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Genomic Scan of the Inbreeding Depression for Litter Size in Two Varieties of Iberian Pigs

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Abstract: Inbreeding depression is expected to be more pronounced in fitness-related traits, such as pig litter size. Recent studies have suggested that the genetic determinism of inbreeding depression may be heterogeneous across the genome. Therefore, the objective of this study is to conduct a genomic scan across the pig autosomal genome to detect the genomic regions that control inbreeding depression for litter size in two varieties of Iberian pigs (Entrepelado and Retinto). The datasets consist of 2,069 (338 sows) and 2,028 (327 sows) records for litter size (Total Number Born and Number Born Alive) for the Entrepelado and Retinto varieties. All sows were genotyped using the Geneseek GGP PorcineHD 70 K. We employed the Unfavorable Haplotype Finder software to extract runs of homozygosity (ROHs) and conducted a mixed model analysis to identify highly significant differences between homozygous and heterozygous sows for each specific ROH. A total of 8 genomic regions located on SSC2, SSC5, SSC7, SSC8, and SSC13, were significantly associated with inbreeding depression, housing some relevant genes such as FSHR, LHCGR, CORIN, AQP6, and CEP120.

Keywords: Iberian pig; litter size; inbreeding depression

1. Introduction

The most significant consequence of inbreeding in the phenotypic performance of livestock populations is the occurrence of inbreeding depression [1]. Theoretically, inbreeding depression arises from two genetic mechanisms, the impact from recessive mutations and the loss of contributions from of over-dominance genes [2]. This phenomenon is particularly evident in traits related to fitness, such as pig litter size [3,4]. Traditionally, inbreeding has been quantified using genealogical information [5]. However, the advent of high-throughput genotyping technologies has introduced a valuable tool for unraveling the genetic basis of inbreeding depression. Several studies [6–8] have indicated that its genetic determination is distributed unevenly across the genome.

One widely used method to detect identical-by-descent (IDB) genomic segments is through runs of homozygosity (ROH) [9]. ROH are completely homozygous segments of an individual's genome. Howard et al. [10] have proposed a strategy for identifying genomic regions associated with inbreeding depression by contrasting the phenotypic performance of individuals carrying specific ROH with those lacking them, employing a mixed model analysis [11].

In the context of Iberian pig populations, the non-uniform effects of inbreeding depression across the genome has been observed in a closed experimental flock of the Guadyerbas variety [7]. However, due to the large genetic diversity among the strains of the Iberian pig [12,13], variations in the genetic determinants of inbreeding depression may exist. Hence, the objective of this study is to investigate the genomic architecture of inbreeding depression effects in two commercial varieties of

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Iberian pigs (Entrepelado and Retinto). The study also aims to pinpoint potential candidate genes located within the most relevant genomic regions.

2. Materials and Methods

The dataset utilized comprises 2,069 records (pertaining to 338 sows) in the Entrepleado variety and 2,028 records (related to 327 sows) in the Retinto for both TNB – Total Number Born- and NBA – Number Born Alive-. In conjunction with this, a pedigree that contains the genetically interconnected individuals has been incorporated, with a total of 581 individuals for Entrepelado and 541 individuals for Retinto. The mean phenotypic performance values for TNB and NBA are summarized in Table 1.

Table 1. Mean and Standard Deviation (between brackets) of TNB (Total Number Born) and NBA (Number Born Alive) in the Entrepelado and Retinto varieties.

Population	TNB	NBA
Entrepelado	7.96 (1.93)	7.70 (1.88)
Retinto	8.27 (2.18)	7.99 (2.17)

Each sow was genotyped using the Geneseek GGP PorcineHD 70 K chip. Subsequent to genotyping, genotypic data underwent filtration using PLINK [14]. Filters were applied to ensure individual and SNP call rates exceeding 95%, with inclusion restricted to autosomal chromosomes. This process resulted in a collective sum of 57,450 SNP markers. Instances of missing genotypic data were rectified utilizing the FImpute software [15]. The allocation of SNP markers across the autosomal chromosomes in the Sscrofa 11.1 assembly is detailed in Table 2.

Table 2. Chromosome (SSC), number of SNP markers (Nm) and base pairs covered (bp).

SSC	Nm	bp
1	5,442	274,315,671
2	3,713	151,610,480
3	3,308	132,657,669
4	3,557	130,773,976
5	2,716	104,477,606
6	4,368	170,802,600
7	3,563	121,758,423
8	3,324	138,930,735
9	3,513	139,386,589
10	2,477	69,319,537
11	2,178	79,072,521
12	2,295	60,834,034
13	4,146	208,240,759
14	3,812	141,719,266
15	3,281	140,404,164
16	2,205	79,282,526
17	1,968	63,391,207
18	1,607	55,752,892

Firstly, we formulate a mixed linear model to assess variance components and calculate the inbreeding depression through the gradient of a covariate associated with the percentage of individual heterozygosity measured as the number of heterozygous SNPs per individual × 100 divided by the total number of SNPs.

The model we postulated for both varieties is as follows:

$$\boldsymbol{y} = \boldsymbol{f}d + \boldsymbol{Xb} + \boldsymbol{Th} + \boldsymbol{Zu} + \boldsymbol{Wp} + \boldsymbol{e} \quad (1)$$

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where **y** represents the vector comprising phenotypic records (specifically TNB and NBA), **f** is a vector encompassing individual heterozygosity and **b** accounts for the vector of systematic effects, which incorporates order of parity -5 levels (1st, 2nd, 3rd, 4th and beyond). Additionally, h is a vector of random herd-year-season -96 levels in Entrepelado and 113 in Retinto -, **u** denotes the vector of additive genetic random effects, **p** is the permanent environmental sow effect and **e** stands for the vector of residuals. Moreover, *d* serves as a covariate of the relationship between individual heterozygosity and phenotypic performance. The matrices **X**, **T, Z** and **W** are the corresponding incidence matrices. The genomic relationships (**G**) among the additive genetic effects (**u**) were calculated using the Single-Step approach [16,17]. For the estimation of variance components, the Average Information Residual Maximum Likelihood [18] was adopted, utilizing the airemlf90 software [19].

Secondly, the Unfavorable Haplotype Finder software [10] was employed with the aim of selecting ROH comprising at least 15 SNP markers, which were shared by a minimum of 5% and a maximum of 95% of individuals within the population. The algorithm's details are expounded in [10]. Concluding this step, the blupf90+ software [19] was utilized to quantify the phenotypic impact associated with the presence or absence of each identified ROH. This analytical model relied upon the variance components previously estimated and encompassed the same systematic, permanent environmental, and additive genetic effects. Significance was determined through a one-sided t-test.

3. Results and Discussion

3.1. Variance components estimation

The results of the variance component estimation are presented in Table 3.

Table 3. Restricted Maximum Likelihood estimates (and sampling variance) of the additive (σ_a^2), permanent environmental (σ_p^2), herd-year-season (σ_h^2) and residual (σ_e^2) variance.

	Entrepelado		Retinto	
	TNB	**NBA**	**TNB**	**NBA**
σ_a^2	0.145 (0.085)	0.098 (0.071)	0.165 (0.092)	0.225 (0.110)
σ_p^2	0.366 (0.097)	0.341 (0.089)	0.283 (0.11)	0.291(0.116)
σ_h^2	0.170 (0.545)	0.129 (0.071)	0.317 (0,085)	0.172 (0,061)
σ_e^2	2.901 (0.101)	2.853 (0.099)	3.908 (0.138)	3.796 (0.134)

The estimates of (co) variance components were similar to ones provided by Srihi et al. [20]. Given the estimates of the variance components, the estimates of the covariate with the percentage of heterozygosity were 0.055 ± 0.026 (p=0.017) and 0.057 ± 0.028 (p=0.021) for NBA and TNB in Entrepelado and 0.077 ± 0.051 (p=0.065) and 0.067 ± 0.050 (p=0.090) for NBA and TNB in Retinto. In all traits and populations there was an increase of litter size as the percentage of heterozygosity increases leading to significant results in the Entrepelado population.

3.2. Runs of Homozygosity (ROH) identification

We have identified 43,188 and 35,175 runs of homozygosity (ROHs) consisting of more than 15 SNP within the Entrepelado and the Retinto varieties, respectively. Figure 1 illustrates the distribution of ROH sizes.

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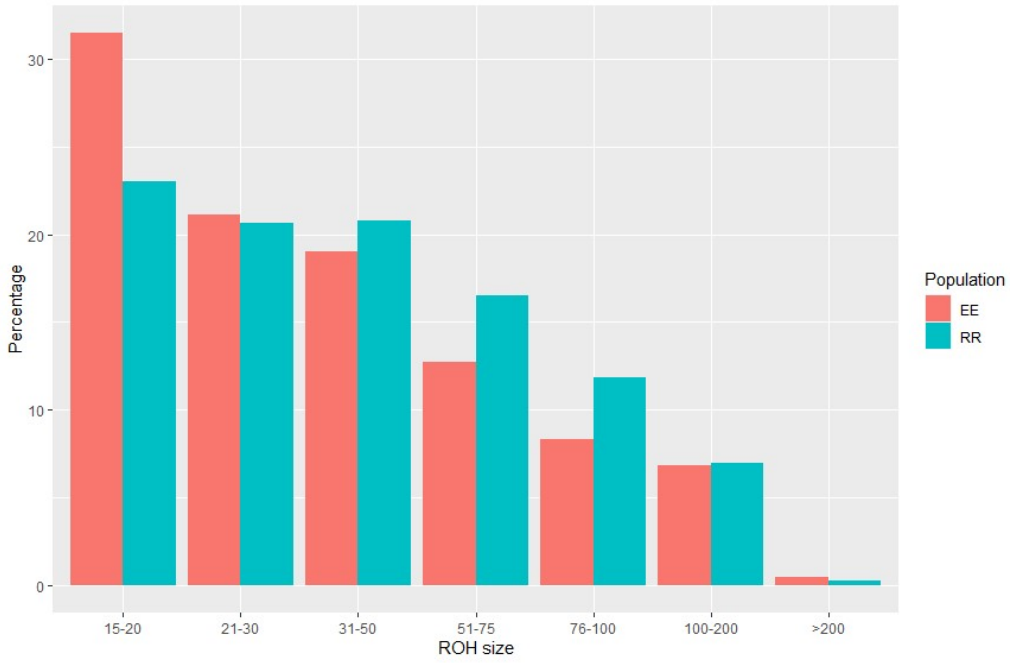

Figure 1. Distribution of the ROH sizes (by SNP number) in the Entrepelado (EE) and Retinto (RR) populations.

This distribution highlights the prevalence of short ROHs in the Entrepelado population, in contrast to the right-skewed distribution observed in the Retinto population. This observation may suggest the presence of more recent inbreeding within the Retinto population. The average size of ROHs in each population was 25.79 SNPs (±18.00) for Entrepelado and 35.96 SNPs (±24.30) for Retinto. Furthermore, the average percentage of an individual's genome covered by ROHs, considering overlapping regions between ROHs, was 26.87% (±3.78%) for Entrepelado and 40.74% (±3.20%) for Retinto.

3.3. ROH segments and inbreeding depression

Among all detected ROH, we were able to identify 20,143 (Entrepelado) and 26,771 (Retinto) ROH shared for at least 5% and at most 95% of individuals, composed by more than 15 SNP, in which is expected to find the most part of the variance source due to the ROHs effect. Therefore, we solved 20,143 and 26,771 mixed model equations for Entrepelado and Retinto, respectively. The objective was to obtain estimates of the effects related to the presence or absence of each specific ROH. The distributions of these effect estimates, pertaining to TNB and NBA, are illustrated in Figure 2 for both populations.

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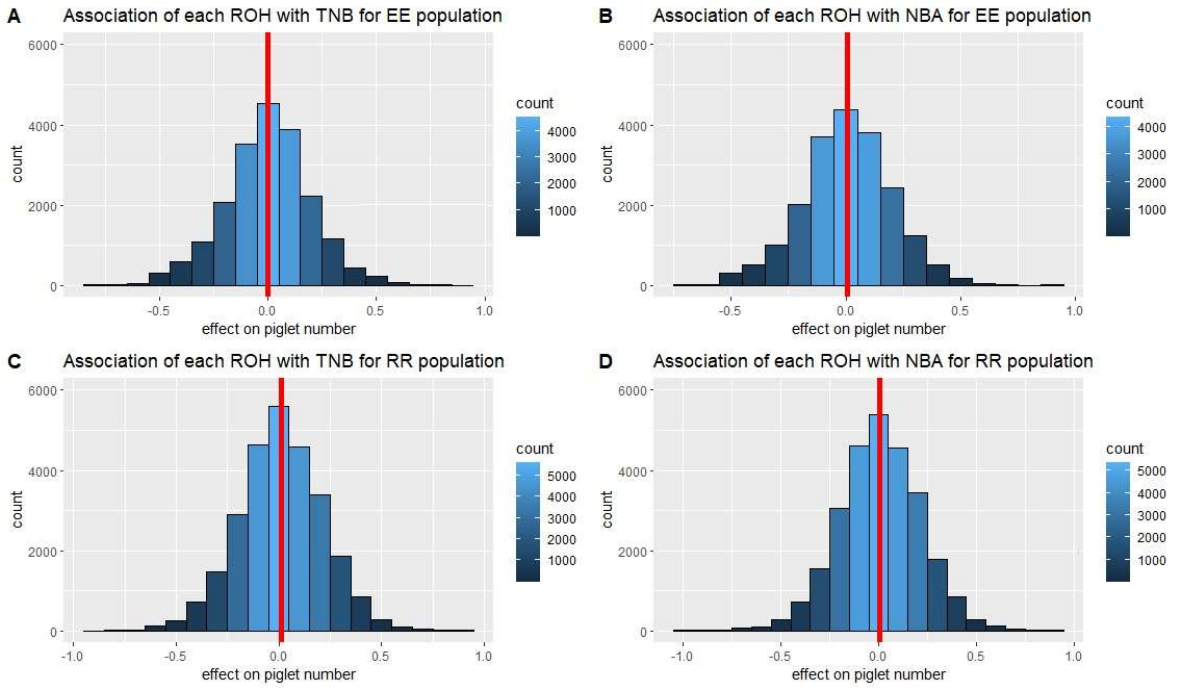

Figure 2. Distribution of the estimates of effects associated with presence or absence of ROH for TNB (Total Number Born) and NBA (Number Born Alive) in Entrepelado and Retinto.

The average estimate of the effects was consistently close to zero across all scenarios indicating that most of ROH were not associated with inbreding depression. The genomic regions associated with inbreeding depression ($p < 0.05$) encompassed 1,123 and 1,533 runs of homozygosity (ROH) for NBA in Entrepelado and Retinto, respectively, while for TNB, they numbered 1,197 and 1,453 regions. These findings represent a proportion of significant ROH that ranged from 5.4% (for RR and TNB) to 5.9% (for EE and TNB), slightly higher than expected by random.

These significant ROHs exhibit heterogeneous distribution across all chromosomes for both populations, as depicted in Figures 3 and 4. Among these ROHs, 14 and 3 ROHs boast a particularly striking significance, with p-values below 0.001. The ROHs associated with p-values lower than 0.001 are presented in Tables 3 and 4 for the Entrepelado and Retinto populations, respectively.

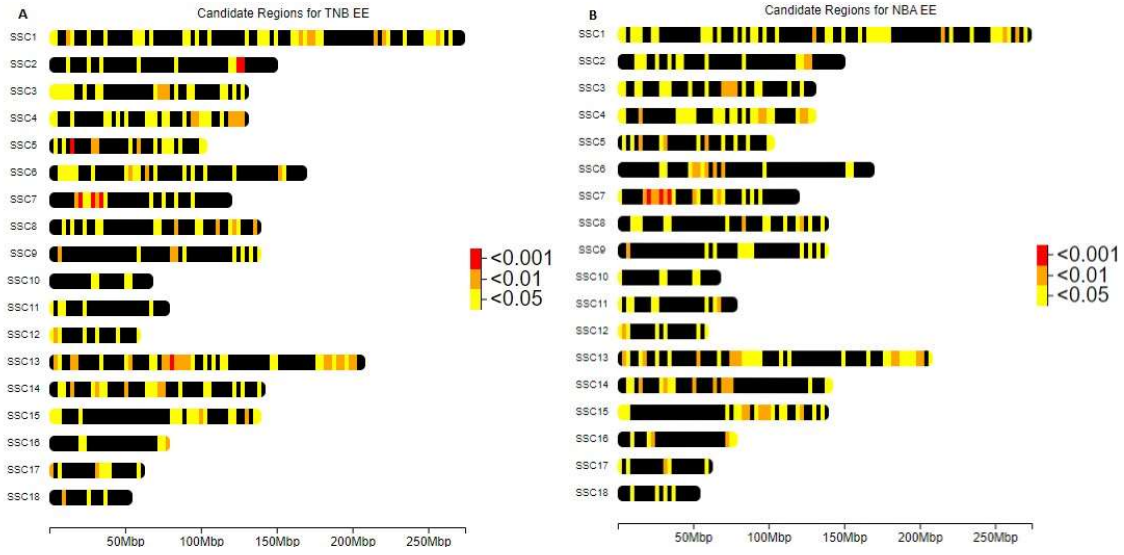

Figure 3. Distribution of ROHs with p-value lower than 0.05, 0.01 and 0.001 in Entrepelado (EE) population for (A) Total Number of Borns Borns (TNB) and (B) Number of Borns Alive (NBA).

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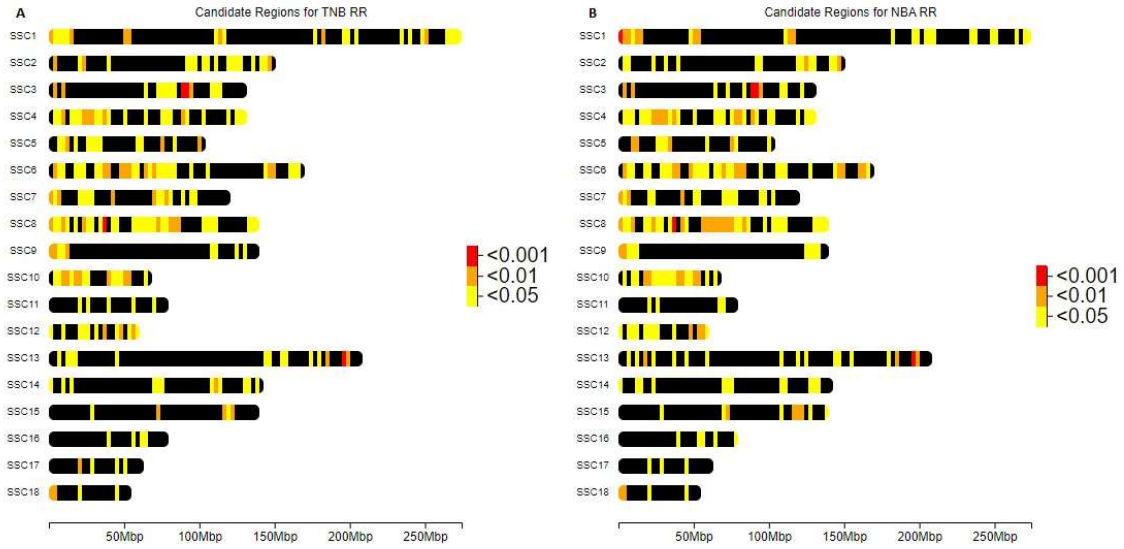

Figure 4. Distribution of ROHs with p-value lower than 0.05, 0.01 and 0.001 in Retinto (RR) population for (A) Total Number of Borns (TNB) and (B) Number of Borns Alive (NBA).

Table 3. Chromosome (SSC), base pair position (bp), effect on number of piglets for Number of Borns Alive (Piglets NBA), effect on number of piglets for Total Number of Borns (Piglets TNB), p-value for Number of Borns Alive (p-value NBA), p-value for Total Number of Borns (p-value TNB) and candidate genes within the genomic regions.

SSC	bp(c)	Piglets (NBA)	Piglets (TNB)	p-value (NBA)	p-value (TNB)	Genes
SSC2	126,506,354 – 126,841,331	-0.5428	-0.6264	2.23E-03	8,83E-04	CEP120
	126,516,022 – 126,857,203	-0.5358	-0.6239	1.28E-03	4,08E-04	
	126,562,142 – 126,971,814	-0.4838	-0.5840	3.46E-03	9,82E-04	
	126,700,457 – 127,000,238	-0.4935	-0.5904	2.69E-03	7,87E-04	
	126,777,258 – 127,080,032	-0.4838	-0.5840	3.46E-03	9,82E-04	
	127,096,117 – 127,764,704	-0.6088	-0.7179	2.75E-03	9,18E-04	
	127,492,532 – 127,890,446	-0.6305	-0.7393	1.70E-03	5,36E-04	
SSC5	15,871,592 – 16,9148,74	-0.6141	-0,7758	5,00E-03	9,12E-04	ATF1
	16,134,195 – 16,852,941	-0.5377	-0,7575	1,10E-02	9,73E-04	AQP5 AQP6 RACGAP1
SSC7	20,074,761 – 20,586,603	-0,5995	-0,5869	7,23E-05	2,03E-04	GMNN
	28,252,780 – 28,721,664	-0,6691	-0,6795	3,54E-05	6,34E-05	
	35,594,623 – 36,062,243	-0,6175	-0,6308	6,97E-04	9,86E-04	BAG2 RAB23
	20,074,761 – 20,586,603	-0,5995	-0,5869	7,23E-05	2,03E-04	
SSC13	80,594,858 – 81,214,645	-0,4839	-0,5679	2,28E-03	7,63E-04	CLSTN2
	80,682,896 – 81,329,857	-0,4929	-0,5693	1,72E-03	6,53E-04	

Table 4. Chromosome (SSC), base pair position (bp), effect on number of piglets for Number of Borns Alive (Piglets NBA), effect on number of piglets for Total Number of Borns (Piglets TNB), p-value and FDR for Number of Borns Alive (p-value/FDR NBA), p-value and FDR for Total Number of Borns (p-value/FDR TNB), and candidate genes within the genomic regions.

SSC	bp(c)	Piglets (NBA)	Piglets (TNB)	p-value (NBA)	p-value (TNB)	Genes
SSC1	632,758 – 18,69,413	-0,9609	-0,7750	1,35E-04	1,41E-03	
	734,657 – 1,869,413	-0,9609	-0,6383	1,35E-04	4,82E-03	
	989,159 – 13,58,337	-0,9609	-0,7750	1,35E-04	1,41E-03	
	1,189,180 – 1,471,069	-0,8987	-0,7750	2,31E-04	1,41E-03	
	1,189,180 – 2,160,998	-0,8174	-0,7244	5,59E-04	2,06E-03	

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SSC3	88,968,396 – 91,465,748	-0,5929	-0,5525	7,53E-04	1,26E-03	FSHR LHCGR GTF2A1L
	90,580,151 – 91,903,672	-0,6761	-0,6322	1,69E-04	3,08E-04	
	91,082,368 – 92,692,939	-0,6931	-0,6421	1,60E-04	3,29E-04	
	91,240,698 – 91,903,672	-0,6388	-0,6003	2,88E-04	4,70E-04	
	91,265,546 – 92,395,629	-0,6761	-0,6322	1,69E-04	3,08E-04	
	91,381,281 – 92,692,939	-0,6608	-0,6010	2,64E-04	6,37E-04	
	91,381,281 – 92,437,314	-0,6458	-0,5931	2,75E-04	5,88E-04	
	91,381,281 – 91,966,239	-0,6102	-0,5632	4,53E-04	8,66E-04	
SSC8	37,024,885 – 37,966,306	-1,0159	-0,8876	6,77E-05	3,58E-04	GABRB1
	37,513,284 – 38,036,453	-0,8992	-0,7349	2,59E-04	2,00E-03	CORIN
SSC13	196,187,718 - 196,460,966	-0,6757	-0,6677	9,06E-04	8,51E-04	USP16 CFAP298
	196,216,549 – 196,471,450	-0,7089	-0,6826	3,89E-04	4,98E-04	

The regions identified in NBA are also shared with TNB results and are located on SSC7, having the lowest p-values. These regions span between 20,074,761 and 20,586,603 base pairs (bp), wherein proximity to QTLs associated with pig litter size [21,22] is noted. Within this region lies the GMNN (Geminin DNA Replication Inhibitor) gene, whose encoded protein plays an essential role in embryo development and implantation [23]. Additionally, in the genomic region spanning from 28,252,780 to 28,721,664 bp, we find the genes BAG2 (BAG Cochaperone 2) and RAB23 (RAB23, Member RAS Oncogene Family). The former is potentially linked to infertility, as mediated inhibition of CHIP expression contributes to endometriosis [24], and the latter has been associated with litter size, as evidenced by GWAS in Bama Xiang pigs [25], and with failure during reproduction at puberty in a F2 population crossbreed of Duroc and Erhualian pigs [26]. Lastly, no associations with litter size were detected in the genomic region spanning from 80,682,896 to 81,329,857 bp.

The remaining regions with p-values lower than 0.001 in TNB are distributed across SSC2 (7 ROHs), SSC5 (2 ROHs), and SSC13 (2 ROHs) in a contiguous manner. Within SSC2, spanning from 126,506,354 to 127,890,446 bp, we identified the CEP120 (Centrosomal Protein 120) gene, which has been associated with maternally derived aneuploidy [27]. In the SSC5 region (15,871,592 – 16,914,874 bp), we find the ATF1 (Activating Transcription Factor 1) gene, known to be involved in the estrogenic signaling pathway [28]. This region also includes AQP5 and AQP6 (Aquaporin 5 and Aquaporin 6), which have been suggested as markers for male infertility in livestock [29]. AQP5 is overexpressed in granulosa cells and flattened follicle cells of the primordial follicles in the ovary and in the oviduct [30], while it is downregulated in pigs infected by Porcine Reproductive and Respiratory Syndrome [31]. We also identified RACGAP1 (Rac GTPase Activating Protein 1), the inhibition of which is required in vitro for human embryonic trophoblast invasion into endometrial stromal cells [32]. Lastly, in the SSC13 region spanning from 80,594,858 to 81,329,857 bp, the only related gene found was CLSTN2 (Calsyntenin 2), which has been proposed as a potential candidate gene in Erhualian pigs [22,33] and in sheep after conducting a GWAS [34].

In the case of the RR population, we identified 17 ROHs with p-values lower than 0.001 in NBA, 10 of which were shared with TNB, as detailed in Table 4. The region with the lowest p-value is situated on SSC8, spanning from 37,024,885 to 37,966,306 base pairs (bp), with p-values of 6.77×10^{-5} in NBA and 3.58×10^{-04} in TNB. Additionally, within SSC8, there is another ROH ranging from 37,513,284 to 38,036,453 bp with a low p-value exclusive to NBA. Within this SSC8 region, several noteworthy genes are located, including GABRB1 (Gamma-Aminobutyric Acid Type A Receptor Subunit Beta1), which plays a role in inhibiting GnRH neurons. This inhibition is essential for the production of the GnRH hormone, which in turn is crucial for the synthesis of LH (luteinizing hormone) and FSH (follicle-stimulating hormone), both of them are crucial for reproduction [35–37], CORIN (Corin, Serine Peptidase), up-regulated in the decidua of the pregnant uterus which suggests a potential role during pregnancy [38], and it has been proposed as a candidate gene for calving easiness in dairy and beef cattle [39]. This SSC8 region is also associated with QTLs linked to

reproduction traits, such as litter size in the Chinese Erhualian pig breed [22] and the number of stillborn piglets in Shaziling Pigs [40]. Furthermore, regions on SSC3 and SSC13 were shared between NBA and TNB and contain genes like FSHR (Follicle Stimulating Hormone Receptor) and LHCGR (Luteinizing Hormone Receptor), both critical in regulating female reproductive processes. Additionally, GTF2A1L (General Transcription Factor IIA Subunit 1 Like) may play an important role in testis biology and male infertility [41]. On SSC13 at positions 196,187,718 – 196,471,450, near a QTL for litter size [22], lies the USP16 (Ubiquitin Specific Peptidase 16) gene, responsible for regulating embryonic stem cell gene expression [42]. In this region, CFAP298 (Cilia And Flagella Associated Protein 298) has been described, with a mutation known to cause infertility in human patients [43]. Lastly, there is a region with p-values lower than 0.001 in NBA at SSC1 spanning from 632,758 to 2,160,998 bp, although no specific relationships with reproductive traits were identified.

4. Conclusion

The results of this study indicate the presence of inbreeding depression in litter size traits of two strains of Iberian pigs. Furthermore, the distribution of the inbreeding depression effects is heterogeneous along the genome, and the architecture of inbreeding depression differs between populations. Additionally, we were able to identify eight genomic regions significantly associated with inbreeding depression that contain several relevant genes such as FSHR, LHCGR, CORIN, AQP6, CEP120.

Author Contributions: Conceptualization, L.V.; methodology, C. H. R., H. S., D. L-C. and L.V.; software, C. H. R., L. V.; validation, C. H. R., H. S. and D. L-C.; formal analysis, C. H. R., H. S. and D. L-C.; investigation, C. H. R., H. S. and D. L-C.; resources, J. C. and N. I. E.; data curation, S. N. and N. I. E.; writing—original draft preparation, C. H. R. and L. V.; writing—review and editing, N. I. E. and J. C.; visualization, C. H. R., H. S., D. L-C..; supervision, L. V.; project administration, J. C. and N. I. E.; funding acquisition, J. C. and N. I. E. All authors have read and agreed to the published version of the manuscript.

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