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Comparative analysis of genomic inbreeding parameters and runs of homozygosity islands in several fancy and meat rabbit breeds

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Abstract

Runs of homozygosity (ROH) are defined as long stretches of DNA homozygous at each polymorphic position. The proportion of genome covered by ROH and their length are indicators of the level and origin of inbreeding. In this study, we analysed SNP chip datasets (obtained using the Axiom OrcunSNP Array) of a total of 702 rabbits from 12 fancy breeds and four meat breeds to identify ROH with different approaches and calculate several genomic inbreeding parameters. The highest average number of ROH per animal was detected in Belgian Hare (~150) and the lowest in Italian Silver (~106). The average length of ROH ranged from 4.001 ± 0.556 Mb in Italian White to 6.268 ± 1.355 Mb in Ermine. The same two breeds had the lowest (427.9 ± 86.4 Mb, Italian White) and the highest (921.3 ± 179.8 Mb, Ermine) average values of the sum of all ROH segments. More fancy breeds had a higher level of genomic inbreeding (as defined by ROH) than meat breeds. Several ROH islands contain genes involved in body size, body length, pigmentation processes, carcass traits, growth, and reproduction traits (e.g.: *AOX1*, *GPX5*, *IFRD1*, *ITGB8*, *NELL1*, *NR3C1*, *OCA2*, *TRIB1*, *TRIB2*). Genomic inbreeding parameters can be useful to overcome the lack of information in the management of rabbit genetic resources. ROH provided information to understand, to some extent, the genetic history of rabbit breeds and to identify signatures of selection in the rabbit genome.

KEYWORDS
genetic variability, *Oryctolagus cuniculus*, ROH, signature of selection, SNP

INTRODUCTION

The domestication process of the European rabbit (*Oryctolagus cuniculus*), usually referred as domestic rabbit or simply rabbit, relied on wild populations that colonised the South of France (Zeuner, 1963). The process probably started quite recently, in a period that spanned from the high middle age to the 15th and 16th centuries. In the early stages, domestication of this species may have been mainly associated with the activities of French monasteries and castles (Callou, 2002; Zeder, 2012; Zeuner, 1963). Subsequently, more undefined trajectories continued that followed the spread of the rabbit in north-central Europe, resulting in the constitution of

modern breeds (reviewed in Fontanesi, 2021a; Fontanesi, Utzeri, et al., 2021). The domesticated rabbit genetic pool became slightly different from the wild counterparts in terms of allele frequencies at many regulatory sites, affecting brain and neuronal development, with potential impact on the behaviour of the animals, so that, they could be more easily handled and bred in captivity (Carneiro et al., 2014). Then, human driven artificial selection, which led to modern rabbit breeds, mainly worked on exterior traits (e.g. coat colours, body size, ear length). The result was that a broad phenotypic diversity distinguished many breeds valued by fancy breeders who continued to create additional breeds, lines, or strains by introgressing specific features and creating new combinations of traits (Boucher et al., 2021; Fontanesi, 2021a, 2021b). Most fancy breeds are named after their colouration or other distinctive exterior features and specific standards have been defined by breeders' organisations or societies, present in several countries (Boucher et al., 2021; Whitman, 2004). Some of these breeds are recognised by more than one breeders' society or association while other breeds are recognised only at the national level. More recently, specialised meat lines have been also constituted by selecting the animals for performance and production traits.

Both fancy breeds (with their close or semi-close national organisations and structures, with few exchanges of animals with other countries) and meat lines (where selection is usually carried out within close nuclei) can be considered small populations genetically. Therefore, to properly manage these populations, it is important to monitor their level of inbreeding. An increased level of autozygosity leads to inbreeding depression and the occurrence of deleterious recessive alleles in homozygous state, which mainly impairs reproductive performances that are usually quite poor, particularly in fancy rabbit purebreds (Boucher et al., 2021).

In diploid organisms, inbreeding (traditionally indicated with F_{PED} when based on pedigree information) can be defined as the probability that, at a randomly selected locus, the two alleles derived from the maternal and the paternal sides are identical by descent (Wright, 1922). This definition of F_{PED} can be extended as the proportion of all loci of an individual's genome that is identical by descent. In a population, the level of inbreeding is estimated by averaging all F_{PED} individual values.

In rabbits, where pedigree recording systems and DNA-based methods to control parentage are not well established, it is not always possible to calculate F_{PED}; when this parameter is calculated, its reliability is usually quite low. In addition, it is also needed to consider that the method of calculation of F_{PED} uses a few assumptions that generate approximations in the calculation of the inbreeding level (Knief et al., 2017; Leutenegger et al., 2003; Wang, 2016). Genomic tools now available also in *O. cuniculus* [including a reference genome and a single nucleotide polymorphism (SNP) array; Carneiro et al., 2014; Fontanesi, Bovo, et al., 2021] can provide alternative avenues to overcome the limits of F_{PED}, which are quite relevant in many rabbit populations.

As already reported in several other species, the level of autozygosity of an animal can be estimated by obtaining the genotype status at thousands of polymorphic sites by genotyping SNPs covering the whole genome (Kristensen et al., 2010). Inbreeding related parameters can be calculated using genome-wide SNP data (Kardos et al., 2015; Leutenegger et al., 2003; Schiavo, Bovo, Bertolini, Tinarelli, et al., 2020; VanRaden, 2008). Runs of homozygosity (ROH), defined as continuous chromosome stretches in which all loci have a homozygous genotype (Gibson et al., 2006), if used to sum up the proportion of the genome in autozygosity state, can provide a quite precise measure of genomic inbreeding of an individual animal (F_{ROH}; Kardos et al., 2015; Peripolli et al., 2017). At the population level, some other characteristics of ROH (the patterns of ROH distribution across the chromosomes, the average length of ROH and the average proportion of the genome covered by ROH) can provide some indications to infer the genetic history of the populations (Ceballos et al., 2018). ROH can also be useful to identify signatures of selection: a high frequency of ROH in certain chromosome regions (defined as ROH islands or ROH hotspots) highlights reduced haplotype variability that spans loci under artificial selection or natural selection, as already reported in several livestock species (Bertolini et al., 2018; Gorssen et al., 2020; Grilz-Seger et al., 2019; Mastrangelo et al., 2018; Peripolli et al., 2017; Purfield et al., 2017; Schiavo et al., 2021; Schiavo, Bovo, Bertolini, Dall'Olio, et al., 2020).

In this study we analysed SNP array datasets from a total of 16 fancy and meat rabbit breeds to identify ROH and calculate several genomic inbreeding parameters. Then, we evaluated the occurrence of ROH islands in the genome of some of these breeds to identify putative signatures of selection that might be derived by different selection histories and structures of these rabbit genetic resources.

MATERIALS AND METHODS

Animals

Biological specimens (hair roots or buccal swaps) were sampled from a total 712 rabbits from four meat and 12 fancy rabbit breeds. Three meat breeds (Italian White, $n = 256$; Italian Spotted, $n = 93$; Italian Silver, $n = 20$) and all fancy breeds (Belgian Hare, $n = 24$; Burgundy Fawn, $n = 6$; Champagne d'Argent, $n = 19$; Checkered Giant, $n = 79$; Coloured Dwarf, $n = 20$; Dwarf Lop, $n = 20$; Ermine, $n = 20$; Giant Grey, $n = 27$; Giant White, $n = 20$; Rex, $n = 19$; Rhinelander, $n = 28$; and Thuringian, $n = 9$) were from the national Herd Book maintained by the

Italian Rabbit Breeders Association (ANCI). One meat breed (n = 52) was from another albino white nucleus of Gruppo Martini spa selected for meat production, indicated hereafter as Commercial Meat line. All breeds from the ANCI Herd Book had the standard breed characteristics. The description of the breeds is reported in Table S1. All animals included in this study were selected to avoid highly related individuals (no full- or half-sibs).

SNP genotyping and population genomic analyses

DNA extraction was carried out using the Wizard Genomic DNA Purification kit (Promega Corporation). DNA from each rabbit was then genotyped with the Affymetrix Axiom OrcunSNP Array (Thermo Fisher Scientific/Affymetrix Inc.), which can analyse 199 692 SNPs. Quality control of the genotyping data was made with the Axiom Analysis Suite that led to discard low quality SNPs and with PLINK v.1.9 software (Chang et al., 2015) using the following filtering criteria: SNPs and animals with more than 10% missed genotyping information were excluded from the analysis; only autosomal SNPs located in unique positions were considered. We avoided filtering the dataset based on minor allele frequency because this procedure could lead to the underestimation of the coverage in ROH (Meyermans et al., 2020). Finally, a total of 43 529 SNPs and 702 animals remained for further analyses (Table S1).

Genotyping data were used to produce multidimensional scaling (MDS) plots with the first three dimensions computed with PLINK v.1.9 software (Chang et al., 2015). The effective population size (N_e) for recent and distant generations was calculated with default parameters using SNeP software (Barbato et al., 2015).

Runs of homozygosity and other genomic inbreeding measures

PLINK v.1.9 software (Chang et al., 2015) was used to identify ROH. No pruning based on linkage disequilibrium was performed to avoid biases that could be derived from this procedure (Marras et al., 2015; Meyermans et al., 2020). A minimum length of 1 Mb was chosen to detect ROH. This threshold may exclude short and common ROH determined by markers in linkage disequilibrium (Ferenčaković, Sölkner, et al., 2013; Marras et al., 2015). To tune the parameters in PLINK based on the characteristics of the genotyping tool and of the reference genome OryCun2.0, which has a limited N50, four approaches were used to identify ROH by varying and combining a few calling parameters: (i) Approach 1—the minimum density of SNPs in a genome window was one SNP every 100 kb, the maximum gap between consecutive SNPs was 1000 kb, the minimum number of consecutive homozygous SNPs included in the ROH was 15, the number of heterozygous SNPs that were allowed in the ROH was zero, and the minimum length that constituted the ROH was 1 Mb; (ii) Approach 2—the minimum density of SNPs in a genome window was one SNP every 100 kb, the maximum gap between consecutive SNPs was 1000 kb, the minimum number of consecutive homozygous SNPs included in the ROH was 15, the number of heterozygous SNPs that were allowed in the ROH was two, and the minimum length that constituted the ROH was 1 Mb; (iii) Approach 3—the minimum density of SNPs in a genome window was one SNP every 100 kb, the maximum gap between consecutive SNPs was 1000 kb, the minimum number of consecutive homozygous SNPs included in the ROH was 50, the number of heterozygous SNPs that were allowed in the ROH was zero, and the minimum length that constituted the ROH was 1 Mb; (iv) Approach 4—the minimum density of SNPs in a genome window was one SNP every 100 kb, the maximum gap between consecutive SNPs was 1000 kb, the minimum number of consecutive homozygous SNPs included in the ROH was 50, the number of heterozygous SNPs that were allowed in the ROH was two, and the minimum length that constituted the ROH was 1 Mb. All parameters used for the four approaches are summarised in Table S2. Identified ROH were then grouped into five size classes according to their physical length (Ferenčaković, Hamzić, et al., 2013; Kirin et al., 2010; Schiavo, Bovo, Bertolini, Tinarelli, et al., 2020; Schiavo et al., 2021): ROH1–2 (ROH ≥1 Mb and <2 Mb); ROH2–4 (ROH ≥2 Mb and <4 Mb); ROH4–8 (ROH ≥4 Mb and <8 Mb); ROH8–16 (ROH ≥8 Mb and <16 Mb); ROH >16 (ROH ≥16 Mb). Other ROH parameters were also computed for each rabbit: (i) number of ROH (nROH); (ii) the average length of ROH (L_{ROH}, in Mb); (iii) the sum of all ROH segments (S_{ROH}, in Mb). The average values of those parameters were also calculated for each breed.

Genomic inbreeding measures (F_{ROH}) were obtained as the proportion of the autosomal genome covered by ROH. According to multiple detected ROH classes with length >1 Mb classes, >4, >8 and >16 Mb, the inbreeding coefficients F_{ROH1}, F_{ROH4}, F_{ROH8}, and F_{ROH16} were obtained respectively. Moreover, the individual F_{ROH} values were averaged to calculate the final inbreeding coefficients values for each rabbit breed included in the study.

Furthermore, using PLINK software version 1.9 (Chang et al., 2015) with the ported functions of GCTA software v. 1.92 (Yang et al., 2011), other different inbreeding coefficients were also considered. These measures included: (i) the genomic inbreeding coefficient F_{HOM} based on the difference between observed and expected number of homozygous genotypes; (ii) the estimate based on the variance-standardised relationship minus 1 (F_{hat1}); (iii) the inbreeding coefficient based on the genomic relationship matrix F_{GRM} (VanRaden et al., 2011; this parameter is equivalent to F_{hat1}, even if scaled in a different

way); (iv) the excess of homozygosity-based inbreeding estimate (F_{hat2}); (v) the estimate based on correlation between uniting gametes (F_{hat3}). Finally, we estimated the Pearson correlation coefficients (r) and the Spearman's rank correlation coefficients (ρ) between all pairs of inbreeding coefficients.

ROH islands and annotation of genome regions

The identification of ROH islands was obtained as follows: (i) the frequency of SNPs observed in ROH was calculated for a given breed by counting the number of samples with a ROH including this SNP within the given breed divided by the total number of genotyped animals of that breed; (ii) a percentile threshold of the frequency of an SNP in ROH was calculated: the percentile-based threshold was defined considering the top 1% of SNPs observed in a ROH in each breed. Adjacent SNPs, having a frequency of ROH occurrences over or equal to the identified thresholds, and with a distance ≤1 Mb between them, constituted ROH islands (Schiavo et al., 2021).

The identified ROH islands were annotated with the BEDTOOL v.2.17 (https://bedtools.readthedocs.io/) by retrieving the mapped genes form OryCun2.0 NCBI's GFF file. Functional enrichment analysis was carried out with Enrichr (Chen et al., 2013) via Fisher's exact test. Analyses was performed according to the following databases: GWAS catalog 2019, KEGG Human database 2019, MGI mammalian phenotype level dataset 2019 and the biological process branch of gene ontology (GO). The analysis was executed for each breed specifically by using the set of genes mapped with ROH islands as input. Additionally, the statistically over-represented terms were considered if at least two input genes from two or more different ROH islands were involved and if the adjusted p value is <0.05.

RESULTS

Population genomic parameters

The tri-dimensional MDS-plot of Figure S1 graphically represents the genomic information and relationships of the rabbit breeds based on the SNP dataset. Rabbits belonging to the same breed were distinguishably clustered together. Two of the meat breeds (Italian White and the Commercial meat breed) were grouped separately among other breeds. Other breeds that share the same or similar morphological traits were grouped closely together. For example: Ermine rabbits (from a breed with animals of small size) were close to the Coloured Dwarf breed; and the three giant breeds (Checkered Giant, Giant Grey, and Giant White) were grouped together. All other breeds could not be clearly resolved with this approach.

The estimated effective population size (N_e) of the 16 rabbit breeds is reported in Table S3. Italian White, Giant Grey, and the Commercial Meat breeds had the highest values (at the 13th past generation: 94, 88, and 73 respectively). The lowest N_e was in Ermine, Thuringian, and Burgundy Fawn breeds (34, 25, and 18 respectively). It is worth to note that Burgundy Fawn and Thuringian were the breeds with the lowest number of analysed rabbits (Table S1), which could have biased this parameter.

ROH in 16 rabbit breeds

Several parameters can be adjusted to call ROH (e.g. number of missed genotypes, number of heterozygous SNP allowed in ROH, the minimum size of a ROH and so on), which can affect the final outputs. We used different parameters to call ROH that were summarised in four approaches (Table S2). We tested them as there is no general rule established in this context (Peripolli et al., 2017) and we needed to tune parameters based on the characteristics of the genotype dataset and of the reference genome OryCun2.0, which has a N50 length for the contigs only equal to 64.65 kb. By modifying the number of allowed heterozygous SNPs, the parameter that was mainly affected was the average length of the ROH, as expected. The approaches that allowed up to two heterozygous SNPs (Approaches 2 and 4) had larger average length of ROH than the approaches that did not allow any heterozygous SNPs (Approaches 1 and 3). The correlations of the two groups of approaches for different ROH measures was, however, very high (from 0.761 to 0.774, for the average length of ROH; from 0.882 to 0.886 for nROH; from 0.909 to 0.921 for S_{ROH}; Tables S5–S7). Among these parameters, S_{ROH} is directly related to F_{ROH} measures, suggesting that the general overview obtained by different approaches is similar in terms of interpretation of the results for the analysed breeds. Looking at the plots that show changes of S_{ROH} values related to different thresholds of a minimum number of consecutive homozygous SNPs to call ROH (ranging from 5 to 70) in the four breeds that had the highest number of genotyped animals (Figure S2), it emerged that S_{ROH} started to drop when more than 20 SNPs were considered. In addition, on average, 15 consecutive SNPs would approximately cover 1 Mb of the rabbit genome, which is only 10 times higher than the N50 length of the contigs for the used reference genome. Another element to be considered is the efficiency of the genotyping platform for which the useful SNPs, after all filtering, were about 1/5 of the total number of genotyped SNPs. Based on these considerations, among the tested approaches to call ROH, Approach 2, which uses a minimum number of 15 consecutive homozygous SNPs to call ROH and a maximum of two heterozygous SNPs in the homozygous SNP-windows, was considered the most appropriate approach in our case, as it can better

overcome some of the current limitations of the genomic tools available in rabbits mentioned above (Fontanesi, Utzeri, & Ribani, 2021). Therefore, all main ROH results were based on this approach, even if full information obtained with the other approaches is reported in Tables S1 and Figures S1 mentioned below.

Table 1 provides an overview of the ROH identified in the investigated breeds, using Approach 2, whereas a comparative analysis among breeds and approaches is reported in Table S4. The average nROH per animal ranged from 105.5 (but with a quite large SD: 32.0) in Italian Silver to 150.2 ± 13.2 in Belgian Hare. The average length of ROH (L_{ROH}) ranged from 4.001 ± 0.556 Mb (Italian White) to 6.268 ± 1.355 Mb (Ermine). The same two breeds had the lowest (427.9 ± 86.4 Mb, Italian White) and the highest (921.3 ± 179.8 Mb, Ermine) sum of all ROH segments (S_{ROH}) values. The correlation for these three ROH statistics (i.e. nROH, L_{ROH}, and S_{ROH}) determined with the four different approaches are reported in Tables S5–S7. Correlations for these parameters determined with Approach 2 between the same parameters calculated with the other approaches ranged from 0.882 to 0.996 (for nROH), from 0.773 to 0.998 (for L_{ROH}), and from 0.909 to 0.999 (for S_{ROH}). The highest correlations were between approaches that used the same maximum number of allowed heterozygous SNPs included in the homozygous window (i.e. Approach 2 vs Approach 4 and Approach 1 vs Approach 3), whereas the minimum number of SNPs included in the window (15 or 50) did not have any relevant effects of the considered ROH parameters (Tables S5–S7).

Figure 1 represents the correlation plots between nROH and S_{ROH} over all individuals of each of the 16 rabbit breeds. Homogeneous correlation plots are evident in Champagne d'Argent, Coloured Dwarf, Commercial Meat, and Giant Grey indicating that most animals within these breeds had similar ROH parameters (nROH, L_{ROH}, and S_{ROH}). In contrast, a heterogeneous distribution was observed in Checkered Giant, Dwarf Lop, Italian Silver, Italian Spotted, and Rex breeds, whereas all other breeds had intermediate patters (excluding the breeds for which a small number of animals was genotyped).

The proportion of ROH of different class length for the 16 breeds is shown in Figure 2. Table S8 reports the detailed numbers. Ermine, Coloured Dwarf, and Italian Silver had the highest proportion of long ROH (>16 Mb) about (7%, 6%, and 5% respectively). Short ROH classes (1–4 Mb) were more frequent in the three giant breeds, in the Commercial Meat and Italian White breeds (Table S8).

The largest ROH in term of length was identified in Ermine and Coloured Dwarf (87.4 Mb on OCU3). These two breeds also included animals with the largest S_{ROH} values in the whole dataset: a Coloured Dwarf rabbit had an S_{ROH} of 1304.3 Mb and an Ermine rabbit had an S_{ROH}

TABLE 1 General statistics of runs of homozygosity (ROH) identified in 16 rabbit breeds based on Approach 2 to call ROH

Breed[a]	nROH (SD)[b]	S_{ROH} (SD)[c]	L_{ROH} (SD)[d]
Belgian Hare (BH)	150.167 (13.160)	735.907 (118.772)	4.887 (0.661)
Burgundy Fawn (BF)	140.500 (7.259)	780.595 (54.878)	5.583 (0.644)
Champagne d'Argent (CdA)	144.579 (9.703)	752.809 (227.838)	5.209 (1.588)
Checkered Giant (CG)	123.853 (26.457)	516.631 (166.303)	4.066 (0.919)
Coloured Dwarf (CD)	124.600 (8.929)	708.714 (199.047)	5.651 (1.369)
Commercial Meat (CM)	120.250 (13.038)	496.797 (92.528)	4.118 (0.617)
Dwarf Lop (DL)	127.800 (12.060)	639.518 (147.462)	5.026 (1.242)
Ermine (ER)	148.100 (12.345)	921.273 (179.834)	6.268 (1.355)
Giant Grey (GG)	131.444 (15.621)	553.177 (151.441)	4.146 (0.767)
Giant White (GW)	115.550 (36.309)	530.500 (196.406)	4.386 (1.128)
Italian Silver (ISI)	105.526 (32.039)	548.743 (254.836)	4.894 (1.543)
Italian Spotted (ISP)	113.946 (16.143)	584.898 (130.293)	5.064 (0.870)
Italian White (ITW)	105.819 (16.797)	427.869 (86.400)	4.001 (0.556)
Rex (RE)	118.389 (25.741)	569.153 (197.209)	4.674 (1.098)
Rhinelander (RH)	133.538 (16.962)	620.754 (166.506)	4.599 (1.005)
Thuringian (TH)	123.667 (44.755)	544.502 (231.073)	4.103 (1.105)

Note: General statistics of ROH detected using the other approaches (Approaches 1, 3 and 4) are reported in Table S4.

[a]The acronym of the breed used in other figures is reported within brackets.

[b]nROH: the average total number of ROH and the standard deviation (SD) calculated for each breed.

[c]L_{ROH}: the average length of ROH (in Mb) considering all length classes and the SD calculated for each breed.

[d]S_{ROH}: the average sum of all ROH segments (in Mb) by animals considering all length classes and the SD calculated for each breed.

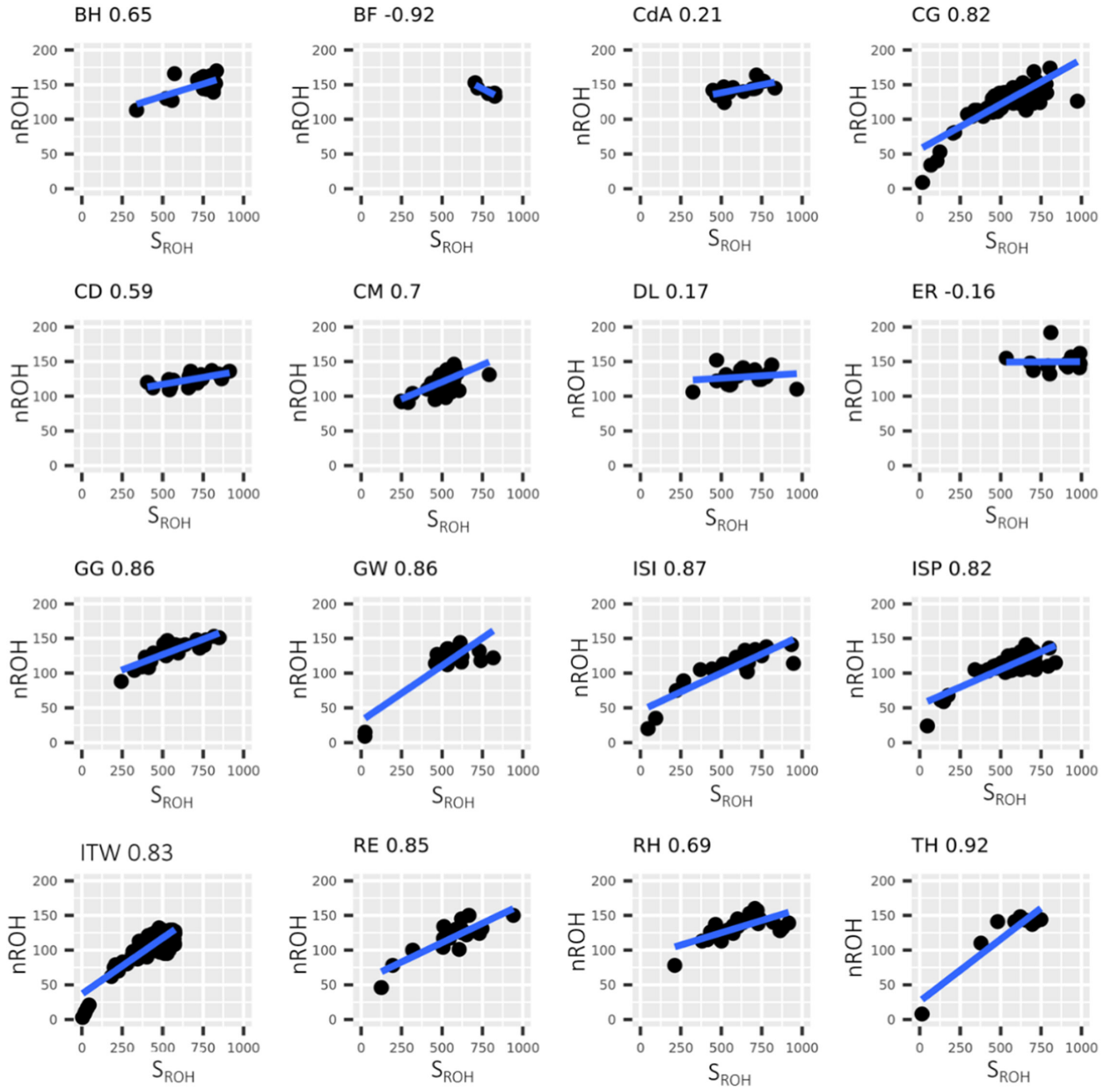

FIGURE 1 Correlation plots between numbers of runs of homozygosity (nROH: *y* axis) and the average sum of all ROH segments (S_{ROH}: *x* axis) for the 16 rabbit breeds including all animals. Acronyms of the breeds and are defined in Table 1. Pearson correlation coefficient between nROH and S_{ROH} is reported beside the acronym of each breed.

of 1226.5 Mb, which indicate that almost half of their genomes were covered by ROH. The same Ermine rabbit had also the largest number of ROH that we identified in this study (n = 192). Table S9 reports the minimum and maximum values observed for nROH, L_{ROH}, and S_{ROH} in all breeds.

Genomic inbreeding parameters

The mean and standard deviation of genomic inbreeding parameters calculated using different classes of ROH length (F_{ROH1} to F_{ROH16}) are reported in Table 2 (for a complete evaluation, Table S10 includes results obtained with all four approaches). With Approach 2, Italian White and Commercial Meat rabbits had the lowest F_{ROH1} values (0.219 and 0.255 respectively). The highest F_{ROH1} values were obtained in Ermine, Burgundy Fawn, and Champagne d'Argent (0.472, 0.400 and 0.385 respectively). Considering the inbreeding parameters based on only medium-long ROH (F_{ROH4}, F_{ROH8}, and F_{ROH16}), the values decreased in all breeds, as expected, but with remarkable differences. For example, in the Commercial Meat rabbits, F_{ROH16} dropped 14 times whereas in Ermine, this parameter dropped only 3.9 times compared to the corresponding F_{ROH1} values. Considering the breeds with at least 20 genotyped animals, the highest F_{ROH16} values are reported in Ermine,

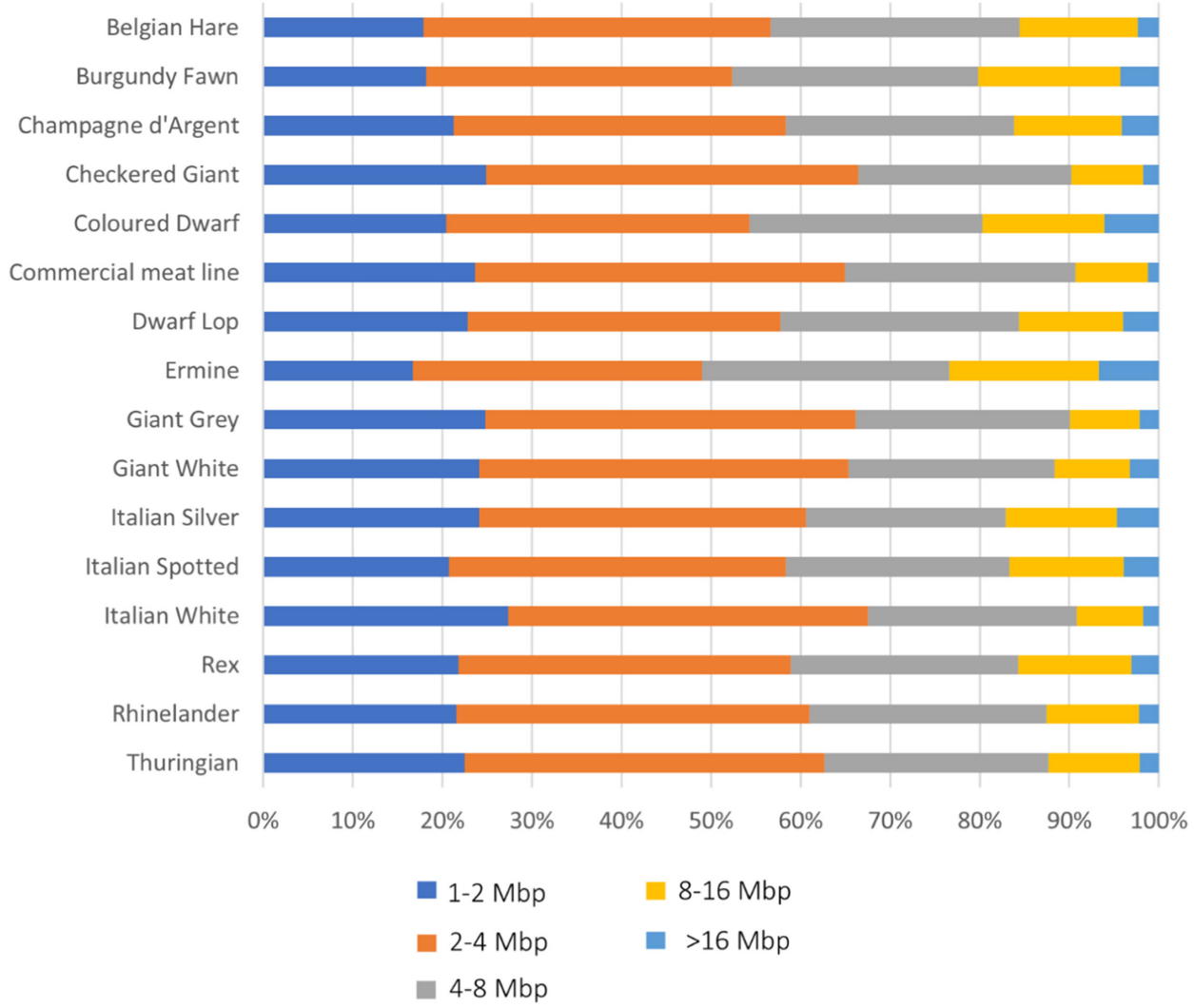

FIGURE 2 Proportion of runs of homozygosity in 16 rabbit breeds of different runs of homozygosity (ROH) categories were defined according to physical size: 1–2, 2–4, 4–8, 8–16, and >16 Mb, identified as ROH1–2, ROH2–4, ROH4–8, ROH8–16, and ROH >16 respectively.

Coloured Dwarf, and Dwarf Lop (0.122, 0.092, and 0.060 respectively). The lowest F_{ROH16} values are reported in Commercial Meat rabbits and Italian White (0.019 and 0.022 respectively). The distribution of the F_{ROH} values of the different length classes obtained with Approach 2 is reported in the boxplots of Figure 3.

Other five genomic inbreeding parameters have been calculated (F_{hat1}, F_{hat2}, F_{hat3}, F_{GRM}, and F_{HOM}). The average values of F_{hat1}, F_{hat2}, F_{hat3}, and F_{HOM} were negative in all breeds except two breeds for F_{hat1} (Coloured Dwarf and Dwarf Lop) or four breeds for F_{hat2}, F_{hat3}, and F_{HOM} (Coloured Dwarf, Dwarf Lop, Ermine, and Rex), even if with large standard deviation (Table S11). These results could be interpreted, to some extent, that in the breeds with positive values, rabbits were on average more related to each other than what happened for the rabbits of the breeds with negative values, even if there was a large within-breed variability for all parameters. Distribution plots of all these other five inbreeding parameters in the 16 rabbits breeds are shown in Figures S3 and S4.

Figure S5 shows the chromosomal F_{ROH} distribution among the analysed rabbit breeds. The highest chromosomal F_{ROH} (F_{CROH}) is reported in the Ermine breed (OCU16, F_{CROH} = 0.603). The commercial rabbit breed had the lowest F_{CROH} (OCU6, F_{CROH} = 0.076).

Table S12 reports the correlation between all considered genomic inbreeding measures calculated within each breed. The correlation between the F_{ROH} parameters based on different ROH length was high in all breeds. Correlation values ranged from 0.997 in Champagne d'Argent (F_{ROH1} vs F_{ROH4}) to 0.582 in Italian White (F_{ROH1} vs F_{ROH16}). Values were higher between close length classes than between distant close classes: that means that correlations in all breeds were higher if, for example, F_{ROH1} and F_{ROH4} were considered in the pairwise analysis than if F_{ROH1} was compared with F_{ROH8} or F_{ROH16}. This was expected considering the distribution of ROH classes (Figure 3) and the progressive reduction of longer ROH in all breeds. However, this drop of correlation was less relevant in Champagne d'Argent, Coloured Dwarf,

TABLE 2 The mean (standard deviation) of genomic inbreeding parameters calculated using different classes of ROH length (F_{ROH1} to F_{ROH16}), identified based on Approach 2

Breed	F_{ROH1}	F_{ROH4}	F_{ROH8}	F_{ROH16}
Belgian Hare	0.377 (0.061)	0.270 (0.065)	0.150 (0.052)	0.042 (0.029)
Burgundy Fawn	0.400 (0.028)	0.306 (0.040)	0.196 (0.055)	0.072 (0.037)
Champagne d'Argent	0.386 (0.117)	0.282 (0.0132)	0.177 (0.129)	0.080 (0.090)
Checkered Giant	0.265 (0.085)	0.164 (0.077)	0.081 (0.061)	0.027 (0.037)
Coloured Dwarf	0.363 (0.102)	0.280 (0.109)	0.189 (0.103)	0.092 (0.074)
Commercial Meat	0.255 (0.047)	0.159 (0.405)	0.044 (0.032)	0.019 (0.025)
Dwarf Lop	0.328 (0.076)	0.239 (0.087)	0.143 (0.087)	0.060 (0.055)
Ermine	0.472 (0.092)	0.381 (0.104)	0.263 (0.113)	0.122 (0.079)
Giant Grey	0.284 (0.078)	0.178 (0.077)	0.089 (0.062)	0.032 (0.034)
Giant White	0.272 (0.101)	0.179 (0.081)	0.104 (0.069)	0.049 (0.47)
Italian Silver	0.281 (0.131)	0.205 (0.120)	0.139 (0.100)	0.064 (0.062)
Italian Spotted	0.300 (0.067)	0.217 (0.064)	0.136 (0.050)	0.054 (0.033)
Italian White	0.219 (0.044)	0.133 (0.037)	0.065 (0.030)	0.022 (0.019)
Rex	0.292 (0.101)	0.206 (0.097)	0.122 (0.075)	0.040 (0.034)
Rhinelander	0.318 (0.085)	0.217 (0.088)	0.116 (0.073)	0.038 (0.048)
Thuringian	0.279 (0.118)	0.182 (0.096)	0.097 (0.061)	0.030 (0.024)

Note: Table S10 includes results obtained with all four approaches.

and Ermine where all correlations were >0.90. The correlation between F_{ROH} parameters and all other genomic inbreeding parameters were high and consistent over all breeds only with the F_{HOM} values (Table 12). For example, correlation between F_{ROH1} and F_{HOM} ranged from 0.974 (Giant Grey) to 0.783 (Dwarf Lop) and correlation between F_{ROH16} and F_{HOM} ranged from 0.939 (Champagne d'Argent) to 0.597 (Belgian Hare). Correlations between the other parameters were not always consistent across breeds (excluding F_{hat1} and F_{GRM}, which are equivalent parameters), with a large range that in some cases spanned extreme values (Tables S12 and S13).

ROH islands

As the interpretation of the results in terms of potential signature of selection is much more reliable when many animals are genotyped (Ceballos et al., 2018), we considered in more details the information derived from the four breeds (Checkered Giant, Commercial Meat, Italian Spotted, and Italian White) for which more than 50 animals were genotyped (summarising information for all 16 breeds are presented in Tables S14 and S15). Manhattan plots reporting ROH islands in these four breeds are shown in Figure 4. In these breeds, ROH islands were identified in a total of 11 autosomes, which covered about 10–20 Mb of the rabbit genome (10.5 Mb in Checkered Giant, 10.5 Mb in Commercial Meat, 17.7 Mb in Italian Spotted, and 19.7 Mb in Italian White). Considering overlapping ROH islands between breeds, a total of 22 independent ROH islands were detected (Table 3). A few ROH islands were identified in all four breeds (on OCU3, position ~140.60–142.31 Mb) or in three breeds (on OCU7 and OCU15) or in two breeds (on OCU7, two in OCU12 and on OCU15). Among the list of the annotated genes included in the ROH islands, some interesting candidate genes, which might provide some potential functional relationships with phenotypic features of the corresponding breeds, could be identified. Several ROH islands contains genes involved in body size, body length, carcass traits, and growth and reproduction traits as previously determined in humans or in other livestock species, including the rabbit (Table 3). Considering that most of the ROH islands were identified in meat breeds, it is interesting to note that several genes included in these regions have been already reported to be associated with production traits mainly relevant in meat species (Table 3). Some of these genes are also associated to relevant human traits. For example, the ROH island on OCU3 identified in all four breeds encompasses eight different genes, including the *tribbles pseudokinase 1* (*TRIB1*) gene. TRIB1 is a signalling regulator protein involved in the activation and suppression of the various interacting signalling pathways. Mutations in the human gene are strongly associated with several fat deposition traits, serum metabolite contents, including triglyceride and cholesterol levels. Another member of this gene family, *tribbles pseudokinase 2* (*TRIB2*) gene, is included in another ROH located on OCU2 and identified in Italian Spotted. The ROH island on OCU17 identified in Checkered Giant contains the *oculocutaneous albinism type 2* (*OCA2*) gene, which is involved in the pigmentation processes (Donnelly et al., 2012).

The genome enrichment analysis for the genes within ROH islands showed over-representation of several GO terms (Table S16), although some of the GO terms could not pass the significance threshold (adjusted *p* value

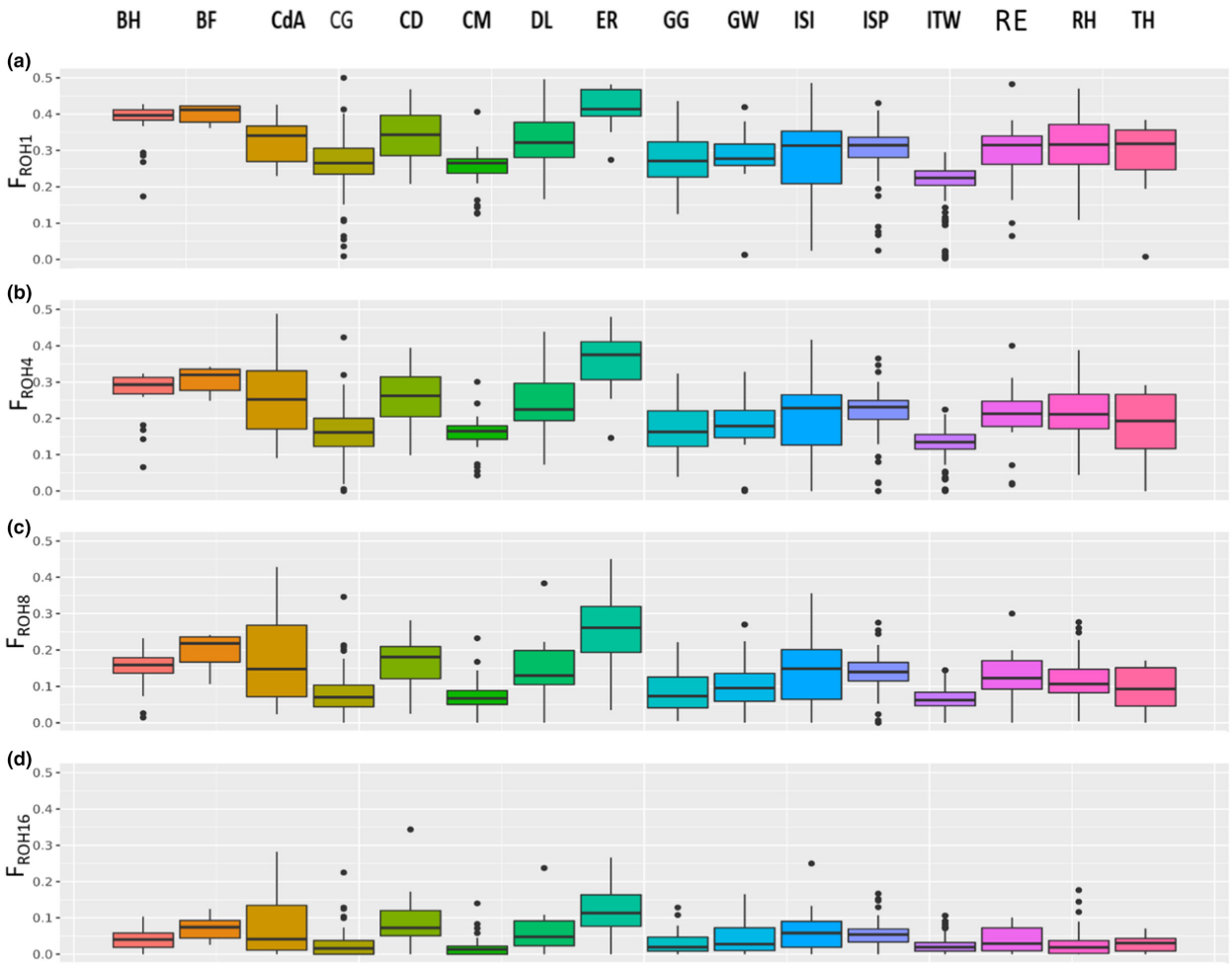

FIGURE 3 Boxplots of the F_{ROH} distribution in the 16 rabbit breeds: (a) F_{ROH1}; (b) F_{ROH4}; (c) F_{ROH8}; (d) F_{ROH16}. Acronyms of the breeds are explained in Table 1.

<0.05). Considering the four breeds mentioned above, Checkered Giant had three enriched terms (atrial fibrillation, basal cell carcinoma, body mass index) and the Commercial Meat line had two enriched terms (breast cancer; systolic blood pressure) whereas no enriched term was identified in Italian White and Italian Spotted. Among the enriched terms identified in the other 12 breeds, it was interesting to note that in Dwarf Lop an enriched term was 'height' (Table S16).

DISCUSSION

Fancy rabbit breeds, with their national breeders' associations and close structures, and meat rabbit lines, with their close nuclei in which a small number of animals are performance tested, are interesting examples of livestock populations where inbreeding should be carefully managed. Empirical experience of practitioners working with rabbits indicates that pedigree recording is usually not very precise (mainly in fancy breeds) which calls for alternative approaches to obtain good estimation of inbreeding. As already demonstrated in many livestock species, genomic analyses could overcome the low accuracy of the pedigree registrations and the biased assumptions that underline the pedigree-based estimations of inbreeding (Howard et al., 2017; Schiavo, Bovo, Bertolini, Dall'Olio, et al., 2020; Schiavo, Bovo, Bertolini, Tinarelli, et al., 2020). In rabbit, however, the routine application of SNP genotyping is still in its infancy, mainly due to the high genotyping cost compared to the value of the animals and to a lower level of organisation of the breeder/breeding industries than what has been already achieved in other livestock species. Applications of genome-wide analyses in domestic rabbit populations have been mainly carried out to understand the effect of selection pressures over generations and to identify genes affecting exterior traits and QTL for economically relevant traits, with limited use of the genomic information for other purposes (Bovo et al., 2021; Carneiro et al., 2021; Laghouaouta et al., 2020; Liu et al., 2021; Sosa-Madrid, Hernández, et al., 2020; Sosa-Madrid, Santacreu, et al., 2020; Sosa-Madrid, Varona, et al., 2020).

In this study we genotyped, with an SNP array, rabbits belonging to 16 breeds and populations and used

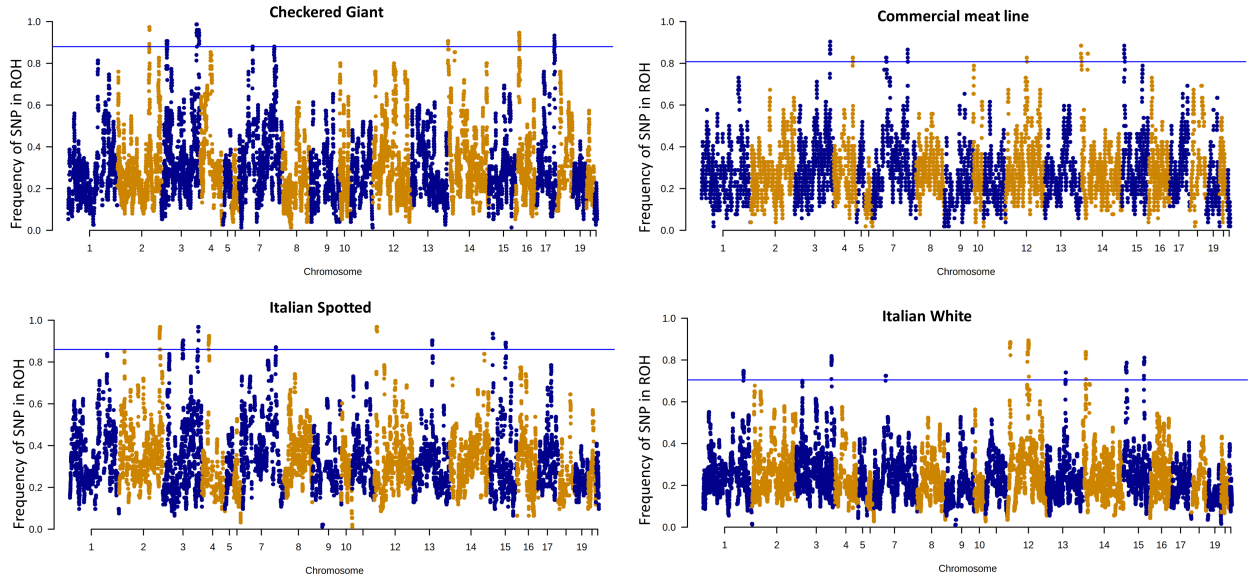

FIGURE 4 Manhattan plots showing runs of homozygosity (ROH) islands in Checkered Giant, Commercial Meat, Italian Spotted, and Italian White breeds. The blue line indicates the frequency corresponding to the top 1% of most frequent SNPs in the population.

this information to estimate genomic inbreeding parameters, including different ROH measures (mainly used for the comparative analyses), and to detect signatures of selection defined by ROH hotspots. As there are no established general rules or guidelines to detect ROH with SNP genotyping data, we tested four approaches, which varied the number of consecutive homozygous SNPs and the maximum number of allowed heterozygous SNPs and reported all obtained results that, however, are highly similar. It is also worth mentioning that some biases in detecting ROH could be potentially derived by the low N50 value of the current available rabbit reference genome and by the genotyping tool that we used, including the density of the filtered SNPs.

The most problematic breed in terms of inbreeding level of the population was Ermine (a rare breed) that had the highest F_{ROH} values and a small *Ne*. Among the other fancy breeds, Checkered Giant had the lowest F_{ROH} values, indicating a low level of inbreeding. This reflects the fact that rabbits with the Checkered Giant breed-specific spotted patterns are actually heterozygous animals at the *English spotting* locus (with genotype *En/en*; Fontanesi et al., 2014). These animals can be obtained by cross-breeding programmes, which might increase the level of heterozygosity at many other loci. All three meat breeds had, in general, lower F_{ROH} values than most of the remaining fancy breeds, suggesting that their nuclei are well managed using pedigree recording information to monitor inbreeding.

Among the other genomic inbreeding parameters that we considered in this study (F_{hat1}, F_{hat2}, F_{hat3}, F_{GRM} and F_{HOM}) only F_{HOM} was consistent across breeds and with high to moderate correlation with F_{ROH} measures. This general picture is consistent to what we already reported in pigs where F_{HOM} produced similar results of F_{ROH} (Schiavo, Bovo, Bertolini, Dall'Olio, et al., 2020; Schiavo, Bovo, Bertolini, Tinarelli, et al., 2020).

Among the inbreeding genomic measures that we calculated, ROH characteristics can be interpreted to infer the genetic structure of the analysed breeds (Ceballos et al., 2018). Starting from the basic ROH parameters, it is possible to read interesting elements. Standard deviation of ROH measures (Table 1) was not very high compared to what we reported in a similar study for different autochthonous and cosmopolitan pig breeds in Europe (Schiavo et al., 2021). This parameter, together with the results of MDS-plot (Figure S1), might suggest that few substructures or subpopulations were sampled in the analysed breeds. Other population genomic statistics should be estimated to better evaluate these aspects. It would be also interesting to genotype a larger number of animals per breed, including rabbits sampled in different countries but of the same breeds to better evaluate these aspects.

The genetic history of the investigated rabbit breeds can be also inferred, to some extent, from other ROH measures (Ceballos et al., 2018). Following the assumption that recent inbreeding usually generates long ROH whereas short ROH have a common ancestral origin (Ceballos et al., 2018; Kirin et al., 2010), it seems that most rabbit breeds included in this study could be mainly ascribed to the second condition. Their genome was covered by many ROH per animal (on average, from about 106 in Italian Silver to about 150 in Belgian Hare) but with quite short L_{ROH} (on average, from ~4 Mb in Italian White to ~6.3 Mb in Ermine). This overview does not change if the approach used to call ROH is changed (we used four approaches; see Table S4). This general picture in these rabbit breeds is, however, the opposite to what we already observed in a study in which we investigated

TABLE 3 Runs of homozygosity islands identified in Checkered Giant, Commercial Meat, Italian Spotted, and Italian White breeds

OCU[a]	Position[b]	Breed	No. of SNPs[c]	No. of genes[d]	Candidate genes (reference)[e]
1	159375514_160629747	Italian White	64	5	*NELL1* (Falker-Gieske et al., 2019)
2	127462270_128281278	Checkered Giant	43	3	*VRK2, FANCL* (Paredes-Sánchez et al., 2020)
2	161490031_163738094	Italian Spotted	54	5	*TRIB2* (Brunes et al., 2021; Fernandes et al., 2021)
3	21908055_22417201	Checkered Giant	23	6	–
3	24713065_25428869	Checkered Giant	45	4	*NR3C1* (Muráni et al., 2010; Reyer et al., 2014)
3	78265347_81591625	Italian Spotted	42	10	*ARMC1* (Zhou et al., 2016)
3	140599855_142305209	Checkered Giant, Commercial meat, Italian Spotted, Italian White	77, 75, 70, 72	8	*TRIB1* (Brunes et al., 2021)
3	149885379_151645140	Checkered Giant	60	4	*ZFAT* (Grilz-Seger et al., 2019)
4	26298302_28844406	Italian Spotted	71	9	–
4	75359078_76496009	Commercial Meat	60	4	*ELK3* (de Lima et al., 2020)
7	47708504_49611011	Italian White	26	10	*IFRD1* (Sorbolini et al., 2017)
7	54842373_57319254	Checkered Giant, Commercial Meat	77, 152	3	–
7	140627212_142319237	Checkered Giant, Commercial Meat, Italian Spotted	19, 55, 32	14	*AOX1* (Casto-Rebollo et al., 2021)
10	5642931_6250075	Commercial	14	3	*ITGB8* (Casto-Rebollo et al., 2021)
12	8595729_11116965	Italian Spotted, Italian White	48, 48	143	*GPX5* (Barranco et al., 2016)
12	78307373_84406018	Italian White, Commercial Meat	60, 15	12	*NDUFAF4* (An et al., 2018)
13	74396836_7641130675693348	Italian Spotted, Italian White	39, 54	18	*PKN2* (Fontanesi et al., 2012)
15	12623264_15388967	Italian White, Commercial Meat, Italian Spotted	59, 56, 52	12	*DCLK2* (Sahana et al., 2013)
15	63477042_65101796	Italian Spotted	58	4	*WDFY3* (Chang et al., 2018)
15	84153011_85988883	Commercial Meat, Italian White	7, 57	3	*TECRL* (Weng et al., 2016)
16	14715459_16455125	Checkered Giant	53	5	–
17	75881549_77977324	Checkered Giant	76	20	*OCA2* (Donnelly et al., 2012)

[a]*Orycolagus cuniculus* chromosome.

[b]Starting and ending nucleotide position of the ROH region. Partially overlapped ROH in different breeds have been combined in a single ROH region.

[c]Number of SNPs in the ROH identified in the corresponding breeds.

[d]Number of genes annotated in the OryCun2.0 genome version included in the ROH region.

[e]Candidate genes, potentially affecting production traits (such as growth, carcass and meat traits, feed efficiency, reproduction traits, body size, behaviour, and related traits) and other exterior traits, as identified from a literature survey in other livestock species (the cited references report the effects of the polymorphisms in the indicated genes). *NELL1*: carcass traits; *VRK2, FANCL*: behaviour; *TRIB2*, feed efficiency, growth rate; *NR3C1*: carcass composition, meat quality traits and *stress response*; *ARMC1*: fat deposition; *TRIB1*: feed efficiency; *ZFAT*: body size; ELK3: feed efficiency; *IFRD1*: growth rate; *AOX1* and *ITGB8*: litter size (in rabbit); *GPX5*: male reproduction trait; *NDUFAF4*: organ size; *PKN2*: fat deposition; *DCLK2*: feed efficiency; *WDFY3*: carcass traits; *TECRL*: fat deposition; *OCA2*: pigmentation.

ROH in European pig breeds, where nROH was much lower on average per breed with a larger average size of L_{ROH}, mainly due to recent inbreeding events and bottlenecks (Schiavo et al., 2021). Therefore, it seems that in most of these rabbit breeds, within breed identical-by-descent chromosome segments might be shared by old ancestors. This interpretation is in agreement with the need, in many fancy breeds, to keep fixed originally defined breed-specific features that have, in several cases, a monogenic or oligogenic determinism and that have been considered the original and basic elements of the standard of the breeds (Fontanesi 2021).

Another study used ROH to estimate genomic inbreeding parameters in a rabbit line established in the 1980s and selected for reproduction and growth traits (Rodríguez-Ramilo et al., 2020). Despite ROH were calculated in different ways, the F_{ROH}, estimated by summing up the values obtained from size ranges, is similar

to what we obtained in the meat breeds. This might suggest again that fancy rabbit breeds should be considered apart from other rabbit lines where inbreeding is usually avoided to maintain high production performances.

Runs of homozygosity were also investigated in rabbits by Casto-Rebollo et al. (2021) for another purpose. These authors used ROH to identify signatures of divergent selection in a rabbit population from which divergent lines for environmental variance of litter size were established. In that study, ROH were more effective than other population genomic parameters to identify signatures of selection. We also analysed ROH to identify hotspots of selection in the 16 investigated breeds. Due to the low number of animals genotyped for a few breeds, we mainly focused our attention on the breeds for which more than 50 animals were investigated. Some of the ROH islands detected in Checkered Giant, Commercial Meat, Italian Spotted, and Italian White breeds contained genes involved in growth, body size and several other important traits in meat species, including carcass, meat and fat deposition traits, growth rate, feed efficiency, stress sensitivity, and reproduction traits. It was interesting to mention the ROH island encompassing the *OCA2* genome region that was identified in Checkered Giant (also known as Papillion). This gene encodes for the homologue of the mouse p (pink-eyed dilution) that is believed to be a melanocyte-specific transporter with an essential role in normal pigmentation. The role of this gene is, however, not completely well defined yet. In another study that involved Checkered Giant rabbits (Ballan et al., 2022), using F_{ST} parameters we confirmed the signature of selection in this gene region. Therefore, it is quite remarkable that two independent types of analyses (i.e. ROH islands and F_{ST}) pointed out this region in this breed that is mainly defined by a peculiar spotted pattern derived by an heterozygous state at the *English spotting* locus, which is associated with alleles at the *KIT proto-oncogene, receptor tyrosine kinase* (*KIT*) gene (Fontanesi et al., 2014). Potential interactions between the *OCA2* and *KIT* gene products, not yet well established, could be needed to determine the Checkered Giant spotted design. It will be also interesting to compare the results of the two methods that we have applied thus far to identify signatures of selection (i.e. the ROH island method described in this study and the F_{ST} method previously reported; Ballan et al., 2022) with other methods that can be implemented using the same SNP datasets.

Genomic analyses in the rabbit can be important for several reasons that span from practical aspects, including the management of small populations with genomic inbreeding parameters, to the exploration of the large genetic diversity that is present across breeds and within breeds and that can be used to identify the genetic architecture underlining phenotype diversity. Runs of homozygosity can complement other methods that are used to interpret the genetic history of livestock populations and to detect signatures of selection in rabbit breeds, exploiting unique genetic resources available from fancy breeders and breeding industries.

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CONFLICT OF INTEREST

The authors declare they do not have any competing interests.

DATA AVAILABILITY STATEMENT

Genotyping data can be shared after the signature of an agreement on their use with the University of Bologna and ANCI.

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REFERENCES

An, B., Xia, J., Chang, T., Wang, X., Miao, J., Xu, L. et al. (2018) Genome-wide association study identifies loci and candidate genes for internal organ weights in Simmental beef cattle. *Physiological Genomics*, 50, 523–531.

Ballan, M., Bovo, S., Schiavo, G., Schiavitto, M., Negrini, R. & Fontanesi, L. (2022) Genomic diversity and signatures of selection in meat and fancy rabbit breeds assessed using high density SNP information. *Genetics Selection Evolution*, 54, 3.

Barbato, M., Orozco-terWengel, P., Tapio, M. & Bruford, M.W. (2015) SNeP: a tool to estimate trends in recent effective population size trajectories using genome-wide SNP data. *Frontiers in Genetics*, 6, 109.

Barranco, I., Tvarijonaviciute, A., Perez-Patino, C., Vicente-Carrillo, A., Parrilla, I., Ceron, J.J. et al. (2016) Glutathione peroxidase 5 is expressed by the entire pig male genital tract and once in the seminal plasma contributes to sperm survival and in vivo fertility. *PLoS One*, 11, e0162958.

Bertolini, F., Cardoso, T.F., Marras, G., Nicolazzi, E.L., Rothschild, M.F. & Amills, M. (2018) Genome-wide patterns of homozygosity provide clues about the population history and adaptation of goats. *Genetics Selection Evolution*, 50, 59.

Boucher, S., Garreau, H., Boettcher, P. & Bolet, G. (2021) Rabbit breeds and lines and genetic resources. In: *The genetics and genomics of the rabbit*. Wallingford: CAB International, pp. 23–37.

Bovo, S., Schiavo, G., Utzeri, V.J., Ribani, A., Schiavitto, M., Buttazzoni, L. et al. (2021) A genome-wide association study for the number of teats in European rabbits (*Oryctolagus cuniculus*) identifies several candidate genes affecting this trait. *Animal Genetics*, 52, 237–243.

Brunes, L.C., Baldi, F., Lopes, F.B., Lôbo, R.B., Espigolan, R., Costa, M.F. et al. (2021) Weighted single-step genome-wide

association study and pathway analyses for feed efficiency traits in Nellore cattle. *Journal of Animal Breeding and Genetics*, 138, 23–44.

Callou, C. (2002) *De la garenne au clapier. Étude archéozoologique du Lapin en Europe occidentale*. Paris, France: Mémoires do Muséum National d'Histoire Naturelle, p. 189.

Carneiro, M., Rubin, C.J., Di Palma, F., Albert, F.W., Alföldi, J., Martinez, B.A. et al. (2014) Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. *Science*, 345, 1074–1079.

Carneiro, M., Vieillard, J., Andrade, P., Boucher, S., Afonso, S., Blanco-Aguiar, J.A. et al. (2021) A loss-of-function mutation in RORB disrupts saltatorial locomotion in rabbits. *PLoS Genetics*, 17, e1009429.

Casto-Rebollo, C., Argente, M.J., García, M.L., Blasco, A. & Ibáñez-Escriche, N. (2021) Selection for environmental variance of litter size in rabbits involves genes in pathways controlling animal resilience. *Genetics Selection Evolution*, 53, 59.

Ceballos, F.C., Joshi, P.K., Clark, D.W., Ramsay, M. & Wilson, J.F. (2018) Runs of homozygosity: windows into population history and trait architecture. *Nature Review Genetics*, 19, 220–234.

Chang, C.C., Chow, C.C., Tellier, L.C.A.M., Vattikuti, S., Purcell, S.M. & Lee, J.J. (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*, 4, 7.

Chang, T., Xia, J., Xu, L., Wang, X., Zhu, B., Zhang, L. et al. (2018) A genome-wide association study suggests several novel candidate genes for carcass traits in Chinese Simmental beef cattle. *Animal Genetics*, 49, 312–316.

Chen, E.Y., Tan, C.M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G.V. et al. (2013) Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, 14, 128.

de Lima, A.O., Koltes, J.E., Diniz, W.J., de Oliveira, P.S., Cesar, A.S., Tizioto, P.C. et al. (2020) Potential biomarkers for feed efficiency-related traits in nelore cattle identified by co-expression network and integrative genomics analyses. *Frontiers in Genetics*, 11, 189.

Donnelly, M.P., Paschou, P., Grigorenko, E., Gurwitz, D., Barta, C., Lu, R.B. et al. (2012) A global view of the OCA2-HERC2 region and pigmentation. *Human Genetics*, 131, 683–696.

Falker-Gieske, C., Blaj, I., Preuß, S., Bennewitz, J., Thaller, G. & Tetens, J. (2019) GWAS for meat and carcass traits using imputed sequence level genotypes in pooled F2-designs in pigs. *G3: Genes, Genomes, Genetics*, 9, 2823–2834.

Ferenčaković, M., Hamzić, E., Gredler, B., Solberg, T.R., Klemetsdal, G., Curik, I. et al. (2013) Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. *Journal of Animal Breeding and Genetics*, 130(4), 286–293.

Ferenčaković, M., Sölkner, J. & Curik, I. (2013) Estimating autozygosity from high-throughput information: effects of SNP density and genotyping errors. *Genetics Selection Evolution*, 45, 42.

Fernandes A.C., da Silva V.H., Goes C.P., Moreira G.C.M., Godoy T.F., Ibelli A.M.G., Peixoto, J. de O., Cantão M.E., Ledur M.C., de Rezende F.M. & Coutinho L.L. (2021) Genome-wide detection of CNVs and their association with performance traits in broilers. *BMC Genomics* 22, 354.

Fontanesi, L. (2021a) Rabbit genetic resources can provide several animal models to explain at the genetic level the diversity of morphological and physiological relevant traits. *Applied Sciences*, 11, 373.

Fontanesi, L. (2021b, 2021) Genetics and molecular genetics of coat colour in the European rabbit. In: Fontanesi, L. (Ed.) *The genetics and genomics of the rabbit*. Wallingford: CAB International, pp. 84–103.

Fontanesi, L., Bovo, S. & Schiavo, G. (2021, 2021) The genome of the European rabbit and genomic tools. In: Fontanesi, L. (Ed.) *The genetics and genomics of the rabbit*. Wallingford: CAB International, pp. 38–42.

Fontanesi, L., Schiavo, G., Galimberti, G., Calò, D.G., Scotti, E., Martelli, P.L. et al. (2012) A genome wide association study for backfat thickness in Italian Large White pigs highlights new regions affecting fat deposition including neuronal genes. *BMC Genomics*, 13, 583.

Fontanesi, L., Utzeri, V.J. & Ribani, A. (2021) The evolution, domestication and world distribution of the European rabbit (*Oryctolagus cuniculus*). In: Fontanesi, L. (Ed.) *The genetics and genomics of the rabbit*. Wallingford: CAB International, pp. 1–22.

Fontanesi, L., Vargiolu, M., Scotti, E., Latorre, R., Faussone Pellegrini, M.S., Mazzoni, M. et al. (2014) The *KIT* gene is associated with the *English spotting* coat color locus and congenital megacolon in Checkered Giant rabbits (*Oryctolagus cuniculus*). *PLoS One*, 9, e93750.

Gibson, J., Morton, N.E. & Collins, A. (2006) Extended tracts of homozygosity in outbred human populations. *Human Molecular Genetics*, 15, 789–795.

Gorssen, W., Meyermans, R., Buys, N. & Janssens, S. (2020) SNP genotypes reveal breed substructure, selection signatures and highly inbred regions in Piétrain pigs. *Animal Genetics*, 51, 32–42.

Grilz-Seger, G., Druml, T., Neuditschko, M., Mesarič, M., Cotman, M. & Brem, G. (2019) Analysis of ROH patterns in the Noriker horse breed reveals signatures of selection for coat color and body size. *Animal Genetics*, 50, 334–346.

Howard, J.T., Pryce, J.E., Baes, C. & Maltecca, C. (2017) Invited review: inbreeding in the genomics era: inbreeding, inbreeding depression, and management of genomic variability. *Journal of Dairy Science*, 100, 6009–6024.

Kardos, M., Luikart, G. & Allendorf, F.W. (2015) Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. *Heredity*, 115, 63–72.

Kirin, M., McQuillan, R., Franklin, C.S., Campbell, H., McKeigue, P.M. & Wilson, J.F. (2010) Genomic runs of homozygosity record population history and consanguinity. *PloS one*, 11, e13996.

Knief, U., Kempenaers, B. & Forstmeier, W. (2017) Meiotic recombination shapes precision of pedigree- and marker-based estimates of inbreeding. *Heredity*, 118, 239–248.

Kristensen, T.N., Pedersen, K.S., Vermeulen, C.J. & Loeschcke, V. (2010) Research on inbreeding in the "omic" era. *Trends in Ecology and Evolution*, 25, 44–52.

Laghouaouta, H., Sosa-Madrid, B.S., Zubiri-Gaitán, A., Hernández, P. & Blasco, A. (2020) Novel genomic regions associated with intramuscular fatty acid composition in rabbits. *Animals*, 10, 2090.

Leutenegger, A.L., Prum, B., Génin, E., Verny, C., Lemainque, A., Clerget-Darpoux, F. et al. (2003) Estimation of the inbreeding coefficient through use of genomic data. *American Journal of Human Genetics*, 73, 516–523.

Liu, C., Wang, S., Dong, X., Zhao, J., Ye, X., Gong, R. et al. (2021) Exploring the genomic resources and analysing the genetic diversity and population structure of Chinese indigenous rabbit breeds by RAD-seq. *BMC Genomics*, 22, 573.

Marras, G., Gaspa, G., Sorbolini, S., Dimauro, C., Ajmone-Marsan, P., Valentini, A. et al. (2015) Analysis of runs of homozygosity and their relationship with inbreeding in five cattle breeds farmed in Italy. *Animal Genetics*, 46, 110–121.

Mastrangelo, S., Sardina, M.T., Tolone, M., Di Gerlando, R., Sutera, A.M., Fontanesi, L. et al. (2018) Genome-wide identification of runs of homozygosity islands and associated genes in local dairy cattle breeds. *Animal*, 12, 2480–2488.

Meyermans, R., Gorssen, W., Buys, N. & Janssens, S. (2020) How to study runs of homozygosity using plink? A guide for analyzing medium density SNP data in livestock and pet species. *BMC Genomics*, 21, 94.

Muráni, E., Ponsuksili, S., d'Eath, R.B., Turner, S.P., Kurt, E., Evans, G. et al. (2010) Association of HPA axis-related genetic variation with stress reactivity and aggressive behaviour in pigs. *BMC Genetics*, 11, 74.

Paredes-Sánchez, F.A., Sifuentes-Rincón, A.M., Casas, E., Arellano-Vera, W., Parra-Bracamonte, G.M., Riley, D.G. et al. (2020) Novel genes involved in the genetic architecture of temperament in Brahman cattle. *PLoS One*, 15, e0237825.

Peripolli, E., Munari, D.P., Silva, M.V.G.B., Lima, A.L.F., Irgang, R. & Baldi, F. (2017) Runs of homozygosity: current knowledge and applications in livestock. *Animal Genetics*, 48, 255–271.

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

Reyer, H., Ponsuksili, S., Wimmers, K. & Murani, E. (2014) Association of N-terminal domain polymorphisms of the porcine glucocorticoid receptor with carcass composition and meat quality traits. *Animal Genetics*, 45, 125–129.

Rodríguez-Ramilo, S.T., Reverter, A., Sánchez, J.P., Fernández, J., Velasco-Galilea, M., González, O. et al. (2020) Networks of inbreeding coefficients in a selected population of rabbits. *Journal of Animal Breeding and Genetics*, 137, 599–608.

Sahana, G., Kadlecová, V., Hornshøj, H., Nielsen, B. & Christensen, O.F. (2013) A genome-wide association scan in pig identifies novel regions associated with feed efficiency trait. *Journal of Animal Science*, 91, 1041–1050.

Schiavo, G., Bovo, S., Bertolini, F., Dall'Olio, S., Nanni Costa, L., Tinarelli, S. et al. (2020) Runs of homozygosity islands in Italian cosmopolitan and autochthonous pig breeds identify selection signatures in the porcine genome. *Livestock Science*, 240, 104219.

Schiavo, G., Bovo, S., Bertolini, F., Tinarelli, S., Dall'Olio, S., Nanni Costa, L. et al. (2020) Comparative evaluation of genomic inbreeding parameters in seven commercial and autochthonous pig breeds. *Animal*, 14, 915–920.

Schiavo, G., Bovo, S., Muñoz, M., Ribani, A., Alves, E., Araújo, J.P. et al. (2021) Runs of homozygosity provide a genome landscape picture of inbreeding and genetic history of European autochthonous and commercial pig breeds. *Animal Genetics*, 52, 155–170.

Sorbolini, S., Bongiorni, S., Cellesi, M., Gaspa, G., Dimauro, C., Valentini, A. et al. (2017) Genome wide association study on beef production traits in Marchigiana cattle breed. *Journal of Animal Breeding and Genetics*, 134, 43–48.

Sosa-Madrid, B.S., Hernández, P., Blasco, A., Haley, C.S., Fontanesi, L., Santacreu, M.A. et al. (2020) Genomic regions influencing intramuscular fat in divergently selected rabbit lines. *Animal Genetics*, 51, 58–69.

Sosa-Madrid, B.S., Santacreu, M.A., Blasco, A., Fontanesi, L., Pena, R.N. & Ibáñez-Escriche, N. (2020) A genomewide association study in divergently selected lines in rabbits reveals novel genomic regions associated with litter size traits. *Journal of Animal Breeding and Genetics*, 137, 123–138.

Sosa-Madrid, B.S., Varona, L., Blasco, A., Hernández, P., Casto-Rebollo, C. & Ibáñez-Escriche, N. (2020) The effect of divergent selection for intramuscular fat on the domestic rabbit genome. *Animal*, 14, 2225–2235.

VanRaden, P.M. (2008) Efficient methods to compute genomic predictions. *Journal of Dairy Science*, 91, 4414–4423.

VanRaden, P.M., Olson, K.M., Null, D.J. & Hutchison, J.L. (2011) Harmful recessive effects on fertility detected by absence of homozygous haplotypes. *Journal of Dairy Science*, 94, 6153–6161.

Wang, J. (2016) Pedigrees or markers: which are better in estimating relatedness and inbreeding coefficient? *Theoretical Population Biology*, 107, 4–13.

Weng, Z., Su, H., Saatchi, M., Lee, J., Thomas, M.G., Dunkelberger, J.R. et al. (2016) Genome-wide association study of growth and body composition traits in Brangus beef cattle. *Livestock Science*, 183, 4–11.

Whitman, B.D. (2004) *Domestic rabbits & their histories: breeds of the world*. Overland Parks, KS: Leathers Publishing.

Wright, S. (1922) Coefficients of inbreeding and Relationship. *American Naturalist*, 56, 330–338.

Yang, J., Lee, S.H., Goddard, M.E. & Visscher, P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. *American Journal of Human Genetics*, 88, 76–82.

Zeder, M.A. (2012) The domestication of animals. *Journal of Anthropological Research*, 68, 161–190.

Zeuner, F.E. (1963) *A history of domesticated animals*. Evanston: Harper & Row Publishers.

Zhou, L., Ji, J., Peng, S., Zhang, Z., Fang, S., Li, L. et al. (2016) A GWA study reveals genetic loci for body conformation traits in Chinese Laiwu pigs and its implications for human BMI. *Mammalian Genome*, 27, 610–621.

SUPPORTING INFORMATION

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

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