2, the nROHet value of MOD (3.46±1.98) halved compared to ROHet1, whereas the values of the other two breeds showed only a slightly decrease. In the other two approaches, the nROHet drastically increased, probably leading to overestimated values that could be considered less reliable. These large numbers could be associated with the permissiveness of the involved methods (i.e., lowest minimum length, less minimum SNPs and, in the case of ROHet3, more homozygous SNPs allowed). As mentioned before, the comparison of ROHet results with the reports in literature is complicated by the differences in the analyzed datasets (e.g., breed or SNP densities), the parameters used to define the heterozygote regions, and softwares. In fact, very different mean numbers of ROHet per animal could be found in literature: 47.7 in Romosinuano (Hidalgo et al., 2021), 30±5.8 (Kenny et al., 2022), and 121.5 in Austrian Pinzgauer (Ferenčaković et al., 2016). On the contrary, nROHet similar to the values computed in the present study were reported by Biscarini et al. (2020), who found that the majority of the animals showed a nROHet between 3 and 9, whereas Lashmar et al. (2022) reported a slightly lower value (about 3 ROHet per animal). In any case, most of the available studies agree that ROHet are usually more rare and shorter than ROHom (Biscarini et al., 2020).

As expected, the average length decreased as the minimum length and the minimum SNP parameters used for the detection decreased, with the magnitude of these changes varying according to the different approach and breed. In the ROHet1, the breed with the largest average length was the CIN (0.68±0.22), whereas in the ROHet2 approach the highest value was found for MOD and CIN (0.68±0.24 and 0.68±0.22, respectively). Finally, almost stable values were observed across breeds in the ROHet3 (0.37 or 0.38) and ROHet4 (0.23) approaches. Biscarini et al. (2020) reported an average ROHet length of 0.70 Mb, quite higher compared to the results of the present study, whereas Lashmar et al. (2022) reported more similar values to those obtained here using ROHet1 and ROHet2 approaches (0.62 Mb). On the contrary, Kenny et al. (2022) reported a very different average length, equal to 0.16±0.08 Mb.

Table 3 shows the correlations among the nROHet and the average ROHet length computed using the four different approaches. The correlations were always positive and significantly different from zero, suggesting a quite consensus among the approaches. As far as nROHet is concerned, the largest correlation was found between ROHet3 and ROHet4 for MOD (0.98), whereas the lowest value was observed between ROHet2 and ROHet4 for IS (0.13). The highest and lowest correlations for meanMb were computed between ROHet1 and ROHet2 for CIN (0.99) and between ROHet2 and ROHet3 for CIN (0.19), respectively.

In general, the number of ROHet identified by the different methods showed larger correlations compared to the average ROHet length (Table 3). Moreover, correlations computed for IS tended to be lower than those computed for the other two breeds.

Table 3. Correlations among different approaches for nROHet and meanMb.

	All breeds		IS		MOD		CIN	
	nROHet[1]	meanMb_{het}[2]	nROHet	meanMb_{het}	nROHet	meanMb_{het}	nROHet	meanMb_{het}
Approaches[3]								
ROHet1 – ROHet2	0.23	0.80	0.28	0.77	0.52	0.46	0.91	0.99
ROHet1 – ROHet3	0.37	0.23	0.56	0.22	0.89	0.29	0.56	0.21
ROHet1 – ROHet4	0.50	0.23	0.86	0.24	0.87	0.27	0.62	0.27
ROHet2 – ROHet3	0.46	0.23	0.17	0.22	0.50	0.24	0.47	0.19
ROHet2 – ROHet4	0.42	0.29	0.13	0.24	0.50	0.33	0.50	0.26
ROHet3 – ROHet4	0.94	0.55	0.76	0.44	0.98	0.62	0.94	0.48

All correlations were significantly different from 0 ($P<0.05$).

[1] nROHet: number of ROHet per animal.

[2] meanMb_{HET}: mean ROHet length.

[3] Parameters for ROHet1: PRI = minSNP 20, minlenght 500.000, maxOpp 2, maxMiss 6; MOD = minSNP 18, minlenght 500.000, maxOpp 3, maxMiss 5; CIN = minSNP 20, minlenght 500.000, maxOpp 2, maxMiss 5.

Parameters ROHet2: minSNP 20, minlenght 500.000, maxOpp 2, maxMiss 1.

Parameters ROHet3: minSNP 15, minlenght 250.000, maxOpp 3, maxMiss 2.

Parameters ROHet4: minSNP 10, minlenght 10.000, maxOpp 0, maxMiss 2.

Analysis of shared homozygote and heterozygote regions

Since ROHet1 and ROHet2 approaches gave results more consistent with the literature, the regions found with these two methods and shared among animals were considered (Table 4). The two approaches showed the same three shared ROHet on BTA4, BTA12, and BTA23. In addition, two (BTA18 and BTA20) and seven (BTA6, 8, 20, 23, and 27) other shared ROHet were found only in ROHet1 and ROHet2 approaches, respectively. It is interesting to note that Mulim et al. (2022) investigated the ROHet that appeared in at least 10% of the animals and two of the identified ROHet, on BTA23, were similar to the regions found in the present study on BTA23. Moreover, regions on BTA23 starting in the same point of the shared ROHet (i.e., 0.02Mb) were highlighted also with the ROHom analysis. In particular, on BTA23 (at 0.02-1.58Mb) the most shared ROHom was found in 45 animals (5 IS, 2 MOD, and 38 CIN). In this region, the *KH RNA Binding Domain Containing, Signal Transduction Associated 2* (*KHDRBS2*) gene was mapped. This gene has been associated with reproductive performance, and in particular with age at first calving, in Sanmartinero cattle (De León et al., 2019), with pregnancy status in Brahman cattle (Reverter et al., 2016), and with environmental adaptation in beef cattle (Rowan et al., 2021). As mentioned before, the same gene was found into a ROHet by Mulim et al. (2022).

Table 4. ROHet found in common among the breeds considering the first two approaches (based on Purfield et al., 2012 and Mulim et al., 2022).

Approach	BTA	Mb	Animals	IS	MOD	CIN	Genes
ROHet1	4	48.43 – 48.96	12 (1.2%)[1]	3 (0.8%)	2 (0.7%)	7 (2.2%)	*COG5*, *GPR22*, *DUS4L*, *BCAP29*, *SLC26A4*, *CBLL1*, *SLC26A3*, *DLD*
ROHet2			10 (1%)	5 (1.3%)	3 (1%%)	2 (0.6%)	
ROHet2	6	80.19 – 80.92	7 (0.7%)	3 (0.8%)	2 (0.7%)	2 (0.6%)	*EPAH5*
ROHet2	8	46.02 – 46.67	10 (1%)	3 (0.8%)	3 (1%)	4 (1.2%)	*MAMDC2*, *SMC5*
ROHet1 ROHet2	12	41.42 – 41.97	18 (1.8%)	6 (1.5%)	3 (1%)	9 (2.8%)	–
ROHet1	18	34.88 – 35.39	6 (0.6%)	2 (0.5%)	2 (0.7%)	2 (0.6%)	*SLC9A5*, *PLEKHG4*, *KCTD19*, *LRRC36*, *TPPP3*, *ZDHHC1*, *HSD11B2*, *ATP6V0D1*, *AGRP*, *RIPOR1*, *CTCF*, *CARMIL2*, *ACD*, *PARD6A*, *ENKD1*, *GFOD2*, *RANBP10*, *TSNAXIP1*, *CENPT*, *THAP11*, *NUTF2*, *EDC4*, *NRN1L*, *PSKH1*
ROHet1	20	30.82 – 31.40	16 (1.6%)	2 (0.5%)	3(1%)	11(3.4%)	*NNT*, *PAIP1*, *TMEM267*, *CCL28*
ROHet2		71.14 – 71.72	17 (1.7%)	4 (1%)	4(1.3%)	9 (2.8%)	*TERT*, *SLC6A18*, *SLC6A19*, *SLC12A7*, *NKD2*, *TRIP13*, *BRD9*, *TPPP*, *CEP72*, *SLC9A3*, *EXOC3*, *AHRR*
ROHet1	23	0.02 – 1.00	22 (2.2%)	12 (3.1%)	8 (2.6%)	2 (0.6%)	*KHDRBS2*
ROHet2			30 (2.9%)	12 (3.1%)	16 (5.3%)	2 (0.6%)	
ROHet2		0.02 – 1.53	25 (2.5%)	10 (2.6%)	9 (3%)	6 (1.9%)	
ROHet2		0.02 – 0.96	33 (3.2%)	11 (2.8%)	8 (2.6%)	14 (4.3%)	
ROHet2		0.18 – 0.87	149 (14.6%)	33 (8.4%)	9 (3%)	107 (33%)	
ROHet2	27	18.08 – 18.61	9 (0.9%)	3 (0.8%)	2 (0.7%)	4 (1.2%)	–

Values within the parenthesis represent the percentage of animals sharing the particular ROHet on the total number of animals of that breed.

The ROHet shared in both approaches (Table 4) mapped nine genes: eight genes were mapped on BTA4 (*COG5*, *GPR22*, *DUS4L*, *BCAP29*, *SLC26A4*, *CBLL1*, *SLC26A3*, and *DLD*), no genes in BTA12, and only one gene in the region found on BTA23 (*KHDRBS2*). The *G Protein-Coupled Receptor 22* (*GPR22*) gene had not previously found in cattle, but it was associated with coat color in goats (Wan et al., 2023). The *Component Of Oligomeric Golgi Complex 5* (*COG5*) gene was found associated with immune traits, and in particular involved in neutrophil response (Freeze and Schachter, 2009). The *B Cell Receptor Associated Protein 29* (*BCAP29*) gene was reported to be mastitis related (Gorji et al., 2019). Irano et al. (2016) reported *SLC26A4*, *CBLL1*, *SLC26A3*, and *DLD* as associated with scrotal circumference in Nellore cattle. In addition, the *Cbl Proto-Oncogene Like 1* (*CBLL1*) gene was found involved in milk composition and cheese-making properties (Sanchez et al., 2019), and *Solute Carrier Family 26 Member 3* (*SLC26A3*) associated with feed efficiency, in particular with volatile fatty acids absorption (Elolimy et al., 2018).

As far as the ROHet found using only the ROHet1 approach were considered, twenty-four genes were mapped in the region found on BTA18, and four in the region on BTA20. Nineteen of them (*SLC9A5, PLEKHG4, KCTD19, LRRC36, TPPP3, ZDHHC1, HSD11B2, ATP6V0D1, AGRP, RIPOR1, CTCF, CARMIL2*, *ACD*, *PARD6A*, *ENKD1*, *GFOD2*, *TSNAXIP1*, *NUTF2*, and *PSKH1*) were reported related to meet traits in Canadian beef cattle (Sood et al., 2023). Eighteen genes (*PLEKHG4*, *TPPP3, HSD11B2, ATP6V0D1, AGRP, CTCF, ACD*, *PARD6A*, *ENKD1*, *GFOD2*, *RANBP10*, *TSNAXIP1*, *CENPT*, *THAP11*, *NUTF2*, *EDC4*, *NRN1L*, and *PSKH1*) were found involved in the determination of meat color in Nellore cattle (Marín-Garzón et al., 2021). Three genes of these (*AGRP*, *RIPOR1*, and *CTCF*) had been associated with feed efficiency, in particular with dry matter intake and residual feed intake (Mota et al., 2023), and the *ATPase H+ Transporting V0 Subunit D1* (*ATP6V0D1*) gene with residual feed intake of beef cattle (Kong et al., 2016). Two genes were reported associated with reproductive performance: the *Nuclear Transport Factor 2* (*NUTF2*) was reported involved in early pregnancy (Forde et al., 2015), and the *Zinc Finger DHHC-Type Containing 1* (*ZDHHC1*) associated with fertility, in particular with *corpus luteum* function, in Holstein cows (Moore et al., 2016).

All the genes mapped on BTA20 were reported associated with milk production. Three of them (*PAIP1*, *TMEM267*, and *CCL28*) were found associated with milk protein percentage in Holstein (Pedrosa et al., 2021). In addition, *Poly(A) Binding Protein Interacting Protein 1* (*PAIP1*) was associated with milk yield (Tribout et al., 2020), and *C-C Motif Chemokine Ligand 28* (*CCL28*), with lactation persistency (Do et al., 2017). In the latter study also *Nicotinamide Nucleotide Transhydrogenase* (*NNT*) was found associated with lactation persistency, and the same gene was reported related to fat and protein percentage in Holstein cattle milk (Prakapenka et al., 2021).

As far as the ROHet found using only the ROHet2 approach were considered, one gene was mapped on BTA6, two on BTA8, and twelve in the region on BTA20.

The *EPH Receptor A5* (*EPHA5*), mapped on BTA6, was previously associated with female traits in Iranian Holstein (Mohammadi et al., 2020) and with feed conversion ratio in Nellore cattle (de Almeida Santana et al., 2016).

On BTA8, *MAM Domain Containing 2* (*MAMDC2*) was found involved in average daily gain in Angus and Charolais cattle (Mukiibi et al., 2019), and *Structural Maintenance Of Chromosomes 5* (*SMC5*) with udder conformation traits (Nazar et al., 2022) and with oocyte development (Walker and Biase, 2020).

Among the twelve genes mapped on BTA20, some of them inhere investigated were previously reported to be involved in immunity traits. The *Solute Carrier Family 6 Member 18* (*SLC6A18*) and *Telomerase Reverse Transcriptase* (*TERT*) genes have been associated with hoof health traits, in particular with sole hemorrhage, in Holstein (Butty et al., 2021). The *TERT* was also associated with somatic cell score (Durán Aguilar et al., 2017). The *NKD Inhibitor Of WNT Signaling Pathway 2* (*NKD2*) was reported to be a candidate gene for tick resistance (Santos et al., 2022), and the *Solute Carrier Family 6 Member 19* (*SLC6A19*) was associated with resistance to intestinal warm in Angus cattle (Li et al., 2015), and with resistance to *mycobacterium avium* subsp. *Paratuberculosis* in Holstein (Hempel et al., 2016). Moreover, these two latter genes, with also *SLC12A7*, *TRIP13*, *BRD9*, *TPPP*, *CEP72*, *SLC9A3*, *EXOC3*, and *AHRR*, were reported as involved in milk production in German Black Pied cattle (Korkuć et al., 2021). In addition, *NKD2* was associated also with meat tenderness in beef cattle (Muniz et al., 2021), and the *Thyroid Hormone Receptor Interactor 13* (*TRIP13*) with age at puberty in Nellore cattle (Stafuzza et al., 2020). Finally, *CEP72*, *SLC9A3*, and *EXOC3* were reported associated with feed efficiency in Nellore (Olivieri et al., 2016).

Comparison between ROHet and ROHom

In order to compare the results about ROHom and ROHet, the approach based on Purfield et al. (2012) was considered since it was used for both techniques. Correlations between nROHom and nROHet and between ROHom and ROHet average lengths are shown in Table 5.

Table 5. Correlations between number and average lengths computed for ROHom and ROHet.

	IS	**MOD**	**CIN**
nROHom vs nROHet	**-0.38**	**0.53**	-0.04
$\text{meanMb}_{\text{hom}}$ vs $\text{meanMb}_{\text{het}}$	0.10	-0.002	0.003

Correlations in bold were significantly different from 0 ($P<0.05$).

The correlation between the numbers of regions were significant for IS and MOD (Table 5). For IS, the correlation (-0.38) was negative, as expected, since the two techniques are considered opposite. On the contrary, for MOD, the correlation was positive and moderate (0.53). The correlations between average lengths were not significantly different from 0, suggesting that these two parameters are not related to each other.

The ROHom and ROHet based coefficients are reported in Table 6. F_{ROHet} values were always very low (0.002), whereas F_{ROHom} values (from 0.04 to 0.06) were similar to those reported in literature. In particular, previous studies on the Simmental cattle breed reported F_{ROHom} of 0.08±0.04 in Poland (Szmatola et al., 2016), 0.09±0.02 in Austria (Ferenčaković et al., 2011), and 0.07±0.02 in Simmental bulls from various European countries (Cesarani et al., 2021). Also the values computed for MOD and

CIN were similar to the reports available in literature: a value of 0.07±0.06 was already reported for Modicana by Cesarani et al. (2018), and estimates of 0.06±0.05 for Modicana and 0.05±0.06 for Cinisara by Mastrangelo et al. (2016). In our knowledge, in literature there is not any other study about F_{ROHet} in cattle.

The correlations between the two coefficients were computed. As expected because of the antagonism between ROHom and ROHet, it resulted negative for IS (Table 6). MOD showed a moderate positive correlation (0.40), reflecting the positive correlation between nROHom and nROHet. Correlations were significantly different from zero for IS and MOD, but not for CIN (Table 6).

Table 6. ROHom and ROHet based coefficients and their correlation in the five considered breeds.

Breed	F_{ROHom}	F_{ROHet}	Correlation
IS	0.06±0.03	0.002±0.002	**-0.35**
MOD	0.05±0.05	0.002±0.002	**0.40**
CIN	0.04±0.05	0.002±0.001	-0.10

Correlations in bold were significantly different from 0 (P<0.05).

ROHom and ROHet islands

ROHom and ROHet count per SNP are graphically presented in Miami plots (Figure 1). All considered breeds had the largest SNP_{ROHet} values on BTA23, where the ROHom and ROHet shared among animals were found (Table 4).

The ROHom islands identified through the SNP_{ROHom} values are reported in Table 7; these regions were located on only two chromosomes – BTA6 (two islands for IS and one for MOD) and BTA5 (five islands for CIN) – and mapped a total of 35 genes. The main functions associated to the genes located in the ROHom islands regarded morphological traits (i.e., hip height), feed efficiency (e.g., residual feed intake and body weight gain), meat quality (fat composition and *Longissimus dorsi* weight), milk production (milk yield, milk fat synthesis), immunity (response to paratuberculosis, and Charcot-Marie-Tooth disease), and reproduction (i.e., sterility, oocyte development, endometrial function, testicular development, perinatal mortality, early pregnancy, estrous behavior, pregnancy rate, embryo implantation, spermatogenesis, and semen quality).

Among the genes mapped in the identified regions, a cluster of 13 genes (*AASDH*, *PPAT*, *PAICS*, *HOPX*, *REST*, *POLR2B*, *IGFBP7*, *RFX4, RIC8B*, *BTBD11*, *PRDM4*, *ASCL4*, and *RTCB*) was reported to be associated with feed efficiency traits in Nellore (Brunes et al., 2021), and another cluster composed by 5 genes (*PKP2*, *YARS2*, *DNM1L*, *FGD4*, and *BICD1*) with early pregnancy in Nellore heifers (Oliveira et al., 2017).

Table 7. Genes found in the ROHom islands.

Breed	BTA	Mb	nSNPs^1	Genes	Associated traits	Reference
IS	6	38.64 – 38.68	14	–		
		71.11 – 72.50	61	*EXOC1L*	–	
				EXOC1	Fat composition, linoleic and oleic acid content	Bruscadin et al., 2022 Poleti et al., 2020
				CEP135	Hip height Milk yield	Zhang et al., 2017 Rui et al., 2013
				KIAA1211	Early pregnancy Azoospermia and sterility in cattle-yak	Oliveira et al., 2017 Zhao et al., 2021
				AASDH	Feed efficiency	Brunes et al., 2021
				PPAT	Residual feed intake, body weight gain, and feed efficiency *Longissimus dorsi* weight	Brunes et al., 2021 Liang et al., 2023
				PAICS	Residual feed intake, body weight gain, and feed efficiency Oocyte development	Brunes et al., 2021 Walker and Biase, 2020
				SRP72	Cold stress response	Hu et al., 2021
				ARL9	Testicular development	Yuan et al., 2023
				THEGL	–	
				HOPX	Residual feed intake, body weight gain, and feed efficiency Perinatal mortality	Brunes et al., 2021 Purfield et al., 2020

Breed	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
					Response to *Mycobacterium avium* subsp. *Paratuberculosis*	Doherty et al., 2016
				SPINK2	Spermatogenesis and semen quality in buffaloes	Binsilla et al., 2021
				REST	Residual feed intake, body weight gain, and feed efficiency	Brunes et al., 2021
				NOA1	Juvenile mortality	Guintard et al., 2022
				POLR2B	Residual feed intake and body weight gain	Brunes et al., 2021
					Estrous behavior	Kommadath et al., 2013
				IGFBP7	Residual feed intake and body weight gain	Brunes et al., 2021
					Coat and teat color	Fan et al., 2014
					Pregnancy rate	Zamorano-Algandar et al., 2017
MOD	6	38.97 – 39.50	132	–		
CIN	5	70.05 – 70.37	16	*RFX4*	Residual feed intake	Brunes et al., 2021
					Milking temperament	Abo-Ismail et al., 2014
				RIC8B	Residual feed intake	Brunes et al., 2021
					Response to *Mycobacterium avium* subsp. *Paratuberculosis*	Malvisi et al., 2020
				TMEM263	Dwarfism in chicken	Xiong et al., 2023
				MTERF2	Reproductive biological processes	Oliveira et al., 2019
				CRY1	Milk fat percentage	Pedrosa et al., 2021
					Carcass traits	Zhang et al., 2022
					Oocyte development	Amano et al., 2010
		70.79 – 70.84	4	*BTBD11*	Residual feed intake	Brunes et al., 2021

Breed	**BTA**	**Mb**	**nSNPs[1]**	**Genes**	**Associated traits**	**Reference**
					Fertility	Jiang et al., 2019
		70.90 – 71.43	31	*BTBD11*	Residual feed intake	Brunes et al., 2021
					Fertility	Jiang et al., 2019
				PWP1	Oocyte development	Walker and Biase, 2020
				PRDM4	Residual feed intake	Brunes et al., 2021
				ASCL4	Residual feed intake	Brunes et al., 2021
					Milk fat synthesis in bovine mammary epithelial cells	Fan et al., 2020
				RTCB	Residual feed intake	Brunes et al., 2021
				BPIFC	–	
				FBXO7	–	
				SYN3	Hip height	An et al., 2019
				TIMP3	Hip height	An et al., 2019
					Endometrial function	Sponchiado et al., 2017
		71.48 – 71.50	4	*SYN3*	Hip height	An et al., 2019
		76.78 – 77.60	37	*PKP2*	Early pregnancy	Oliveira et al., 2017
					Embryo implantation	Sugimoto et al., 2013
				YARS2	Early pregnancy	Oliveira et al., 2017
				DNM1L	Early pregnancy	Oliveira et al., 2017
					Response to *Mycobacterium avium* subsp. *Paratuberculosis*	Marino et al., 2017
					Heat stress response	Dou et al., 2022

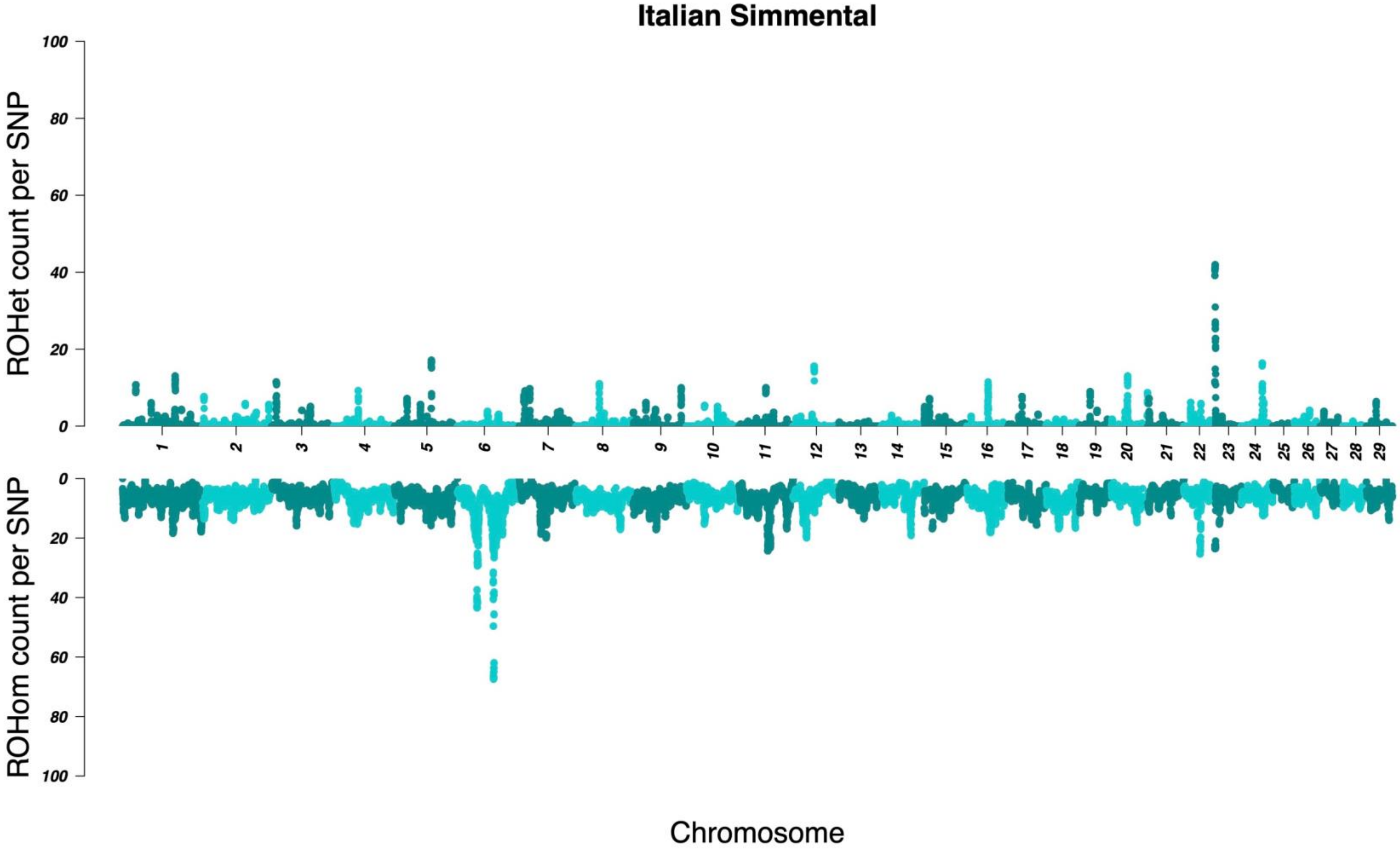
Breed	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
				FGD4	Early pregnancy	Oliveira et al., 2017
					Charcot-Marie-Tooth disease	Reynolds et al., 2021
				BICD1	Early pregnancy	Oliveira et al., 2017

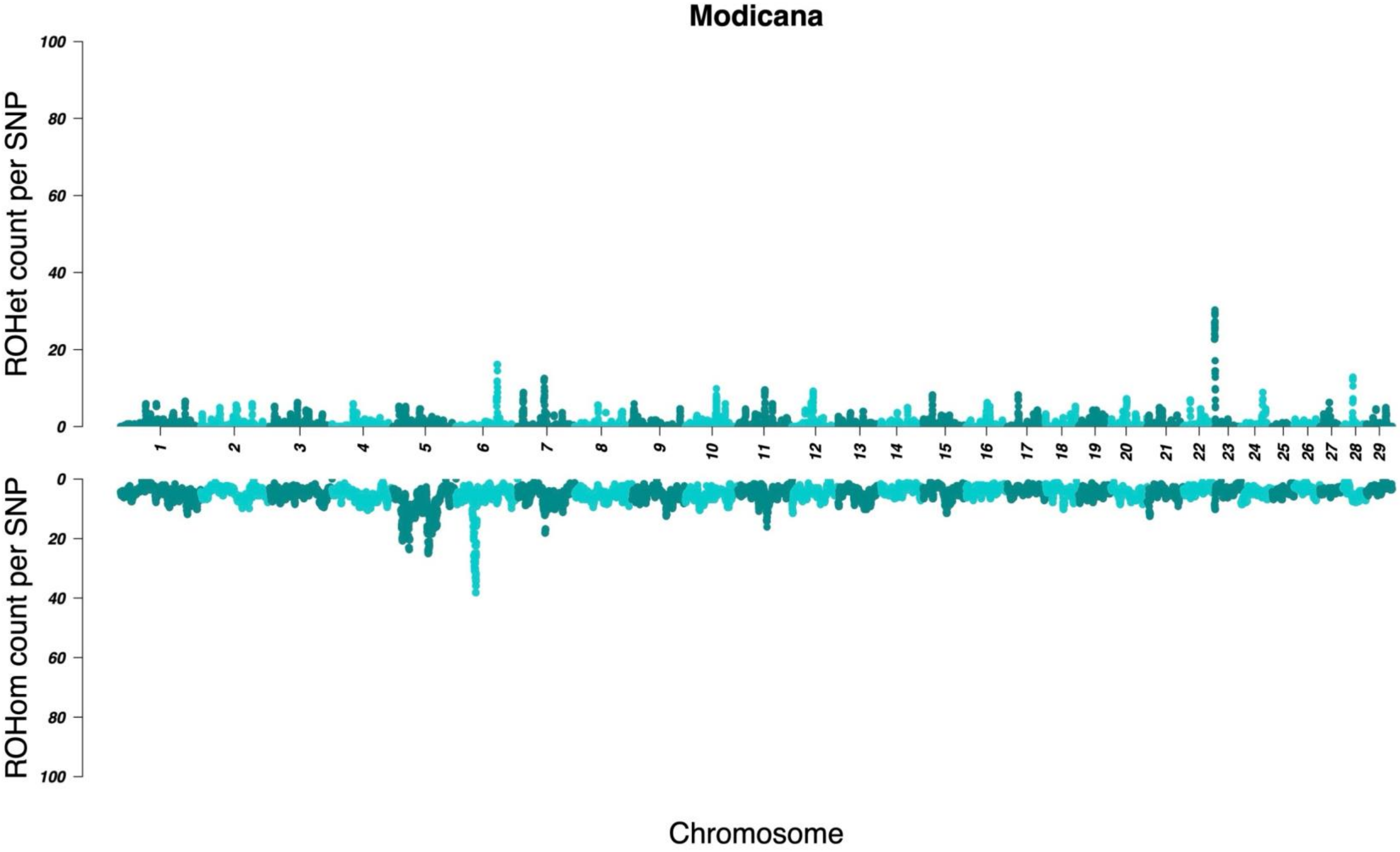
[1] number of SNPs inside the ROHet.

Table 8 shows the identified ROHet islands and Figure 2 graphically shows the overlapping rate among breeds. Again, all investigated breeds showed a peak on BTA23 (Figure 2); the regions slightly changed within breed, but all shared the genomic region located at 0.18-1.58 Mb. This region mapped the *KHDRBS2* gene, already mentioned for the most shared ROHet and ROHom. Moreover, MOD and CIN showed a similar ROHet island on BTA7: the CIN island was larger, but both shared the region from 51.63 to 52.22 Mb. In this region seventeen genes were located (Table 8). The genes mapped in the ROHet island located on BTA4 (Table 8) were found also in one of the ROHet shared among animals (Table 4). As reported in Table 8, most of the genes found within the ROHet islands were previously reported to be associated in livestock with milk and meat production, immune traits (e.g., mastitis response and resistance, neutrophil response, and resistance to intestinal nematodes), reproduction (e.g., fertility, gestation length, scrotal circumference, and age at first calving), and fitness performances (e.g., response to heat and cold stress or environmental adaptation). Milk production traits included lactation persistency, milk composition, and cheese-making properties, whereas meat production traits referred to meat quality, marbling score, intramuscular fat, tenderness and texture of meat, and dry matter intake.

A total of 13 genes (*EIF4EBP3*, *SRA1*, *APBB3*, *SLC35A4*, *CD14*, *TMCO6*, *NDUFA2*, *IK*, *WDR55*, *DND1*, *HARS1*, *HARS2*, *ZMAT2*) were reported to be associated with thoracic circumference (Guzman et al., 2020), milk protein yield (Lázaro et al., 2021), and lactation persistency (Lázaro et al., 2023) in buffaloes. Another cluster composed by ten genes (*PCDHA13, PCDHA3*, *ZMAT2*, *TMCO6*, *NDUFA2*, *IK*, *WDR55*, *DND1*, *HARS1*, and *HARS2*) has been associated with intramuscular fat in Nellore cattle

(Berton et al., 2022). Moreover, other two little clusters, each composed by three genes (*NRBF2*, *JMJD1C*, and *REEP3; SLC26A4*, *CBLL1*, and *SLC26A3*), were associated with feed efficiency in mid-lactation (Hardie et al., 2017) and scrotal circumference (Irano et al., 2016), respectively.


Italian Simmental
ROHet count per SNP
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
ROHom count per SNP
0
20
40
60
80
100
Chromosome


Modicana
ROHet count per SNP
100
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22
23
24
25
26
27
28
29
ROHom count per SNP
0
20
40
60
80
100
Chromosome

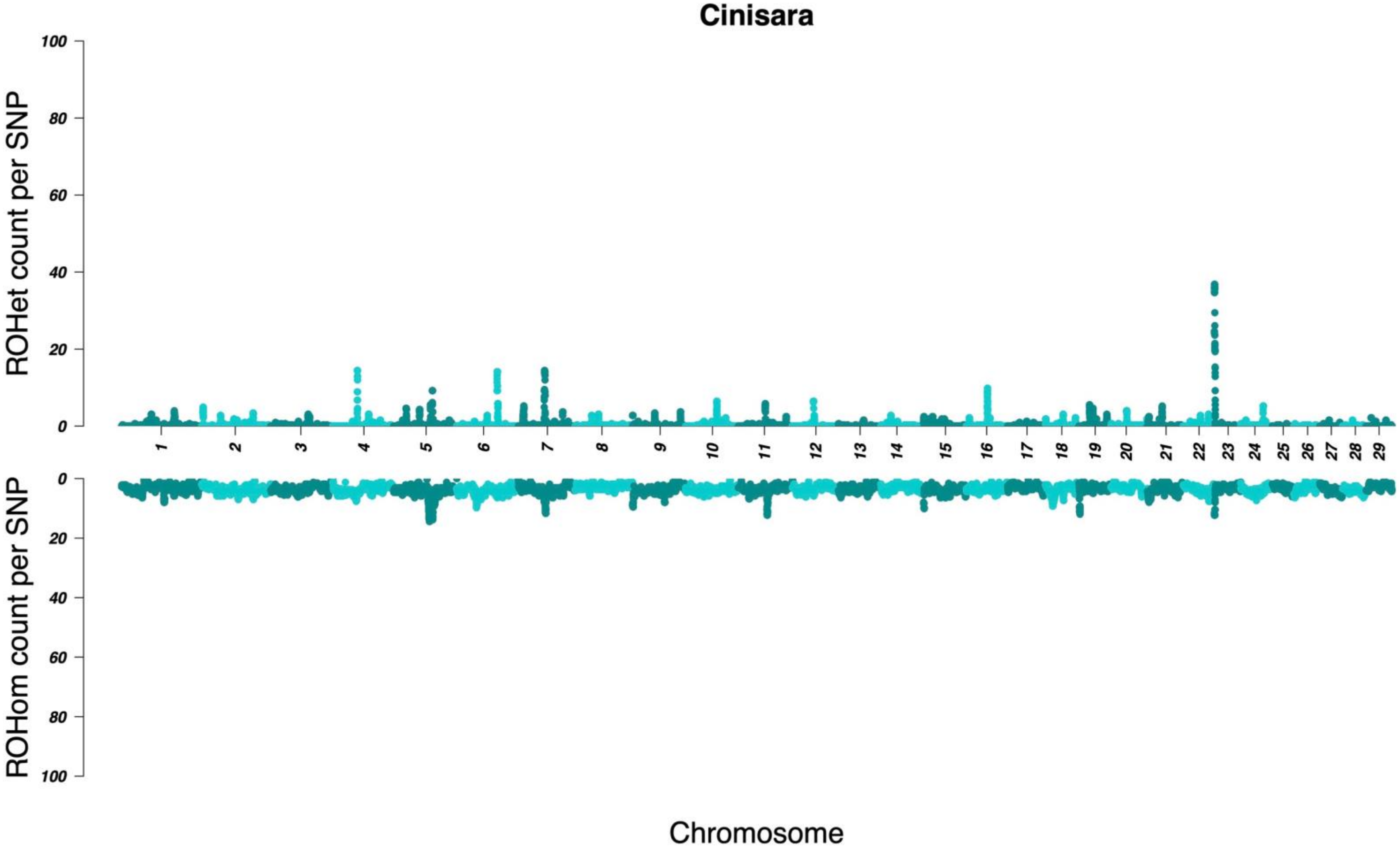

Figure 1. Miami plots of ROH and ROHet for each breed.

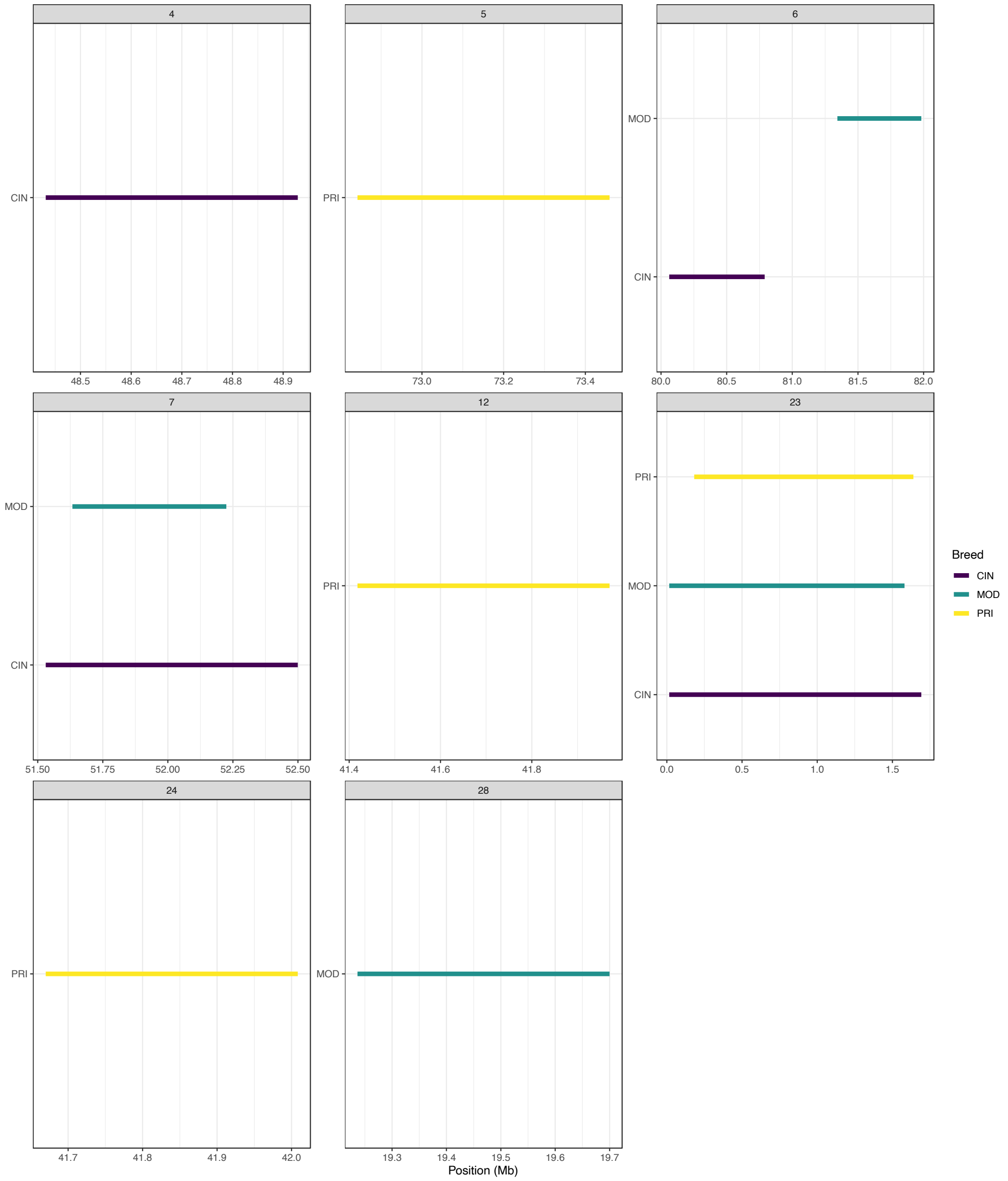

Figure 2. ROHet islands identified per chromosome and their overlapping among breeds.

Table 8. Genes found in the ROHet islands.

Group	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
IS	5	72.84 – 73.46	28	*ISX*	Marbling score	Ryu and Lee, 2016
	12	41.42 – 41.97	20	–		
	24	41.67 – 42.01	16	*PPP4R1*	Plasma urea concentration in Santa Inês sheep	de Souza et al., 2021
				RAB21	Physiological response to heat stress	De León et al., 2019
				TXNCD2	Spermatogenesis in cattle-yak	Cao et al., 2022
				VAPA	–	
MOD	6	81.34 – 81.98	26	–		
	28	19.24 – 19.70	16	*NRBF2*	Feed efficiency in mid-lactation	Hardie et al., 2017
					Black-white pigmentation	Li et al., 2014
				JMJD1C	Feed efficiency in mid-lactation	Hardie et al., 2017
					Fertility and reproduction traits	Chen et al., 2022
				REEP3	Feed efficiency in mid-lactation	Hardie et al., 2017
					Residual feed intake	Sherman et al., 2010
MOD and CIN	7	51.63 – 52.22	10	*ANKHD1*	Lactation persistency in buffaloes	Lázaro et al., 2023
				EIF4EBP3	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Fertility	Moore et al., 2016

Group	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
				SRA1	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
				APBB3	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
				SLC35A4	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Dry matter intake in beef steers	Kern et al., 2016
					Regulation of mastitis	Lu et al., 2023
				CD14	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Mastitis resistance	Selvan et al., 2014
					Milk production	Beecher et al., 2010
					Immune response to trypanosome	Morenikeji et al., 2020
				TMCO6	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023

Group	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
					Intramuscular fat	Berton et al., 2022
				NDUFA2	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
				IK	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
					Resistance to intestinal nematodes	Araujo et al., 2009
				WDR55	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
				DND1	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
				HARS1	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021

Group	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
					Somatic cell score	Lu et al., 2023
				HARS2	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
					Somatic cell score	Lu et al., 2023
				ZMAT2	Thoracic circumference in buffaloes	Guzman et al., 2020
					Milk protein yield in buffaloes	Lázaro et al., 2021
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Intramuscular fat	Berton et al., 2022
					Fertility	Fonseca et al., 2020
					Oocyte development	Walker and Biase, 2020
				PCDHA13	Thoracic circumference in buffaloes	Guzman et al., 2020
					Gestation length	Fang et al., 2019
					Intramuscular fat	Berton et al., 2022
				PCDHA3	Thoracic circumference in buffaloes	Guzman et al., 2020
					Intramuscular fat	Berton et al., 2022
				PCDHB1	Marbling, tenderness, and texture of meat	Rezende et al., 2021

Group	BTA	Mb	nSNPs¹	Genes	Associated traits	Reference
					Gestation length	Chen et al., 2021
CIN	4	48.43 – 48.93	19	*COG5*	Neutrophil response	Freeze and Schachter, 2009
				GPR22	Coat color in goats	Wan et al., 2023
				DUS4L	–	
				BCAP29	Mastitis related	Gorji et al., 2019
				SLC26A4	Scrotal circumference	Irano et al., 2016
				CBLL1	Scrotal circumference	Irano et al., 2016
					Milk composition and cheese-making properties	Sanchez et al., 2019
				SLC26A3	Scrotal circumference	Irano et al., 2016
					Feed efficiency	Elolimy et al., 2016
	6	80.06 – 80.79	20		–	
	7	51.53 – 52.50	18	*HBEGF*	Heat adaptation	Andrade et al., 2022
				SLC4A9	Thoracic circumference in buffaloes	Guzman et al., 2020
					Lactation persistency in buffaloes	Lázaro et al., 2023
					Volatile fatty acids absorption	Liu et al., 2022
				PCDHB8	–	
				PCDHB14	Meat quality	Leal-Gutiérrez et al., 2018
				PCDHB11	Meat quality	Leal-Gutiérrez et al., 2018
				SLC25A2	Ribeye area	Silva et al., 2020
				TAF7	Cold adaptation	Huang et al., 2023

Group	BTA	Mb	nSNPs[1]	Genes	Associated traits	Reference
				PCDHGA2	Mastitis resistance	Yang et al., 2014
				PCDHGB4	Fertility	Tarekegn et al., 2021
				PCDHGA8	Fertility	Tarekegn et al., 2021
IS	23	0.18 – 1.64	54	*KHDRBS2*	Age at first calving	De León et al., 2019
MOD	23	0.02 – 1.58	60		Pregnancy status	Reverter et al., 2016
CIN	23	0.02 – 1.69	62		Environmental adaptation	Rowan et al., 2021

[1]number of SNPs inside the ROHet.

Conclusions

In the present study, four different approaches reported in the literature were compared for ROHet detection. The results confirmed that ROHet features are strongly affected by the adopted parameters; only two out of four approaches showed values consistent with the literature. Differences between ROHom and ROHet were observed: ROHet are lower and shorter compared to ROHom, but the correlations were not so clear. A similar region located on BTA23 was found by analyzing both shared ROHom and ROHet, other than ROHet islands. Future investigations are needed to better clarify the relationship between the two techniques considered and to study the biological importance of the region found on BTA23.

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CHAPTER 4

Relationship between runs of homozygosity and heterozygosity in the Italian Simmental cattle genotyped at different densities

Abstract

Runs of Homozygosity (ROHom) are continuous stretches of homozygous loci that can reflect genome autozygosity. On the contrary, Runs of Heterozygosity (ROHet) are heterozygosity-rich regions that can contain genes resilient to genetic drift. The diversity in these regions could benefit survival rates, fertility, and other fitness traits. The detection of these two types of genomic regions (ROHom and ROHet) is influenced by the parameters used to define them and the number of available SNPs. The present study aimed at testing the effect of chip density and minimum length in detecting ROHom and ROHet in the Italian Simmental cattle breed. A sample of 897 Italian Simmental cattle were genotyped in low-density (50k SNP; 397 individuals), medium-density (140k SNP; 348 individuals), or high-density (800k SNP; 152 individuals). ROHom and ROHet were detected using the consecutive runs method. The number of ROHom and ROHet per animal (nROHom and nROHet, respectively) and their average length ($meanMb_{hom}$ and $meanMb_{het}$) were calculated. Runs shared by more than one animal and the number of times a particular SNP was inside a run were also computed (SNP_{ROHom} and SNP_{ROHet}). The nROHom increased, whereas their average length decreased, as the chip density increased. On the contrary, the pattern for nROHet was not so clear. The largest number of ROHet per individual was observed at medium-density. A total of 15 SNPs (located on BTA6) exceeded the 99th percentile of the SNP_{ROHom} distribution, whereas only two (located on BTA21) exceeded that percentile of the SNP_{ROHet} distribution. The genomic region on BTA6 mapped the *SLIT2* gene, whereas on BTA21, the *UBE3A*, *SNRPN*, and *SNURF* genes were found. The most shared ROHom and ROHet were found at 38.43-39.46 Mb on BTA6 and at 0.18-0.87 Mb on BTA23, respectively. In the latter, the *KHDRBS2* gene was mapped. The results confirmed that ROHom can be better detected using a higher chip density, whereas further studies are needed to understand the findings about ROHet better.

Keywords

selection signatures, BeadChip density, dual-purpose cattle breed.

Introduction

Runs of Homozygosity (ROHom) are continuous stretches of homozygous loci firstly recognized by Broman and Weber (1999) in human populations. They can be considered as indicators of the level of autozygosity of the genome (McQuillan et al., 2008) because the homozygous segments may have been inherited from common ancestors, i.e., the so-called Identical by Descent (IBD) (Purfield et al., 2012). Therefore, their length is a temporal indicator of the inbreeding occurrence (Kirin et al., 2010): recent inbreeding results in longer ROHom, whereas old inbreeding is associated with shorter ROHom, respectively, because recombination events tend to break the segments during the progression of the generations. Indeed, ROHom can be used to calculate inbreeding based on the level of autozygosity of the genome (Ferenčaković et al., 2013a). ROHom occurrence can be also related to selection, both natural and artificial, since homozygous genotypes can arise from the fixation of favorable alleles at selected loci and the linkage disequilibrium can extend the variation of allele frequency to the neighboring loci ("hitchiking effect"), thus increasing the degree of homozygosity and the portion of the genome covered by ROHom (Macciotta et al., 2021). For this reason, the investigation of shared ROHom within a population may allow to identify regions potentially under selection related to production traits, adaptation to environment, or breed-specific traits (Mastrangelo et al., 2018). In contrast to the definition of ROHom, Runs of Heterozygosity (ROHet) cannot be defined as true runs but rather as heterozygosity-rich regions (Marras et al., 2018). The ROHet were first investigated by Williams et al. (2016) in the Chillingham white, a UK local cattle breed, that exhibited a low level of polymorphisms compared to commercial breeds. Heterozygous loci where not homogeneously distributed across the genome, but clustered in specific regions of the genome, named ROHet. This suggested that those regions could contain genes resilient to genetic drift and with possible effects on fitness traits. Indeed, the diversity in these heterozygous regions could favor benefit survival rate, fertility, and other fitness traits (Mc Parland et al., 2009). For this reason, ROHet islands can be investigated to search for balancing selection, defined as the natural selection that maintains genetic diversity via heterozygote advantage (Fijarczyk and Babik, 2015).

The ROHet is a quite recent concept. After the first study of Williams et al. (2016), other studies on ROHet have been carried out in cattle (Ferenčaković et al., 2016; Biscarini et al., 2020; Hidalgo et al., 2021; Mulim et al., 2022), sheep (Selli et al., 2021), goats (Li et al., 2022), turkey (Marras et al., 2018), horses (Santos et al., 2021; Santos et al., 2023), and pigs (Chen et al., 2022; Ruan et al., 2022; Bordonaro et al., 2023). Overall, there is a lack of consensus on the criteria to be used to define ROHet; in fact, one main challenge in ROHet investigation is the choice of parameters (i.e., minimum run length, number of consecutive, homozygous, and missing SNP allowed). The ROHom tend to be more abundant in inbreed and artificially selected populations (Kim et al., 2018), whereas, in theory, ROHet might be more common in outbred or less selected populations. Usually, dual-purpose cattle breeds have been subjected to a lower selection pressure compared to specialized breeds, so it is reasonable to expect a larger level of heterozygosity of their genome. The Italian Simmental is a dual-purpose breed, farmed mostly in small herds in the mountainous areas of Northeastern Italy (Cesarani et al., 2020). It is the third cattle breed in Italy considering the number of farmed animals (www.vetinfo.it, Sistema Informativo Veterinario 2022). Therefore, it could represent a middle ground regarding artificial selection. The aim of this study was to investigate the differences in the detection of ROHom and ROHet using scenarios in which runs were calculated using different run lengths and densities of genotyping in Italian Simmental cattle.

Materials and methods

Data

A sample of 897 Italian Simmental cattle were considered in this study: 397 were genotyped with low (50K), 348 with medium (140K) and 152 with high-density (800K) chip array, respectively.

Quality control was performed separately for each beadchip density. SNP were retained for the analysis if: there was no statistical deviation from the Hardy-Weinberg Equilibrium (HWE), Minor Allele Frequency (MAF) was > 0.01, animal and SNP call rates were > 95%. Additionally, SNPs mapped on sexual chromosomes or unmapped according to the ARS-UCD1.3 were discarded. After quality control, 43,431 SNPs were retained for the 50K, 113,042 for the 140K, and 583,637 for the 800K chip arrays, respectively.

Runs detection

Consecutive runs were computed using the "*detectruns*" R package (Biscarini et al., 2019). To minimize the number of false positive runs, the minimum number of SNPs that constituted a ROHom was computed using the following formula (Purfield et al., 2012):

$$nSNP_{ROH} = \frac{\log_e \frac{\alpha}{n_s . n_i}}{\log_e(1 - het)} \qquad [1]$$

where n_s is the number of SNPs per individual, n_i is the number of individuals, α is the percentage of false positive (0.05), and *het* is the average heterozygosity. Equation [1] was used also to compute the minimum number of SNPs that constituted a ROHet, considering homozygosity instead of heterozygosity in the denominator.

For both ROHom and ROHet, the number of missing SNP allowed in a run was computed by using the non-missing rate in the denominator of equation [1] instead of the heterozygosity, whereas the number of opposite SNPs (i.e., heterozygotes while computing ROHom or homozygotes while computing ROHet) allowed were computed as the ratio between the minimum number of SNP in a ROHom and the minimum number of SNP in a ROHet. Table 1 shows the parameters adopted to define ROHom and ROHet at different densities.

Table 1. Parameters used to define runs of homozygosity and runs of heterozygosity.

	ROHom			ROHet		
Density	Min. SNP	Opposite	Missing	Min. SNP	Opposite	Missing
50k	49	-	4	18	3	4
140k	48	-	5	20	2	5
800k	50	-	5	20	2	5

Four different scenarios of minimum run length were calculated for every chip array density: 0.25, 0.5, 1 and 2 million base pairs (Mb) respectively.

The number of ROHom and ROHet per animal (nROHom and nROHet, respectively) and run length average ($\text{meanMb}_{\text{hom}}$ and $\text{meanMb}_{\text{het}}$) per animal were computed. To test the effect of the minimum length on these parameters, the following model was adopted:

$$y = \mu + L + e \qquad [2]$$

where y is the considered parameter (i.e., nROHom, nROHet, $\text{meanMb}_{\text{hom}}$, or $\text{meanMb}_{\text{het}}$), μ is the overall mean, L is the cross-classified fixed effect of the scenario of run minimum length (i.e., 0.25, 0.50, 1, and 2 Mb), and e is the residual.

A region starting and ending exactly at the same position was identified as a unique run (Cesarani et al., 2018; Macciotta et al., 2021). These regions found in at least two animals were classified as repeated ($\text{ROHom}_{\text{REP}}$ or $\text{ROHet}_{\text{REP}}$) and those falling in 99th percentile of the distribution were considered of interest. Finally, the number of animals a given SNP was included in a run was computed ($\text{SNP}_{\text{ROHom}}$ and $\text{SNP}_{\text{ROHet}}$, for the homozygous and heterozygous runs, respectively). The SNPs exceeding the 99th percentile of these distributions were considered as part of ROHom or ROHet islands.

Using online databases, the genes (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) and the QTLs (https://www.animalgenome.org) mapped in the most frequent ROHom and ROHet and the ROHom and ROHet islands were retrieved.

Linkage Disequilibrium computation

Using Haploview software (Barrett et al., 2005) the squared correlation coefficient of allele frequencies at pair of loci (r^2) was computed for SNPs within 1000kb of distance (McKay et al., 2007) as measure for the Linkage Disequilibrium (LD) levels. The computed r^2 values were visualized as a function of inter-marker distance.

Results and Discussion

Runs of homozygosity

The number of ROHom and their average length are reported in the Table 2. The number of ROHom per animal increased and the average length decreased as the genotyping density increased. This is because a higher SNP density improves the detection resolution, by reducing the detection of false ROHom (Ferenčaković et al., 2013b). Moreover, the parameters used to identify a ROHom in the three different densities (Table 1) could have also had an impact. In fact, the average nROHom (across the minimum lengths) detected at the low-density (16.71) was 11 times lower than the average value (189.10) found using the high-density chip. On the contrary, the average ROHom length decreased 3.4 times moving from low-density (6.2Mb, across minimum length) to high-density chip (1.8Mb, across minimum length). Thus, when a high-density chip is involved in the analysis, the long ROHom are divided in more smaller regions, as already observed by Hillestad et al. (2018).

A significant effect ($P<0.05$) of the minimum run length scenario has been observed across the three different beadchip densities (Table 2). In general, as the minimum run length increased (i.e., from 0.25 to 0.5, 1, and 2 Mb), the nROHom decreased, whereas their average length increased, respectively. This trend was also observed by Ferenčaković et al. (2011) in a study on the Austrian Fleckvieh cattle. However, the magnitude of variation of nROHom across minimum length scenarios greatly differed among densities (Table 2). For the 50k SNP beadchip, the differences were not relevant, whereas the nROHom drastically decreased from 0.25 to 2Mb in the medium- (i.e., 140k SNP) and high-density chip (i.e., 800k SNP).

Table 2. Runs of homozygosity (ROHom) detected at three chip array densities using four different minimum lengths.

	SNP				ROHom				
Density	Mininum	Opposite	Missing	Mininum Length	Total	Unique	Animals	nROHom	MeanMb$_{hom}$
50k	49	0	4	0.25	6,972	5,759	394	17.70±6.31^{A}	5.87±1.84^{C}
				0.5	6,779	5,702	394	17.21±6.23^{AB}	6.03±1.83^{BC}
				1	6,450	5,529	394	16.37±6.18^{BC}	6.30±1.84^{AB}
				2	6,124	5,337	394	15.54±6.15^{C}	6.57±1.88^{A}
140k	48	0	5	0.25	19,969	15,373	348	57.38±19.15^{A}	2.67±0.99^{C}
				0.5	19,013	14,921	348	54.65±18.65^{A}	2.79±1.01^{BC}
				1	17,305	13,777	347	49.87±17.59^{B}	2.99±1.07^{B}
				2	7,667	6,674	337	22.75±10.47^{C}	4.88±1.72^{A}
800k	50	0	5	0.25	75,851	53,921	152	499.02±75.13^{A}	0.63±0.10^{D}
				0.5	25,765	21,101	152	169.51±38.98^{B}	1.19±0.21^{C}
				1	9,788	8,868	151	64.82±22.09^{C}	2.05±0.38^{B}
				2	3,408	3,284	148	23.03±11.37^{D}	3.31±0.61^{A}

Different superscript letters within a column within Chip density indicate significant difference for $P<0.05$.

Of particular interest is the nROHom trend in the highest density: as the minimum run length doubled, the number of ROHom per individual approximately halved. A possible explanation could be that a higher SNP density allows for a better detection of short ROHom compared to a low density beadchip (Hillestad et al., 2018).

The largest differences in the nROHom among chip densities (Table 2) was observed for the lower minimum lengths (i.e., ≤ 1Mb), whereas they tended to markedly decrease in the scenario of 2Mb run minimum length. The same trend was found by Ferenčaković et al. (2013b) in Brown Swiss, Pinzgauer and Tyrol Grey cattle breeds. The lower detection power of the low SNP density for short ROHom could be one of the main causes of this result (Szmatola et al., 2016; Purfield et al., 2012). Larger minimum lengths to define a run, such as 4 Mb as proposed by some authors (Ferenčaković et al., 2013b; Mastrangelo et al., 2016; Kukučková et al., 2017), were not considered in our study since ROHet were found only up to 0.5 Mb and a comparison between the two types of runs (i.e., ROHom and ROHet) would not be possibile. The pattern of number of SNP per ROHom was not significantly affected by the minimum length scenario at low SNP density (Table 1), whereas at the medium- and high-density it increased as the minimum length increased. A comparison with previous reports for Simmental cattle is hampered by the difference in defining parameters adopted to define a ROHom. Two studies on Italian Simmental (Cesarani et al., 2021; Marras et al., 2015), using a 50k beadchip with at least 15 SNP and a length of 1 Mb as minimum thresholds, found nROHom of 77.47 ± 15.23 and 94.30 ± 12.20, respectively. A similar value (i.e., 96.79 ± 13.37 ROHom per animal) was reported also by Ferenčaković et al. (2011) in Austrian Simmental bulls genotyped using the 50k chip array density and again at least 15 SNPs covering 1 Mb to define a

ROHom. These figures, markedly higher than those reported in the present study for the 50K SNP density, are clearly due to the smaller minimum number of SNP included in a run.

Runs of heterozygosity

The detection of ROHet across different scenarios showed a rather cryptic pattern (Table 3). The chip density with the largest number of ROHet per animal was the140k for the lower minimum lengths (i.e., 0.25 and 0.5 Mb), whereas the lowest value was for the 50k. At high-density, ROHet were detected only at 0.25 and 0.50 Mb as minimum length. A study carried out on horses reported ROHet for the 0–2 Mb class, whereas no runs were detected for longer classes (> 2Mb) (Santos et al., 2021). In each beadchip density, the number of ROHet per animal decreased as the minimum length increased (Table 3). The average ROHet length showed an opposite pattern. Moving from the low- to the high-density, the average length of ROHet decreased in the lower minimum lengths (i.e., 0.25 and 0.5), in agreement with the results reported by Mulim et al. (2022), whereas the values were very similar in the 1Mb and 2Mb minimum lengths. Similar results were reported in studies on cattle (Biscarini et al., 2020) and pigs (Chen et al., 2022). As for ROHom, a higher density of SNP may facilitate the detection of shorter heterozygous homozygosity segments. Biscarini et al. (2020) reported an increase in the number and average size of ROHet when increasing the number of missing and/or homozygous SNPs allowed inside the region, but using a low-density chip. All the above reported figures highlight the great influence of the parameters (i.e., the minimum length, the number of genotyping errors allowed and the minimum number of SNPs) and of the density of genotyping in run detection

(Biscarini et al., 2020; Hidalgo et al., 2021; Mulim et al., 2022; van Marle-Köster et al., 2022).

Table 3. Runs of heterozygosity (ROHet) detected at three chip array densities using four different minimum lengths.

	SNP				ROHet				
Density	Mininum	Opposite	Missing	Mininum Length	Total	Unique	Animals	nROHet	Mean length (Mb
50k	18	3	4	0.25	4,300	2,341	395	10.89±4.57^{A}	0.90±0.11^{D}
				0.5	3,554	2,040	395	9.00±4.15^{B}	1.02±0.11^{C}
				1	1,545	923	388	3.98±2.62^{C}	1.30±0.19^{B}
				2	57	34	50	1.14±0.50^{D}	2.27±0.21^{A}
140k	20	2	5	0.25	9,357	5,405	348	26.89±18.43^{A}	0.51±0.05^{D}
				0.5	3,735	2,140	348	10.73±7.74^{B}	0.68±0.09^{C}
				1	216	91	165	1.31±0.61^{C}	1.55±0.41^{B}
				2	46	7	44	1.05±0.21^{C}	2.29±0.06^{A}
800k	20	2	5	0.25	3,924	1,693	152	25.82±5.24^{A}	0.34±0.02^{B}
				0.5	310	119	139	2.23±1.09^{B}	0.70±0.09^{A}
				1					
				2					

Different superscript letters within a column within Chip density indicate significant difference for $P<0.05$.

Comparison between ROHom and ROHet

Being obtained on different animals, results reported in the present study for different SNP densities may be affected by a sampling bias. In order to check this hypothesis, analyses were repeated using only the 152 animals genotyped with the 800K: densities of 140K and 50K were then mimicked by retaining only the markers included in the lower densities. Results of ROHom and ROHet, both for average number and length are in agreement with those reported for the different sets of animals of the full data set (Supplementary Table 1). Moreover, in order to investigate a possible effect of different linkage disequilibrium (LD) levels in the three considered datasets, the r^2 values were computed between markers within 1,000kb of distance. The LD analysis showed no differences among the three densities (Supplementary Figure 1).

Table 4 shows the correlations between ROHom and ROHet statistics for different genotyping densities and minimum run lengths. As expected, negative values were obtained in almost all the cases, since these two regions are opposed by definition. However, a defined pattern could not be detected among the different combinations of SNP density and minimum run length. As far as the number of runs is concerned, the correlation was statistically different from zero only in the 2Mb at low-density, in the 0.25Mb and 0.5Mb scenarios at medium-density and in the 0.25Mb at high-density, respectively. For the average length, only the correlation of the 0.25Mb scenario at the medium-density (-0.12) was significantly different from zero.

Table 5 shows the correlation between scenarios for the occurrence of a SNP in a ROHom or in a ROHet, respectively. As expected, the correlations were larger within each density and decreased as the chip density increased. Overall, the correlations for

ROHom were larger than those computed for ROHet, reflecting the more consistent results obtained for the detection of ROHom.

Table 4. Correlations of minimum length with the number of regions and their average length in Mb or SNP for ROHom and ROHet defined at different chip array densities.

	Number of regions	**Region length (Mb)**
Low density		
0.25 Mb	-0.04^{NS}	-0.02^{NS}
0.5 Mb	-0.02^{NS}	-0.07^{NS}
1 Mb	-0.05^{NS}	-0.05^{NS}
2 Mb	-0.35*	-0.12^{NS}
Medium density		
0.25 Mb	-0.43***	-0.12*
0.5 Mb	-0.38***	-0.07^{NS}
1 Mb	-0.07^{NS}	-0.02^{NS}
2 Mb	-0.09^{NS}	-0.02^{NS}
High density		
0.25 Mb	-0.22**	0.04^{NS}
0.5 Mb	-0.05^{NS}	0.10^{NS}
1 Mb		
2 Mb		

NS = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001.

Table 5. Correlations between the number of times a SNP was inside a ROHom (above diagonal) or ROHet (below diagonal) among the different scenarios.

		50k				**140k**				**800k**			
Density	**Min. length**	**0.25**	**0.5**	**1**	**2**	**0.25**	**0.5**	**1**	**2**	**0.25**	**0.5**	**1**	**2**
50k	0.25		0.99	0.97	0.93	0.51	0.52	0.52	0.53	0.31	0.41	0.49	0.51
	0.5	1		0.99	0.95	0.53	0.54	0.54	0.55	0.32	0.42	0.50	0.53
	1	0.93	0.93		0.98	0.55	0.55	0.56	0.57	0.32	0.43	0.52	0.54
	2	0.79	0.79	0.87		0.55	0.56	0.56	0.59	0.33	0.43	0.52	0.56
140k	0.25	0.47	0.47	0.45	0.39		0.97	0.93	0.79	0.46	0.59	0.65	0.52
	0.5	0.42	0.42	0.43	0.38	0.66		0.98	0.84	0.46	0.61	0.68	0.54
	1	0.32	0.32	0.36	0.37	0.49	0.82		0.87	0.45	0.61	0.69	0.56
	2	0.37	0.37	0.4	0.38	0.41	0.67	0.82		0.37	0.5	0.59	0.59
800k	0.25	0.24	0.24	0.27	0.29	0.42	0.67	0.71	0.49		0.80	0.60	0.46
	0.5	0.01	0.01	0.02	0.05	0.2	0.35	0.45	0.03	0.72		0.83	0.63
	1	–	–	–	–	–	–	–	–	–	–		0.80
	2	–	–	–	–	–	–	–	–	–	–	–	

Genes and QTL

A total of 147 and 22 regions exceeded the 0.1% of the ROHom$_{\text{REP}}$ and ROHet$_{\text{REP}}$ distribution, respectively (Supplementary Table 2). Since ROHom are potentially due to selection (Kim et al., 2013; Gorssen et al., 2021) and ROHet could be to balancing selection (Fijarczyk and Babik, 2015), both can be used to identify selection signatures. For this reason, it is of particular interest to investigate regions shared among different populations, which may harbor important genes.

The most shared ROHom was detected in 34 animals (4% of the total number of individuals), and it was located at 38.43-39.46 Mb on BTA6. This genomic region, where no genes were mapped, overlapped with 283 QTLs (Supplementary Table 3). In particular, 52 QTLs were related to milk production, 152 with meat production, 75 with conformation traits, and 4 with other phenotypes (Supplementary Table 3). The most repeated ROHet was found in 89 animals (about 10% of the total number of animals) and it was located at 0.18-0.87 Mb on BTA23. In this region the *KH RNA Binding Domain Containing, Signal Transduction Associated 2 (KHDRBS2*) gene was mapped. This gene was found in a heterozygosity enriched region by Mulim et al. (2022) and it has been reported to be associated with reproductive traits, such as age at first calving in Sanmartinero cattle (De Léon et al., 2019), calving ease (Paim et al., 2020), and pregnancy status in Brahman cattle (Reverter et al., 2016). Moreover, this genomic region overlapped with 16 QTLs: 5 related to milk production, 4 to conformation, 5 to reproduction, and 3 with other phenotypes (Supplementary Table 3).

Figures 1-3 and 4-6 report the Manhattan plot of the SNP$_{\text{ROHom}}$ and SNP$_{\text{ROHet}}$, respectively. A total of 29,871 SNPs was at least one time within a ROHom in all the

12 scenarios (i.e., 4 minimum lengths for 3 densities) and fifteen SNPs exceeded the 99th percentile of the SNP_{ROHom} distribution in all scenarios (Table 6). All these significant SNPs were mapped on BTA6 between 38.46 and 39.82 Mb and could be considered as only one large ROHom island. The *Slit Guidance Ligand 2* (*SLIT2*) gene was mapped in this region. This gene has been previously reported to be involved in several weight traits: in particular, internal organ (especially spleen) weight in Simmental cattle (An et al., 2018); bone weight in beef cattle (Niu et al., 2021); birth, yearling, and weaning weights in US Red Angus cattle (Smith et al., 2022); birth weight in US Gelbvieh cattle (Smith et al., 2019). Moreover, the same gene was also associated with the infection of tropical theileriosis parasite (Larcombe et al., 2022) and it has been identified both in clinical and subclinical ketosis (Soares et al., 2021). Finally, it has been found associated with female fertility in the Nordic Red cattle (Höglund et al., 2015). The ROHom island defined by the fifteen SNPs that exceeded the 99th percentile of the SNP_{ROHom} harbors 387 QTLs. In particular, 62, 231, 83, and 8 QTLs were related to milk production, meat production, conformation, and reproduction, respectively (Supplementary Table 3).

Table 6. SNP exceeding the 99th percentile of the SNP_{ROHom} and SNP_{ROHet} distribution in each scenario.

		Region		Low density (50k)				Medium density (140k)				High density (800k)			
	SNP	BTA	bp	0.25	0.5	1	2	0.25	0.5	1	2	0.25	0.5	1	2
ROHom	Hapmap30134-BTC-034283	6	38,464,203	21.41	21.41	21.41	21.41	73.85	67.82	50.29	25.57	70.39	70.39	42.11	22.37
	ARS-BFGL-NGS-112812	6	38,627,070	21.41	21.41	21.41	21.41	73.56	67.82	50.29	25.29	71.05	71.05	42.76	23.03
	BTA-100891-no-rs	6	38,689,886	21.41	21.41	21.41	21.41	74.43	68.39	50.86	25.29	73.03	73.03	42.76	23.03
	Hapmap43470-BTA-114677	6	38,746,212	21.41	21.41	21.41	21.41	74.43	68.10	50.86	25.29	73.03	73.03	42.76	23.03
	Hapmap27083-BTC-041166	6	38,825,835	21.41	21.41	21.41	21.41	73.28	68.68	50.86	25.29	73.03	73.03	42.76	23.03
	Hapmap23507-BTC-041133	6	38,845,992	21.41	21.41	21.41	21.41	72.70	69.25	50.86	25.29	73.03	73.03	42.76	23.03
	Hapmap27529-BTC-050639	6	39,034,201	21.41	21.41	21.41	21.41	74.43	72.13	51.44	25.86	73.03	73.03	42.76	23.03
	Hapmap53940-rs29026121	6	39,216,868	21.41	21.41	21.41	21.41	68.97	67.82	47.99	26.15	70.39	70.39	40.79	23.03
	Hapmap27537-BTC-060891	6	39,257,620	21.16	21.16	21.16	21.16	56.90	55.46	44.25	23.28	55.26	55.26	40.13	23.03
	Hapmap27849-BTC-071108	6	39,503,443	21.16	21.16	21.16	21.16	42.24	34.20	29.31	22.41	41.45	31.58	29.61	23.68
	Hapmap33339-BTC-071052	6	39,529,973	20.15	20.15	20.15	20.15	39.94	31.90	27.30	21.55	40.79	30.92	28.95	23.68
	Hapmap26618-BTC-070864	6	39,597,740	20.15	20.15	20.15	20.15	37.36	29.31	25.00	20.40	41.45	30.26	28.29	23.68
	ARS-BFGL-NGS-113801	6	39,688,028	19.90	19.90	19.90	19.90	39.94	29.31	25.00	20.40	41.45	29.61	28.29	23.68
	Hapmap32513-BTC-066089	6	39,753,141	20.15	20.15	20.15	20.15	40.52	29.60	25.29	20.40	40.79	28.95	27.63	23.03
	Hapmap26842-BTC-035606	6	39,816,133	20.15	20.15	20.15	20.15	40.52	29.60	25.29	20.40	40.79	28.95	27.63	23.03
ROHet	ARS-BFGL-NGS-53975	21	2,151,256	19.65	19.65	19.65	2.27	20.11	20.11	19.54	12.36	41.45	3.29		
	Hapmap52072-rs29018920	21	2,333,804	19.65	19.65	19.65	2.27	20.11	20.11	19.54	12.36	40.79	1.32		

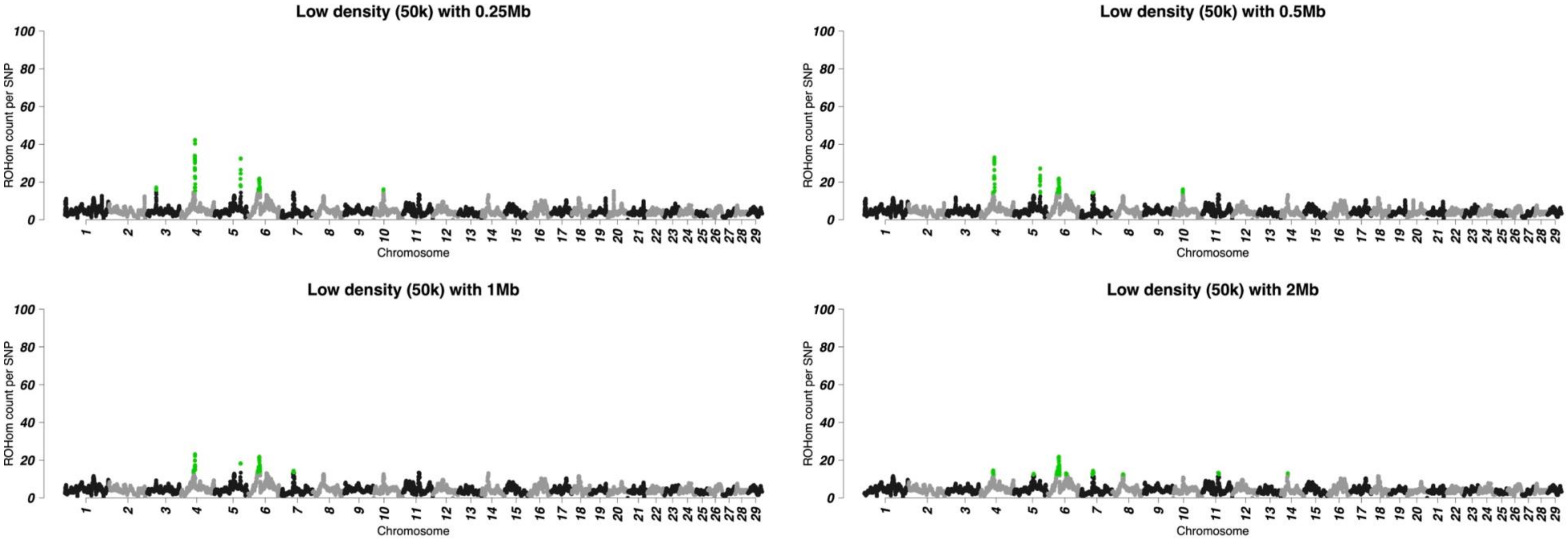

Figure 1. Manhattan plot of the SNP_{ROHom} detected in the low-density scenario (50k SNP). Green dots represent SNP with a SNP_{ROHom} value exceeding the 99th percentile of its distribution.

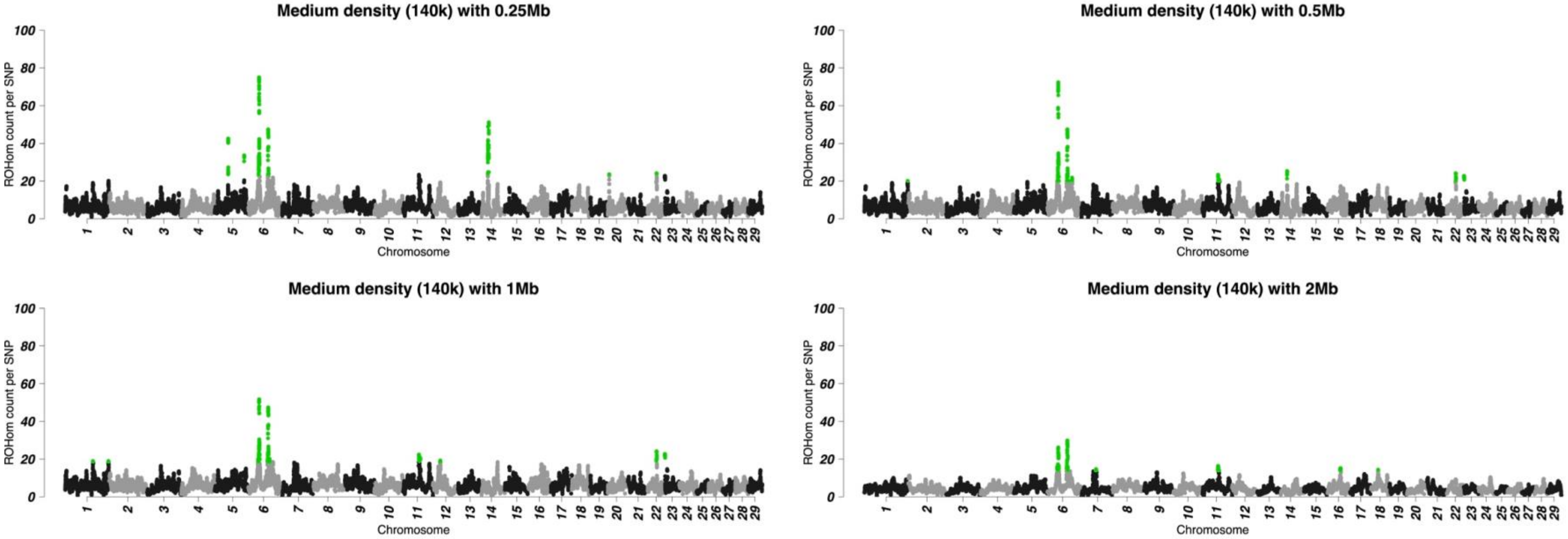

Figure 2. Manhattan plot of the SNP_{ROHom} detected in the medium-density scenario (140k SNP). Green dots represent SNP with a SNP_{ROHom} value exceeding the 99th percentile of its distribution.

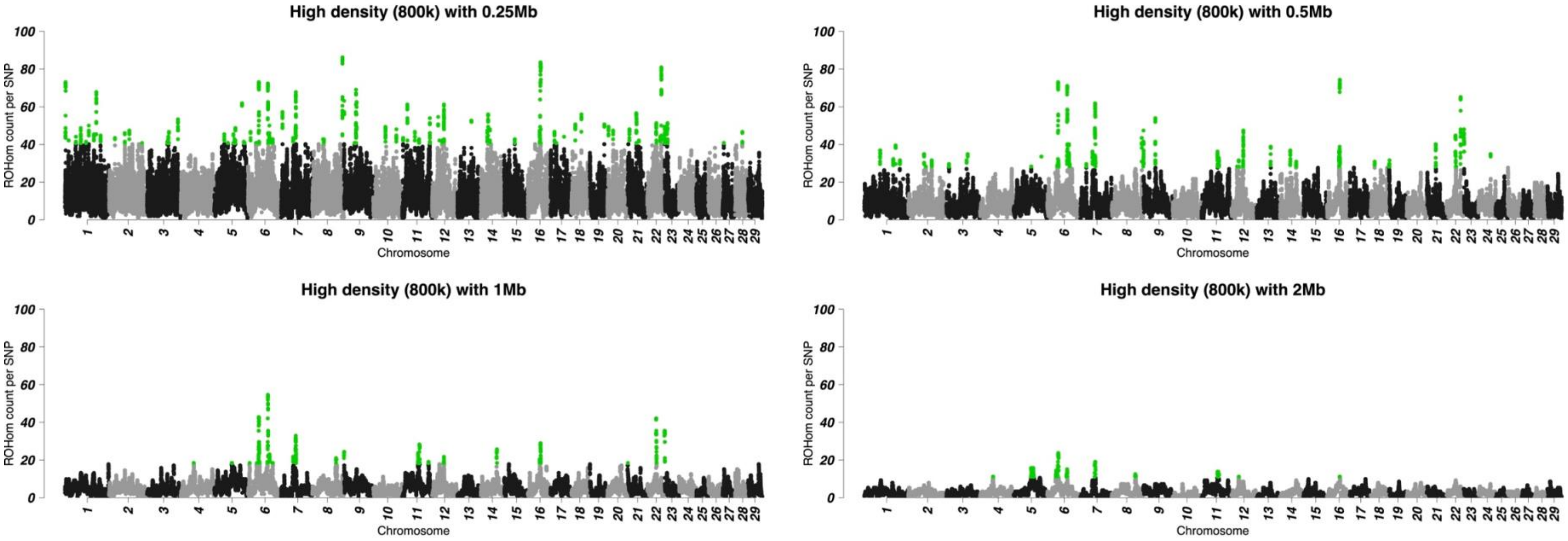

Figure 3. Manhattan plot of the $\text{SNP}_{\text{ROHom}}$ detected in the high-density scenario (800k SNP). Green dots represent SNP with a $\text{SNP}_{\text{ROHom}}$ value exceeding the 99th percentile of its distribution.

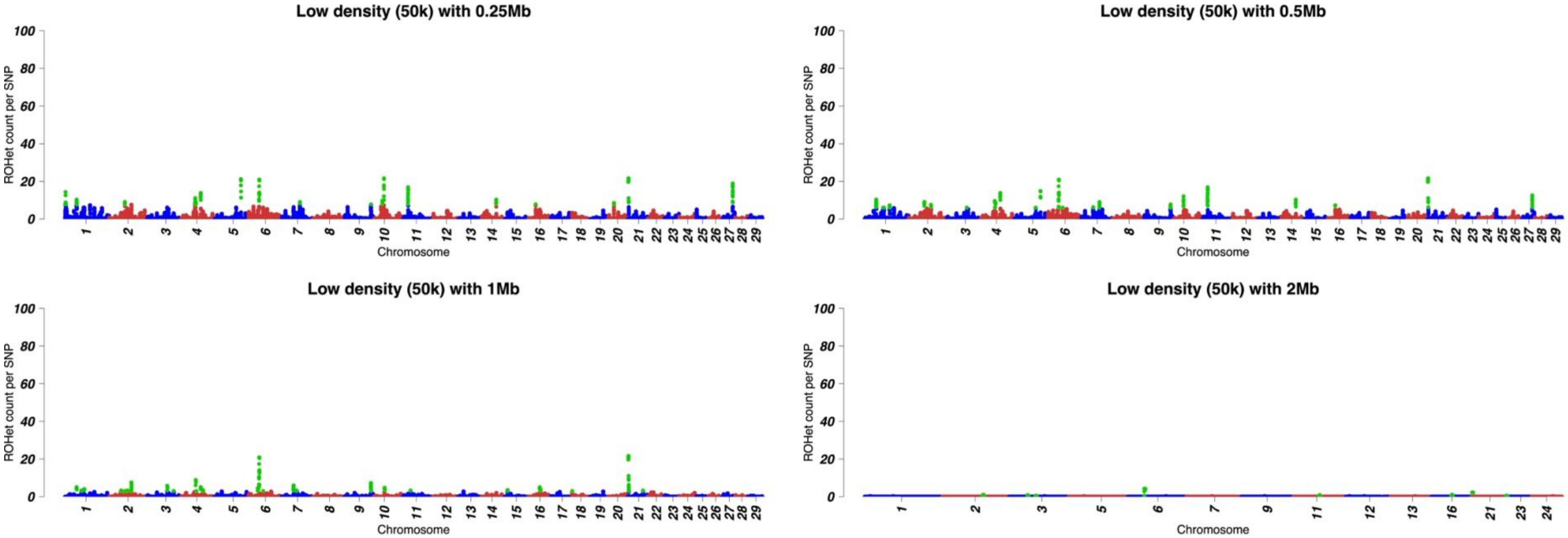

Figure 4. Manhattan plot of the SNP$_{\mathrm{ROHet}}$ detected in the low-density scenario (50k SNP). Green dots represent SNP with a SNP$_{\mathrm{ROHet}}$ value exceeding the 99th percentile of its distribution.

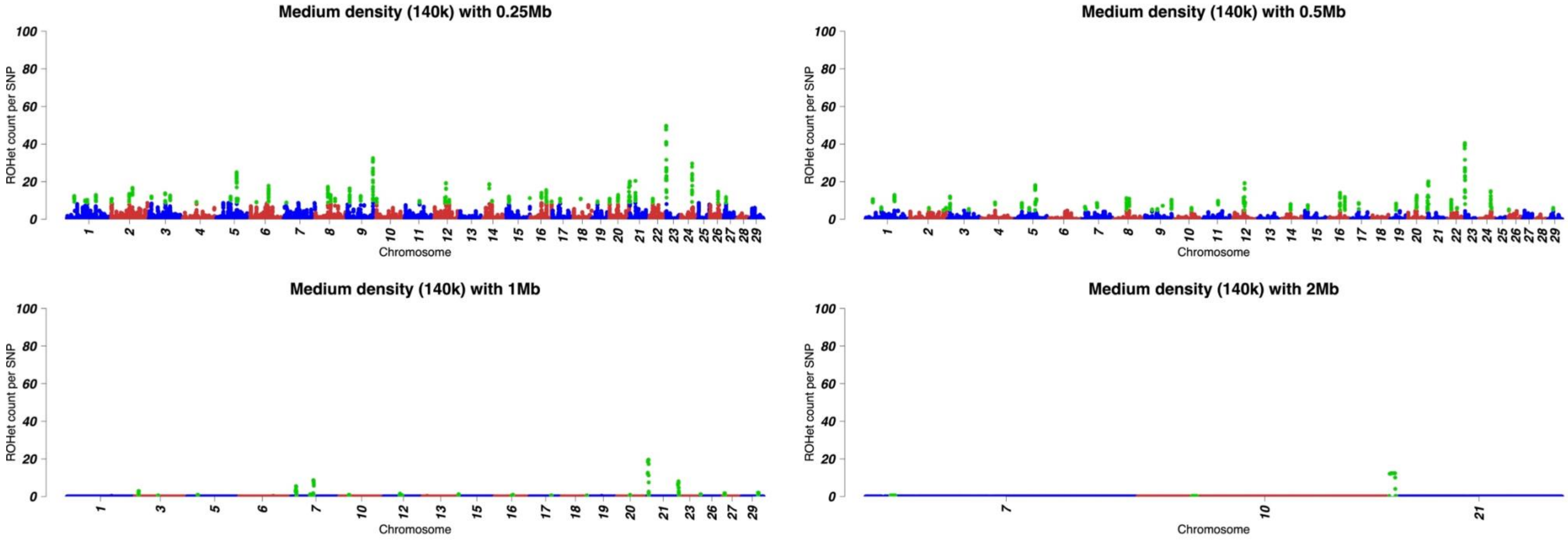

Figure 5. Manhattan plot of the SNP$_{\text{ROHet}}$ detected in the medium-density scenario (140k SNP). Green dots represent SNP with a SNP$_{\text{ROHet}}$ value exceeding the 99th percentile of its distribution.

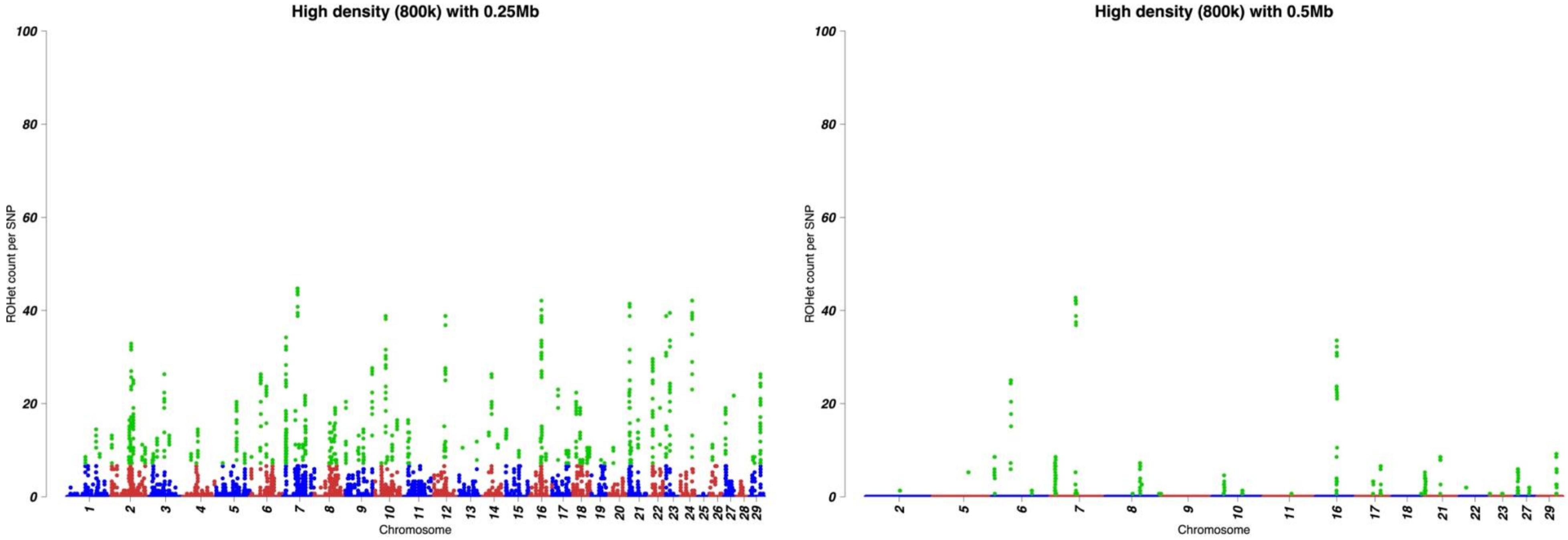

Figure 6. Manhattan plot of the SNP$_{\mathrm{ROHet}}$ detected in the high-density scenario (800k SNP). Green dots represent SNP with a SNP$_{\mathrm{ROHet}}$ value exceeding the 99th percentile of its distribution.

Only two SNPs occurred at least one time in a ROHet in all the 10 scenarios (excluding 1 and 2Mb at high-density for which no ROHet were found). These two markers were located at 2.15-2.33 Mb on BTA21, and they exceeded the 99th percentile in all scenarios (Table 6). Near to these markers, three genes were mapped: *Ubiquitin Protein Ligase E3A* (*UBE3A*), *Small Nuclear Ribonucleoprotein Polypeptide N* (*SNRPN*), and *SNRPN Upstream Open Reading Frame* (*SNURF*). The *UBE3A* gene was previously found to be associated with calving ease in cattle (Pausch et al., 2011; Mészáros et al., 2016), and highly expressed in the *Longissimus dorsi* of different beef cattle breeds (Liu et al., 2020). The same gene cluster (i.e., *UBE3A*, *SNRPN*, and *SNURF*) was associated with cattle temperament by Costilla et al. (2020) and with the occurrence of early pregnancy in Nellore cattle (Irano et al., 2016). As Suzuki et al. (2009) pointed out, the *SNRPN* and *SNURF* genes have been widely studied in mice and humans, constituting a bicistronic gene. The genomic regions highlighted by the two SNPs that exceeded the 99th percentile of $\text{SNP}_{\text{ROHet}}$ overlapped 234 QTLs (Supplementary Table 2) reported in the literature related to milk (7 QTLs), meat (6 QTLs), and reproduction (220 QTLs).

Conclusion

In the present study, genotypes from the Italian Simmental cattle breed were used to investigate the impact of chip density and minimum length required to detect a ROHom or a ROHet. The results confirmed that by improving the chip density, the detection of ROHom is more reliable. Still, at the same time new questions were raised related to the cryptic impact on ROHet detection. Genes and QTLs mapped in the highlighted ROHet were mainly associated with reproduction traits, whereas the gene and the QTLs associated with the ROHom were predominantly involved in meat production traits. The results of the present study strengthened the usefulness of these techniques to analyze selection signatures and their biological meaning. The comparison between these two techniques and a deeper analysis of ROHet deserve future investigations.

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Appendix Chapter 4

Relationship between runs of homozygosity and heterozygosity in the Italian Simmental cattle genotyped at different densities

Supplementary Table 1. Runs of homozygosity (ROHom) and Runs of heterozygosity (ROHet) detected at three chip array densities on the same 152 animals using four different minimum lengths.

Supplementary Table 2. List of the ROHom and ROHet in the top 0.1% of the distribution of repeated runs.

Supplementary Table 3. List of the QTLs annotated in literature retrieved in the genomic regions highlighted by the most shared ROHom and ROHet or by the SNPs exceeding the 99th percentile of the SNP_{ROHom} and SNP_{ROHet} distributions.

Supplementary Figure 1. Linkage Disequilibrium (LD) decay in the three considered densities.

Supplementary Table 1. Runs of homozygosity (ROHom) and Runs of heterozygosity (ROHet) detected at three chip array densities on the same 152 animals using four different minimum lengths.

		ROHom					ROHet				
Density	**Min. Length**	**Total**	**Unique**	**Animals**	**nROHom**	**Mb**	**Total**	**Unique**	**Animals**	**nROHet**	**Mb**
50k	0.25	1,998	1,862	151	13.23±5.36	6.64±6.01	1,290	1,050	151	8.54±7.35	1.10±0.35
	0.5	1,953	1,830	151	12.93±5.30	6.79±6.00	1,268	1,031	151	8.40±7.29	1.11±0.34
	1	1,889	1,785	151	12.51±5.31	6.99±6.00	718	588	147	4.88±4.47	1.32±0.31
	2	1,844	1,742	149	12.38±5.14	7.12±6.01	32	28	26	1.23±0.59	2.34±0.22
140k	0.25	8,656	7,401	152	56.95±15.05	2.59±3.09	3,033	2,142	152	19.95±15.51	0.49±0.17
	0.5	8,191	7,129	152	53.89±14.69	2.72±3.13	1,184	796	152	7.79±5.99	0.64±0.17
	1	7,397	6,504	152	48.66±14.04	2.92±3.23	30	24	27	1.11±0.32	1.44±0.33
	2	3,207	2,980	151	21.24±7.82	4.86±4.16			–		
800k	0.25	75,851	53,921	152	499.02±75.13	0.63±0.10	3,924	1,693	152	25.82±5.24	0.34±0.02
	0.5	25,765	21,101	152	169.51±38.98	1.19±0.21	310	119	139	2.23±1.09	0.70±0.09
	1	9,788	8,868	151	64.82±22.09	2.05±0.38			–		
	2	3,408	3,284	148	23.03±11.37	3.31±0.61			–		

Supplementary Table 2. List of the ROHom and ROHet in the top 0.1% of the distribution of repeated runs.

Density	Mininum length (Mb)	BTA	Position (Mb)	Animals
		4	49.65-50.80	14
		4	49.69-50.03	14
	0.25	14	22.98-26.47	15
		4	50.22-50.80	16
		4	50.53-50.80	20
		1	3.91-5.10	10
		2	0.19-3.05	10
		5	92.84-93.95	12
	0.5	4	49.65-50.80	14
		14	22.98-26.47	15
		4	50.22-50.80	16
50k		1	3.91-5.10	10
		2	0.19-3.05	10
	1	5	92.84-93.95	12
		4	49.65-50.80	14
		14	22.98-26.47	15
		24	41.49-45.35	8
		6	38.46-42.87	8
		6	64.28-62.12	8
		2	0.19-3.05	10
	2	14	22.98-26.47	15
		12	33.78-39.02	7
	4	24	41.19-45.35	8
		6	38.46-42.87	8
		14	23.42-23.93	14
		17	57.76-59.03	14
		6	38.46-39.46	14
		9	58.06-59.97	14
		20	4.6-4.86	15
		21	44.8-46.15	15
140k	0.25	6	71.04-72.48	15
		14	23.93-24.28	17
		14	26.62-26.95	17
		6	38.43-39.22	18
		6	39.46-39.82	21
		14	26.62-26.94	26
		5	48.64-49.03	26

Density	Mininum length (Mb)	BTA	Position (Mb)	Animals
		5	105.93-106.3	31
		23	0.02-1.58	32
		6	38.43-39.46	34
	0.5	12	21.35-22.89	13
		3	113.43-114.77	13
		4	38.88-40.24	13
		5	105.8-106.33	13
		14	23.42-23.93	14
		17	57.76-59.03	14
		6	38.46-39.46	14
		9	58.06-59.97	14
		21	44.8-46.15	15
		6	71.04-72.48	15
		6	38.43-39.22	18
		23	0.02-1.58	32
		6	38.43-39.46	34
	1	11	60.97-62.5	12
		17	55.45-56.59	12
		17	74.03-75.13	12
		1	3.02-4.83	12
		12	21.35-22.89	13
		3	113.43-114.77	13
		4	40.24-0	13
		17	57.76-59.03	14
		9	59.97-0	14
		21	44.8-46.15	15
		6	72.48-0	15
		23	0.02-1.58	32
		6	39.46-0	34
	2	6	37.08-39.22	8
		6	38.43-40.72	8
		17	72.53-75.13	9
		2	1.07-4.51	9
		7	110.1-112.61	9
		6	70.86-73.12	11
	4	11	55.88-60.28	6
		11	55.90-60.28	6
		17	49.89-57.70	6
800k	0.25	10	43.4-43.77	14
		11	65.59-65.9	14

Density	Mininum length (Mb)	BTA	Position (Mb)	Animals
		18	14.13-14.57	14
		19	52.53-52.78	14
		21	3.05-3.41	14
		22	28.82-29.16	14
		22	33.49-34.16	14
		28	33.17-33.42	14
		6	8.96-9.31	14
		7	51.61-52.6	14
		9	0.62-1.04	14
		12	36.48-36.76	15
		14	39.45-39.76	15
		16	44.04-44.3	15
		18	35.97-36.3	15
		1	139.36-139.93	15
		1	1.56-2.02	15
		22	30.43-30.77	15
		22	50.34-50.6	15
		6	39.46-39.82	15
		6	83.08-83.35	15
		7	69.06-69.48	15
		8	107.84-108.14	15
		13	50.23-50.72	16
		21	31.51-31.77	16
		24	2.91-3.2	16
		2	20.79-21.18	16
		6	8.89-9.31	16
		7	87.6-87.96	16
		1	12.54-12.8	17
		5	99.58-100.09	17
		9	38.03-38.3	17
		9	43.02-43.52	17
		20	3.58-3.85	18
		6	93.94-94.22	18
		7	52.53-53.43	18
		12	51.9-52.16	19
		16	81.27-81.55	19
		7	112.3-112.63	19
		6	76.56-76.89	20
		23	9.06-9.37	21

Density	Mininum length (Mb)	BTA	Position (Mb)	Animals
		5	106.01-106.3	21
		5	75.63-76.05	21
		3	110.9-111.21	22
		9	58.83-59.11	22
		11	55.02-55.28	23
		11	63.87-64.17	23
		14	33.07-33.47	23
		16	45.04-45.66	25
		2	72.6-72.98	26
		5	99.58-99.94	28
		9	45.39-45.72	29
		16	46.48-46.75	31
	0.5	13	24.65-25.17	11
		23	0.01-1.53	11
		24	44.1-44.86	11
		9	0.01-1.04	11
		5	31.78-32.53	12
		6	35.61-36.59	12
		23	0.01-1.59	13
		23	0.01-0.96	13
		3	76.35-76.87	13
		8	107.66-108.18	13
		22	33.49-34.16	14
		7	51.61-52.6	14
		1	139.36-139.93	15
		5	99.58-100.09	17
		9	43.02-43.52	17
		7	52.53-53.43	18
		16	45.04-45.66	25
	1	21	0.01-1.66	8
		11	61-62.54	9
		6	71.25-72.37	10
		23	0.01-1.53	11
		9	0.01-1.04	11
		23	0.01-1.59	13
	2	16	45.04-47.05	4
		18	15.61-17.66	4
	4	8	56.63-61.65	3

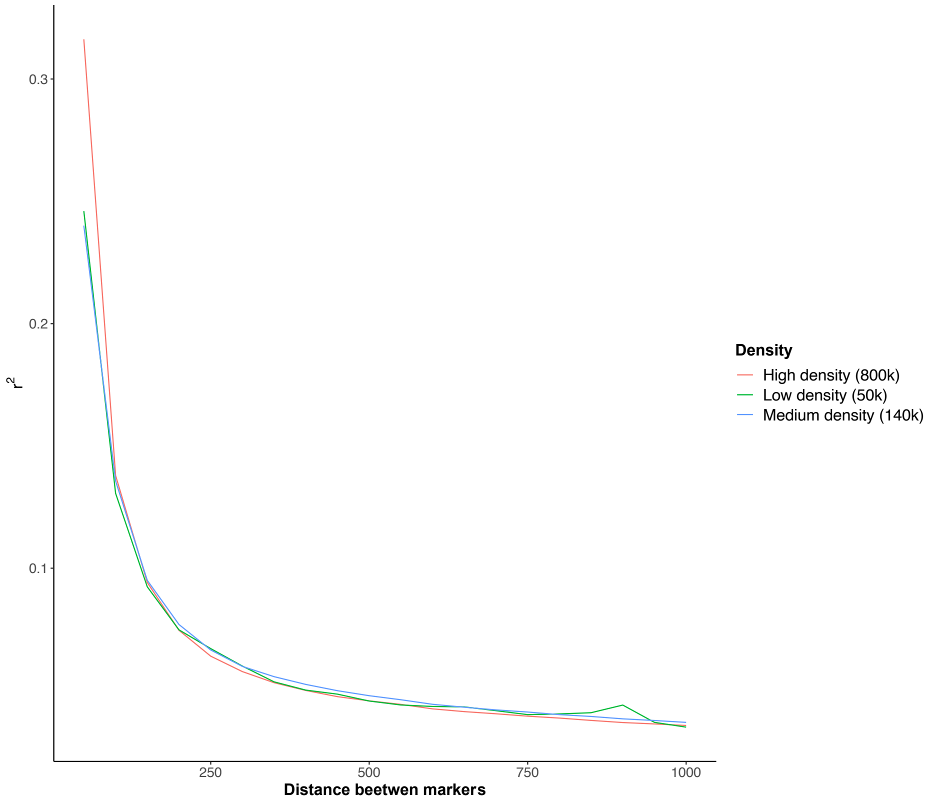
Supplementary Table 3. List of the QTLs annotated in literature retrieved in the genomic regions highlighted by the most shared ROHom and ROHet or by the SNPs exceeding the 99th percentile of the $\mathrm{SNP_{ROHom}}$ and $\mathrm{SNP_{ROHet}}$ distributions.

		QTL			Reference
Parameter	**Region**	**Category**	**Trait**	**N**	
$\mathrm{ROHom_{REP}}$	BTA6 38,428,952- 39,461,621	Milk	Milk yield	1	Silva et al., 2011
			Milk fat percentage	18	Olsen et al., 2016 Ashwell et al., 2004 Olsen et al., 2004 van den Berg et al., 2020
			Milk fat yield	1	Silva et al., 2011
			Milk protein percentage	26	van den Berg et al., 2020 Nayeri et al., 2016 Olsen et al., 2016 Olsen et al., 2004
			Milk potassium content	3	Buitenhuis et al., 2015
			Curd firming rate	1	Bertelsen et al., 2016
			Somatic cell score	1	Daetwyler et al., 2008
			Dairy form	1	Sahana et al., 2015
		Meat	Body weight	27	Kneeland et al., 2004 Snelling et al., 2010 Akanno et al., 2018 Lu et al., 2013
			Metabolic body weight	48	Zhang et al., 2020
			Body weight gain	6	Snelling et al., 2010
			Average daily gain	28	Zhang et al., 2020 Sorbolini et al., 2017
			Carcass weight	10	Wang et al., 2020 Akanno et al., 2018
			Dry matter intake	19	Zhang et al., 2020
			Longissimus muscle area	6	Wang et al., 2020 Akanno et al., 2018
			Biceps brachii weight	5	Song et al., 2016
			Tenderness score	1	Leal-Gutiérrez et al., 2020
			Connective tissue amount	1	Leal-Gutiérrez et al., 2020
			Lean meat yield	1	Doran et al., 2014

		QTL			Reference
Parameter	Region	Category	Trait	N	
		Conformation	Bone weight	69	Niu et al., 2021 Miao et al., 2018 Chang et al., 2018 Chang et al., 2019
			Liver weight	3	An et al., 2018
			Udder attachment	1	Schrooten et al., 2004
			Teat placement - front	1	Schrooten et al., 2004
			Rump angle	1	Sahana et al., 2015
		Immunity	Bovine tuberculosis susceptibility	2	Richardson et al., 2016
		Reproduction	Length of productive life	1	Daetwyler et al., 2008
			Temperament	1	Chen et al., 2020
$\text{ROHet}_{\text{REP}}$	BTA23 181,959-868,795	Milk	Milk fat percentage	1	Cole et al., 2011
			Milk fat yield	1	Cole et al., 2011
			Milk protein percentage	1	Cole et al., 2011
			Dairy form	1	Cole et al., 2011
			Somatic cell score	1	Cole et al., 2011
		Conformation	Foot angle	1	Cole et al., 2011
			Teat length	1	Cole et al., 2011
			Rear leg placement - side view	1	Cole et al., 2011
			Strength	1	Cole et al., 2011
		Reproduction	Calving ease	2	Cole et al., 2011
			Stillbirth	1	Cole et al., 2011
			Pregnancy rate	1	Cole et al., 2011
			Length of productive life	1	Cole et al., 2011
			Net merit	1	Cole et al., 2011
			Methane production	1	Calderón-Chagoya et al., 2019
$\text{SNP}_{\text{ROHet}}$	BTA21 1,901,256-2,583,804	Milk	Milk yield	1	Rodriguez-Zas et al., 2002
			Milk kappa-casein percentage	1	Buitenhuis et al., 2016
			Milking speed	5	Marete et al., 2018
		Meat	Body weight	3	Snelling et al., 2010 Veerkamp et al., 2012 Saatchi et al., 2014
			Muscle potassium content	1	Mateescu et al., 2017
			Marbling score	1	Leal-Gutiérrez et al., 2020
			Shear force	1	Leal-Gutiérrez et al., 2020

		QTL			Reference
Parameter	Region	Category	Trait	N	
		Reproduction	Age at puberty	1	Melo et al., 2019
			Calving ease	219	Frischknecht et al., 2017 Pausch et al., 2011
		Conformation	Stature	1	Bouwman et al., 2018
SNP$_{ROHom}$	BTA6 38,214,203- 40,066,133	Milk	Milk protein percentage	33	Russo et al., 2012 van den Berg et al., 2020 Nayeri et al., 2016 Olsen et al., 2016 Jiang et al., 2019
			Milk fat percentage	20	Olsen et al., 2016
			Milk fat yield	1	Nayeri et al., 2016
			Milk potassium content	3	Buitenhuis et al., 2015 Sanchez et al., 2021
			Milk C18 index	2	Gebreyesus et al., 2019
			Somatic cell score	1	Ilie et al., 2021 Daetwyler et al., 2008
			Curd firming rate	1	Bertelsen et al., 2016
			Dairy form	1	Sahana et al., 2015
		Meat	Body weight	36	Snelling et al., 2010 Akanno et al., 2018 Lu et al., 2013
			Metabolic body weight	33	Zhang et al., 2020 Lu et al., 2013
			Body weight gain	27	Snelling et al., 2010
			Average daily gain	40	Zhang et al., 2020 Sorbolini et al., 2017 Akanno et al., 2018
			Dry matter intake	33	Zhang et al., 2020
			Carcass weight	22	Wang et al., 2020 Akanno et al., 2018 Keogh et al., 2021 Nishimura et al., 2012
			Longissimus muscle area	12	Wang et al., 2020 Akanno et al., 2018
			Conformation score	16	Sahana et al., 2015
			Biceps brachii weight	6	Song et al., 2016
			Tenderness score	1	Leal-Gutiérrez et al., 2020

		QTL			Reference
Parameter	**Region**	**Category**	**Trait**	**N**	
			Connective tissue amount	1	Leal-Gutiérrez et al., 2020
			Lean meat yield	1	Doran et al., 2014
			Fat thickness at the 12th rib	1	Nalaila et al., 2012
			Marbling score	2	Takasuga et al., 2007 Leal-Gutiérrez et al., 2020
		Conformation	Bone weight	74	Niu et al., 2021 Miao et al., 2018 Chang et al., 2018 Chang et al., 2019
			Liver weight	3	An et al., 2018
			Spleen weight	3	An et al., 2018
			White spotting on belly	1	Liu et al., 2009
			White spotting on back	1	Liu et al., 2009
			Rump angle	1	Sahana et al., 2015
		Reproduction	Interval from first to last insemination	1	Höglund et al., 2015
			Calving index	4	Sahana et al., 2015
			Male fertility	1	Blaschek et al., 2011
			Length of productive life	2	Daetwyler et al., 2008
		Immunity	Bovine tuberculosis susceptibility	2	Richardson et al., 2016
			Temperament	1	Chen et al., 2020



Supplementary Figure 1. Linkage Disequilibrium (LD) decay in the three considered densities.

CHAPTER 5

General conclusion

The need to safeguard the biodiversity and, thus, to maintain the genetic diversity is constantly gaining great relevance because of the new environmental challenges that humanity will be face. Local breeds represent a reservoir of genes fundamental for adaptation to poor environment, rusticity, resilience, and disease resistance. On the contrary, the specialized cattle breeds have almost lost these fitness traits because of the high selection pressure, resulting in high performances and profitability, rather than plasticity, and so less adaptable to possible future challenges.

In the present thesis, some metrics were used to investigate genetic diversity among cattle breeds based on particular features of their genome. In particular, the analysis on uninterrupted sequences of homozygous or heterozygous SNP was carried out to enhance structural diversity and to infer the selective history of different genomic regions.

In the first experimental contribution, genomic data of different breeds belonging to the subspecies *Bos taurus taurus* and *Bos taurus indicus*, and their hybrids, living in different regions of the world, were considered. The analysis of Runs of Homozygosity (ROH) allowed to identify selection signatures in genomic regions where genes related to various environmental adaptation processes were mapped.

In the second study, other than analyzing ROH in three Italian dual-purpose cattle breeds, Runs of Heterozygosity (ROHet) were also investigated. Being a quite new technique, a standard method for the detection of ROHet does not exist yet and, thus, four different approaches retrieved in literature were tested. The relationship between ROH and ROHet, which theoretically are opposed by definition, was investigated, and the results opened new interesting questions for further studies.

In the third study, technical issues on ROH and ROHet detection were deepened comparing different parameters (i.e., minimum length required to define a region) and different genotyping densities. When changing parameters and densities, the trend of ROH statistics was clear, whereas for ROHet the results were quite cryptic. Even in this study, the relationship between the two techniques was not as clear as expected. ROH and ROHet were analyzed to search for selection signatures: ROH were mainly associated with production traits, whereas heterozygous regions were predominantly related to reproduction traits. These findings confirm that ROH can be mainly due to directional selection and ROHet to balancing selection.