**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 autozygosity leads to inbreeding depression and the occurrence of deleterious recessive alleles in homozygous state, which mainly impairs reproductive performances and production traits.

In diploid organisms, inbreeding (traditionally indicated with $F_{\text{PED}}$ 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 $F_{\text{PED}}$ 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 $F_{\text{PED}}$ 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 $F_{\text{PED}}$; 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 $F_{\text{PED}}$ 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 $F_{\text{PED}}$, 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 ($F_{\text{ROH}}$; 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; Gorskens 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 OrecunSNP 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; 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 \((n_{ROH})\); (ii) the average length of ROH \((L_{ROH})\); (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 \(F_{ROH1}, F_{ROH2}, F_{ROH4}, F_{ROH8}, F_{ROH16}\) 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 \((F_{hat})\); (iii) the inbreeding coefficient based on the genomic relationship matrix \(F_{GRM}\) (VanRaden et al., 2011; this parameter is equivalent to \(F_{hat}\), even if scaled in a different
way); (v) the estimate based on correlation between unifying gametes ($F_{hat2}$); (vi) the estimate based on correlation between unifying gametes ($F_{hat3}$). Finally, we estimated the Pearson correlation coefficients ($\rho$) and the Spearman's rank correlation coefficients ($\rho$) between all pairs of in-breeding 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 bedtools 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 $S^{'ROH}$). 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 (LROH) 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 (SROH) values. The correlation for these three ROH statistics (i.e. nROH, LROH, and SROH) 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 LROH), and from 0.909 to 0.999 (for SROH). 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 SROH 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, LROH, and SROH). 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 patterns (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 SROH values in the whole dataset: a Coloured Dwarf rabbit had an SROH of 1304.3 Mb and an Ermine rabbit had an SROH of 1304.3 Mb.
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.
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,
and Ermine where all correlations were >0.90. The correlation between $F_{\text{ROH}}$ parameters and all other genomic inbreeding parameters were high and consistent over all breeds only with the $F_{\text{HOM}}$ values (Table 12). For example, correlation between $F_{\text{ROH1}}$ and $F_{\text{HOM}}$ ranged from 0.974 (Giant Grey) to 0.783 (Dwarf Lop) and correlation between $F_{\text{ROH16}}$ and $F_{\text{HOM}}$ ranged from 0.939 (Champagne d’Argent) to 0.597 (Belgian Hare). Correlations between the other parameters were not always consistent across breeds (excluding $F_{\text{hat1}}$ and $F_{\text{GRM}}$, 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 contain 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 I* (*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

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

**Note:** Table S10 includes results obtained with all four approaches.
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...
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 \( N_e \). 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 \( En/\bar{en} \); 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...
RUNS OF HOMOZYGOITY IN RABBITS

ROH in European pig breeds, where nROH was much lower on average per breed with a larger average size of $L_{\text{ROH}}$, 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-Ramílo et al., 2020). Despite ROH were calculated in different ways, the $F_{\text{ROH}}$, estimated by summing up the values obtained from size ranges, is similar

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

<table>
<thead>
<tr>
<th>OCUa</th>
<th>Positionb</th>
<th>Breed</th>
<th>No. of SNPs</th>
<th>No. of genes</th>
<th>Candidate genes (reference)e</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>159375514_160629747</td>
<td>Italian White</td>
<td>64</td>
<td>5</td>
<td>NELL1 (Falker-Gieske et al., 2019)</td>
</tr>
<tr>
<td>2</td>
<td>127462270_128281278</td>
<td>Checkered Giant</td>
<td>43</td>
<td>3</td>
<td>VRK2, FANCL (Paredes-Sánchez et al., 2020)</td>
</tr>
<tr>
<td>2</td>
<td>161490031_163738094</td>
<td>Italian Spotted</td>
<td>54</td>
<td>5</td>
<td>TRIB2 (Brunes et al., 2021; Fernandes et al., 2021)</td>
</tr>
<tr>
<td>3</td>
<td>21908055_2247201</td>
<td>Checkered Giant</td>
<td>23</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>24713065_25428869</td>
<td>Checkered Giant</td>
<td>45</td>
<td>4</td>
<td>NR3C1 (Muráni et al., 2010; Reyer et al., 2014)</td>
</tr>
<tr>
<td>3</td>
<td>78265347_81591625</td>
<td>Italian Spotted</td>
<td>42</td>
<td>10</td>
<td>ARMC1 (Zhou et al., 2016)</td>
</tr>
<tr>
<td>3</td>
<td>14059855_142305209</td>
<td>Checkered Giant, Commercial meat, Italian Spotted, Italian White</td>
<td>77, 75, 70, 72</td>
<td>8</td>
<td>TRIB1 (Brunes et al., 2021)</td>
</tr>
<tr>
<td>3</td>
<td>149885379_151645140</td>
<td>Checkered Giant</td>
<td>60</td>
<td>4</td>
<td>ZFAT (Grilz-Seger et al., 2019)</td>
</tr>
<tr>
<td>4</td>
<td>26298302_28844406</td>
<td>Italian Spotted</td>
<td>71</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>75359078_76496009</td>
<td>Commercial Meat</td>
<td>26</td>
<td>10</td>
<td>IFRD1 (Sorbolini et al., 2017)</td>
</tr>
<tr>
<td>7</td>
<td>54842373_57319254</td>
<td>Checkered Giant, Commercial Meat</td>
<td>77, 152</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>140627212_14239237</td>
<td>Checkered Giant, Commercial Meat, Italian Spotted</td>
<td>19, 55, 32</td>
<td>14</td>
<td>AOX1 (Casto-Rebollo et al., 2021)</td>
</tr>
<tr>
<td>10</td>
<td>5642931_6250075</td>
<td>Commercial</td>
<td>14</td>
<td>3</td>
<td>ITGB8 (Casto-Rebollo et al., 2021)</td>
</tr>
<tr>
<td>12</td>
<td>8595729_11116965</td>
<td>Italian Spotted, Italian White</td>
<td>48, 48</td>
<td>143</td>
<td>GPX5 (Barranco et al., 2016)</td>
</tr>
<tr>
<td>12</td>
<td>78307373_84406018</td>
<td>Italian White, Commercial Meat</td>
<td>60, 15</td>
<td>12</td>
<td>NDUFAF4 (An et al., 2018)</td>
</tr>
<tr>
<td>13</td>
<td>74396836_764130675693348</td>
<td>Italian Spotted, Italian White</td>
<td>39, 54</td>
<td>18</td>
<td>PKN2 (Fontanesi et al., 2012)</td>
</tr>
<tr>
<td>15</td>
<td>12623264_15388967</td>
<td>Italian White, Commercial Meat, Italian Spotted</td>
<td>59, 56, 52</td>
<td>12</td>
<td>DCLK2 (Sahana et al., 2013)</td>
</tr>
<tr>
<td>15</td>
<td>63477042_65101796</td>
<td>Italian Spotted</td>
<td>58</td>
<td>4</td>
<td>WDFY3 (Chang et al., 2018)</td>
</tr>
<tr>
<td>15</td>
<td>84153011_85988883</td>
<td>Commercial Meat, Italian White</td>
<td>7, 57</td>
<td>3</td>
<td>TECRL (Weng et al., 2016)</td>
</tr>
<tr>
<td>16</td>
<td>14715459_16455125</td>
<td>Checkered Giant</td>
<td>53</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>75881549_77977324</td>
<td>Checkered Giant</td>
<td>76</td>
<td>20</td>
<td>OCA2 (Donnelly et al., 2012)</td>
</tr>
</tbody>
</table>

aOrycolagus cuniculus chromosome.  
bStarting and ending nucleotide position of the ROH region. Partially overlapped ROH in different breeds have been combined in a single ROH region.  
cNumber of SNPs in the ROH identified in the corresponding breeds.  
dNumber of genes annotated in the OryCun2.0 genome version included in the ROH region.  
eCandidate 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.
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 Papillon). 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 underlying 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.

**ACKNOWLEDGMENTS**

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 – MiPAAF), by the University of Bologna RFO 2019-2021 programmes and by Gruppo Martini spa (project Cun-Genomics).

**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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**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., Schiavo, G., Bovo, S., Schiavitto, M., Negrini, R., Frabetti, A. et al. (2022) Comparative analysis of genomic inbreeding parameters and runs of homozygosity islands in several fancy and meat rabbit breeds. *Animal Genetics*, 00, 1–14. Available from: [https://doi.org/10.1111/age.13264](https://doi.org/10.1111/age.13264)