



Persistence of autozygosity in crossbreds between autochthonous and cosmopolitan breeds of swine: a simulation study



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ABSTRACT

Crossbreeding might be a valid strategy to valorize local pig breeds. Crossbreeding should reduce homozygosity and, as a consequence, yield hybrid vigor for fitness and production traits. This study aimed to quantify the persistence of autozygosity in terminal crossbred pigs compared with purebreds and, in turn, identify genomic regions where autozygosity's persistence would not be found. The study was based on genotyping data from 20 European local pig breeds and three cosmopolitan pig breeds used to simulate crossbred offspring. This study consisted of two steps. First, one hundred matings were simulated for each pairwise combination of the 23 considered breeds (for a total of 276 combinations), ignoring the sex of the parent individuals in order to generate purebred and crossbred matings leveraging all the germplasm available. Second, a few preselected terminal-maternal breed pairs were used to mimic a realistic terminal crossbreeding system: (i) Mora Romagnola (boars) or Cinta Senese (boars) crossed with Large White (sows) or Landrace (sows); (ii) Duroc (boars) crossed with Mora Romagnola (sows) or Cinta Senese (sows). Runs of homozygosity was used to estimate genome-wide autozygosity (F_{ROH}). Observed F_{ROH} was higher in purebreds than in crossbreds, although some crossbred combinations showed higher F_{ROH} than other purebred combinations. Among the purebreds, the highest F_{ROH} values were observed in Mora Romagnola and Turopolje (0.50 and 0.46, respectively). F_{ROH} ranged from 0.04 to 0.16 in the crossbreds Alentejana \times Large White and Alentejana \times Iberian, respectively. Persistence of autozygosity was found in several genomic segments harboring regions where quantitative trait loci (QTLs) were found in the literature. The regions were enriched in QTLs involved in fatty acid metabolism and associated with performance traits. This simulation shows that autozygosity persists in most breed combinations of terminal crosses. Results suggest that a strategy for crossbreeding is implemented when leveraging autochthonous and cosmopolitan breeds to obtain most of the hybrid vigor.

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Implications

Crossbreeding schemes are routinely applied in many pig production systems, but only some breeds are commonly employed in these programs. Using real high-density Single Nucleotide Polymorphism datasets, this study simulated, at the genome level, the effect of crossbreeding between pairs of 23 European pig breeds, including 20 local breeds. The results provided a genomic landscape picture of many regions that lost or maintained autozygosity in the pairwise combination. This information could be further

exploited to properly design crossbreeding programs based on local breeds as parental lines.

Introduction

Crossbreeding is the mating between individuals of different lines, breeds, or populations. This mating system can be utilized to reduce the inbreeding levels of the offspring. Another benefit of crossbreeding is the expression of heterosis, also known as hybrid vigor. Hybrid vigor is a phenomenon in which the crossbred individuals' performances surpass the parental lines' average performances (Duenk et al., 2021). Hybrid vigor is the opposite of

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inbreeding depression origin, and inbreeding tends to cause more gene pairs in an individual to be homozygous. In contrast, crossbreeding tends to cause more gene pairs to be heterozygous. Crossbreeding is routinely used in many commercial pig production systems because, in general, the offspring is more robust, with better growth and carcass traits, resulting in improved performances (Sørensen et al., 2008). Interestingly, pig crossbreds show better fertility (Dragomir, 2013; Praew Thiengpimol, 2017), improving animal welfare and sustainability. A typical crossbreeding program is based on three pig breeds where a boar line (from Duroc or Pietrain) is crossed with crossbred sows (obtained by crossing Large White and Landrace pigs) (Christensen et al., 2015). In a few countries, crossbreeding schemes also include some local breeds: for example, in Italy, Cinta Senese is sometimes crossed with cosmopolitan breeds, for instance, with Large White (Franci et al., 2005 and 2007), and in Spain, Iberian pigs are crossed with Duroc lines (Ortiz et al., 2021), to obtain superior terminal crossbreds that constitute interesting examples of niche pork value chains. These examples can support the exploitation of crossbreeding as a potential strategy in the conservation program of autochthonous pig genetic resources.

Runs of homozygosity (ROH) have been frequently used to investigate the level of autozygosity within and across breeds. ROH are continuous stretches of homozygous genotypes (Peripolli et al., 2017). It is known as that autozygosity could be led to unfavorable effects on economic traits as reported by Tao et al. (2023) who analyzed 126 pig traits related to carcass, meat quality and body conformation and all decreased in inbreeding depression. Persistence of ROH in crossbred pigs has been detected in two-way Landrace × Large White crossbred populations (Gomez-Raya et al., 2019), in three-way Duroc × (Pietrain × Large White) crosses (Ganteil et al., 2021), and have been inferred from simulated three-way Duroc × (Landrace × Large White) crosses (Howard et al., 2016). Persistence of ROH was discussed also in a very recent study which analyzed the crosses of Large White × Chinese local breed (Tao et al., 2023). These results suggest that the same allotypes segregate in different pig breeds and will therefore be found in the homozygous state in the crossbred offspring. Investigating why the same haplotypes could be quite frequent in other breeds would be interesting. Some hypotheses could be raised: (i) high level of linkage disequilibrium and, in turn, very low recombination rate in some genomic regions; (ii) ancestral haplotypes that are maintained in the populations because they are associated with some artificially selected traits; (iii) introgression by recent admixtures (sometimes unknown) between pig populations; (iv) ascertainment biases in the design of the single nucleotide polymorphism genotyping tools. All four hypotheses could have different downstream interpretations and potential use of the information, mainly when autochthonous pig breeds are considered in crossbreeding programs. In this context, on the other hand, it would also be interesting to obtain a general picture of the genomic regions that would be heterozygous in the crossbreds derived from different pig breeds. These regions might indicate the expected heterosis of the final crossbred product.

The aims of this study were (i) to quantify the loss of autozygosity in simulated crossbred pigs compared to purebred pigs and identify the best potential crosses in terms of loss of autozygosity and (ii) to obtain a landscape genomic picture of regions potentially affected by the loss autozygosity (i.e., high frequency of heterozygosity) in pig crossbreeding programs. For these simulations, we used high-density Single Nucleotide Polymorphisms (SNPs) chip genotyping data available from 23 European local and cosmopolitan pig breeds, making this study, to the best of our knowledge, the most comprehensive simulation of crossbred pigs thus far reported that could provide some interesting hints on the different mentioned hypotheses reported above and, on

the possibility, to apply in practice crossbreeding schemes that involve local pig breeds.

Material and methods

Animal sampling and population structure investigation

This study included 1 141 individuals belonging to 23 pig breeds collected in the frame of the European project TREASURE (<https://treasure.kis.si/>). Twenty were European local breeds (n = 984) from nine countries (Croatia: Black Slavonian, Turopolje (TUR); France: Basque, Gascon; Germany: Schwabisch-Hällisches Schwein (SCH); Italy: Apulo Calabrese, Casertana, Cinta Senese (CIN), Mora Romagnola (MOR), Nero Siciliano, Sarda (SAR); Lithuania: Indigenous Wattle, White Old Type (LIW); Portugal: Alentejana (ALE), Bísara; Serbia: Moravka, Swallow-Bellied Mangalitsa (SWM); Slovenia: Krškopolje; Spain: Iberian (IBE), Majorcan Black), and three Italian cosmopolitan breeds (n = 157, Duroc (DUR), Landrace (LAN), and Large White (LAR)). Details on sampling methods, number of animals for each breed and population structure and differentiation (e.g. Principal component analysis, Admixture analysis) are reported in previous studies (Muñoz et al., 2019; Dadousis et al., 2022). All individuals were genotyped with the GGP-70K HD porcine chip containing 68 516 SNPs. Only autosomal SNPs with minor allele frequency ≥ 0.01 and individuals with less than 10% of missing genotypes were retained for population structure analyses.

To quantify the genetic distance of the 23 purebred populations, a pairwise F_{ST} (Fixation index) estimate on SNPs that passed the quality control for each combination of breeds was calculated using PLINK software (Chang et al., 2015) using the `--fst` flag.

Simulation of crossbred individuals and autozygosity detection

Beagle software (v.5.4) (Browning et al., 2021) was used for phasing genotypes of purebreds using default software parameters (i.e., burn-in = 3, iterations = 12, and number of model states used to estimate genotype phase = 280). A set of phased genotypes was created for each breed. Then, the following steps were applied for creating offspring genotypes (in pure-breeding or cross-breeding): (1) parents were sampled from the respective breed set, regardless of their sex; (2) two gametes were formed from each parent and autosome, as two strings of alleles in haploidy with length equal to the number of markers in that chromosome; (3) the probability of parental vs recombinant gametes as well as the region-specific recombination rate was generated following the map provided by Johnsson et al. (Johnsson et al., 2021), which was used to sample the number of recombination events as well as their location, per autosome; (4) one of the two gametes was chosen to be passed to the offspring, with equal probability; (5) zygotes for offspring were formed by pairing parents' haploid gametes, per autosome. All zygotic genotype samples were labeled with the breed combination of origin, sire, and dam, and then stacked in a single dataset for further analysis.

ROH were used to estimate autozygosity. ROH were detected with the R package *detectRUNS* v. 0.9.5 (Biscarini et al., 2019). The genomic data pruning was not applied for that analysis to better identify homozygous regions. The consecutive SNP-based detection method was preferred to avoid the detection of artificial ROH shorter than the window chosen (Marras et al., 2015). The parameters applied to detect a ROH were: (i) the minimum number of consecutive SNPs was set to 30; (ii) the minimum ROH length required was 1 Mbp; (iii) the maximum gap between consecutive homozygous SNPs was 1 Mbp; (iv) the maximum number of opposite genotypes in the run was set to 1; (v) the maximum

number of missing genotypes allowed was 1. The amount of ROH was compared between crossbreds and purebreds and tested through a Poisson regression model, where the dependent variable was the count of ROH and the independent variable was the type of crossbreeding (purebred or crossbred).

The genomic inbreeding (F_{ROH}) was calculated as suggested by McQuillan et al. (2008):

$$F_{ROH} = \frac{\sum L_{ROH}}{L_{genome}}$$

where $\sum L_{ROH}$ was the sum of the length of all ROH found in an individual, and L_{genome} was the total autosome genome length. The F_{ROH} was used to define the degree of autozygosity in each purebred (actual data) and in each pair of crossbred combinations (simulated data, as defined above).

Mapping the loss of autozygosity

Italian purebred pairs and their crossbreds were chosen to investigate potential regions involved in the loss of autozygosity. Italy was the country with more representative data because the highest number of local breeds here analyzed came from Italy ($n = 6$); in addition, several of these Italian breeds were already involved in crossbreeding while some others were reared in pure-breeding, exhibited shallow effective population sizes (Muñoz et al., 2019); this made mandatory to take into account crossbreeding as a possible strategy. The breeds included in this part of the work were Apulo Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano, and Sarda as local breeds, and Large White, Landrace, and Duroc as cosmopolitan breeds.

Each genomic region was tested for systematically losing autozygosity between purebreds and crossbreds through a logit logistic regression. The variables included in the model were defined as follows: a matrix $n \times m$ was built, where n corresponded to each offspring (27 600 rows) and m represented each marker (57 417 columns). Each SNP value was considered equal to 1 if it was included in any ROH, and 0 if it was not (dependent variable). The purebred offspring group were coded as 0 and the crossbred offsprings as 1 (independent variable). Consequently, the logit logistic regression applied was the following:

$$\text{logit}(p_i) = a + bX_i + e_{ilm}$$

where p_i is the probability of the i^{th} SNP on of an individual to belong to an ROH; a is the intercept; X is the l^{th} group, which was assigned 1 if the individual is a crossbred and 0 if the individual is a purebred; b is the regression coefficient, which connects the group with the probability of an SNP to be in an ROH.

Statistically significant loss of autozygosity was considered with a P-value less than 0.0001 to investigate the most important regions. Genomic segments surrounding each significant consecutive SNP, resulting from logistic regression, were mapped on the QTL pig database available at <https://www.animalgenome.org/cgi-bin/QTLdb/SS/index>. It has been decided to include 250 kbp upstream and downstream of each significant region in the QTL investigation. With this approach, QTLs that run out close to the beginning or start close to the end of the region analyzed were considered. The QTLs were listed to investigate which regions are characterized by a loss of autozygosity.

The practical and real scenario of crossbreeding

Some of the breeds included in this study were chosen to perform further analysis, i.e., to simulate as much as possible a real situation of breeding herds, where specific crossbreds were preferred, and a maternal line and a terminal line were defined. The sex of the

genotype pigs was inferred using PLINK 1.9 software –check-sex and --impute-sex functions with default parameters (Chang et al., 2015): these functions compare sex assignments in the dataset with those imputed from X chromosome inbreeding coefficients and values smaller than 0.2 are reported as females, and values larger than 0.8 were evaluated males. Individuals assigned to males or females have been retained for the crosses. In this step, the zygote simulation process described above was modified such that, in step 1, parents were sampled conditionally on their sex. Different scenarios were simulated: (i) crossbreds where local breeds were used as a maternal line, i.e., Duroc \times Cinta Senese and Duroc \times Mora Romagnola; (ii) crossbreds where local breeds were used as a terminal line, i.e., Cinta Senese \times Landrace, Cinta Senese \times Large White, Mora Romagnola \times Landrace and Mora Romagnola \times Large White. Matings were drawn such that all the males and all females were represented as parents of the offspring generated.

Results

Animal sampling and population structure investigation

The number of animals and SNPs which passed the quality control (QC) are reported in Table 1. Only six animals were excluded from the analysis for low data quality. The number of SNPs that passed the QC ranged from 52 863 in SAR to 53 801 in TUR. These markers were used to calculate the pairwise F_{ST} .

Values of F_{ST} that tend towards 0 mean complete sharing of genetic material, while values that tend towards 1 mean a complete divergence between populations. The pairwise F_{ST} detected here ranged from 0.03 to 0.29. From the heatmap in Fig. 1, it is clear that the Mora Romagnola and Turopolje breeds showed extreme levels of differentiation with F_{ST} values larger than 0.18 in all comparisons. Mora Romagnola had higher estimates with Turopolje,

Table 1
Number of Single Nucleotide Polymorphisms (SNPs) and pigs after the quality control for each breed.

Breed	N.SNPs	N.Animals
ALE	53 436	48
APU	53 283	53
BAS	53 463	39
BLA	53 279	49
BIS	52 999	48
CAS	53 338	53
CIN	53 472	53
DUR	52 991	53
GAS	53 336	48
IBE	53 434	48
KRS	53 305	52
LAN	53 382	52
LAR	53 254	52
LIN	53 164	48
LIW	53 221	48
MAJ	53 085	48
MOR	53 504	48
MKA	53 309	50
NER	53 334	48
SAR	52 863	48
SCH	53 261	49
SWM	53 648	50
TUR	53 801	50

Abbreviations: ALE = Alentejana; APU = Apulo Calabrese; BAS = Basque; BLA = Black Slavonian; BIS = Bisara; CAS = Casertana; CIN = Cinta Senese; DUR = Duroc; GAS = Gascon; IBE = Iberian; KRS = Krskopolje; LAN = Landrace; LAR = Large White; LIN = Lithuanian Native; LIW = Lithuanian White Old Type; MAJ = MajorcanBlack; MOR = Mora Romagnola; MKA = Moravka; NER = Nero Siciliano; SAR = Sarda; SCH Schwäbisch-Hällisches Schwein; SWM = Swallow-Bellied Mangalitsa; TUR = Turopolje.

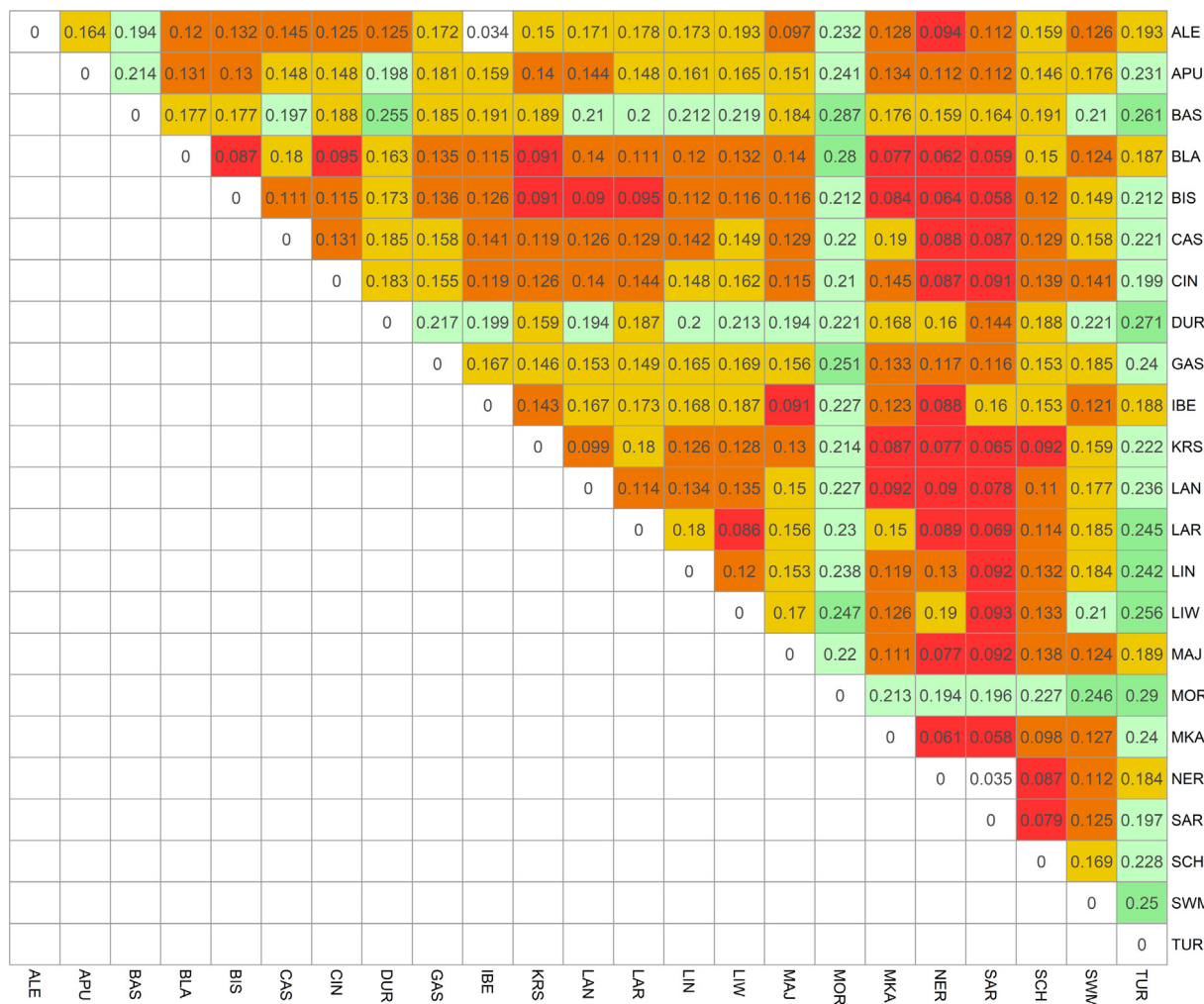


Fig. 1. Heatmap of pairwise Fixation index (F_{ST}) estimates of each pig crossbred, where ALE = Alentejana; APU = Apulo Calabrese; BAS = Basque; BLA = Black Slavonian; BIS = Bisara; CAS = Casertana; CIN = Cinta Senese; DUR = Duroc; GAS = Gascon; IBE = Iberian; KRS = Krskopolje; LAN = Landrace; LAR = Large White; LIN = Lithuanian Native; LIW = Lithuanian White Old Type; MAJ = Majorcan Black; MOR = Mora Romagnola; MKA = Moravka; NER = Nero Siciliano; SAR = Sarda; SCH Schwäbisch-Hällisches Schwein; SWM = Swallow-Bellied Mangalitsa; TUR = Turopolje.

Basque, and Black Slavonian ($F_{ST} \sim 0.29$) and lower values with two Italian local breeds, Sarda and Nero Siciliano, with F_{ST} equal to 0.19, which demonstrated a high degree of divergence between populations. Turopolje resulted in F_{ST} values close to 0.19 when compared with Duroc and with three local breeds, Black Slovenian, Majorcan Black, and Nero Siciliano; for the rest of the populations, greater values have been detected. Nero Siciliano and Sarda were the two breeds that were more distant between them and the other pig populations. Generally, they had values close to 0.10, which decreased to 0.05–0.06 with Black Slavonian, Bisara, Krskopolje, and Moravka. The pairwise F_{ST} in Alentejana – Iberian showed the lowest values found in this study (0.03).

Simulation of crossbred individuals and autozygosity detection

In total, 27 600 crossbred individuals were simulated based on 68 516 SNPs. The number of ROH detected with the parameters set ranged from 17 532 in Duroc, followed by Basque ($n = 17 020$) and Alentejana ($n = 14 935$) purebreds, to $\sim 4 500$ in Iberian \times Landrace, Alentejana \times Large White and Duroc \times Schwäbisch-Hällisches Schwein crossbreds (Supplementary Table S1). Fig. 2 shows the ROH distribution reported for purebreds and crossbreds. The amount of ROH between crossbreds and purebreds results statistically sig-

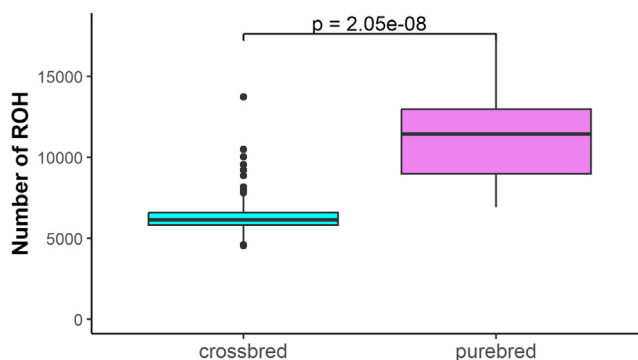


Fig. 2. Number of Runs of Homozygosity (ROH) distribution in pig purebreds and crossbreds.

nificant, with a P -value equal to 2.06×10^{-8} . Indeed, it is evident that autozygosity plunged in crossbred animals; nevertheless, it was not gotten rid of since autozygosity persisted, and some combinations of crossbreds with high autozygosity were identified as outliers.

Also, according to chromosome distribution, the ROH number was evaluated between purebreds and crossbreds (Supplementary

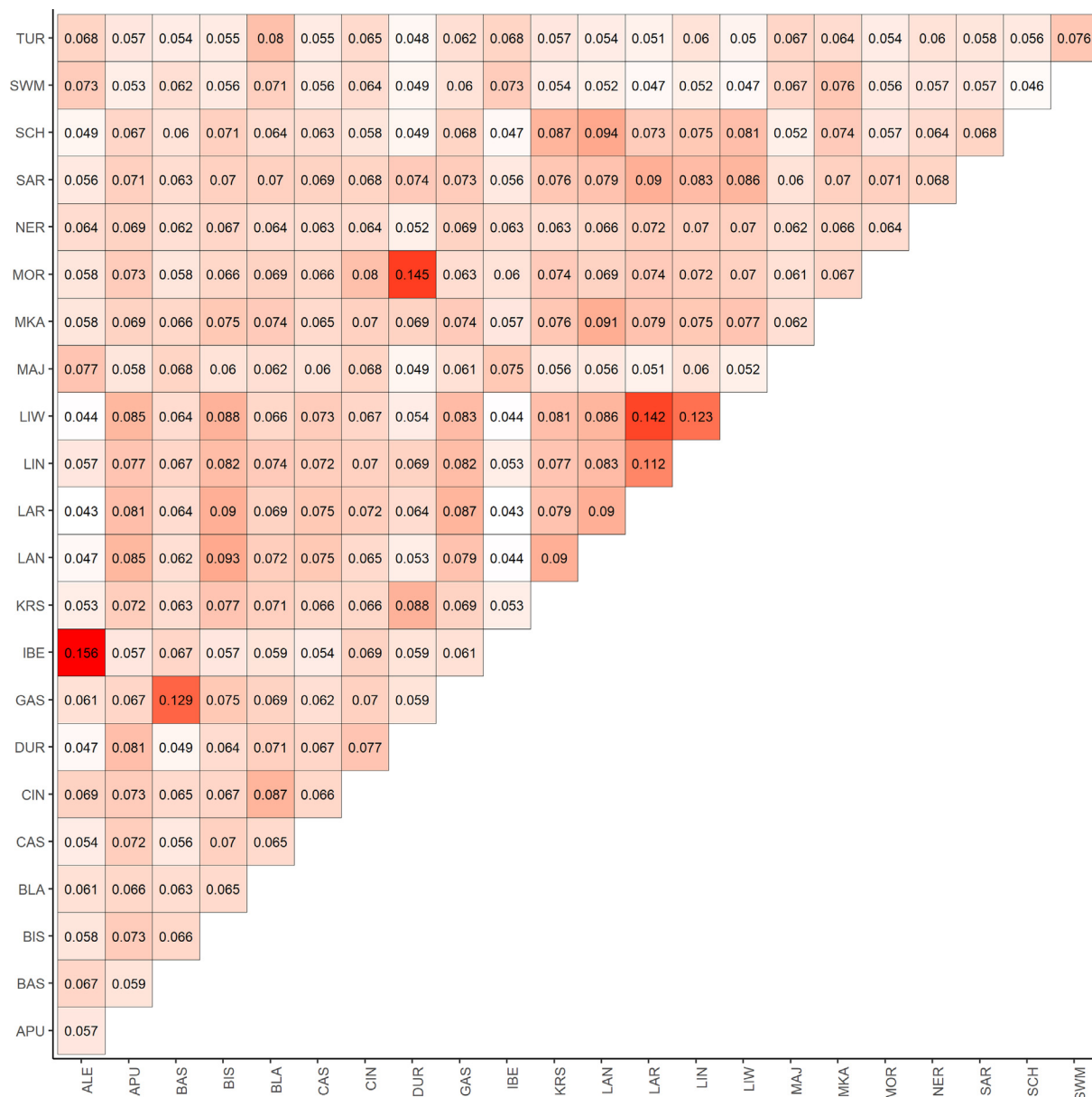


Fig. 3. Heatmap of pairwise genomic inbreeding (F_{ROH}) estimates in pig crossbreds, where ALE = Alentejana; APU = Apulo Calabrese; BAS = Basque; BLA = Black Slavonian; BIS = Bisara; CAS = Casertana; CIN = Cinta Senese; DUR = Duroc; GAS = Gascon; IBE = Iberian; KRS = Krskopolje; LAN = Landrace; LAR = Large White; LIN = Lithuanian Native; LIW = Lithuanian White Old Type; MAJ = Majorcan Black; MOR = Mora Romagnola; MKA = Moravka; NER = Nero Siciliano; SAR = Sarda; SCH Schwäbisch-Hällisches Schwein; SWM = Swallow-Bellied Mangalitsa; TUR = Turopolje.

Figs. S1 and S2). What appeared evident was the presence of outliers in this case, too, in crossbreds compared to purebreds; this means that the individual variability within-group was considerable and/or that some crossbreds provided higher estimates of autozygosity compared to the mean of the other combinations. In general, the trend of autozygosity was similar in the two groups across chromosomes, although purebreds showed higher ROH numbers. *Sus scrofa* chromosomes most covered by ROH were autosomes 1, 6, 13, and 14.

Although the autozygosity level varied among breed combinations, these levels were much lower than those found in simulated purebred offspring. Indeed, the highest averaged values were found for Mora Romagnola ($F_{ROH} \sim 0.50$) and Turopolje ($F_{ROH} = 0.47$) purebreds, followed by Basque (0.43). Four were the combinations with greater values of averaged autozygosity, namely Alentejana vs Iberian ($F_{ROH} = 0.16$), Duroc vs Mora Romagnola, and Large White vs Lithuanian White Old Type ($F_{ROH} = 0.14$ for both), and Basque

vs Gascon ($F_{ROH} = 0.13$) (Fig. 3, Supplementary Table S2). From the heatmap in Fig. 3, it is easy to characterize the breed combinations that created the lowest values of F_{ROH} , identifying the best theoretical crossbred. The combinations which produced autozygosity lower than 0.05 were ALE_LAR, IBE_LAR, IBE_LAN, IBE_LIW, ALE_LIW, SCH_SWM, LAR_SWM, LIW_SWM and IBE_SCH. Interestingly, Iberian, Alentejana, and Large White were often involved in the crossbreds mentioned above with lower F_{ROH} , and this makes sense because the Iberian and Alentejana breeds are phenotypically divergent from the Large White, and it is well known that there is no introgression of Large White alleles into these breeds.

Mapping the loss of autozygosity

A logistic regression was performed for specific pairs of crossbreds compared to purebreds to test which genomic regions could be involved in the loss of autozygosity if that crossbreeding was

used. The populations chosen were Italian local populations and the cosmopolitan breeds (Large White, Landrace, Duroc). Many significant SNPs (P -value < 0.0001) resulted between crossbreds and purebreds. Each potential region of interest established by consecutive significant SNPs was investigated for the presence of QTL, which was done for each crossbred combination. The complete list of QTLs found is reported in [Supplementary Table S3](#); 710 QTLs have been identified. [Table 2](#) describes the number of QTLs in the regions identified from the applied methodology for each evaluated crossbred. When Landrace is used, it can highlight in the detected regions the highest number of QTLs identified if crossed with Apulo Calabrese, Nero Siciliano, and Sarda; Large White vs Sarda presented loss of autozygosity in regions containing 53 QTLs, while Cinta Senese resulted as the cross with greater QTLs when Duroc was used.

[Table 3](#) represents common QTLs identified among local \times cosmopolitan crossbreds in genomic regions that probably would be involved in the loss of autozygosity. When Large White was used, four QTL were common to all the six combinations with local breeds. If Landrace was considered, we found six QTLs in common, while crossbreds with Duroc showed no QTLs shared between all the six combinations. QTLs found in common between crosses of LAN or LAR with local breeds were related to fatty acid content and growth, which means that a loss of autozygosity characterized these traits.

Practical scenario of crossbreeding

The number of individuals predicted as males, females, and unknown is reported in [Table 4](#). The sex prediction allowed to elaborate a different number of possible mating combinations for each crossbred due to the number of males and females being different for each purebred included in this study. The number of possible mating combinations elaborated was consequently 440 for CIN vs LAR (10 males \times 44 females, respectively), 470 for CIN vs LAN (10 males \times 47 females, respectively), 616 for MOR vs LAR (14 males \times 44 females, respectively), 658 for MOR vs LAN (14 males \times 47 females, respectively), 1 600 for DUR vs CIN (50 males \times 32 females, respectively), and 1 450 for DUR vs MOR (50 males \times 29 females, respectively).

When sex was considered, a higher mean of F_{ROH} was detected in all the six crossbreds analyzed. The greatest variability was observed in Mora Romagnola when used as a terminal maternal line with other male breeds (MOR_LAN, MOR_LAR). Indeed, F_{ROH} passed from \sim 0.07 ([Supplementary Table S2](#), [Fig. 4](#)) to \sim 0.10 if Mora Romagnola was crossed with Landrace and Large White, respectively. In the other four crossbreds, the differences between F_{ROH} were smaller. [Fig. 4](#) underlines the F_{ROH} distribution, and some matings should be avoided because they may increase autozygosity, and others should be preferred because they led to lower values of F_{ROH} . From that, it is clear that even if crossbred-

Table 2

Quantitative Traits loci (QTLs) identified for each cross in pigs, located in statistically significant regions characterized by loss of autozygosity (P -value < 0.0001).

Breed	N. QTLs		
	DUR	LAN	LAR
APU	29	53	45
CAS	14	39	30
CIN	52	45	31
MOR	19	31	31
NER	30	54	50
SAR	34	65	53

Abbreviations: APU = Apulo Calabrese; CAS = Casertana; CIN = Cinta Senese; DUR = Duroc; LAN = Landrace; LAR = Large White; MOR = Mora Romagnola; NER = Nero Siciliano; SAR = Sarda.

ing decreases autozygosity as expected, individual variability is an essential factor to consider.

Discussion

A few simulation studies on crossbreds derived from local livestock breeds have mainly focused on the features of the final products that can increase profitability ([Tsukahara et al., 2011](#); [Stock et al., 2021](#)). Previous studies have investigated the 23 pig breeds included in this study at the genome level and obtained information on their genetic architecture and distance. Taking into account the genetic parameters underlined by previous authors ([Muñoz et al., 2019](#); [Schiavo et al., 2021](#); [Dadousis et al., 2022](#)), crossbreeding could be hypothesized as a method to encourage farmers to rear local breeds, indirectly minimizing autozygosity; but it is first necessary to estimate how much autozygosity would decrease depending on the breed combination. This question was the motivation for this study; the question was reinforced because it was found a persistence of autozygosity in pig crosses, also if local breeds were used ([Tao et al., 2023](#)). It complements the previous studies by giving information for a practical application, namely, using crossbreeding in pigs. A persistence in autozygosity has been revealed, especially in specific crossbred combinations. This could be for many reasons. First of all, some breeds showed high levels of linkage disequilibrium (LD) in pure-breeding, as [Muñoz et al. \(2019\)](#) demonstrated, but to be the unique cause of persistence, LD should be in both breeds used in crossbreeding and, in the same measure. We found some genomic segments involved in the loss of autozygosity in more than one crossbred, suggesting that ancestral haplotypes could be maintained in the populations because associated with natural or artificial selection or because of the introgression by recent admixtures between pig populations. Indeed, we have to consider ascertaining biases in the design of the single nucleotide polymorphism genotyping tools, considering that the SNP chip is built on commercial breeds.

Animal sampling and population structure investigation

F_{ST} statistic measures the extent of genetic differentiation between breeds/populations. The F_{ST} estimates here detected confirmed, with some exceptions, the population structure described by [Muñoz et al. \(2019\)](#), [Bovo et al. \(2020\)](#), and [Dadousis et al. \(2022\)](#), who used other approaches to characterize these pig populations. Principal component analysis, admixture, and other approaches ([Muñoz et al., 2018](#); [Bovo et al., 2020](#); [Dadousis et al., 2022](#)) identified Duroc and Mora Romagnola as the breeds that diverged most from the rest of the analyzed samples; here, Mora Romagnola, Duroc but also Turopolje, followed by Bisara, were the pure breeds that seemed to be more divergent. The closeness between Duroc and Mora Romagnola was not revealed by F_{ST} analysis, while the similarity between Large White and Lithuanian breeds was confirmed. In agreement with admixture analysis, ([Dadousis et al., 2022](#)) and F_{ST} based on whole-genome sequencing ([Bovo et al., 2020](#)), Sarda and Nero Siciliano showed low values of pairwise F_{ST} , confirming that a significant level of introgression derived by the same cosmopolitan breeds characterizes these two populations. However, it is important to remember that the pairwise F_{ST} detected here ranged from 0.03 to 0.30, reporting values very far from 1, the maximum degree of population differentiation. Nevertheless, [Hall \(2022\)](#) identified the pairwise F_{ST} benchmark range for SNP data between 0.15 and 0.22, indicating breed differentiation. Here, only 31 of 276 unique combinations had pairwise F_{ST} estimates up to 0.22 (dark green in [Fig. 1](#)), confirming that a proportion of ancestry is shared among the studied breeds.

Table 3

. Quantitative Traits loci (QTLs) name and unique ID located in genomic regions where crossbred individuals show significantly lower autozygosity than pig purebreds (P-value < 0.0001).

CHR	Start Position	End Position	QTL name	QTL ID	Cosmopolitan breed used
7	16929995	16929999	Mean corpuscular volume QTL	127949	LAN
7	17049495	17049499	Saturated fatty acid content QTL	101869	
14	122040584	122040588	Palmitic acid content QTL	131811	
14	122937626	122937630	Oleic acid content QTL	133928	
9	7560120	7560124	Average daily gain QTL	194101	LAR
9	7629457	7629461	Feed conversion ratio QTL	139990	
14	117949585	117949589	Stearic acid content QTL	133426	
14	117949585	117949589	Palmitoleic acid content QTL	132790	

Abbreviations: CHR = Chromosome; LAN = Landrace; LAR = Large White.

Table 4

Number of imputed male, female and unknown individuals on the five pig breeds chosen to simulate practical scenarios in Italy.

Breed	N.Male	N.Female	N.Unknown sex
CIN	10	32	0
MOR	14	29	5
DUR	50	3	0
LAN	0	47	5
LAR	4	44	4

Abbreviations: CIN = Cinta Senese; DUR = Duroc; LAN = Landrace; LAR = Large White; MOR = Mora Romagnola.

Low differentiation was expected between Iberian and Alentejana, as also demonstrated by previous authors from genetic distance analyses reported with SNP chip data (Muñoz et al., 2019)

and whole-genome sequencing data (Bovo et al., 2020) for the same breeds. In addition, the pairwise F_{ST} has been previously calculated based on 32 polymorphisms located on 26 major genes for these two breeds, and the values were very similar to those found in this study (0.030 and 0.034, respectively) (Muñoz et al., 2018). This supports the hypothesis that Alentejana and Iberian are genetically highly close.

Simulation of crossbred individuals and autozygosity detection

Broman and Weber (1999) were the first authors to suggest that ROHs are likely to be autozygous and, although ROH can arise for several reasons, the primary cause of ROH is believed to be inbreeding. Consequently, in these last years, ROH has become almost a gold standard for investigating genomic inbreeding.

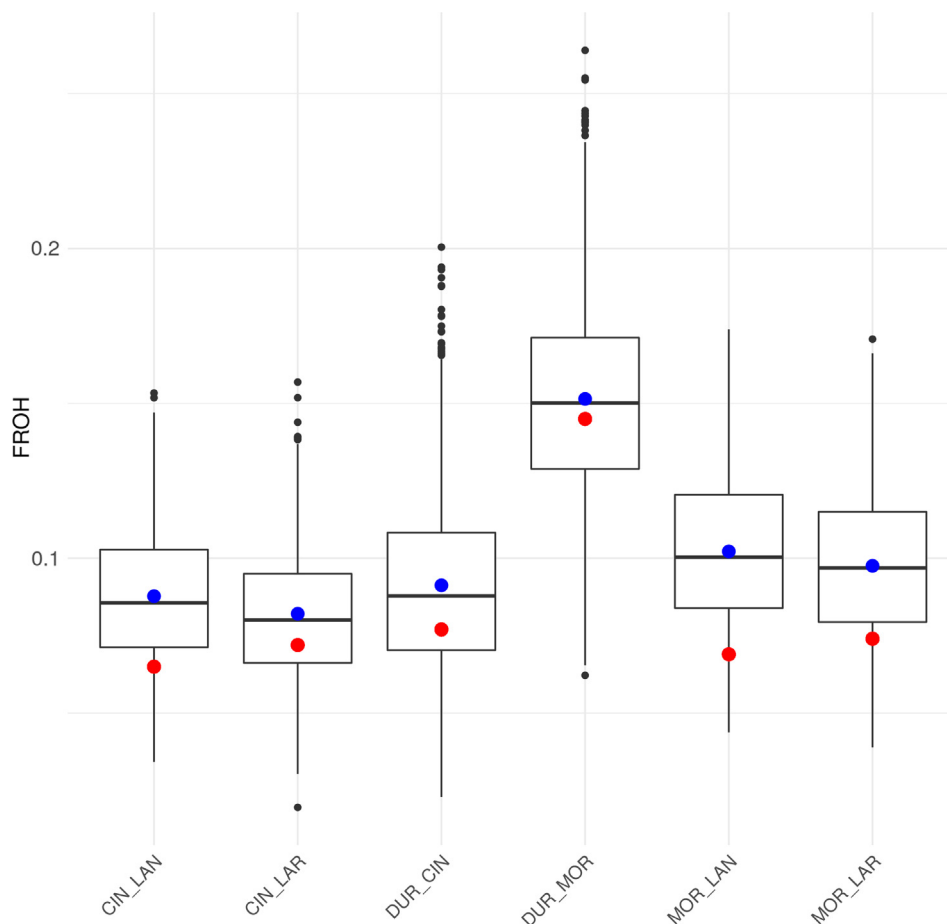


Fig. 4. Genomic inbreeding distribution (F_{ROH}) of each combination of crosses in pigs, where CIN = Cinta Senese; DUR = Duroc; LAN = Landrace; LAR = Large White; MOR = Mora Romagnola. The red line was the mean of population when the entire data were used, in blue, the line of the real averaged F_{ROH} if crossbreds would be performed.

Inbreeding increase is unavoidable in populations under selection, as only a subset of individuals is used for breeding, but also in local breeds where artificial insemination is not widely used, and the effective population size can be extremely reduced. Therefore, describing what happens to autozygosity if crossbreeding is used between local and cosmopolitan purebreds could be interesting. Howard et al. (2016) showed that long stretches of ROH present in the parents persist in crossbred animals, but these authors simulated crossbreds including only cosmopolitan breeds, i.e., Large White, Landrace, and Duroc.

In the present study, the autozygosity falls strongly in most crossbreds. The maximum value of F_{ROH} in purebreds was ~ 0.50 found in Mora Romagnola and Turopolje, while the minimum estimate was 0.09 for Sarda and Nero Siciliano. On the other hand, the maximum F_{ROH} in crossbreds was 0.15, and the minimum value was 0.04. These results confirm our hypothesis of a significantly lower average genomic inbreeding in crosses between local and cosmopolitan pig breeds. The extent by which the average autozygosity decreased depended on the breeds crossed, but it generally was in line with the genetic distance between breeds used as parents (Fig. 1). Interestingly, we found that the pairwise combination of breeds that showed the highest level of F_{ST} was different from the combination that produced the lowest F_{ROH} . The decrease in autozygosity was likely related to phenotypic traits. The crossbreds with the lowest F_{ROH} were the combinations to one hand of Large White, Landrace, Lithuanian White Old Type or Schwäbisch-Hällisches Schwein and, on the other hand, Iberian, Alentejana or Swallow-Bellied Mangalitsa. Practically, white pig vs black pig breeds. Worthy of attention is that some of the purebreds mentioned above showed high estimates of autozygosity (Supplementary Table S2 in decreasing order: Swallow-Bellied Mangalitsa, Lithuanian White Old Type, Large White, Alentejana, Landrace, Schwäbisch-Hällisches Schwein, Iberian were purebreds with greater F_{ROH} , respectively), but if they were used in specific crossbreds, they contributed to produce the lowest autozygosity.

Mapping the loss of autozygosity

Artificial selection has resulted in phenotypic changes in domestic pigs for some traits, including behavior, body composition, reproduction, and coat color. Consequently, some genomic regions and haplotypes are fixed in the populations. Crossbred animals represent a mosaic of genomic regions inherited from different breeds, and depending on which one a SNP-allele was inherited, it could have different effects (Sevillano et al., 2019). This is because QTLs may be in linkage disequilibrium with different SNPs depending on which parental breed the QTL was inherited; in addition, some alleles did not segregate in purebreds but could segregate in crossbreds. Here, it has been possible to simulate which genomic regions might be interested in the loss of autozygosity in crossbred cosmopolitan \times local breeds. Only duplicated QTLs have been described and analyzed between Landrace/Large White/Duroc vs Apulo Calabrese/Casertana/Cinta Senese/Mora Romagnola/Nero Siciliano and Sarda.

No QTLs located in regions where autozygosity was potentially lost were found in common in the different crossbreds when Duroc was the cosmopolitan breed used. Genes undergoing selective pressure were highlighted as related to the fast growth rate and the high lean ratio of Duroc (Yu et al., 2020). However, it is also true that Dadousis et al. (2022) found that in an unsupervised scenario of cross-validation, Duroc was assigned to Sarda, defining these breeds more similar to each other, causing fewer lost regions than expected. For Large White and Landrace, two groups of QTLs in regions potentially lost in autozygosity were defined: fatty acid content-related and growth-related traits. These latter results align with other studies, e.g., it has been determined that Large White

showed growth rate, feed efficiency, and backfat depth higher than the local pig breed analyzed (Basque) (Alfonso et al., 2005). Another finding is that Apulo Calabrese was characterized by reduced growth and carcass performance (Aboagye et al., 2020), and if crossed, it could increase the heterozygosity in those regions that cosmopolitan breeds have under selection. Several studies highlighted that local pig breeds showed a different fatty acid composition than cosmopolitan pig breeds, which is well described in the review by Poklucar et al. (2020). So, discussing the second group of QTLs identified, i.e., fatty acid content and intramuscular fat, it is important to take into account that intramuscular fat (IMF) content is highly correlated with the sensory acceptability of pork and with several other traits like water holding capacity and tenderness, and breeding of modern pig breeds resulted in lower intramuscular fat deposition. Indeed, a higher IMF was found in the local Pulawska pig breed than in Polish Large White pigs (Wojtysiak and Połtowicz, 2014) and Korean native black pigs, compared to Landrace (Park et al., 2007). Finally, oleic and palmitic fatty acids seemed to be higher in local breeds compared to cosmopolitan breeds (Poklucar et al., 2020), while stearic fatty acid was mainly detected in Large White and Landrace than in other cosmopolitan breeds (Popova et al., 2020), confirming the reasons why these QTLs were associated with the regions of loss of autozygosity.

Practical scenario of crossbred

The analysis of a more practical and real situation was performed because of the known positive effect of crossbreeding, but it is fundamental to include also the within-breed (and crossbred) variability to investigate the loss of autozygosity in a pool of chosen crossbreds. This is because a great within-breed variability generally characterizes local breeds, but even within farms (Fabbri et al., 2020). This makes it challenging to identify which crossbred indeed minimizes autozygosity because, as Fig. 4 identified, some offsprings reported considerably higher autozygosity than the mean, and others showed interesting low values of autozygosity. These latter simulated individuals should be taken into account in a real mating scheme. In addition, from this analysis, it seemed that Cinta Senese should be used both as a boar in crossbreds with white pig breeds (Landrace and Large White) and as a maternal line. Mora Romagnola provided a great inbreeding level in both approaches, suggesting that this breed is involved in a wide autozygosity even if crossed with the Duroc breed, although the F_{ST} estimate of their crosses was high.

Conclusion

Crossbreeding could be hypothesized as a method to minimize autozygosity. Twenty-three pig breeds were crossed, and autozygosity for each combination was evaluated. Inbreeding coefficients were lower in crossbreds than in purebreds, except for Alentejana vs Iberian, Duroc vs Mora Romagnola, Large White vs Lithuanian White Old Type, and Basque vs Gascon, which showed the greatest estimates; this is in line with the genetic distances between breeds. Specific QTL regions resulted involved in the loss of autozygosity, namely those regions under positive selection in cosmopolitan breeds and regions related to rusticity in local breeds. The results obtained in this study represent a valuable tool for valorizing local pig biodiversity and for elaborating mating schemes with a well-defined background of these populations and their simulated crossbreds.

Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2023.101070>.

Ethics approval

Blood samples were collected from each institution by specialized professionals, following standard guidelines. No interventions with animals were applied that would require ethical protocols (according to Directive 2010/63/EU-2010).

Data and model availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its [supplementary materials](#). The raw genetic datasets generated during the current study are available from the corresponding author upon reasonable request. The data were not deposited in an official repository.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

None.

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References

- Aboagye, G., Zappaterra, M., Pasini, F., Dall'Olio, S., Davoli, R., Nanni Costa, L., 2020. Fatty acid composition of the intramuscular fat in the longissimus thoracis muscle of Apulo-Calabrese and crossbred pigs. *Livestock Science* 232, 103878.
- Alfonso, L., Mourot, J., Insausti, K., Mendizabal, J.A., Arana, A., 2005. Comparative description of growth, fat deposition, carcass and meat quality characteristics of Basque and Large White pigs. *Animal Research* 54, 33–42.
- Biscarini, F., Cozzi, P., Gaspa, G., Marras, G., 2019. detectRUNS: Detect Runs of Homozygosity and Runs of Heterozygosity in Diploid Genomes. [accessed on 18 December 2023];CRAN (The Compr. R Arch. Network) 2018 Available online: <https://rdrr.io/cran/detectRUNS/f/vignettes/detectRUNS.vignette.Rmd>.
- Bovo, S., Ribani, A., Muñoz, M., Alves, E., Araujo, J.P., Bozzi, R., Čandek-Potokar, M., Charneca, R., Di Palma, F., Etherington, G., Fernandez, A.I., García, F., García-Casco, J., Karolyi, D., Gallo, M., Margeta, V., Martins, J.M., Mercat, M.J., Moscatelli, G., Núñez, Y., Quintanilla, R., Radović, Č., Razmaite, V., Riquet, J., Savić, R., Schiavo, G., Usai, G., Utzeri, V.J., Zimmer, C., Ovilo, C., Fontanesi, L., 2020. Whole-genome sequencing of European autochthonous and commercial pig breeds allows the detection of signatures of selection for adaptation of genetic resources to different breeding and production systems. *Genetics Selection Evolution* 52, 33.
- Broman, K.W., Weber, J.L., 1999. Long homozygous chromosomal segments in reference families from the centre d'Etude du polymorphisme humain. *American Journal of Human Genetics* 65, 1493–1500.
- Browning, B.L., Tian, X., Zhou, Y., Browning, S.R., 2021. Fast two-stage phasing of large-scale sequence data. *American Journal of Human Genetics* 108, 1880–1890.
- 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.
- Christensen, O.F., Legarra, A., Lund, M.S., Su, G., 2015. Genetic evaluation for three-way crossbreeding. *Genetics Selection Evolution* 47, 98.
- Dadousis, C., Muñoz, M., Óvilo, C., Fabbri, M.C., Araujo, J.P., Bovo, S., Potokar, M.Č., Charneca, R., Crovetto, A., Gallo, M., García-Casco, J.M., Karolyi, D., Kušec, G., Martins, J.M., Mercat, M.-J., Pugliese, C., Quintanilla, R., Radović, Č., Razmaite, V., Ribani, A., Riquet, J., Savić, R., Schiavo, G., Škrlep, M., Tinarelli, S., Usai, G., Zimmer, C., Fontanesi, L., Bozzi, R., 2022. Admixture and breed traceability in European indigenous pig breeds and wild boar using genome-wide SNP data. *Scientific Reports* 12, 1–13.
- Dragomir, L., 2013. Reproductive traits in relation to crossbreeding in pigs. *African Journal of Agricultural Research* 8, 2166–2171.
- Duenk, P., Bijma, P., Wientjes, Y.C.J., Calus, M.P.L., 2021. Review: optimizing genomic selection for crossbred performance by model improvement and data collection. *Journal of Animal Science* 99, skab205.
- Fabbri, M.C., Dadousis, C., Bozzi, R., 2020. Estimation of linkage disequilibrium and effective population size in three Italian autochthonous beef breeds. *Animals* 10, 1034.
- Franci, O., Bozzi, R., Pugliese, C., Acciaioli, A., Campodoni, G., Gandini, G., 2005. Performance of Cinta Senese pigs and their crosses with Large White. 1 Muscle and subcutaneous fat characteristics. *Meat Science* 69, 545–550.
- Franci, O., Pugliese, C., Acciaioli, A., Bozzi, R., Campodoni, G., Sirtori, F., Pianaccioli, L., Gandini, G., 2007. Performance of Cinta Senese pigs and their crosses with Large White 2. Physical, chemical and technological traits of Tuscan dry-cured ham. *Meat Science* 76, 597–603.
- Ganteil, A., Rodriguez-Ramilo, S.T., Ligonesche, B., Larzul, C., 2021. Characterization of autozygosity in pigs in three-way crossbreeding. *Frontiers in Genetics* 11, 584556.
- Gomez-Raya, L., Rauw, W.M., Dunkelberger, J.R., Dekkers, J.C.M., 2019. Autozygosity and genetic differentiation of landrace and large white pigs as revealed by the genetic analyses of crossbreds. *Frontiers in Genetics* 10, 739.
- Hall, S.J.G., 2022. Genetic Differentiation among Livestock Breeds—Values for Fst. *Animals* 12, 1115.
- Howard, J.T., Tiezzi, F., Huang, Y., Gray, K.A., Maltecca, C., 2016. Characterization and management of long runs of homozygosity in parental nucleus lines and their associated crossbred progeny. *Genetics Selection Evolution* 48, 91.
- Johnsson, M., Whalen, A., Ros-Freixedes, R., Gorjanc, G., Chen, C.-Y., Herring, W.O., de Koning, D.-J., Hickey, J.M., 2021. Genetic variation in recombination rate in the pig. *Genetics Selection Evolution* 53, 54.
- Marras, G., Gaspa, G., Sorbolini, S., Dimauro, C., Ajmone-Marsan, P., Valentini, A., Williams, J.L., Macciotta, N.P.P., 2015. Analysis of runs of homozygosity and their relationship with inbreeding in five cattle breeds farmed in Italy. *Animal Genetics* 46, 110–121.
- McQuillan, R., Leutenegger, A.-L., Abdel-Rahman, R., Franklin, C.S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., Macleod, A.K., Farrington, S.M., Rudan, P., Hayward, C., Vitart, V., Rudan, I., Wild, S.H., Dunlop,

- M.G., Wright, A.F., Campbell, H., Wilson, J.F., 2020. Runs of homozygosity in European populations. *American Journal of Human Genetics* 83, 359–372.
- Muñoz, M., Bozzi, R., García, F., Núñez, Y., Geraci, C., Crovetto, A., García-Casco, J., Alves, E., Škrlep, M., Charneca, R., Martins, J.M., Quintanilla, R., Tibau, J., Kušec, G., Djurkin-Kušec, I., Mercat, M.J., Riquet, J., Estellé, J., Zimmer, C., Razmaite, V., Araujo, J.P., Radović, Č., Savić, R., Karolyi, D., Gallo, M., Čandek-Potokar, M., Fontanesi, L., Fernández, A.L., Óvilo, C., 2018. Diversity across major and candidate genes in European local pig breeds. *PLOS ONE* 13, e0207475.
- Muñoz, M., Bozzi, R., García-Casco, J., Núñez, Y., Ribani, A., Franci, O., García, F., Škrlep, M., Schiavo, G., Bovo, S., Utzeri, V.J., Charneca, R., Martins, J.M., Quintanilla, R., Tibau, J., Margeta, V., Djurkin-Kušec, I., Mercat, M.J., Riquet, J., Estellé, J., Zimmer, C., Razmaite, V., Araujo, J.P., Radović, Č., Savić, R., Karolyi, D., Gallo, M., Čandek-Potokar, M., Fernández, A.L., Fontanesi, L., Óvilo, C., 2019. Genomic diversity, linkage disequilibrium and selection signatures in European local pig breeds assessed with a high density SNP chip. *Scientific Reports* 9, 13546.
- Ortiz, A., Tejerina, D., García-Torres, S., Gaspar, P., González, E., 2021. Performance and carcass quality traits of Iberian × Duroc crossbred pig subject to gender and age at the beginning of the free-range finishing phase. *Animal* 15, 100324.
- Park, B.Y., Kim, N.K., Lee, C.S., Hwang, I.H., 2007. Effect of fiber type on postmortem proteolysis in longissimus muscle of Landrace and Korean native black pigs. *Meat Science* 77, 482–491.
- Peripolli, E., Munari, D.P., Silva, M.V.G.B., Lima, A.L.F., Irgang, R., Baldi, F., 2017. Runs of homozygosity: current knowledge and applications in livestock. *Animal Genetics* 48, 255–271.
- Poklukar, K., Čandek-Potokar, M., Batorek Lukač, N., Tomažin, U., Škrlep, M., 2020. Lipid deposition and metabolism in local and modern pig breeds: A review. *Animals* 10, 424.
- Popova, T., Nakev, J., Rodriguez, J.M.L., 2020. Quality of meat in purebred pigs involved in crossbreeding schemes. II. Fatty acid composition of m. Longissimus thoracis. *Bulgarian Journal of Agricultural Science* 26, 1292–1296.
- Praew Thiengpimol, S.T., 2017. Reproductive performance of purebred and crossbred landrace and large white sows raised under Thai commercial swine herd. *Thammasat International Journal of Science and Technology* 22, 2.
- Schiavo, G., Bovo, S., Muñoz, M., Ribani, A., Alves, E., Araújo, J.P., Bozzi, R., Čandek-Potokar, M., Charneca, R., Fernandez, A.L., Gallo, M., García, F., Karolyi, D., Kušec, G., Martins, J.M., Mercat, M.-J., Núñez, Y., Quintanilla, R., Radović, Č., Razmaite, V., Riquet, J., Savić, R., Usai, G., Utzeri, V.J., Zimmer, C., Óvilo, C., Fontanesi, L., 2021. Runs of homozygosity provide a genome landscape picture of inbreeding and genetic history of European autochthonous and commercial pig breeds. *Animal Genetics* 52, 155–170.
- Sevillano, C.A., Bovenhuis, H., Calus, M.P.L., 2019. Genomic evaluation for a crossbreeding system implementing breed-of-origin for targeted markers. *Frontiers in Genetics* 10, 418.
- Sørensen, M.K., Norberg, E., Pedersen, J., Christensen, L.G., 2008. Invited Review: Crossbreeding in dairy cattle: A Danish perspective. *Journal of Dairy Science* 91, 4116–4128.
- Stock, J., Esfandiyari, H., Hinrichs, D., Bennewitz, J., 2021. Implementing a genomic rotational crossbreeding scheme to promote local dairy cattle breeds—A simulation study. *Journal of Dairy Science* 104, 6873–6884.
- Tao, L., Wang, L.-G., Adeola, A.C., Zhang, L.-C., Li, L.-W., Li, Q.-L., Cen, D.-J., Yan, C., Ma, Z.-S., Wang, L.-X., Xie, H.-B., Zhang, Y.-P., 2023. Associations of autozygosity with economic important traits in a cross of Eurasian pigs. *Journal of Genetics and Genomics* 50, 216–220.
- Tsukahara, Y., Oishi, K., Hirooka, H., 2011. Development and application of a crossbreeding simulation model for goat production systems in tropical regions. *Journal of Animal Science* 89, 3890–3907.
- Wojtysiak, D., Połtowicz, K., 2014. Carcass quality, physico-chemical parameters, muscle fibre traits and myosin heavy chain composition of m. longissimus lumborum from Puławska and Polish Large White pigs. *Meat Science* 97, 395–403.
- Yu, J., Zhao, P., Zheng, X., Zhou, L., Wang, C., Liu, J.-F., 2020. Genome-wide detection of selection signatures in Duroc revealed candidate genes relating to growth and meat quality. *G3: Genes|Genomes|Genetics* 10, 3765–3773.