

# Genomic regions underlying positive selection in local, Alpine cattle breeds

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## Abstract

We used genome-wide SNP data from 18 local cattle breeds from six countries of the Alpine region to characterize population structure and identify genomic regions underlying positive selection. The geographically close breeds Evolèner, Eringer, Valdostana Pezzata Nera, and Valdostana Castana were found to differ from all other Alpine breeds. In addition, three breeds, Simmental, and Original Braunvieh from Switzerland and Pinzgauer from Austria built three separate clusters. Of the 18 breeds studied, the intra-alpine Swiss breed Evolèner had the highest average inbreeding based on runs of homozygosity ( $F_{ROH}$ ) and the highest average genomic relationship within the breed. In contrast, Slovenian Cika cattle had the lowest average genomic inbreeding and the lowest average genomic relationship within the breed. We found selection signatures on chromosome 6 near known genes such as *KIT* and *LCORL* explaining variation in coat color and body size in cattle. The most prominent selection signatures were similar regardless of marker density and the breeds in the data set. In addition, using available high-density SNP data from 14 of the breeds we identified 47 genome regions as ROH islands. The proportion of homozygous animals was higher in all studied animals of local breeds than in Holstein and Brown Swiss cattle, the two most important commercial breeds in the Alpine region. We report ROH islands near genes related to thermoregulation, coat color, production, and stature. The results of this study serve as a basis for the search for causal variants underlying adaptation to the alpine environment and other specific characteristics selected during the evolution of local Alpine cattle breeds.

## KEYWORDS

diversity, selection signature, runs of homozygosity, cattle

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## INTRODUCTION

Cultivated landscapes cover most of the Alps, a high mountain range in Central Europe, and are of great cultural, political, ecological, and socio-economic importance (Streifeneder, 2010). The majority is permanent grassland, traditionally used by ruminants in extensive or semi-extensive livestock production (Battaglini et al., 2014). Alpine livestock production has traditionally been based on small herds of local dual-purpose breeds housed in cowsheds located in the valley in winter and moved to the high pastures in summer (Battaglini et al., 2014). Today's alpine cattle breeds are assumed to be adapted to the harsh climatic conditions in the mountains, such as the sparse food resources and the long distances expected to be traversed to reach summer pastures above 2000 m (Del Bo et al., 2001).

This adaptation is understood to be a result of several fundamental events in the history of local Alpine cattle breeds, starting with the domestication of the humpless taurine cattle (*Bos taurus*) 10 000–11 000 years ago in the Fertile Crescent region (Frantz et al., 2020; McHugo et al., 2019). The domestication of cattle was followed by several major migratory events, with the Neolithic settlement of Europe from 6000 to 4000 BC and the introduction of cattle interpreted as the most consequential migration for the creation of European cattle breeds (Zhang et al., 2020b). It is generally assumed that cattle spread via the *Mediterranean* and *Danube* routes (Ajmone-Marsan et al., 2010; Cubric-Curik et al., 2021; Edwards et al., 2011). Based on mitochondrial DNA variation, Scheu et al. (2015) concluded that the genetic history of domestic cattle consisted of a small, localized domestication process followed by a relatively straightforward series of spasmodic expansion episodes that led to a serial dilution of genetic diversity from the Near East to western and northern Europe. Recently, it has been suggested, that Bronze Age Alpine cattle are linked to modern cattle, particularly in Italy (Granado et al., 2021). Despite sparsity of documentation on the diversity of European cattle up to the 18th century, it seems plausible that local developments led to a number of different cattle types such as the Alpine spotted and Alpine brown cattle, which already existed in the Middle ages (Felius et al., 2011). Since the 18th century, the differences between local cattle types have increased due to the formation of breeds with explicit breeding objectives (Felius et al., 2014, 2015). Today, breeding programs of Alpine cattle focus on the dual purpose of milk and meat production, product quality, and robustness (Table S1). In addition, fighting ability remains an important trait in the breeding of certain local cattle breeds such as the Swiss Eringer (ER), as well as the Valdostana Castana (CAST) and Valdostana Pezzata Nera (VPN), which are found in Italy (Flury et al., 2010; Sartori et al., 2020).

The genetic diversity of some Alpine cattle breeds has been studied using microsatellite genotypes (Del Bo

et al., 2001; Simčič et al., 2013). Del Bo et al. (2001) underlined the known relationship between the three local cattle breeds from the Italian Aosta valley (CAST, VPN, and Valdostana Pezzata Rossa (VPR)) and ER and Evolèner (EV) from the canton of Valais in Switzerland. In the last decade, genome-wide single nucleotide polymorphism (SNP) data have advanced the characterization of the genetic diversity of local cattle breeds (Decker et al., 2014). A comprehensive study of 50k SNP genotypes of 23 local, Alpine breeds found a geographical gradient along the alpine east–west axis and confirmed genetic proximity between animals of the same breed from different countries (Senczuk et al., 2020). The higher genetic diversity and lower degree of genomic inbreeding in local Alpine Swiss cattle breeds (ER, Simmental (SIM) and Original Braunvieh (OB)) compared to single-purpose dairy breeds was explained by the greater use of natural service sires in local breeds (Signer-Hasler et al., 2017). Strillacci et al. (2020) presented genetic diversity and genomic inbreeding estimates based on runs of homozygosity ( $F_{ROH}$ ) for CAST, VPN, and VPR cattle using 728 animals with 150k SNP data genotypes.

This study aimed to combine available genome-wide SNP data from 18 local Alpine cattle breeds originating from six countries to characterize population structure and identify genomic regions underlying positive selection. A particular interest was to determine the relationship between the two local Swiss cattle breeds ER and EV with other breeds of the Alpine area, especially with the geographically adjacent breeds from the Aosta valley in Italy. We therefore investigated the genomic relationships within and between these 18 breeds. In addition, we present ROH islands and signatures of selection using genome-wide SNP data at medium and high marker densities to highlight genomic regions potentially related to the evolution and adaptation of local Alpine cattle breeds.

## MATERIALS AND METHODS

An interactive map showing the geographical locations and corresponding countries of the committee offices of the 18 cattle breeds considered in this study (Table S1) has been created with the R-package leaflet and can be found in Appendix S1. A more detailed description of the breeds, such as DAD-IS risk status, number of breeding females, major use(s), and phenotypic characteristics (body size, milk yield, and coat color) can be found in Table S1.

### Genotypes

For this study, medium-density SNP genotypes of 2235 animals from 18 local Alpine cattle breeds were available. All SNP markers were mapped to the ARS-UCD1.2

reference genome (Rosen et al., 2020). The available medium-density SNP genotypes were filtered for a call rate of at least 90% in SNP and animals. Markers with a minor allele frequency of <1% in all 18 breeds were also removed. To achieve a balanced data set, we randomly selected 150 animals from the four breeds OB, SIM, ER, and EV with a comparatively high number of genotyped animals. This resulted in Data 1 containing 32 649 autosomal SNP per animal and 1292 animals from 18 breeds (Table 1).

In addition, high-density SNP genotypes were available for animals from 14 of the 18 breeds considered. First, we filtered for a call rate of 90% in SNP and animals, resulting in 712 225 autosomal SNP and 1678 animals. We also added a filter for MAF of less than 1% for all 14 breeds. Finally, this resulted in Data 2 consisting of 660 097 autosomal SNP per animal and 1678 animals from 14 breeds (Table 1). For ROH-derivation we used the genotypes from 712 225 SNP (before MAF-filtering) and 1678 animals (see above). To compare ROH islands of these 14 local breeds with ROH islands of two commercial breeds, the genotypes for the 712 225 autosomal SNP were considered using randomly selected high-density SNP genotypes of 167 Brown Swiss

(BS) and 170 Holstein (HO) herdbook animals from the current Holstein- and Brown Swiss breeding programs in Switzerland (Data 3).

## Population structure

Data 1 was used for population structure analysis. Pairwise genomic relationships (PIHAT) were calculated using PLINK1.9 --genome and graphically represented as a level plot from R library “lattice” showing the pairwise genomic relationship between two individuals within and between breeds. Genetic distances were calculated using PLINK1.9 --distance-matrix. We then performed a principal component analysis (PCA) using cmdscale() function in R. The function ggplot from the “tidyverse” package was used for the visualization of PCA-results. The pairwise  $F_{ST}$  values were calculated using PLINK1.9 --fst. A circular neighbor-joining tree based on the pairwise  $F_{ST}$  values was created using MEGA X (Kumar et al., 2018). Admixture analysis was performed using ADMIXTURE-software (Alexander et al., 2009) and results were plotted using DISTRUCT (Rosenberg et al., 2002).

**TABLE 1** Overview of the Alpine and commercial cattle breeds considered: Number of animals and marker density per data set (local Alpine cattle breeds are in black and commercial breeds in gray).

Breed	Abbr.	Country	# animals Data 1 (32k)	# animals Data 2 (660k)	# animals Data 3 (712k)
Original Braunvieh	OB	CH	150	317	317
Simmental	SIM	CH	150	48	48
Eringer	ER	CH	150	734	734
Evolèner	EV	CH	150	41	41
Brown Swiss	BS	CH	–	–	167
Holstein	HO	CH	–	–	170
Valdostana Castana	CAST	IT	24	24	24
Valdostana Pezzata Nera	VPN	IT	25	25	25
Valdostana Pezzata Rossa	VPR	IT	47	47	47
Grauvieh	GV	AT	120	120	120
Pinzgauer	PI	AT	141	142	142
Pustertaler Sprinzen	PUS	AT	25	25	25
Ennstaler Bergschecken	ENS	AT	25	25	25
Tuxer	TUX	AT	58	58	58
Hinterwälder	HW	DE	14	14	14
Vorderwälder	VW	DE	58	58	58
Murnau Werdenfelser	MWF	DE	47	–	–
Abondance	ABO	FR	42	–	–
Tarentaise	TAR	FR	40	–	–
Cika	CIK	SI	26	–	–
		Total	1292	1678	2015

## Genomic inbreeding

The PLINK1.9 --homozyg option was used to derive runs of homozygosity (ROH). For Data 1, the minimal number of SNP markers to define a ROH was set to 53 and the minimum SNP density was set to 1 per 150 kb. No heterozygous SNP were allowed within a ROH and the number of missing SNPs per window was set to 1. The inbreeding coefficients ( $F_{\text{ROH}}$ ) for the 1292 individuals from Data 1 were calculated according to McQuillan et al. (2008):

$$F_{\text{ROH}} = \sum \frac{L_{\text{ROH}}}{L_{\text{AUTO}}}$$

where  $L_{\text{ROH}}$  represents the total length of the genome fraction of an individual within ROH and  $L_{\text{AUTO}}$  stands for the total length of the autosomal genome and corresponds to 2470825 kb for Data 1.

## Selection signatures

Data 1 and Data 2 were used to identify selection signatures based on  $d_i$ -values.  $d_i$  measures the standardized locus-specific divergence of allele frequencies for breed  $i$  with all other breeds by summing up the standardized locus-specific differences across all breedwise combinations involving breed  $i$  (Akey et al., 2010). Wright's (1943)  $F_{\text{ST}}$  values were calculated for the 153 breed pairs in Data 1 and the 91 breed pairs in Data 2 using PLINK1.9 --fst. Next, the  $d_i$ -values for each breed were calculated according to Akey et al. (2010):

$$d_i = \sum_{j \neq i} \frac{F_{\text{ST}}^{ij} - E[F_{\text{ST}}^{ij}]}{\text{sd}[F_{\text{ST}}^{ij}]}$$

where  $E[F_{\text{ST}}^{ij}]$  and  $\text{sd}[F_{\text{ST}}^{ij}]$  denote the expected value and standard deviation of  $F_{\text{ST}}$  between breeds  $i$  and  $j$ , which were calculated based on all 32649 SNP markers in Data 1 and 660097 SNP markers in Data 2, respectively. The  $d_i$ -values were averaged for SNPs in non-overlapping windows of 0.5 Mb (Data 1) and 0.25 Mb (Data 2). Windows with less than four SNPs were removed. For each breed, windows exceeding the 99th percentile of the breed-specific empirical distribution of  $d_i$  were considered putative selection signals. Genes in the region of these windows were identified in a breed-specific manner using the NCBI annotation release 106 of the ARS-UCD1.2 reference assembly. The top 30 windows with the genes annotated in them were listed (Tables S6 and S7) and combined with the knowledge of breed-specific traits and findings from the literature to select possible candidate genes that might be associated with these selection signatures.

## ROH islands

ROH islands were derived from Data 1 (with the parameters given in the section on genomic inbreeding) and Data 3 using PLINK1.9. In agreement with Meyermans et al. (2020), we did not apply MAF-filtering to Data 3 and used the following parameters to derive ROH: --homozyg-snp 63 and --homozyg-kb 500. ROH islands were defined as genomic regions where the proportion of homozygous animals exceeded the 99th percentile. Based on 1292 individuals from 18 alpine breeds in Data 1, the maximum proportion of homozygous animals for a SNP within a ROH did not exceed 14% (not shown), so we decided to focus on the ROH islands of high-density SNP genotypes (Data 3). Using Data 3, we derived ROH islands by considering all 1678 individuals of 14 alpine cattle breeds. The significant ROH islands among all individuals of the alpine breeds (with proportions of 32.6% or more) contrasted with the ROH islands found in the animals of two commercial breeds (BS and HO) (Data 3).

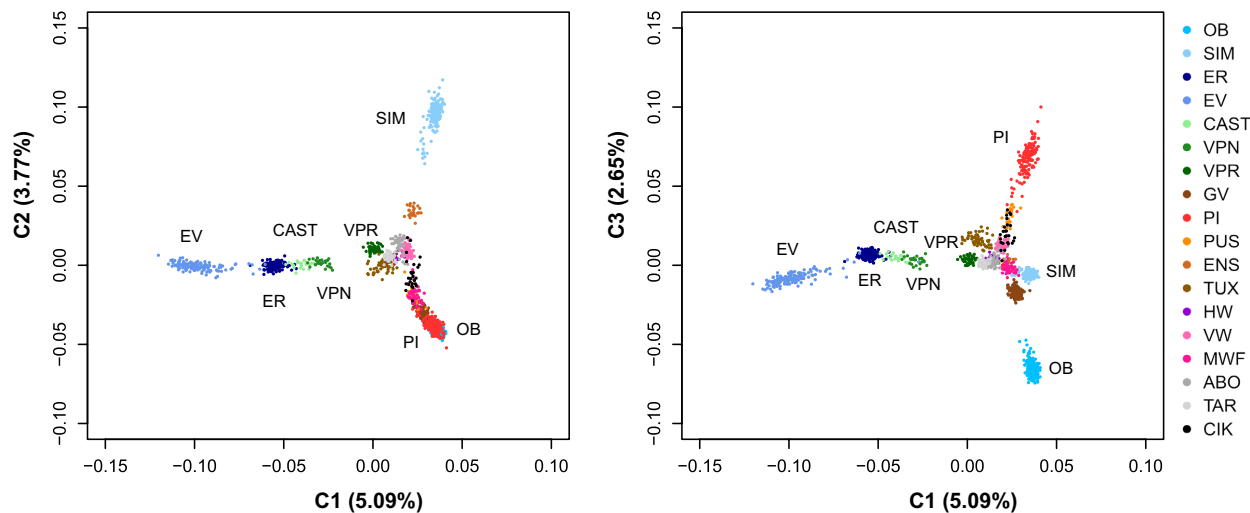
Gene annotation in the identified ROH islands was taken from NCBI annotation release 106 of the ARS-UCD1.2 reference assembly. For breed comparisons, we additionally calculated the average and maximum proportion of individuals having the SNPs of the derived ROH islands in a ROH for each of the 14 local Alpine breeds and the two commercial breeds (Data 3).

## RESULTS

### Population structure

The results of the PCA based on genetic distances and Data 1 are shown in Figure 1. The first component separates the four breeds ER and EV (from Switzerland) and CAST and VPN (from Italy) from the other 14 alpine cattle breeds. The second component separates two breeds, SIM from Switzerland and Ennstaler Bergschecken (ENS) from Austria, from the other breeds, while the third component separates Swiss OB and Austrian Pinzgauer (PI) from the other breeds (Figure 1).

The average genomic relationship was highest within Swiss EV (15%) and lowest in Slovenian Cika (CIK, 4%, Table S2, Data 1). Genomic relationships within and between breeds based on Data 1 are shown in Figure S1. Genomic relationships between breeds are visible between ER, EV, CAST and VPN. Overall, we observed at least one pair of individuals with genomic relationships exceeding 5% in 18 out of 153 breeds pairs (Figure S1, Table S3). Maximum pairwise genomic relationships greater than 20% were observed in individuals from different breeds for EV/VPN (50.8%), PI/CIK (22.7%), and ER/EV (22.3%). The unexpectedly high relationship of 50.8% for one pair of individuals from EV and VPN can



**FIGURE 1** PCA-plot showing population structure based on the first (C1) and second (C2) components (left) and on the first (C1) and third (C3) components (right) of the 18 local Alpine cattle breeds. The first principal component (C1) explains 5.09%, the second (C2) 3.77%, and the third (C3) 2.65% of the observed variation.

be explained by the recent introduction of VPN sires into the EV population.

The average pairwise  $F_{ST}$  values for the 153 breed pairs in Data 1 indicating population differentiation, are shown in Table S4. The overall average  $F_{ST}$  value for all breed pairs was 0.068. The lowest  $F_{ST}$  values were observed for CAST and VPN (0.019) and CAST and ER (0.021), followed by CIK and PI (0.038) and CIK and Pustertaler Sprinzen (PUS, 0.039), while EV and ENS (0.114) had the highest average  $F_{ST}$  value. Except CAST, ER, and VPN, the average  $F_{ST}$  values were highest between EV and the 14 remaining breeds (all  $\geq 0.087$ ). For CAST and VPN, the average  $F_{ST}$  values were highest with SIM (0.085 and 0.086, respectively) and for ER with ENS (0.085). The circular neighbor-joining tree based on  $F_{ST}$  values is shown in Figure S2.

The results from admixture analysis (Figure S3) show that ER, EV, CAST, and VPN are close to each other, with EV occupying a special position among these four breeds. This was visible in the PCA plot (Figure 1), where EV clustered in the group with ER, CAST, and VPN, but somewhat separated from these three breeds. For  $K = 30$  and greater, the algorithm produced the lowest cross-validation error, indicating the optimal value of  $K$  (not shown).

## Genomic inbreeding

Among the 1292 individuals in Data 1, the average  $F_{ROH}$  was 5.0%. The highest average  $F_{ROH}$  per population was found in EV (9.8%), followed by SIM (6.2%) and Tarentaise (TAR, 5.3%), while CIK had the lowest average  $F_{ROH}$  at 1.6% (Figure 2, Table S5).

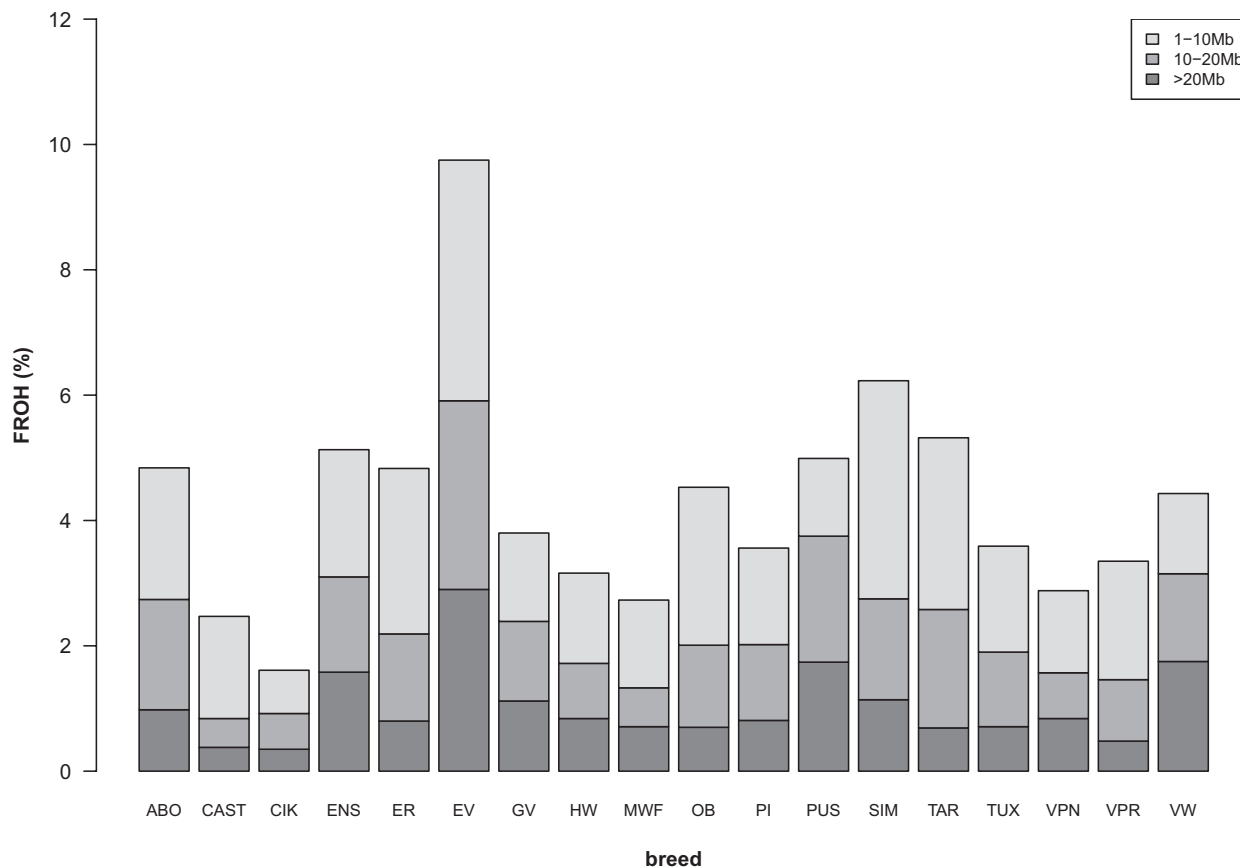
For all 18 breeds, an average of 46.0% of  $F_{ROH}$  came from segments 1–10 Mb long, 30.4% from segments

10–20 Mb long and 23.6% from segments 20 Mb and longer (Table S5). In ENS, PUS, and Vorderwalder (VW), more than 30% of  $F_{ROH}$  (Table S5) originate from segments larger than 20 Mb, indicating recent inbreeding about 2.5 generations ago (Ferenakovic et al., 2013b).

## Selection signatures

Using Data 1, 792 windows of 500 kb exceeded the 99th percentile, resulting in 44 significant windows per breed. Based on Data 2, 1400 significant windows of 250 kb exceeded the 99th percentile, resulting in 100 significant windows per breed. The coordinates of the windows with the 10 highest  $d_i$ -values from Data 1 and Data 2 and the genes annotated in these windows are shown in Table 2. Manhattan plots representing the  $d_i$ -values based on Data 1 and Data 2 for all breeds are shown in Figure S4.

In Data 1, the highest  $d_i$ -value (88.9) in OB was observed for the window on chromosome 11 at 67.7 Mb harboring the genes *GFPT1*, *NFUI*, *AAKI*, and *ANTXR1* (Table 2). The same window exceeded the 99th percentile in PI and Grauvieh (GV, Table S6). The second highest  $d_i$ -value (83.5) was also observed in OB on chromosome 11 at 68.2 Mb and this seven-gene-containing window resulted in significant  $d_i$ -values in PI and Abondance (ABO). The third highest  $d_i$ -value (77.9) was observed in ENS on chromosome 6 at 69.7 Mb, which harbors the genes *CHIC2*, *G SX2*, and *PDGFRA* (Table 2). With a  $d_i$ -value of 60.7, the same window was among the top 10 for VPR (9th rank) and except OB this window was significant in all other 17 breeds (Table 2). The fourth, fifth, and sixth highest  $d_i$ -values were again found in OB on chromosome 11, in the same region resulting in the highest  $d_i$ -values mentioned above (~67.3 Mb to ~68.8 Mb)



**FIGURE 2** Genomic inbreeding ( $F_{ROH}$ ) in 18 alpine cattle breeds. Average  $F_{ROH}$  per breed and relative contribution of the different segment length classes (1–10 Mb, 10–20 Mb, >20 Mb).

and additionally at ~66.2 Mb. The two windows harboring the *LCORL* gene were ranked 7th and 8th for Data 1 and the OB breed, respectively. The 10th-rank window ( $d_i$ -value: 59.8) harboring the genes *KIT* and *KDR* was observed in SIM and exceeded the significance threshold in a total of 12 breeds (Table 2).

With Data 2 the highest  $d_i$ -value (79.2) was observed in OB for a gene-less window on chromosome 5 at 18.6 Mb (Table 2). With the exception of SIM and EV, this window was significant in a total of 12 breeds from Data 2. The 2nd highest  $d_i$ -value (78.6) includes the *PDGFRA* gene and was found in VPR on chromosome 6 at 69.8 Mb. The same window yielded in the 9th highest  $d_i$ -value in SIM and Data 2. In agreement with the results of Data 1, it was significant in all breeds except OB. The third highest  $d_i$ -value (70.2) was found in OB for the window on chromosome 6 at 37.9 Mb. No genes are annotated for this genome segment, but it is close to the 10th ranked window ( $d_i$ -value of 58.48) containing the genes *NCAPG*, *DCAF16*, *LCORL*, and *FAM184B* (Table 2). Ranks 4 to 5 in Data 2 are occupied by windows in the region from 69.5 to 70.3 Mb on chromosome 6 for ENS, which contain several genes including *PDGFRA* and *KIT*. Ranks 6 to 8 were observed for OB on chromosome 6 from 67.3 to 68.0 Mb, harboring seven different genes (Table 2). When comparing the top 30  $d_i$ -values from Data 1 and Data 2,

two additional regions on chromosome 20 (47.7–50.1 Mb) and chromosome 26 (22.3–22.5 Mb) were significant in both data sets. The windows with the 30 highest  $d_i$ -values from Data 1 and Data 2 and the corresponding genome region, breed(s), and annotated genes are shown in Tables S6 and S7. In addition, the windows with the three highest  $d_i$ -values per breed are summarized in Tables S8 (Data 1) and Table S9 (Data 2).

## ROH islands

We examined ROH islands based on 1678 individual high-density SNP genotypes of 14 local cattle breeds (Data 3, Table 1) as possible signatures of selection. In total, 7230 SNP in 47 genomic regions on 18 different chromosomes exceeded the 99th percentile of the fraction of homozygous animals in the breeds studied (Figure 3). The Manhattan plots with the ROH islands for the two commercial breeds BS and HO (Data 3) are presented in supplementary Figure S5.

The 47 regions exceeding the 99th percentile (32.6%) in local Alpine breeds, including the corresponding chromosome, lower and upper limit, the proportion of animals with a SNP in a ROH in different breeds and samples, and the annotated genes are listed in Table S10.

**TABLE 2** Summary of selection signature search. Representation of the 10 windows with the highest  $d_f$ -values in Data 1 (shaded rows) and Data 2 (non-shaded rows), the corresponding chromosome, the mean position in bp, the corresponding  $d_f$ -value, the data set and rank of the  $d_f$ -value in the given data set, the breed for which the given  $d_f$ -value was observed, the number of breeds with significant  $d_f$ -values for this window and data set, the lower and upper limit and the known genes within the windows.

Chr	Lower bound (bp)	Upper bound (bp)	Middle position of window (bp)	$d_f$ -value	Dataset	Rank of $d_f$ -value in the dataset	Breed where $d_f$ -value observed	Number of breeds having significant $d_f$ -values for this window and data set	Annotated genes with known functions
5	18 470 868	18 720 868	18 595 868	79.18	Data2	1	OB	12	None
6	37 074 432	37 574 432	37 324 432	63.22	Data1	8	OB	14	LAP3, MED28, FAM184B, NCAPG, DCAF16, LCOLL
6	37 244 799	37 494 799	37 369 799	58.48	Data2	10	OB	11	NCAPG, DCAF16, LCOLL, FAM184B
6	37 514 322	38 014 322	37 764 322	71.31	Data1	7	OB	1	LCORL
6	37 744 607	37 994 607	37 869 607	70.25	Data2	3	OB	9	None
6	69 519 214	69 769 214	69 644 214	69.77	Data2	4	ENS	8	CHIC2, GSX2, PDGFRA
6	69 476 731	69 976 731	69 726 731	77.87	Data1	3	ENS	17	CHIC2, GSX2, PDGFRA
6	69 476 731	69 976 731	69 726 731	60.69	Data1	9	VPR	17	CHIC2, GSX2, PDGFRA
6	69 720 250	69 970 250	69 845 250	78.64	Data2	2	VPR	13	PDGFRA
6	69 720 250	69 970 250	69 845 250	65.92	Data2	9	SIM	13	PDGFRA
6	70 015 548	70 265 548	70 140 548	68.62	Data2	5	ENS	13	KIT
6	70 113 753	70 613 753	70 363 753	59.83	Data1	10	SIM	12	KIT, KDR
11	65 978 236	66 478 236	66 228 236	72.42	Data1	5	OB	1	None
11	67 019 186	67 519 186	67 269 186	73.35	Data1	4	OB	1	ARHGAP25, BMP10, GKN2, GKN1, ANTXR1, PROKR1
11	67 275 441	67 525 441	67 400 441	67.29	Data2	7	OB	1	GKN2, GKN1, ANTXR1
11	67 475 654	67 725 654	67 600 654	68.00	Data2	6	OB	2	GFPTI, ANTXR1
11	67 464 037	67 964 037	67 714 037	88.93	Data1	1	OB	3	GFPTI, NFUI, AAK1, ANTXR1
11	67 738 304	67 988 304	67 863 304	66.77	Data2	8	OB	2	NFUI, AAK1, GFPTI
11	67 995 599	68 495 599	68 245 599	83.55	Data1	2	OB	3	ANXA4, GMCL1, SNRNP27, MXDI, ASPR1, PCBPI, C11H2orf42
11	68 569 233	69 069 233	68 819 233	71.77	Data1	6	OB	2	SNRPG, EHD3, CAPN14, GALNT14, CAPN13, PCYOXI

To check whether the 47 ROH islands occur exclusively in local Alpine breeds, we calculated the proportions of animals with a SNP in a ROH for the 47 ROH islands and the group of breeds, for each of the 14 native breeds separately and the two commercial breeds (Table S10). The results for the most significant ROH islands (maximum proportion >57% in animals of local breeds) found on chromosomes 3, 5, 7, 11, 14, 15, 16, and 21 (Figure 3) are shown as line plots in Figure 4.

Table 3 shows more detailed information on the eight most significant ROH islands in local Alpine cattle breeds, including the lower and upper limit of the regions, the average proportion of animals having the SNPs from these regions in a ROH in the native breeds, the maximum proportion of animals having SNPs in a ROH in local breeds, and the corresponding average and maximum proportions of animals having SNPs in a ROH in the two commercial breeds; the corresponding maximum proportions in both commercial breeds are listed. Table 3 has been completed by indicating the genes with known functions annotated within the respective ROH islands.

Within the identified 47 ROH islands, we observed three regions where individual breeds reached fractions greater than 90% within breed (Table S10). On chromosome 6, a proportion of 99.7% homozygous animals was observed in OB cattle for the island from 37.0 to 38.0 Mb harboring the genes *LAP3*, *NCAPG*, and *LCORL*. On the same chromosome, a proportion of 96% was observed in ENS for the gene-less island from 75.8 to 76.3 Mb. For the region on chromosome 21 (31.4–32.2 Mb), proportions of 96.6% and 92.1% were observed in ENS and SIM.

## DISCUSSION

All 18 breeds studied are local breeds from the Alps, where transboundary transhumance is widespread

(Liechti & Biber, 2016) and individuals of certain breeds have traditionally been used in other Alpine cattle breeds (Del Bo et al., 2001; Mastrangelo et al., 2020a; Simčič et al., 2013, 2015). The analysis of the population structure based on Data 1 showed that the four inner-alpine breeds EV, ER, VPN, and CAST from the Swiss canton Valais and the Italian Aosta valley were clearly different from all other breeds (Figure 1). In addition, the animals of Swiss SIM and OB, and Austrian PI formed three separate clusters (Figures 1, S3).

Based on the results of the admixture analysis ( $K = 18$ ; Figure S3) the Italian CAST breed consisted mainly of clusters assigned to Swiss ER and Italian VPN cattle. This result is in line with previous studies (Strillacci et al., 2020) and information from the relevant breeding organizations. These three breeds are used for traditional cow fighting and the exchange of breeding individuals is known and documented (Del Bo et al., 2001; Sartori et al., 2015; Strillacci et al., 2020).

Interestingly, breed EV had the highest average  $F_{ST}$ -values (Table S4), but differed least from ER, VPN, and CAST. This finding is also confirmed by the PCA (Figure 1), genomic relationships, the admixture as well as the neighbor-joining tree analysis (Figures S2 and S3). Even though the proximity of the EV breed to the three other breeds from the same geographical region can be observed, the results of the PCA and admixture analysis suggest that some breeding activities have forced the separation from their relatives. Among the 18 breeds studied, EV had the highest average  $F_{ROH}$  (9.8%) and the highest average genomic relationship (15.0%) within the breed. The remarkable level of genomic inbreeding is in line with the results of previous studies (Signer-Hasler et al., 2017) and is not surprising considering that the herdbook population consists of less than 350 breeding females (Table S1). The genetic uniqueness of EV in combination with its risk status and the low diversity within breed underline the urgent need for conservation

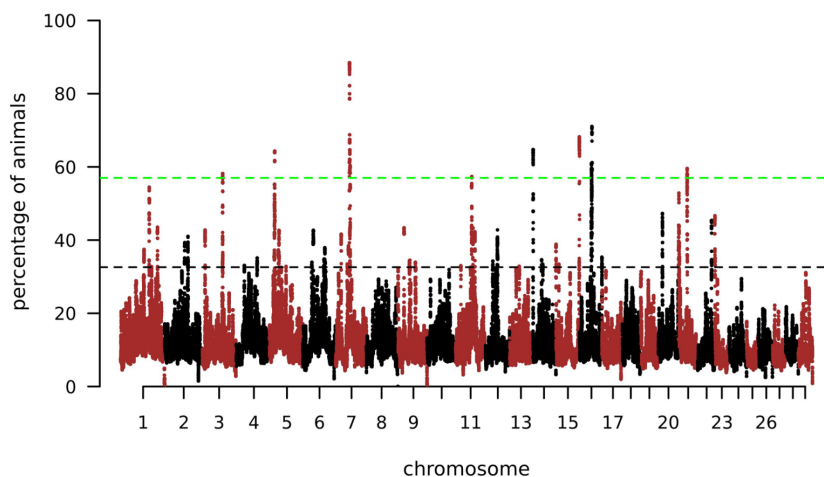
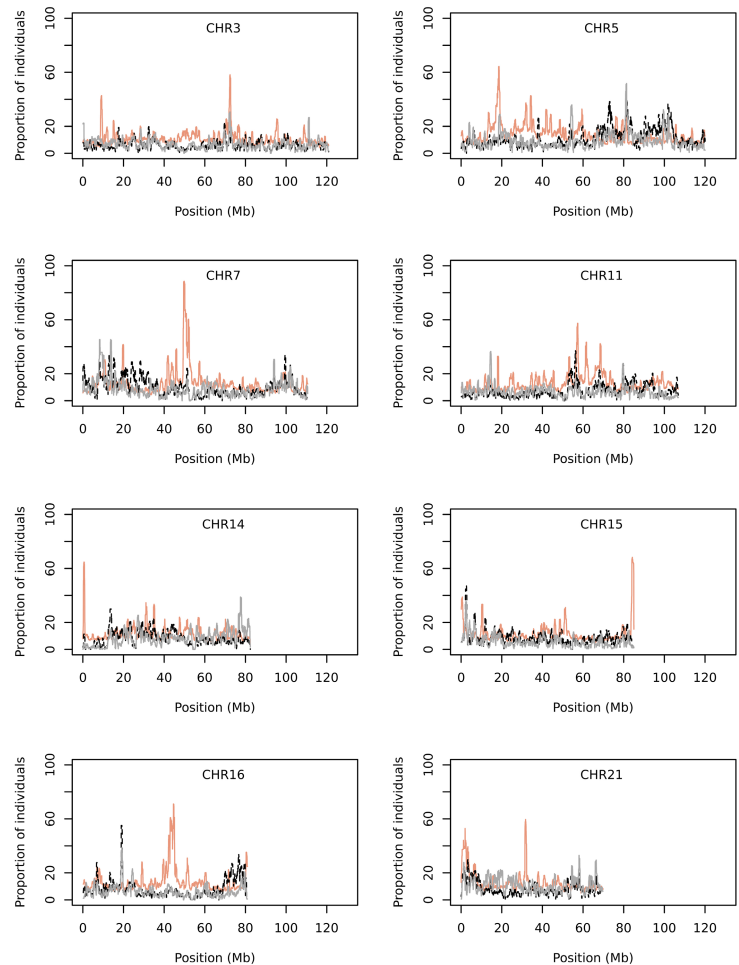


FIGURE 3 Detection of ROH islands. Manhattan plot showing the proportion of animals with the SNP within an ROH with a minimum length of 500kb for the group of all animals of the 14 local cattle breeds from Data 3. The first threshold line (black dashed) is 32.6% (99th percentile), and regions with SNPs exceeding this threshold are interpreted as ROH islands. The second threshold line (green dashed) corresponds to 57% and was set to indicate the eight most significant ROH islands found in the breeds considered.

**FIGURE 4** Line plots showing the proportion of animals with the SNP within a ROH for three different groups: (a) breeds consisting of animals from 14 local Alpine breeds (salmon line) (b) Brown Swiss (black line) and (c) Holstein (grey line) at eight different chromosomes 3, 5, 7, 11, 14, 15, 16, and 21.



programs for this breed. Similarly, ENS, Hinterwolder (HW), and Murnau Werdenfelser (MWF) are the three other breeds for which less than 400 breeding females are reported (Table S1). In ENS, 30% of average  $F_{ROH}$  comes from large segments >20 Mb, indicating recent inbreeding. This is understandable considering the low number of breeding females and the information that only four farms kept ENS animals in the 1990s (Binder, 2016).

## Selection signatures

Alpine cattle breeds are unique genetic resources of the alpine Arch (Mastrangelo et al., 2020b) and it seems worthwhile to understand the genomic regions that were relevant for evolution and adaptation. We investigated selection signatures in alpine local cattle breeds based on  $d_i$ -values using available medium-density SNP data from 18 breeds (Data 1) and high-density SNP data from a subset of 14 breeds (Data 2). The results of the search for selection signatures and ROH islands could be used to identify candidate genes being responsible for the evolution of specific traits in the local livestock breeds studied (e.g. Bhati et al., 2020; Signer-Hasler et al., 2022). Several signatures of selection were similar across the dataset

regardless of marker density and breeds (Table 2). Two of the most prominent signatures were observed on chromosome 6 in the vicinity of known genes such as *KIT* and *LCORL*, which explain the differences in coat color (e.g. white spotting) and stature in cattle. Selection signatures in the region of these genes have been previously described in numerous alpine cattle breeds (Bhati et al., 2020; Ferenakovic et al., 2013b; Rothhammer et al., 2013; Signer-Hasler et al., 2017). We also report a strong selection signature on chromosome 11 at 66 to 69 Mb in Swiss OB cattle. This signature harbors multiple genes and has been described in previous studies based on 50 k SNP genotypes (Rothhammer et al., 2013; Signer-Hasler et al., 2017) and has also been confirmed using WGS data of OB cattle (Bhati et al., 2020). The region from 18.5 to 18.7 Mb on chromosome 5 yielded the highest  $d_i$ -value based on Data 2 (Table 2) but does not harbor any annotated genes by itself. However, this genome region is very close to *KITLG*. It is suggested that the high  $d_i$ -value is due to the vicinity of this candidate gene for coat color (Charlier et al., 1996; Yurchenko et al., 2018). It possibly contains a specific, almost fixed variant in the OB breed that explains the typical brown coat color. We report two additional regions on chromosome 20 (47.7–50.1 Mb) and chromosome 26

**TABLE 3** Details of the most important ROH islands on chromosomes 3, 5, 7, 11, 14, 15, 16, and 21. Presentation of their lower and upper limits, the total percentage of animals of local breeds having the SNPs from this region in a ROH, the maximum percentage in local breeds, the total percentage in the two commercial breeds (BS and HO), the corresponding maximum percentage in both commercial breeds and the genes annotated in the region of ROH islands.

Chr	Lower bound (bp)	Upper bound (bp)	Fraction local	Max fraction local (%)	Fraction BS (%)	Max fraction BS (%)	Fraction HO (%)	Max fraction HO (%)	Annotated genes with known functions
3	71 943 101	72 798 545	47.8	58.2	13.7	16.2	24.9	30.6	None
5	17 109 743	19 084 340	44.8	64.3	10.6	14.4	15.2	28.8	C5H12orf50; C5H12orf29; CEP290; TMTC3; KITLG
7	49 358 289	52 390 627	59.0	88.4	11.3	24.0	7.4	16.5	PKD2L2; FAM13B; WNT8A; NME5; LOC101902951; BRD8; KIF20A; CDC23; GFRA3; CDC25C; SLBP2; FAM53C; KDM3B; REEP2; EGRI; ETFI; HSPA9; CTNNA1; LRRTM2; SIL1; MATR3; PAIP2; SLC23A1; MZBI; PROBI; SPATA24; DNAJC18; ECSCR; SMIM33; TMEM173; UBE2D2; CXXC5; PSD2; NRG2; PURA; IGIP; CYSTM1; PFDNI; HBEGF; SLC4A9; ANKHD1; EIF4EBP3; SRA1; APBB3; SLC35A4; CD14; TMC06; NDUFA2; IK; WDR55; DND1; HARS; HARS2; ZMAT2; PCDHA13; PCDHA3; PCDHBI; PCDHB8; PCDHBI4; PCDHBI
11	56 798 352	57 593 263	49.6	57.3	13.7	21.6	5.4	8.8	REG3G; REG3A
14	272 795	933 738	57.9	64.7	9.6	12.0	3.6	4.7	C14H8orf33; ZNF34; RPL8; ZNF7; COMM5; ARHGAP39; C14H8orf82; LRRC24; LRRCL4; REQL4; MFSD3; GPT; PPP1R16A; FOXH1; KIFC2; CYHR1; TONSL; VPS28; SLC39A4; CPSFI; ADCK5; SLC52A2; FBXL6; TMEM249; SCRT1; DGATI; HSF1; BOPI; SCX; MROHI; HGHI; WDR97; MAFI; SHARPIN; CYCI; GPAAI; EXOSC4; OPLAH; SMPD5; SPATCI; PARP10; GRINA; PLEC; ZNF16
15	83 834 272	84 965 740	59.9	68.2	3.3	7.8	2.0	2.9	OR4A15; OR4C6; OR4A16
16	42 099 866	45 179 523	52.9	71.0	6.0	9.0	7.1	11.8	DISP3; UBIAD1; MTOR; ANGPTL7; EXOSC10; SRM; MASP2; TARDBP; CASZ1; PEX14; DFFA; CORT; CENPS; PGD; KIF1B; UBE4B; RBP7; NMNATI; LZIC; CTNNB1; CLSTN1; PIK3CD; TMEM201; SLC25A33; SPSBI; GPR157; CA6; ENO1; RERE; SLC45A1;
21	31 392 845	32 243 978	51.0	59.5	5.9	8.4	8.9	12.4	ETFA; ISL2; SCAPER; RCN2; PSTPIP1; TSPAN3; PEAK1; TMEM266;

(22.3–22.5 Mb) that were among the best 30  $d_i$ -values in both datasets. Interestingly, the window on chromosome 20 was below the top 30  $d_i$ -values in Austrian PI and PUS cattle and was further significant in Swiss EV (Data 1), Austrian GV (Data 2), German HW (Data 2) and includes the *stanniocalcin 2* gene (*STC2*). This gene has been proposed to explain size reduction in dogs (Rimbault et al., 2013) and variation in average daily gain in beef cattle (Zhang et al., 2020a). Interestingly, a major ROH island was recently reported in its paralog *STC1* in local Swiss goat breeds (Signer-Hasler et al., 2022). The signature on chromosome 26 (22.3–22.5 Mb) harbors the *FGF8* gene, which is thought to be involved in lactation (Kemper et al., 2014) and, in agreement with a previous study, has also been observed in Swiss OB in particular (Signer-Hasler et al., 2017). Overall, the OB breed appears to be overrepresented in the lists (Tables 2, S6, S7) with the top 30  $d_i$ -values (11× in Data 1 and 16× in Data 2) compared to all other breeds. In particular, in three genome regions on chromosomes 6, 11, and 26, OB cattle show remarkable differentiation from all other breeds, resulting in high  $d_i$ -values. The  $d_i$ -statistic is a function of pairwise  $F_{ST}$  values between breed  $i$  and the other breeds in the considered dataset (Akey et al., 2010). When closely related breeds (e.g. ER, CAST, and VPN) are considered as separate breeds, the  $d_i$ -values for such breeds may be underestimated compared to the  $d_i$ -values of clearly distinct breeds such as OB and SIM.

## ROH islands

As highlighted by Mastrangelo et al. (2018), genomic regions subject to selection tend to form ROH islands that exhibit high levels of homozygosity around a selected locus compared to the rest of the genome (Purfield et al., 2017; Szmatoła et al., 2016). We have inferred ROH islands in alpine local cattle breeds as indicators of positive selection (Gorssen et al., 2021; Nothnagel et al., 2010). Based on 50k data, the maximum proportion of animals with a SNP within a ROH was 13.9% (chromosome 6: 37.3–37.7 Mb) among all 1292 individuals, and only in a few breeds could we observe clear ROH islands (not shown). If the genome of an animal contains segments of 1 Mb length, the autozygosity of this individual originates from a common ancestor 50 generations in the past (Ferenčaković et al., 2013b). Therefore, medium-density SNP genotypes are not dense enough for inferring common ROH islands in our diverse group of 18 local Alpine cattle breeds separated before/around breed formation several generations ago. For studies investigating common ROH islands in such a group, the availability of high-density SNP or even whole-genome sequencing data is a prerequisite.

Based on high-density SNP (Data 3) we found the most prominent ROH-island in local Alpine cattle breeds

on chromosome 7 at 49.4–52.4 Mb (Table 3, Table S10). ROH islands in this region have been previously identified in several studies based on medium-density (50–150k) SNP genotypes: e.g. in Modena cattle of northern Italy (Schiavo et al., 2022), in several breeds sampled in Poland (Szmatoła et al., 2016) and in a previous study using genotypes from CAST (Strillacci et al., 2020). In contrast to Szmatoła et al. (2016), we found this ROH island only in local Alpine breeds, but not in the two commercial breeds considered (Table 3, Figure 4, Table S10). The same ROH island was previously described in different studies using high-density SNP genotypes from indicine and taurine cattle breeds (Karimi, 2013) and in seven out of eight local Chinese cattle breeds studied (Xu et al., 2019). This ROH island comprises more than 50 genes (Tables 3, S10), proving that ROH islands are often found in gene-rich regions (Karimi, 2013). Among many others, it harbors the *HSPA9* gene that belongs to the *heat shock protein 70* (*HSP70*) family. Rovelli et al. (2020) reported that the *HSP70* family and other *HSP* genes serve as biomarkers for the selection of heat- and cold-tolerant genotypes in the context of global warming. Variations in the expression of heat shock proteins in cattle and buffalo are associated with heat tolerance and adaptation to different climatic conditions (Kumar et al., 2015). A comparison of *HSP70* gene expression levels in cold- and heat-adapted local goats, for instance, revealed higher levels in cold-adapted goats in summer (Banerjee et al., 2014). Two other ROH islands were observed on chromosome 7 at 45.7–46.2 Mb (Table S10). This genome region was previously described (Karimi, 2013; Nandolo et al., 2018) and includes the *TCF7* gene, which has been associated with climate adaptation of local cattle breeds (Flori et al., 2019) and variation in coat color in two northern Italian cattle breeds (Bertolini et al., 2022).

We report an ROH island on chromosome 14 (0.2 to 0.9 Mb), a genome region that contains more than 40 genes including the well-known *DGATI* gene, which affects milk production traits, having the greatest effect on fat content, especially in Holstein cattle (Grisart et al., 2002). The maximum percentage in this ROH island was 65%, based on the 1678 individuals from Alpine local breeds, compared to the two commercial breeds, 12% and 5% in BS and HO individuals, respectively. Recent results of routine genotyping with custom SNP arrays (Häfliger et al., 2021) show that the four local dairy breeds from Switzerland (OB, SI, ER, EV) are almost homozygous for the milk fat decreasing *DGATI* allele encoding alanine at residue 232 (allele frequency >94%; not shown). The fixation of that unfavorable allele in some taurine breeds such as Austrian PI has already been described (Kaupe et al., 2004). A GWAS on 15 traits linked to milk production, udder health, and udder morphology using whole genome sequence data from bulls showed that the best-associated variants for many traits were found close to *DGATI* (Tribout et al., 2020). Therefore, it has been concluded that there might be other causal

variants in this genome region (Kühn et al., 2004; Tribout et al., 2020). We speculate that the fixation of the trait-decreasing *DGATI* allele in local Alpine breeds could be a hitchhiking effect due to the selection of other causal variants in this region. For example, the *heat shock factor 1 (HSF1)* gene is a direct neighbor of *DGATI* and is also associated with thermoregulation in cattle (Kumar et al., 2015; Rovelli et al., 2020). Further studies using dense marker data are needed to better understand the high level of homozygosity in this genome region and to identify possible candidate genes.

The ROH island on chromosome 5 (17.1–19.1 Mb) harbors *KITLG*, a known candidate gene underlying selection for coat color variation in cattle (Signer-Hasler et al., 2017; Yurchenko et al., 2018). A missense variant in *KITLG* is responsible for the co-dominantly inherited roan phenotype in Belgian Blue and Shorthorn cattle (Seitz et al., 1999). The average proportion of animals with SNPs in this region within a ROH varies between local Alpine breeds. While the average proportion is above 55% for ER, EV, VPN, and VPR (Table S10), it varies between 33% and 42% in CAS, VW, Tuxer (TUX), PI, and OB and is below 18% for the remaining five breeds (ENS, GV, HW, PUS, SIM) and the two commercial breeds. Elevated homozygosity levels for this region and the three cattle breeds from the Italian Aosta valley (VPN, VPR, and CAST) have been previously reported (Strillacci et al., 2020). A GWAS on the UV-protective eye pigmentation area attributed much of the phenotypic variance to QTL regions comprising the genes *KITLG*, *KIT*, and *MITF* (Pausch et al., 2016). Interestingly, the five breeds with increased homozygosity flanking *KITLG* are geographically and genetically close to each other (Figure 1) but show obvious variation in the white spotting phenotype (Table S1). Therefore, it seems plausible that variation in *KITLG* could contribute to the genetic heterogeneity of bovine spotting (Küttel et al., 2019).

The ROH islands on chromosomes 11 (56.8–57.6 Mb), 15 (83.8–84.9 Mb), 16 (42.1–45.1 Mb), and 21 (31.3–32.2 Mb) all have an increased proportion of SNPs being in a ROH in local Alpine breeds, while the average proportions in commercial breeds are below 15% (Figure 4, Tables 3, S10). The region on chromosome 16 has previously been recognized as ROH island in several local European breeds (Karimi, 2013; Mastrangelo et al., 2018; Szmatola et al., 2016). This ROH island is characterized by more than 30 annotated genes (Table 3). In a meta-analysis of selection signatures that compiled data from 90 pure- and crossbred cattle breeds, this region was found to underlie selection for several traits such as mammary gland function, milk production, embryonal development and survival, tropical adaptation, as well as immune system response and regulation (Randhawa et al., 2016).

In contrast, information on the three ROH islands on chromosomes 11, 15, and 21 is sparse. The ROH

island on chromosome 15 was recently described in a study using high-density SNP genotypes from eight Chinese cattle breeds (Xu et al., 2019). In addition to three genes from the olfactory receptor gene family, eight other genes with unknown functions are located in this region. Human studies are currently investigating the possible involvement of olfactory receptor genes in the pathogenesis of obesity (Diels et al., 2020). As far as we know, this is the first study reporting ROH islands on chromosome 11 (56.8–57.6 Mb) and 21 (31.3–32.2 Mb). In particular, the second region on chromosome 21, harboring the genes *ETFA*, *ISL2*, *SCAPER*, *REC2*, *PSTPIPI*, *TSPAN3*, *PEAK1*, and *TMEM266*, seems to be of interest for further studies, as the average proportion is >90% e.g. in the Austrian ENS as well as in the Swiss SIM population. Investigations in other local taurine breeds could clarify whether or not this ROH island occurs privately in local breeds from the alpine arc. We also reported another ROH island (chromosome 6: 75.8–76.3 Mb) where the average proportion in ENS cattle was above 90% (Table S10). However, no genes are annotated in this region either. For another ROH island on chromosome 6 (37.9–38.0 Mb) containing the genes *LAP*, *NCAPG*, and *LCORL* fixation was observed for the OB breed (Table S10), while the average proportions of EV, ER, CAS, VPN, and the two commercial breeds were less than 15%. Extended homozygosity for OB and this region was confirmed by selection signature analysis and described previously (Bhati et al., 2020). As expected from previous studies (Ferenčaković et al., 2013a; Mastrangelo et al., 2018; Mészáros et al., 2015), the average proportions in ENS, GV, PI, and SIM and this region were also considerably high (>57%).

In summary, we have presented overlapping results from the analysis of selection signatures and ROH islands. This is especially true for the signatures from highly differentiated breeds such as OB cattle from Switzerland and ENS cattle from Austria. The correlation between short ROH regions and regions presumed to be under selection based on  $F_{ST}$  has already been previously described by Zhang et al. (2015), who also suspected that such short ROH are the result of inbreeding and selection. Our results provide a basis for the search for putative causal variants for adaptation to the alpine environment and other specific characteristics of local Alpine cattle breeds.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

The SNP data analyzed during the current study were made available by various breeding organizations and research partners from a total of six countries. The SNP data belong to the breeding organizations and are therefore not publicly available. For reproduction of conclusions, the data are available from the corresponding author with the permission of the data provider/owner.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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