Received: 18 March 2023 | Revised: 25 June 2023 | Accepted: 2 July 2023

DOI: 10.1111/jbg.12818

Journal of Animal Breeding and Genetics WILEY

ORIGINAL ARTICLE

Population genomic structures and signatures of selection define the genetic uniqueness of several fancy and meat rabbit breeds

Mohamad Ballan[1] | **Samuele Bovo**[1] | **Francesca Bertolini**[1] | **Giuseppina Schiavo**[1] | **Michele Schiavitto**[2] | **Riccardo Negrini**[3] | **Luca Fontanesi**[1]

[1]Division of Animal Sciences, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

[2]Associazione Nazionale Coniglicoltori Italiani (ANCI), Volturara Appula, Italy

[3]Associazione Italiana Allevatori, Rome, Italy

Correspondence
Luca Fontanesi, Division of Animal Sciences, Department of Agricultural and Food Sciences, University of Bologna, Viale Giuseppe Fanin 46, 40127 Bologna, Italy.
Email: luca.fontanesi@unibo.it

Funding information
Cun-Fu and Cun Fu 2 projects; European Agricultural Fund for Rural Development of the European Union and by the Italian Ministry of Agriculture; Food and Forestry - MASAF; PSRN (Programma di Sviluppo Rurale Nazionale); University of Bologna, Grant/Award Number: RFO 2019-2022

Abstract

Following the recent domestication process of the European rabbit (*Oryctolagus cuniculus*), many different breeds and lines, distinguished primarily by exterior traits such as coat colour, fur structure and body size and shape, have been constituted. In this study, we genotyped, with a high-density single-nucleotide polymorphism panel, a total of 645 rabbits from 10 fancy breeds (Belgian Hare, Champagne d'Argent, Checkered Giant, Coloured Dwarf, Dwarf Lop, Ermine, Giant Grey, Giant White, Rex and Rhinelander) and three meat breeds (Italian White, Italian Spotted and Italian Silver). ADMIXTURE analysis indicated that breeds with similar phenotypic traits (e.g. coat colour and body size) shared common ancestries. Signatures of selection using two haplotype-based approaches (iHS and XP-EHH), combined with the results obtained with other methods previously reported that we applied to the same breeds, we identified a total of 5079 independent genomic regions with some signatures of selection, covering about 1777 Mb of the rabbit genome. These regions consistently encompassed many genes involved in pigmentation processes (*ASIP*, *EDNRA*, *EDNRB*, *KIT*, *KITLG*, *MITF*, *OCA2*, *TYR* and *TYRP1*), coat structure (*LIPH*) and body size, including two major genes (*LCORL* and *HMGA2*) among many others. This study revealed novel genomic regions under signatures of selection and further demonstrated that population structures and signatures of selection, left into the genome of these rabbit breeds, may contribute to understanding the genetic events that led to their constitution and the complex genetic mechanisms determining the broad phenotypic variability present in these untapped rabbit genetic resources.

KEYWORDS

admixture, animal genetic resources, domestication, *Oryctolagus cuniculus*, selection sweep, SNP

1 | INTRODUCTION

The domestication of the European rabbit (*Oryctolagus cuniculus*), known simply as rabbit, has been a recent process, compared to the domestication of other livestock species. It began just over the High Middle Ages in French monasteries and castles using wild populations of the *O. c. cuniculus* subspecies spread in this region after its post-glacial expansion from the Iberian Peninsula (Callou, 2003; Fontanesi et al., 2021; Zeder, 2012; Zeuner, 1963). Then, the domestication process continued in the XV–XVII centuries, with the subsequent dispersion and transport of semi-domesticated/domesticated rabbit populations in the North of Europe and, subsequently, in other European regions (Fontanesi et al., 2021). A genetic bottleneck occurred during this phase due to the use of wild subpopulations and to the limited recurred introgression from the wild forms into the domesticated lineage (Alves et al., 2015; Carneiro et al., 2014). The first waves of this process produced a soft allele frequency modification at many loci distinguishing wild and domestic rabbit populations. This allelic shift mainly occurred in regulatory regions of genes that contributed to the behavioural and reproductive adaptation of the domesticated rabbit stocks to the human environment and needs (Carneiro et al., 2014). Then, the human-driven selection contributed to the domestication process with the constitution of the first rabbit breeds (Boucher et al., 2021; Fontanesi, 2021a). The subsequent result was that a variety of peculiar phenotypic differences were fixed in many breeds which were valued by fancy breeders who continued the selection activities and then created additional breeds, lines and strains by introgressing specific genetic factors to obtain novel genetic combinations (Boucher et al., 2021; Fontanesi, 2021a). Most of the fancy breeds were named according to their characterizing exterior traits (mainly derived by their coat colours and patterns, body size, ear length and pelage features), which became their specific breed standards defined by breeders' associations or societies, constituted in several countries (Boucher et al., 2021; Whitman, 2004). Nowadays, some of these fancy breeds are recognized by all or almost all national breeders' organizations, and a few other breeds, mainly defined by coat colour variants, are recognized only in one or few countries. Specialized meat rabbit lines were more recently developed through the selection activities of breeding companies that mainly valued carcass, growth and reproduction traits.

The selection histories and the genetic events that led to the constitution of many different fancy breeds and meat lines have defined population genetic structures (Alves et al., 2015) and left signatures of selection in their genomes that made possible to identify the genetic factors affecting some of the most relevant phenotypes that characterize several rabbit genetic resources (Fontanesi, 2021a). Using a combination of the candidate gene approach and linkage analysis, we and others identified the causative mutations or the associated markers that explain the allele series at several coat colour loci, including the *Agouti, Albino*, *Brown, Dilute*, *English Spotting* and *Extension* loci (Aigner et al., 2000; Demars et al., 2018; Fontanesi et al., 2006; Fontanesi, Forestier, et al., 2010; Fontanesi, Scotti, et al., 2010, 2014; Fontanesi, Vargiolu, et al., 2014; Lehner et al., 2013; Letko et al., 2020; Utzeri et al., 2014, 2021), and at other loci affecting, for example, the rex hair coat phenotype (Diribarne et al., 2011), a type of dwarfism (Carneiro et al., 2017) and a saltatorial behaviour (Carneiro et al., 2021). In our previous studies, single-nucleotide polymorphisms (SNPs) covering the whole-rabbit genome have been used to identify several other genomic regions under selection in fancy and meat rabbit lines using some classical approaches based on F_{ST}, PCA-based (*PCAdapt*) and runs of homozygosity (ROH) methods that are mainly based on allele frequencies (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022).

The investigation of signature of selection includes other methods and approaches that have been developed for high-density SNP panels and that utilized haplotypes instead of allele frequencies. Many applications in human and animal populations have already demonstrated their complementarities and usefulness to further confirm some previously identified signatures of selection (as proof of concepts) and in extracting additional signatures that could also characterize the populations under investigation (e.g.: González-Rodríguez et al., 2016; Qanbari & Simianer, 2014; Sabeti et al., 2006; Saravanan et al., 2020; Utsunomiya et al., 2013). For example, two frequently applied haplotype-based tests, considered to be complementary to capture signatures of selection (Gautier & Naves, 2011), are the integrated haplotype score (iHS; Voight et al., 2006), which derives from the extended haplotype homozygosity (EHH), and the cross-population extended haplotype homozygosity (XP-EHH; Sabeti et al., 2007), which is based on both EHH and iHS principles and that further extends the possibility to detect haplotype derived information at the genome level. These approaches have been successfully applied but so far they have not been explored in rabbit experiments.

This is a follow-up study of our two previous works based on ROH, F_{ST} and PCA performed with high-density SNP datasets (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022). Here we applied two haplotype-based approaches (iHS and XP-EHH) to identify signatures of selection in the genome of 13 rabbit breeds (10 fancy breeds and 3 meat breeds or lines) with a combination of within breed and across breeds analyses. The results were compared and combined with the signatures

of selection that were previously obtained with the other three methods (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022). We then compiled a more comprehensive pattern of selection sweeps that distinguishes rabbit breeds and that might be useful to reconstruct the genetic history of these animal genetic resources and identify the genetic mechanisms that can explain their broad phenotypic diversity.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Animal samples used in this study were collected following the recommendation of directive 2010/632.1.

2.2 | Animals

Hair roots or buccal swabs were sampled from 645 rabbits of 10 fancy and three meat rabbit breeds. All fancy breeds (Belgian Hare, n. 24; 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; and Rhinelander, n. 28) and meat breeds (Italian White, n. 256; Italian Spotted, n. 93; Italian Silver, n. 20) were from the national Herd Book maintained by the Italian Rabbit Breeders Association (ANCI). All rabbits included in this study were selected to avoid highly related animals (no full- or half-sibs) and had their respective standard breed characteristics. The description of the breeds is reported in Ballan, Bovo, et al. (2022) and summarized in Table S1.

To capture the signature of selection common to more than one breed with similar characteristics, breeds were also grouped according to a few common features (Tables S1 and S2) based on coat colour (completely white *Albino* breeds: Giant White and Italian White; silver breeds: Champagne d'Argent and Italian Silver; spotted breeds: Checkered Giant and Rhinelander), body size (giant breeds: Checkered Giant, Giant Grey and Giant White; dwarf/small breeds: Coloured Dwarf, Dwarf Lop and Ermine) and the use of the breed (meat breeds: Italian White, Italian Spotted and Italian Silver; fancy breeds: all other breeds).

2.3 | Genotyping, data filtering and phasing

DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation). Animals were then genotyped using the Affymetrix Axiom OrcunSNP Array (Affymetrix Inc.) which can analyse 199,692 DNA markers. The genotyping data quality check was made with the Axiom Analysis Suite (Affymetrix Inc.) and PLINK v.1.9 software (Chang et al., 2015). SNPs with a call rate <0.90, unmapped or on sex chromosomes were removed and after filtering, a total number of 139,922 SNPs from 645 rabbits were retained for further analyses. Then, the SNP dataset of all investigated rabbits was phased using SHAPEIT2, with the default parameters (Delaneau et al., 2012).

2.4 | Population structure analyses

To investigate the relationships between the analysed breeds, multidimensional scaling (MDS) analysis was performed after obtaining the matrix of genome-wide identity by state (IBS) pairwise distances using PLINK 1.9 (Chang et al., 2015). ADMIXTURE 3.1 software (Alexander et al., 2009) evaluated population stratification. For this analysis, following Bertolini et al. (2022) and the recommendation of Alexander et al. (2009), the pruning of SNPs in high linkage disequilibrium (LD) was carried out using PLINK 1.9 (Chang et al., 2015) considering –indep-pairwise command with the default parameters (windows size of 1000 kb and r^2 threshold of 0.2). A total of 5858 SNPs were retained (an average of 163 SNPs for each chromosome). Analysis was performed on the pruned dataset considering the number of subpopulations (K) that ranged from 1 to 17 and calculating the cross-validation error (CV) for each K. Pruning is a useful procedure for mitigating the biases that might derive from differences in LD between populations in ancestry estimation methods (Alexander et al., 2009).

2.5 | Haplotype-based methods to detect signatures of selection

The rehh R package V 2.04 (Gautier et al., 2017) was used on the 139,922 phased SNPs to obtain the integrated haplotype scores (iHS; Voight et al., 2006) for each breed and across-population and the extended haplotype homozygosity (XP-EHH; Sabeti et al., 2007) values for breed comparisons and groups of breed comparisons. iHS values were calculated with the unpolarized option, available for this method when it is impossible to define the ancestral status of the alleles at a polymorphic site. Here, absolute values |iHS| were chosen and considered independent from the ancestral allele (Bertolini et al., 2020; Kemper et al., 2014). Signatures of selection were identified based on these absolute values for SNPs

that passed the threshold of the top 98.0th percentile of the empirical distribution with at least three consecutive SNPs within a 350 kb window.

The XP-EHH analysis is based on a single-breed approach, considered for each breed N-1 pairwise XP-EHH comparisons (where *N* is the number of breeds included in the study; $N = 13$). The final XP-EHH score for each SNP was then calculated for each breed, considered the reference population, by computing the mean of the XP-EHH values obtained from these multiple comparisons. XP-EHH analyses for groups of breeds were carried out considering as one reference population all rabbits belonging to the grouped breeds against another population, including all rabbits of the other breeds (Table S2). Therefore, only negative XP-EHH mean values were considered (Maiorano et al., 2018). To detect a signature of selection, all SNPs within the top 95.0th percentile of the distribution of their log *p*-value were retained. Then, regions with at least three consecutive SNPs within a 350 kb window with negative XP-EHH values were considered to identify signatures of selection with this approach.

2.6 | Compilation of signatures of selection in the rabbit genome

Signatures of selection identified with the iHS and XP-EHH analyses derived from this study were combined with signatures of selection obtained in previous studies for the same rabbit breeds genotyped with the same SNP array. These additional signatures of selection were obtained using other approaches: window-based F_{ST}, a principal component analysis-based method obtained with *PCAdapt* (Luu et al., 2017), and ROH islands (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022). Genome coordinates of all these regions were obtained using OryCun2.0 chromosomes. Annotated protein-coding genes within the reported genome windows or that were ± 500 kb apart from them were retrieved with the bedtools v.2.17 (https://github.com/arq5x/bedtools2/) from the OryCun2.0 NCBI's GFF file (NCBI *Oryctolagus cuniculus* Annotation Release 102). Gene enrichment analyses were performed with Enrichr (Chen et al., 2013), using the following databases: GWAS catalog (https://www.ebi.ac.uk/gwas/), KEGG Human database (KEGG, http://www.kegg.jp/), MGI mammalian phenotype level (https://www.informatics.jax.org/vocab/mp_ontology) and the biological process branch of gene ontology (GO:BP; http://geneontology.org/). As input, Enrichr took the whole set of genes ($n = 1493$) mapped within the genome regions identified by more than one method. We considered statistically enriched terms presenting: (i) at least two genes of the input set related to (at least) two different genome regions and (ii) an adjusted *p*-value of <0.05.

3 | RESULTS

3.1 | Population structure of the investigated breeds

The MDS plots, obtained with the genotyped SNPs, including the rabbits of the 13 breeds, are reported in Figure 1. Rabbits belonging to the same breeds were usually clustered, with few exceptions when breeds were not separated. The Italian White breed was clustered apart from all other breeds. Breeds grouped according to the same type of body size, that is, the three giant breeds (Checkered Giant, Giant Grey and Giant White) and the three dwarf/small breeds (Coloured Dwarf, Dwarf Lop and Ermine), and the two breeds with the silver coat colour (Champagne d'Argent, and Italian Silver), were clustered closely together.

Figure 2 shows the results of the ADMIXTURE analysis. Within the 13 subpopulations, the lowest value was obtained at *K* 17, while the CV error reached a first plateau at *K* 11. At *K* 11, the three meat breeds (Italian White, Italian Spotted and Italian Silver) shared some ancestries which were maintained, only in part, at *K* 13 (mainly between Italian Spotted and Italian Silver) and *K* 17 (mainly between Italian White and Italian Spotted). The two silver breeds (Italian Silver and Champagne d'Argent) shared large common ancestries at *K* 11 and *K* 13 and a lower level at *K* 17. An admixed genetic background was observed between Giant White and Giant Grey at all three mentioned Ks, with a much lower level of admixture in Checkered Giant. The three small/dwarf breeds (Coloured Dwarf, Dwarf Lop and Ermine) shared some common ancestries at *K* 11. At *K* 13, common ancestries were high between Coloured Dwarf and Dwarf Lop and between Ermine and Coloured Dwarf; the latest was also maintained at *K* 17. Some common ancestries also emerged between the two dwarf breeds and the Rex breed at *K* 11 which was then reduced at *K* 13. No common ancestries emerged for Rhinelander and Belgian Hare at all three Ks.

A very low level of introgression at the same three Ks was shown by these two breeds, that is, Belgian Hare and Rhinelander. At *K* 13, in addition to the previously mentioned breeds, Champagne d'Argent, Ermine and Dwarf Lop showed the lowest levels of introgression, followed by Giant Grey and Giant White (that were genetically very

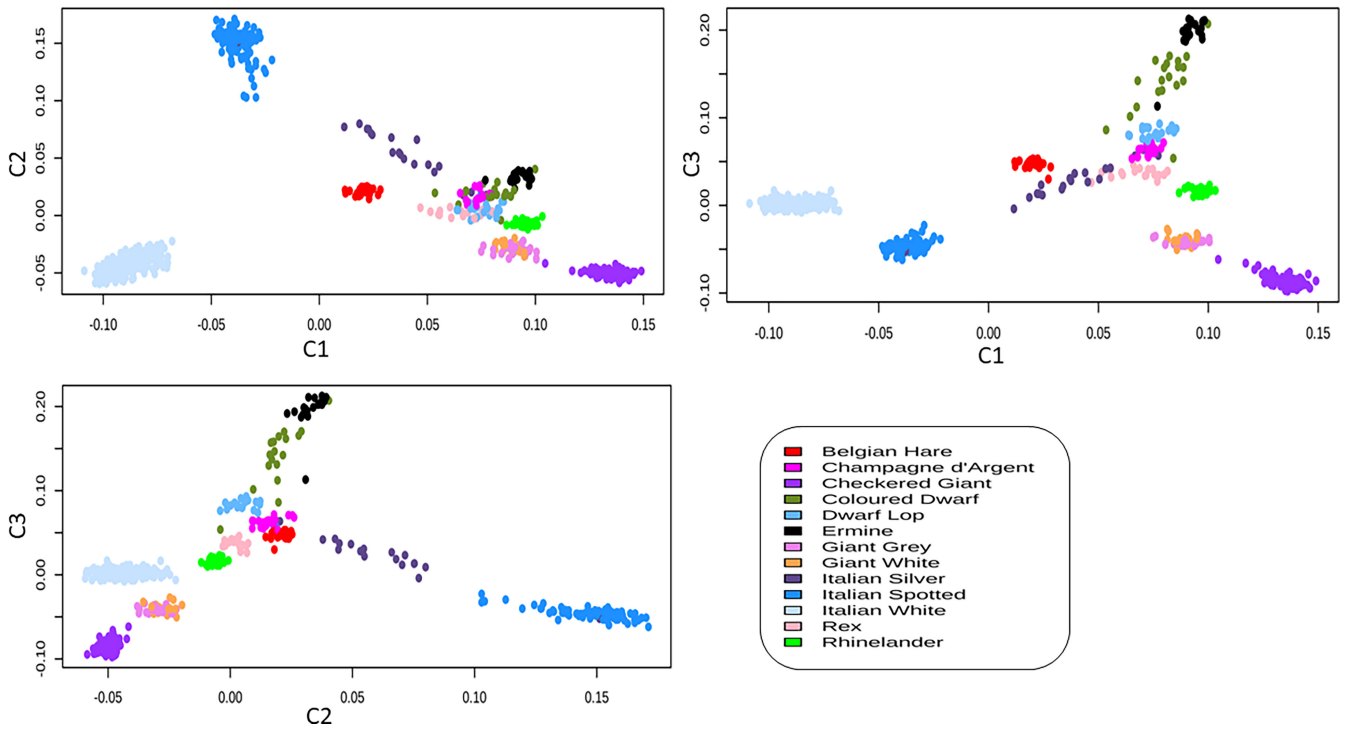

FIGURE 1 Multidimensional scaling plots. The first three components are provided.

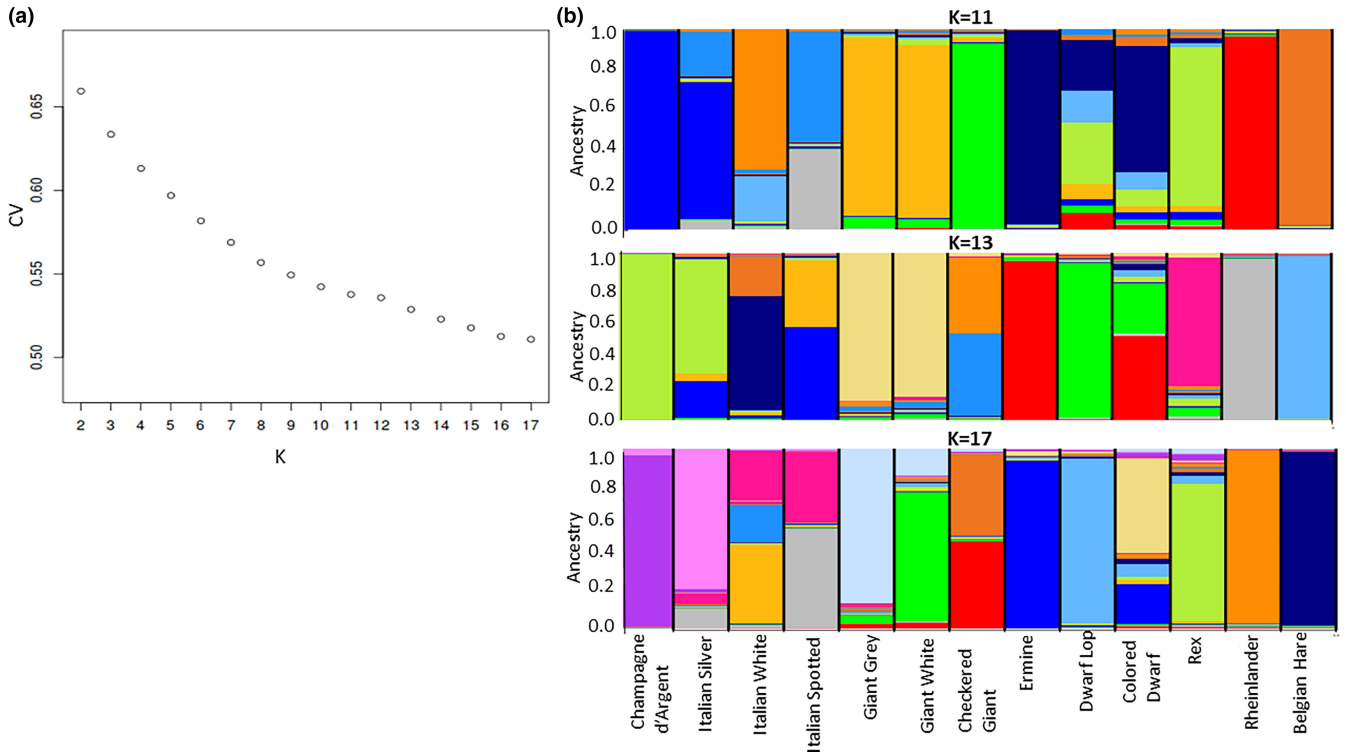

FIGURE 2 Results of the ADMIXTURE analysis. (a) Cross validation (CV) errors with K from 1 to 17. (b) Plot distribution with $K = 11$, 13 and 17 of the averaged admixture ancestries per breed. Putative subpopulations are labelled with a different colour.

similar at this level) and, subsequently, by Rex. All three meat breeds showed two main groups of different ancestries at *K* 11 and *K* 13, which also remained or increased at three groups (for Italian White) at *K* 17. The Coloured Dwarf breed consisted of heterogeneous ancestries, with two main groups at *K* 13 and *K* 17.

3.2 | Signatures of selection identified with the integrated haplotype score |iHS| analyses

Figure S1a shows the Manhattan plots reporting the |iHS| values obtained for all single breeds. The Manhattan plots obtained for the breeds grouped according to their coat colour, body size and use (i.e. meat breeds) are included in Figure 3a. The list of the top 20 genomic windows for |iHS| values identified with the analyses based on single breeds (8 out of 20) or groups of breeds (13 out of 20) is reported in Table 1 (one top value was identified in both analyses). The complete overview of the |iHS| values across single breeds and groups of breeds is reported in Tables S3 and S4 respectively.

The top 20 |iHS| windows were located on OCU1, OCU2, OCU4, OCU7, OCU9, OCU12, OCU15 and OCU16. Many genes involved in growth processes and body morphology are included in these windows. For example, the top |iHS| value, identified in the Italian White breed and in the meat breeds group, highlighted window on OCU2 that encompasses the *ligand dependent nuclear receptor corepressor like* (*LCORL*) gene, which is well known to affect stature and body size in mammals (e.g. Pryce et al., 2011). Another top ranked genomic region of this list, identified on OCU4 in the Checkered Giant breed, contains the *high mobility group AT-hook 2* (*HMGA2*) gene, which is involved in several basic biological processes, including mesenchymal differentiation, adipogenesis and post-natal myogenesis. The genomic region encompassing this gene was also evidenced in the Dwarf Lop breed (Figure S1a) and in the analyses involving two breeds groups: giant breeds and dwarf breeds (Figure 3a). Several other relevant genes in the detected |iHS| regions play roles in growth-related processes and meat production traits (Tables S3 and S4), some of which are also annotated in Figures 3a and Figure S1a. We can mention a few of them, among several others: *discoidin domain receptor tyrosine kinase* 2 (*DDR2*) that plays a role in signal transduction pathways involved in cell adhesion, proliferation and extracellular matrix remodelling; *gonadotropin releasing hormone receptor* (*GNRHR*), that is involved in abnormality of body height; *SET domain containing 7, histone lysine methyltransferase* (*SETD7*), that plays a central role in the transcriptional activation of several genes; *suppressor of cytokine signalling 2* (*SOCS2*), that is involved in mechanisms affecting body height.

Other genomic windows containing genes affecting pigmentation were identified in the analyses: (i) using groups of breeds (*endothelin receptor type A* or *EDNRA*; *endothelin receptor type B* or *EDNRB*; and *KIT proto-oncogene receptor tyrosine kinase* or *KIT*; Figure 3a); (ii) in the single-breed approach (*agouti signaling protein* or *ASIP* in Belgian Hare and Giant Grey; *EDNRA* in Checkered Giant; *EDNRB* in Champagne d'Argent, Coloured Dwarf, Giant White, Italian Spotted and Italian White; *KIT* in

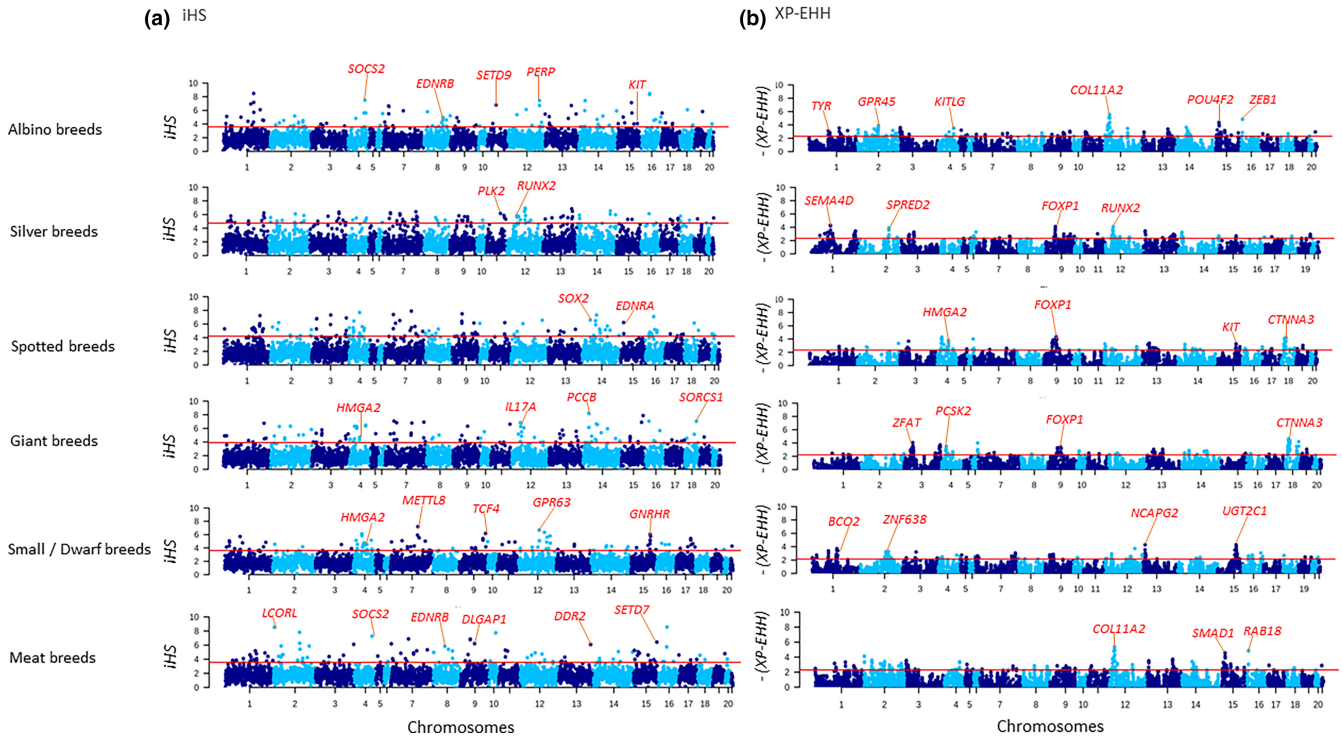

FIGURE 3 The Manhattan plots obtained for the breeds grouped according to their coat colour, body size and use (i.e. meat breeds). (a) Manhattan plots obtained with |iHS| values. (b) Manhattan plots obtained with XP-EHH values.

TABLE 1 List of the top 20 integrated haplotype score measures |iHS| for the 350 kb genomic windows and their closest genes within flanked genomic region of 500 kb, with information on the *Oryctolagus cuniculus* chromosome (OCU) position, identified with the single-breed analysis or the group of breeds analyses. The full information of |iHS| for single-breed approach and groups of breeds approach are presented in Tables S2 and S3 respectively.

|iHS|	OCU:Position	Breeds/groups of breeds[a]	Closest genes	Distance (kb)[b]
7.408	1:122550000–123500000	Italian White	*UBE2I;TAF1D;PANX1;VSTM5;MED17;C1H11orf 54;SMCO4;HEPHL1;CEP295;CCDC67*	100
7.235	1:149500000–150450000	Spotted breeds	*NLRP10,EIF3F,LMO1,TRIM66,TUB,STK33,RIC3*	269
7.821	2:107150000–108100000	Meat production	*REG3G*	142
8.684	2:8450000–9400000	Italian White, Meat production	*LCORL*	45
7.344	4:43800000–44750000	Checkered Giant	*WIF1,MSRB3,HMGA2,LEMD3*	100
7.688	4:46950000–47900000	Spotted breeds	*IFNG,IL26,IL22,RAP1B,SLC35E3,MDM2,MDM1,NUP107,CPM*	162
7.58	4:56400000–57350000	Italian White	*NAV3*	200
7.495	4:72850000–73800000	Albino breeds	*LOC108176670,SOCS2,CRADD,PLXNC1,CEP83*	500
7.266	4:72850000–73800000	Meat production	*SOCS2,CRADD,PLXNC1,CEP83*	400
7.386	7:38550000–39500000	Spotted breeds	*PTPN12,LOC108176821,FGL2,LRRC17,ARMC10, GSAP,CCDC146,FAM185A,FBXL13*	110
7.219	7:47650000–48600000	Giant Grey	*LOC100355039,LRRN3,IMMP2L*	300
7.493	9:42400000–43350000	Spotted breeds	*ZNF717*	128
7.407	9:49750000–50700000	Checkered Giant	*CHMP1B,AFG3L2,CIDEA,MPPE1,LOC108177123, TUBB6,IMPA2,GNAL,LOC108177122*	73
8.472	12:128500000–129450000	Italian White	*HEBP2,PERP,CCDC28A,ARFGEF3,ECT2L, REPS1,NHSL1*	16
7.448	12:128500000–129450000	Albino breeds	*HEBP2,LOC108177357,PERP,CCDC28A,ARFGEF3, ECT2L,REPS1,NHSL1*	500
8.209	14:29100000–30050000	Giant breeds	*MSL2,PCCB,EPHB1,PPP2R3A*	98
7.426	14:30500000–31450000	Albino breeds	*SLC35G2,IL20RB,NCK1*	107
7.948	15:64800000–65750000	Italian White	*ARHGAP24*	500
8.558	16:34000000–34950000	Meat production	*LOC100345607,KCNK1,PCNX2*	495
8.323	16:34700000–35650000	Albino breeds	*MAP10,LOC100345607,NTPCR,PCNX2,SIPA1L2*	0

[a]The breed or group of breeds listed first are reported according to the top |iHS|. Other breeds or groups of breeds are reported when the same region was evidenced over the thresholds in other populations.

[b]The distance between the genomic window and the most relevant or closest gene in kb.

Champagne d'Argent, Ermine, Italian Spotted, Italian White; *OCA2 melanosomal transmembrane protein* or *OCA2* in Checkered Giant; *paired box 2* or *PAX2* in Giant White; *tyrosinase-related protein 1* or *TYRP1* in Dwarf Lop; Figure S1a).

3.3 | Signatures of selection identified with the *XP-EHH* analyses

Results of the single-breed XP-EHH pairwise comparisons are presented in Table S5 and are shown in the Manhattan plots of Figure S1b. Figure 3b and Table S6 report the results of the analyses carried out using the defined groups of breeds. Based on the lists of obtained windows, the results of the XP-EHH analyses were in part overlapping and part complementary with the |iHS| results. For example, the *HMGA2* gene region, already reported in the |iHS| results, emerged again in the Checkered Giant and spotted breed group. Other regions, which included many genes involved in growth, body size and development (according to the information available in humans and other species; e.g. *collagen type XI alpha 2 chain*, *COL11A2*; *non-SMC condensin II complex subunit G2*, *NCAPG2*; *semaphorin 4D*, *SEMA4D*; *SMAD family member 1*, *SMAD1*; *transforming growth factor beta receptor 2*, *TGFBR2*; *zinc finger and AT-hook domain containing*, *ZFAT*), emerged from the within breed comparisons and the analyses based on groups of breeds with this method but not with the |iHS| method (Figure 3b; Figure S1b).

A few regions containing genes affecting pigmentation, identified in the |iHS| analyses, also emerged with the XP-EHH analyses: *KIT* in spotted breeds, Checkered Giant and Ermine; *OCA2* in Checkered Giant; *tyrosinase* (*TYR*) in Italian White (and also in Italian Spotted and the Albino breed comparison, not emerged with the previous method); *EDNRB* in Belgian Hare (a breed that however did not show this region with the previous method). Other regions containing relevant genes involved in pigmentation emerged only with the XPP-EHH method: KIT ligand (*KITLG*) in albino breeds and Italian White; *melanocyte inducing transcription factor* (*MITF*) in Champagne d'Argent.

Another expected signature of selection was identified in the Rex breed in the genomic region of OCU14 that harbours the *lipase H* (*LIPH*) gene, which is responsible for the *Rex*^{1} locus affecting coat structure (Diribarne et al., 2011).

3.4 | Comparison with previous approaches and enrichment analysis

To obtain a complete picture of the signatures of selection identified in the investigated rabbit breeds, we combined results obtained in this study using the iHS and the XP-EHH approaches with the results we previously obtained with other methods: different F_{ST} pairwise analyses, the application of the principal component analysis methodology of *PCAdapt* and the identification of runs of homozygosity (ROH) islands (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022). Figure 4 shows an overview of the genomic regions covered by signatures of selection identified by three, four or five different methods. The complete list of all genomic regions identified with one or more methods in different breeds is reported in Table S7. Considering all methods and all breeds together, a total of 5079 independent regions that might have been under selection were identified, covering a total of 1777 Mb. The most consistent results obtained with three, four or five methods, covered a total of 516, 118 and 22 Mb respectively.

In the single-breed analyses, 12 genomic regions were identified by at least four different methods (Table 2). Again, a few of these regions consistently highlighted some obvious candidate genes affecting breed-specific traits. For example, four different methods made possible to identify in the Italian White breed (an albino breed) the genomic region on OCU1 containing the *TYR* gene whose allele series is responsible of the *Albino* locus (Fontanesi, 2021b). Several other regions might harbour candidate genes explaining other breed-specific phenotypes or domestication-derived traits. The region on OCU3, identified in Giant White, has been associated with the number of teats in rabbits (Bovo et al., 2021). One region of OCU4 identified in Dwarf Lop includes the *kinesin family member 16B* (*KIF16B*) gene involved in early embryonic development and body size (Ueno et al., 2011; Yengo et al., 2022). A region on OCU9 identified in Checkered Giant includes the *protein tyrosine phosphatase*

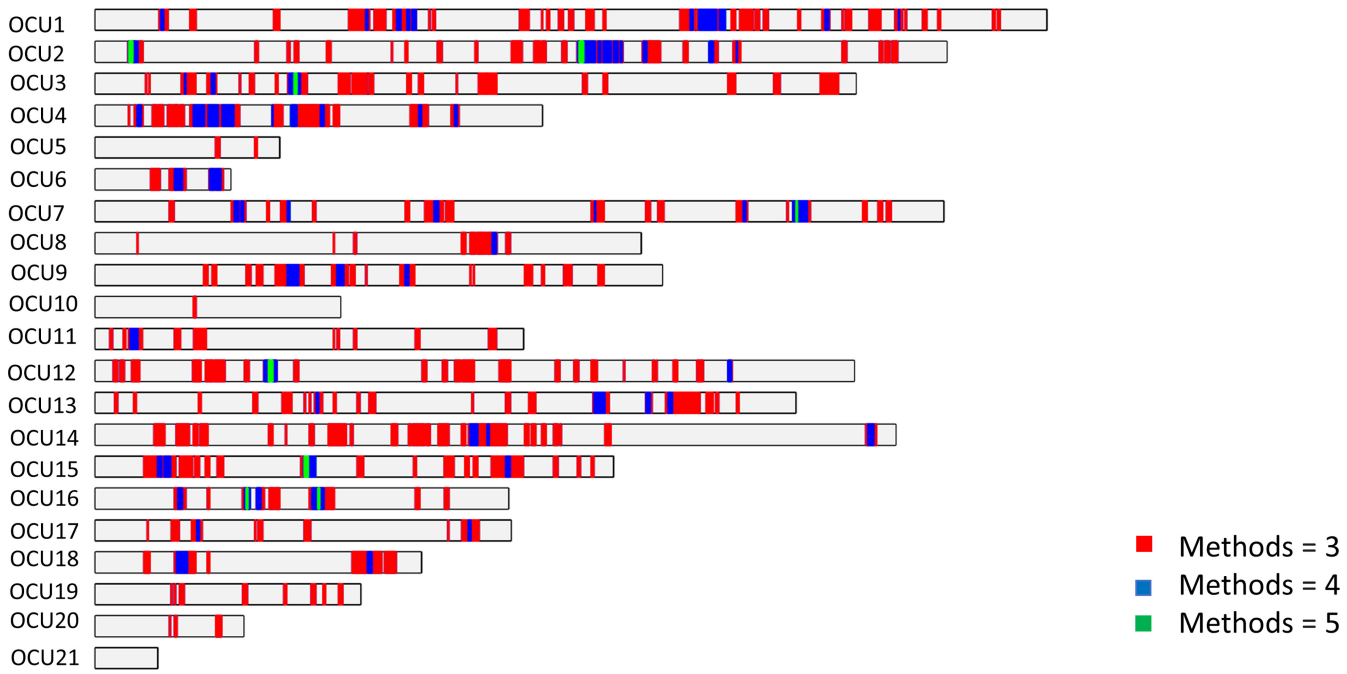

FIGURE 4 An overview of the genomic regions covered by signatures of selection identified by three (reported in red), four (blue) or five (green) different methods. Detailed information is reported in Figure S1.

TABLE 2 List of all regions identified with at least four methods in the single-breed analysis [|iHS| and XP-EHH approaches were reported in this study; the results obtained using F_{ST}, *PCAdap*t and ROH island (ROH) approaches have been obtained from: Ballan, Bovo, et al. (2022); Ballan, Schiavo, et al. (2022)].

Genomic region[a]	Methods	Breeds	Annotated genes[b]	Trait[c]	Reference[ds]
1:127150001–127200000	|iHS|, XP-EHH, *PCAdapt*, F_{ST}	Italian White	*CHORDC1, TRIM77, NOX4,* ***TYR***	Coat colour	Aigner et al. (2000)
3:41000001–41050000	ROH, |iHS|, XP-EHH, PCAdapt, F_{ST}	Giant White	*ADRA1B, TTC1, UBLCP1, IL12B,* ***EBF1****, PWWP2A, RNF145*	Number of teats in rabbits	Bovo et al. (2021)
4:20050001–20100000	|iHS|, XP-EHH, *PCAdapt*, F_{ST}	Dwarf Lop	*KIF16B, MACROD2*	-	-
4:40300001–40350000	|iHS|, XP-EHH, *PCAdapt*, F_{ST}	Checkered Giant	*APOF, ANKRD52, NACA, FAM19A2, STAT2, IL23A, PAN2, PTGES3, RBMS2, TIMELESS, COQ10A, SPRYD4, CNPY2, ATP5B, BAZ2A;MIP, NABP2, SLC39A5, GLS2*	-	-
6:18000001–18050000	|iHS|, XP-EHH, *PCAdapt*, F_{ST}	Italian Silver	*ASPHD1, CORO1A, TBX6, CDIPT, YPEL3, XPO6, SLX1A, PPP4C, SGF29, MAPK3, BOLA2B, DOC2A, KCTD13, IL4R, KIAA0556, MVP, GSG1L, ALDOA, INO80E, SEZ6L2, TAOK2, FAM57B, IL21R, GTF3C1, PRRT2, GDPD3, TMEM219, PAGR1, HIRIP3*	-	-
7:102350001–102400000	ROH, |iHS|, XP-EHH, F_{ST}	Giant White	*FIGN, TRNAF-GAA, KCNH7*	-	-
9:38800001–38850000	|iHS|, XP-EHH, PCAdapt, F_{ST}	Belgian Hare	*EIF4E3, FOXP1, PROK2*	-	-
9:49650001–49700000	|iHS|, XP-EHH, *PCAdapt*, F_{ST}	Checkered Giant	*SEH1L, TUBB6, SPIRE1, ANKRD30B, CEP76, AFG3L2, IMPA2, PSMG2,* ***PTPN2****, GNAL, CHMP1B, PRELID3A, MPPE1, CIDEA*	Crohn's disease	Festen et al. (2011)
9:63250001–63300000	|iHS|, XP-EHH, PCAdapt, F_{ST}	Checkered Giant	*SNRPD1, ESCO1, TRNAK-CUU, MIB1, GATA6, ABHD3, GREBs1L*	-	-
11:50150001–50200000	ROH, |iHS|, *PCAdapt*, F_{ST}	Belgian Hare	*CDH9*	-	-
15:80600001–80650000	ROH, |iHS|, XP-EHH, F_{ST}	Coloured Dwarf	*UGT2C1, UGT2B14, UGT2B16, UGT2B13, UGT2A1*	-	-
17:78450001–78500000	ROH, |iHS|, XP-EHH, F_{ST}	Checkered Giant	*GABRG3,* ***OCA2***	Coat colour	Ballan, Bovo, et al. (2022); Ballan, Schiavo, et al. (2022)

[a]*Oryctolagus cuniculus* chromosome (OCU) and chromosome positions in OryCun2.0 genome version.

[b]Genes included in the genome regions. In bold, candidate genes with described function related to some external and functional traits.

[c]Traits that might be affected by variability in the genes indicated in bold in the previous column.

[d]References related to the involvement of the genes indicated in bold in the mentioned traits.

non-receptor type 2 (*PTPN2*) gene, which also emerged as one of the most relevant peaks in the *PCAdapt* analysis based on all meat rabbit breeds (Ballan, Bovo, et al., 2022). Another consistent region that emerged in Checkered Giant was on OCU17 and included a coat colour gene, the *OCA2* gene.

The results of the enrichment analysis, based on the genes annotated in the chromosome regions where at least two methods detected signatures of selection, are reported in Table S8. Only two GO terms, identified in the Checkered Giant breed (the axial length) and the Giant Grey breed (eye colour), were significantly enriched.

4 | DISCUSSION

The more recent outcome of the domestication process in the rabbit has been the constitution of many different breeds that can be mainly distinguished through their exterior traits, like coat colour and structure, body size and shape (Boucher et al., 2021; Fontanesi, 2021a). These genetic resources, mainly established and maintained by fancy and commercial breeders, may contain the footprints that testify their genetic history and origin in their genome. Several genetic events and breeding practices, including artificial directional selection, bottleneck, genetic drift and introgression, have shaped the genomic architecture and population structures of these breeds. Identifying the signatures of selection left into the genome by the combined action of these events may be useful to understand the genetic mechanisms that determined the peculiar phenotypic characteristics of many of these untapped genetic resources.

We already reported some population genomic information and the identification of signatures of selection in several rabbit breeds using a few approaches (F_{ST} methods, an adapted PCA-based approach and ROH islands) that made possible to identify or confirm genes or genomic regions associated with some relevant phenotypes in the domestic rabbit (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022). Here, we reported additional layers of information obtained by further exploiting high-density genotyping data in the same breeds. Therefore, this is a follow-up study that complement what we already reported (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022). Our population structure investigation that we carried out with SNP genotyping data enlarged and refined the population structure analysis based on microsatellite information carried out in 16 rabbit breeds by Alves et al. (2015), who involved just a few breeds in common with our study (Belgian Hare, Champagne d'Argent or Champagne Silver and Rex) or that might be considered similar or with a close origin respect to our investigated breeds (Netherland Dwarf similar to Coloured Dwarf; Flemish Giant and Hungarian Giant similar to Giant Grey). It is also worth mentioning that the sampled animals in the study of Alves et al. (2015) were from France and other countries, but not Italy.

Admixture analysis that we carried out in our 13 investigated breeds clearly showed that the breeds with similar phenotypic characteristics (body size and coat colour) and, to some extent, the use for meat production, also shared common genetic features that can affect both quantitative traits and monogenic or oligogenic traits, which, in turn, differentiate their populations. This aspect did not emerge from the study of Alves et al. (2015), where breeds could not be grouped based on some common features and the applied methodology did not report any results on macrogroups of breeds.

In our study, two giant breeds (Giant Grey and Giant White) largely shared the same ancestral genetic features that could only be distinguished at the subpopulation level ($K=17$). The third giant breed, Checkered Giant, was quite divergent from the other two. The main characteristic of this breed is its unique spotted coat colour derived from the heterozygous genotype at the *English spotting* locus (Fontanesi, 2021b; Fontanesi, Vargiolu, et al., 2014). Therefore, only planned crosses that follow the classical Mendelian inheritance can produce the required phenotype in the rabbits which, in turn, can respect this breed standard. These needed practices may have also contributed, on one hand, to separate this giant breed from the two other breeds and, on the other hand, to introduce some within genetic heterogeneity, as evidenced at $K=13$ and $K=17$, where two main genetic substructures emerged as components of the genetic pool of the Checkered Giant breed.

The three small/dwarf breeds (Ermine, Dwarf Lop and Coloured Dwarf) shared some common genetic ancestry. Subsequent interbreed stratification was probably able to distinguish more clearly the Ermine (a small body sized breed) and the Dwarf Lop genetic pools (as shown at higher Ks). Some heterogeneity remained in the Coloured Dwarf. As this breed is not fixed for any colour, it might have experienced several introgressions from other morphs increasing its genetic heterogeneity. From the genetic structures of these three breeds, it could be possible to speculate that the general complex and quantitative genetic features that determine the small body size might have been commonly shared by all these three breeds that then were distinguished by some monogenic or oligogenic features determining dwarfism in two of them (Dwarf Lop and Coloured Dwarf), which might further reduce their body size. The ear lop characteristic should have also contributed to genetically separating the Dwarf Lop from the Coloured Dwarf. This is also evident from the analyses of

the signature of selection obtained in this study and our previous study carried out in these two breeds (Ballan, Bovo, et al., 2022). Different genomic regions containing candidate genes might be involved in determining different dwarfisms and the adapted skull structure requested by the ear lop phenotype (Ballan, Bovo, et al., 2022; Carneiro et al., 2017; Fontanesi, 2021c).

The Rex breed, defined by its peculiar coat structure, showed some common ancestry with the two dwarf breeds at $K=11$, suggesting a partially shared genetic background, which then diverged at higher Ks, according to a breed stratification process mainly led by the *Rex*^{l} locus genotype, that, according to the results of signature of selection analyses, we confirmed to be caused by variability at the *LIPH* gene, as previously reported (Diribarne et al., 2011). The Rex breed population analysed with a few microsatellites by Alves et al. (2015) showed some undefined substructures, probably derived by admixture with the New Zealand White breed, suggesting that the genetic material coming from different countries might have experienced some different introgressions. As we did not include in our study the New Zealand White breed, we could not confirm these potential genetic relationships.

As expected, the two silver breeds (Champagne d'Argent and Italian Silver) shared large common ancestral genetic background with some substructures in the Italian Silver breed probably derived by the breeding programme aimed to improve performance traits in this male meat line. Several substructures were also evident in the other two meat breeds at all Ks, which can be attributed, again, to their breeding programmes, which introduced blood of different genetic origin to enlarge their genetic background and maximize the genetic progress.

Rhinelander and Belgian Hare breeds were very well differentiated from all other breeds. Belgian Hare was well separated from all other breeds also in the study of Alves et al. (2015), further suggesting a quite early demographic separation of this breed, as it could be expected from the lean, long and thin body structure (very peculiar features compared to all other breeds) that characterizes Belgian Hare rabbits.

The complete picture of signatures of selection in the rabbit genome that we obtained in this study (by adding the results of the two haplotype-based approaches and combining this information with previous results obtained in the same rabbit breeds; Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022), further confirm and extend the relevance of many genetic features for the genetic differentiation of groups of breeds and single breeds. Considering all breeds and all applied methods, signatures of selection were identified in a total of about 1.8 Gb of the rabbit genome. From this picture, it emerged that a large fraction of the genome experienced some relevant modifications in the process of breed constitution (considering that we analysed only a few breeds over the several tens or hundreds that have been constituted). On average, independent signals per breed covered about 113 Mb and if we considered only the most consistent results, again, on average, at least three methods identified 25 Mb, 8 Mb were identified by at least four methods and 1.2 Mb were identified by at least five methods. These highly consistent regions might contain very important genetic features that potentially should largely contribute to the specific genetic footprints of these breeds. It is also worth noting that as different methods are based on different assumptions, the detected regions that emerged with one or more methods might contain peculiar signatures of selection that could be captured with the combined integration of one or more approaches.

The role of the genes annotated in signatures of selection regions, known from other studies and other species, could be used to derive functional effects linked to some characteristics of the considered breeds or groups of breeds. However, more than one gene is usually annotated in the regions we identified. In some cases, it was possible to highlight the most relevant candidates according to their already well-established roles. For example, all genes involved in pigmentation could be easily highlighted, as most breeds and groups of breeds can be clearly distinguished through their coat colour and colour patterns. The list of these genes identified with the |iHS| and XP-EHH approaches included *ASIP*, *EDNRA*, *EDNRB*, *KIT*, *KITLG*, *MITF*, *OCA2*, *TYR* and *TYRP1* (Figure 3; Figure S1), some of which have also been reported using previously applied methods (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022), others were newly identified in this study. Therefore, the list of pigmentation-relevant genes reported in this study and in the other studies that we carried out (Ballan, Bovo, et al., 2022; Ballan, Schiavo, et al., 2022) is quite large, further indicating that coat colours and colour patterns are very important in establishing and then recognizing a rabbit breed (Fontanesi, 2021a, 2021b). Sequencing of the newly identified genes would be needed to identify other candidate causative mutations explaining coat colour differences between breeds. Here is also interesting to mention the signature of selection identified in Champagne d'Argent on OCU9 in the correspondence of the *MITF* gene, which in humans is involved in the hair greying process (Harris et al., 2018) and melanocyte survival, migration, proliferation and differentiation (Saleem, 2019). This region did not emerge in our previous study based on F_{ST} that, however, highlighted other genomic regions potentially involved in the greying phenotype (Ballan, Bovo, et al., 2022). The *Silver* locus, inferred by classical genetic studies based on colour segregation, has been suggested to be the genetic factor underlying this

peculiar progressive greying phenotype that characterizes this breed (Fontanesi, 2021b). However, the inconsistent results obtained with different signatures of selection approaches, that would indirectly aim to identify the genetic factors affecting the hair greying phenotype in this breed, might indicate that the genetic mechanisms determining this coat colour could be more complex than previously expected.

How we phenotypically grouped the breeds based on body size, which also reflects some common genetic origin (as also evidenced by the results of the ADMIXTURE analysis), indicated interesting patterns of signatures of selection that included some genes already known to affect similar traits in other species. For example, *LCORL* has already been reported to affect body size and stature in humans and several other mammals (Bouwman et al., 2018; Plassais et al., 2019; Pryce et al., 2011; Rubin et al., 2012; Signer-Hasler et al., 2012). Our previous study based on F_{ST} already showed a signature of selection including this gene but in small/dwarf breeds (Ballan, Bovo, et al., 2022). A similar signature of selection in this gene region was also observed in dwarf rabbits by Carneiro et al. (2017). Here, we report signatures of selection including *LCORL* in Italian White rabbits and, in turn in all meat breeds. This result highlighted some putative alternative alleles at this gene region, probably affecting body structure in the opposite direction than that we can infer in the small/dwarf breeds, in line with what has been reported in humans and other mammals (Bouwman et al., 2018; Plassais et al., 2019; Pryce et al., 2011; Rubin et al., 2012; Signer-Hasler et al., 2012). Similar bidirectional signatures of selection were identified in the *HMGA2* gene region that was highlighted in small/dwarf breeds and, with a putative opposite functional effect, also in giant breeds and in spotted breeds (that have large or medium body size). Variability in the *HMGA2* has been consistently associated with body size and stature in humans and several mammals (Bouwman et al., 2018; Boyko et al., 2010; Makvandi-Nejad et al., 2012; Weedon et al., 2007). A large deletion in this gene is the causative mutation of a form of dwarfism in rabbit and where only heterozygous animals at this mutated region are viable (Carneiro et al., 2017). In our previous study based on F_{ST} (Ballan, Bovo, et al., 2022), we could only capture the signature of selection in the giant breeds but not the selection sweep that we identified here with the |iHS| values in the small breed Ermine and the Dwarf Lop breed (and in turn, in the group of small/dwarf breeds). Again, this result confirms the complementarity of the different methods we applied to identify signatures of selection in the rabbit genome. Many other regions, that emerged in the analyses carried out for the small/dwarf and giant breeds, may contain relevant genes involved in defining body size in rabbits, a complex and polygenic trait, as already demonstrated in many other mammals.

Another signature of selection was identified with both |iHS| and XP-EHH methods in Checkered Giant on OCU9 in a region that includes the *PTPN2* gene. This gene region also strongly emerged in our previous study but as a main outlier in the meat breeds (Ballan, Bovo, et al., 2022). This gene has been associated with Crohn's disease, a chronic inflammation involving the whole-digestive tract (Festen et al., 2011). Therefore, *PTPN2* might have an important role in the functionality of the digestive structures. It is worth mentioning here that Checkered Giant rabbits are usually carriers of a genetic defect associated with the *English spotting* locus (probably derived by a mutation in the *KIT* gene), which when in homozygous condition it causes deleterious megacolon in the rabbits but that is usually silent in the heterozygous state (Fontanesi, Vargiolu, et al., 2014). Therefore, it could be speculatively interesting to pair these two genetic elements in Checkered Giant: one at the *PTPN2* gene region, which could play a corrective function of a defective or semi-defective genotype at the second gene region, the *KIT*, to reduce the negative impact of the megacolon in the breed population. Further studies are needed to evaluate this potential epistatic role of *PTPN2* in this context.

The results of the functional enrichment analysis based on the genes included in the combined list of signatures of selection that we were able to obtain for the rabbit genome, even if derived from only 13 rabbit breeds, may indicate that the processes of breed constitution involved many genomic regions, which resulted in the modification of many different functions, with just very few major functional patterns for body size/shape (as derived by the GO term: axial length) and pigmentation processes (as derived by the GO term: eye colour). Therefore, it seems that the second step of the domestication process of the rabbit, that can be considered when breeds started to be constituted/defined, included a shift from the genes/functions of the first stage (genes mainly involved in behavioural and reproduction adaptation; Carneiro et al., 2014), to many other genes and functions with some major modifications that occurred for genes involved in the characterization of exterior traits (morphology and colour), that are mainly targeted by fancy breeders.

5 | CONCLUSIONS

This study further demonstrated that high-throughput genotyping data could be very useful to explore the population structure of rabbit breeds and to obtain information useful to reconstruct their genetic history and sometimes

the complex series of admixture and introgression events that have shaped their genetic structures. It is also interesting to note that the use of different methods to detect signatures of selection highlighted important regions that could not emerge using just one or another approach. Body size and coat colour appeared to be under strong selection, as expected, considering that these traits clearly differ between many rabbit breeds. Therefore, in turn, the signatures of selection left in the genome of rabbit genetic resources emerged to be at least partly consistent with their phenotypic similarities. Moreover, these selection sweeps derived by the genetic events and divergent artificial selection trajectories that have differentiated many genetic resources in the domestic rabbit, provide useful hints to go into more detail and better analyse some genes that could contribute to the genetic variability of external traits in this species and, as a model, also in other mammals. Other approaches can be added to what we reported in our studies to increase further and improve the obtained signature of the selection picture over the rabbit genome. Many other rabbit breeds remain to be investigated at the genome level. Their full characterization may identify additional hints able to explain the large phenotypic diversity in this domesticated species.

AUTHOR CONTRIBUTIONS

Luca Fontanesi designed the study, interpreted the results and obtained funding. Luca Fontanesi, Francesca Bertolini and Mohamad Ballan wrote the paper. Mohamad Ballan, Samuele Bovo, Francesca Bertolini and Giuseppina Schiavo conducted data and bioinformatic analyses. Michele Schiavitto and Riccardo Negrini provided samples and data. Francesca Bertolini, Samuele Bovo and Giuseppina Schiavo contributed to data interpretation. All authors read and approved the submitted version.

ACKNOWLEDGEMENTS

This study was funded by the PSRN (Programma di Sviluppo Rurale Nazionale) Cun-Fu and Cun-Fu 2 projects (co-funded by the European Agricultural Fund for Rural Development of the European Union and by the Italian Ministry of Agriculture, Food and Forestry - MASAF) and by the University of Bologna RFO 2019-2022 programmes.

CONFLICT OF INTEREST STATEMENT

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. All requests should be addressed to luca.fontanesi@unibo.it.

ORCID

Mohamad Ballan https://orcid.org/0000-0003-4585-7502
Samuele Bovo https://orcid.org/0000-0002-5712-8211
Francesca Bertolini https://orcid.org/0000-0003-4181-3895
Giuseppina Schiavo https://orcid.org/0000-0002-3497-1337
Michele Schiavitto https://orcid.org/0000-0002-9367-3823
Riccardo Negrini https://orcid.org/0000-0002-8735-0286
Luca Fontanesi https://orcid.org/0000-0001-7050-3760

REFERENCES

Aigner, B., Besenfelder, U., Müller, M., & Brem, G. (2000). Tyrosinase gene variants in different rabbit strains. *Mammalia Genome*, *11*, 700–702. https://doi.org/10.1007/s003350010120

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*, 1655–1664. https://doi.org/10.1101/gr.094052.109

Alves, J. M., Carneiro, M., Afonso, S., Lopes, S., Garreau, H., Boucher, S., & Ferrand, N. (2015). Levels and patterns of genetic diversity and population structure in domestic rabbits. *PLoS One*, *10*, e0144687. https://doi.org/10.1371/journal.pone.0144687

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. https://doi.org/10.1186/s12711-022-00696-9

Ballan, M., Schiavo, G., Bovo, S., Schiavitto, M., Negrini, R., Frabetti, A., Fornasini, D., & Fontanesi, L. (2022). Comparative analysis of genomic inbreeding parameters and runs of homozygosity islands in several fancy and meat rabbit breeds. *Animal Genetics*, *53*, 849–862. https://doi.org/10.1111/age.13264

Bertolini, F., Moscatelli, G., Schiavo, G., Bovo, S., Ribani, A., Ballan, M., & Fontanesi, L. (2022). Signatures of selection are present in the genome of two close autochthonous cattle breeds raised in the North of Italy and mainly distinguished for their coat colours. *Journal of Animal Breeding and Genetics*, *139*, 307–319. https://doi.org/10.1111/jbg.12659

Bertolini, F., Schiavo, G., Bovo, S., Sardina, M. T., Mastrangelo, S., Dall'Olio, S., & Fontanesi, L. (2020). Comparative selection signature analyses identify genomic footprints in Reggiana cattle, the traditional breed of the Parmigiano-Reggiano cheese production system. *Animal*, *14*, 921–932. https://doi.org/10.1017/S1751731119003318

Boucher, S., Garreau, H., Boettcher, P., & Bolet, G. (2021). Rabbit breeds and lines and genetic resources. In L. Fontanesi (Ed.), *The genetics and genomics of the rabbit* (pp. 23–37). CAB International. https://doi.org/10.1079/9781780643342.0002

Bouwman, A. C., Daetwyler, H. D., Chamberlain, A. J., Ponce, C. H., Sargolzaei, M., Schenkel, F. S., Sahana, G., Govignon-Gion, A., Boitard, S., Dolezal, M., Pausch, H., Brøndum, R. F., Bowman, P. J., Thomsen, B., Guldbrandtsen, B., Lund, M. S., Servin, B., Garrick, D. J., Reecy, J., ... Hayes, B. J. (2018). Meta-analysis of genome-wide association studies for cattle stature

identifies common genes that regulate body size in mammals. *Nature Genetics*, *50*, 362–367. https://doi.org/10.1038/s41588-018-0056-5

Bovo, S., Schiavo, G., Utzeri, V. J., Ribani, A., Schiavitto, M., Buttazzoni, L., & Fontanesi, L. (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. https://doi.org/10.1111/age.13036

Boyko, A. R., Quignon, P., Li, L., Schoenebeck, J. J., Degenhardt, J. D., Lohmueller, K. E., Zhao, K., Brisbin, A., Parker, H. G., vonHoldt, B. M., Cargill, M., Auton, A., Reynolds, A., Elkahloun, A. G., Castelhano, M., Mosher, D. S., Sutter, N. B., Johnson, G. S., Novembre, J., … Ostrander, E. A. (2010). A simple genetic architecture underlies morphological variation in dogs. *PLoS Biology*, *8*, e1000451. https://doi.org/10.1371/journal.pbio.1000451

Callou, C. (2003). *De la garenne au clapier: Étude archéozoologique du lapin en Europe occidentale* (Vol. *189*, p. 356). Mémoires du Muséum national d'Histoire naturelle, 2-85653-545-3.

Carneiro, M., Hu, D., Archer, J., Feng, C., Afonso, S., Chen, C., Blanco-Aguiar, J. A., Garreau, H., Boucher, S., Ferreira, P. G., Ferrand, N., Rubin, C. J., & Andersson, L. (2017). Dwarfism and altered craniofacial development in rabbits is caused by a 12.1 kb deletion at the *HMGA2* locus. *Genetics*, *205*, 955–965. https://doi.org/10.1534/genetics.116.196667

Carneiro, M., Rubin, C. J., Di Palma, F., Albert, F. W., Alföldi, J., Martinez Barrio, A., Pielberg, G., Rafati, N., Sayyab, S., Turner-Maier, J., Younis, S., Afonso, S., Aken, B., Alves, J. M., Barrell, D., Bolet, G., Boucher, S., Burbano, H. A., Campos, R., … Andersson, L. (2014). Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. *Science*, *345*, 1074–1079. https://doi.org/10.1126/science.1253714

Carneiro, M., Vieillard, J., Andrade, P., Boucher, S., Afonso, S., Blanco-Aguiar, J. A., Santos, N., Branco, J., Esteves, P. J., Ferrand, N., Kullander, K., & Andersson, L. (2021). A loss-of-function mutation in RORB disrupts saltatorial locomotion in rabbits. *PLoS Genetics*, *17*, e1009429. https://doi.org/10.1371/journal.pgen.1009429

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, *4*, s13742–015–0047–8. https://doi.org/10.1186/s13742-015-0047-8

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., & Ma'ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*(1), 1–14. https://doi.org/10.1186/1471-2105-14-128

Delaneau, O., Marchini, J., & Zagury, J. F. (2012). A linear complexity phasing method for thousands of genomes. *Nature Methods*, *9*, 179–181. https://doi.org/10.1038/nmeth.1785

Demars, J., Iannuccelli, N., Utzeri, V. J., Auvinet, G., Riquet, J., Fontanesi, L., & Allain, D. (2018). New insights into the melanophilin (*MLPH*) gene affecting coat color dilution in rabbits. *Genes*, *9*, 430. https://doi.org/10.3390/genes9090430

Diribarne, M., Mata, X., Chantry-Darmon, C., Vaiman, A., Auvinet, G., Bouet, S., Deretz, S., Cribiu, E.-P., de Rochambeau, H., Allain, D., & Guérin, G. (2011). A deletion in exon 9 of the *LIPH* gene is responsible for the rex hair coat phenotype in rabbits (*Oryctolagus cuniculus*). *PLoS One*, *6*, e19281. https://doi.org/10.1371/journal.pone.0019281

Festen, E. A., Goyette, P., Green, T., Boucher, G., Beauchamp, C., Trynka, G., Dubois, P. C., Lagacé, C., Stokkers, P. C. F., Hommes, D. W., Barisani, D., Palmieri, O., Annese, V., van Heel, D. A., Weersma, R. K., Daly, M. J., Wijmenga, C., & Rioux, J. D. (2011). A meta-analysis of genome-wide association scans identifies IL18RAP, PTPN2, TAGAP, and PUS10 as shared risk loci for Crohn's disease and celiac disease. *PLoS Genetics*, *7*, e1001283. https://doi.org/10.1371/journal.pgen.1001283

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. https://doi.org/10.3390/app11010373

Fontanesi, L. (2021b). Genetics and molecular genetics of coat colour in the European rabbit. In L. Fontanesi (Ed.), *The genetics and genomics of the rabbit* (pp. 38–42). CAB International. https://doi.org/10.1079/9781780643342.0006

Fontanesi, L. (2021c). Genetics and molecular genetics of morphological and physiological traits and inherited disorders in the European rabbit. In L. Fontanesi (Ed.), *The genetics and genomics of the rabbit* (pp. 120–162). CAB International. https://doi.org/10.1079/9781780643342.000

Fontanesi, L., Forestier, L., Allain, D., Scotti, E., Beretti, F., Deretz-Picoulet, S., Pecchioli, E., Vernesi, C., Robinson, T. J., Malaney, J. L., Russo, V., & Oulmouden, A. (2010). Characterization of the rabbit agouti signaling protein (*ASIP*) gene: Transcripts and phylogenetic analyses and identification of the causative mutation of the nonagouti black coat colour. *Genomics*, *95*, 166–175. https://doi.org/10.1016/j.ygeno.2009.11.003

Fontanesi, L., Scotti, E., Allain, D., & Dall'Olio, S. (2014). A frameshift mutation in the melanophilin gene causes the dilute coat colour in rabbit (*Oryctolagus cuniculus*) breeds. *Animal Genetics*, *45*, 248–255. https://doi.org/10.1111/age.12104

Fontanesi, L., Scotti, E., Colombo, M., Beretti, F., Forestier, L., Dall'Olio, S., Deretz, S., Russo, V., Allain, D., & Oulmouden, A. (2010). A composite six bp in-frame deletion in the melanocortin 1 receptor (*MC1R*) gene is associated with the Japanese brindling coat colour in rabbits (*Oryctolagus cuniculus*). *BMC Genetics*, *11*, 59. https://doi.org/10.1186/1471-2156-11-59

Fontanesi, L., Tazzoli, M., Beretti, F., & Russo, V. (2006). Mutations in the melanocortin 1 receptor (*MC1R*) gene are associated with coat colours in the domestic rabbit (*Oryctolagus cuniculus*). *Animal Genetics*, *37*, 489–493. https://doi.org/10.1111/j.1365-2052.2006.01494.x

Fontanesi, L., Utzeri, V. J., & Ribani, A. (2021). The evolution, domestication and world distribution of the European rabbit (*Oryctolagus cuniculus*). In L. Fontanesi (Ed.), *The genetics and genomics of the rabbit* (pp. 1–22). CAB International. https://doi.org/10.1079/9781780643342.0001

Fontanesi, L., Vargiolu, M., Scotti, E., Latorre, R., Faussone Pellegrini, M. S., Mazzoni, M., Asti, M., Chiocchetti, R., Romeo, G., Clavenzani, P., & De Giorgio, R. (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. https://doi.org/10.1371/journal.pone.0093750

Gautier, M., Klassmann, A., & Vitalis, R. (2017). Rehh 2.0: A reimplementation of the R package rehh to detect positive selection from haplotype structure. *Molecular Ecology Resources*, *17*, 78–90. https://doi.org/10.1111/1755-0998.12634

Gautier, M., & Naves, M. (2011). Footprints of selection in the ancestral admixture of a New World Creole cattle breed. *Molecular*

Ecology, *20*, 3128–3143. https://doi.org/10.1111/j.1365-294X.2011.05163.x

González-Rodríguez, A., Munilla, S., Mouresan, E. F., Cañas-Álvarez, J. J., Díaz, C., Piedrafita, J., Altarriba, J., Baro, J. Á., Molina, A., & Varona, L. (2016). On the performance of tests for the detection of signatures of selection: A case study with the Spanish autochthonous beef cattle populations. *Genetics Selection Evolution*, *48*, 81. https://doi.org/10.1186/s12711-016-0258-1

Harris, M. L., Fufa, T. D., Palmer, J. W., Joshi, S. S., Larson, D. M., Incao, A., Gildea, D. E., Trivedi, N. S., Lee, A. N., Day, C. P., Michael, H. T., Hornyak, T. J., Merlino, G., NISC Comparative Sequencing Program, & Pavan, W. J. (2018). A direct link between MITF, innate immunity, and hair graying. *PLoS Biology*, *16*, e2003648. https://doi.org/10.1371/journal.pbio.2003648

Kemper, K. E., Saxton, S. J., Bolormaa, S., Hayes, B. J., & Goddard, M. E. (2014). Selection for complex traits leaves little or no classic signatures of selection. *BMC Genomics*, *15*, 246. https://doi.org/10.1186/1471-2164-15-246

Lehner, S., Gähle, M., Dierks, C., Stelter, R., Gerber, J., Brehm, R., & Distl, O. (2013). Two-exon skipping within *MLPH* is associated with coat color dilution in rabbits. *PLoS One*, *8*, e84525. https://doi.org/10.1371/journal.pone.0084525

Letko, A., Ammann, B., Jagannathan, V., Henkel, J., Leuthard, F., Schelling, C., Carneiro, M., Drögemüller, C., & Leeb, T. (2020). A deletion spanning the promoter and first exon of the hair cycle-specific *ASIP* transcript isoform in black and tan rabbits. *Animal Genetics*, *51*, 137–140. https://doi.org/10.1111/age.12881

Luu, K., Bazin, E., & Blum, M. G. (2017). Pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *17*, 67–77. https://doi.org/10.1111/1755-0998.12592

Maiorano, A. M., Lourenco, D. L., Tsuruta, S., Ospina, A. M. T., Stafuzza, N. B., Masuda, Y., Filho, A. E. V., Cyrillo, J. N. D. S. G., Curi, R. A., & Silva, J. A. I. D. V. (2018). Assessing genetic architecture and signatures of selection of dual purpose Gir cattle populations using genomic information. *PLoS One*, *13*, e0200694. https://doi.org/10.1371/journal.pone.0200694

Makvandi-Nejad, S., Hoffman, G. E., Allen, J. J., Chu, E., Gu, E., Chandler, A. M., Loredo, A. I., Bellone, R. R., Mezey, J. G., Brooks, S. A., & Sutter, N. B. (2012). Four loci explain 83% of size variation in the horse. *PLoS One*, *7*, e39929. https://doi.org/10.1371/journal.pone.0039929

Plassais, J., Kim, J., Davis, B. W., Karyadi, D. M., Hogan, A. N., Harris, A. C., Decker, B., Parker, H. G., & Ostrander, E. A. (2019). Whole genome sequencing of canids reveals genomic regions under selection and variants influencing morphology. *Nature Communications*, *10*, 1489. https://doi.org/10.1038/s41467-019-09373-w

Pryce, J. E., Hayes, B. J., Bolormaa, S., & Goddard, M. E. (2011). Polymorphic regions affecting human height also control stature in cattle. *Genetics*, *187*, 981–984. https://doi.org/10.1534/genetics.110.12394

Qanbari, S., & Simianer, H. (2014). Mapping signatures of positive selection in the genome of livestock. *Livestock Science*, *166*, 133–143. https://doi.org/10.1016/j.livsci.2014.05.003

Rubin, C. J., Megens, H. J., Barrio, A. M., Maqbool, K., Sayyab, S., Schwochow, D., Wang, C., Carlborg, Ö., Jern, P., Jørgensen, C. B., Archibald, A. L., Fredholm, M., Groenen, M. A. M., & Andersson, L. (2012). Strong signatures of selection in the domestic pig genome. *Proceedings of the National Academy of Sciences of the USA*, *109*, 19529–19536. https://doi.org/10.1073/pnas.1217149109

Sabeti, P. C., Schaffner, S. F., Fry, B., Lohmueller, J., Varilly, P., Shamovsky, O., Palma, A., Mikkelsen, T. S., Altshuler, D., & Lander, E. S. (2006). Positive natural selection in the human lineage. *Science*, *312*, 1614–1620. https://doi.org/10.1126/science.1124309

Sabeti, P. C., Varilly, P., Fry, B., Lohmueller, J., Hostetter, E., Cotsapas, C., Xie, X., Byrne, E. H., McCarroll, S. A., Gaudet, R., Schaffner, S. F., Lander, E. S., & The International HapMap Consortium. (2007). Genome-wide detection and characterization of positive selection in human populations. *Nature*, *449*, 913–918. https://doi.org/10.1038/nature06250

Saleem, M. D. (2019). Biology of human melanocyte development, Piebaldism, and Waardenburg syndrome. *Pediatric Dermatology*, *36*, 72–84. https://doi.org/10.1111/pde.13713

Saravanan, K. A., Panigrahi, M., Kumar, H., Bhushan, B., Dutt, T., & Mishra, B. P. (2020). Selection signatures in livestock genome: A review of concepts, approaches and applications. *Livestock Science*, *241*, 104257. https://doi.org/10.1016/j.livsci.2020.104257

Signer-Hasler, H., Flury, C., Haase, B., Burger, D., Simianer, H., Leeb, T., & Rieder, S. (2012). A genome-wide association study reveals loci influencing height and other conformation traits in horses. *PLoS One*, *7*, e37282. https://doi.org/10.1371/journal.pone.0037282

Ueno, H., Huang, X., Tanaka, Y., & Hirokawa, N. (2011). KIF16B/Rab14 molecular motor complex is critical for early embryonic development by transporting FGF receptor. *Developmental Cell*, *20*, 60–71. https://doi.org/10.1016/j.devcel.2010.11.008

Utsunomiya, Y. T., Pérez O'Brien, A. M., Sonstegard, T. S., Van Tassell, C. P., do Carmo, A. S., Meszaros, G., Sölkner, J., & Garcia, J. F. (2013). Detecting loci under recent positive selection in dairy and beef cattle by combining different genome-wide scan methods. *PLoS One*, *8*, e64280. https://doi.org/10.1371/journal.pone.0064280

Utzeri, V. J., Ribani, A., & Fontanesi, L. (2014). A premature stop codon in the TYRP 1 gene is associated with brown coat colour in the European rabbit (Oryctolagus cuniculus). *Animal Genetics*, *45*, 600–603. https://doi.org/10.1111/age.12171

Utzeri, V. J., Ribani, A., Schiavo, G., & Fontanesi, L. (2021). Describing variability in the tyrosinase (*TYR*) gene, the *albino* coat colour locus, in domestic and wild European rabbits. *Italian Journal of Animal Science*, *20*, 181–187. https://doi.org/10.1080/1828051X.2021.1877574

Voight, B. F., Kudaravalli, S., Wen, X., & Pritchard, J. K. (2006). A map of recent positive selection in the human genome. *PLoS Biology*, *4*, e72. https://doi.org/10.1371/journal.pbio.0040072

Weedon, M. N., Lettre, G., Freathy, R. M., Lindgren, C. M., Voight, B. F., Perry, J. R., Elliott, K. S., Hackett, R., Guiducci, C., Shields, B., Zeggini, E., Lango, H., Lyssenko, V., Timpson, N. J., Burtt, N. P., Rayner, N. W., Saxena, R., Ardlie, K., Tobias, J. H., … Frayling, T. M. (2007). A common variant of HMGA2 is associated with adult and childhood height in the general population. *Nature Genetics*, *39*, 1245–1250. https://doi.org/10.1038/ng2121

Whitman, B. D. (2004). *Domestic Rabbits & Their Histories: Breeds of the world*. Leathers Publishing.

Yengo, L., Vedantam, S., Marouli, E., Sidorenko, J., Bartell, E., Sakaue, S., Graff, M., Eliasen, A. U., Jiang, Y., Raghavan, S., Miao, J., Arias, J. D., Graham, S. E., Mukamel, R. E., Spracklen, C. N., Yin, X., Chen, S.-H., Ferreira, T., Highland, H. H., … Lee, J. Y. (2022). A saturated map of common genetic variants

associated with human height. *Nature*, *610*, 704–712. https://doi.org/10.1038/s41586-022-05275-y

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

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

SUPPORTING INFORMATION

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

How to cite this article: Ballan, M., Bovo, S., Bertolini, F., Schiavo, G., Schiavitto, M., Negrini, R., & Fontanesi, L. (2023). Population genomic structures and signatures of selection define the genetic uniqueness of several fancy and meat rabbit breeds. *Journal of Animal Breeding and Genetics*, *00*, 1–16. https://doi.org/10.1111/jbg.12818