

Type of the Paper Article

The Nero Lucano pig breed: variation of the genetic structure and identification of conserved ROH islands

Paola Di Gregorio *, Annamaria Perna, Adriana Di Trana and Andrea Rando

Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy; paola.digregorio@unibas.it (P.D.G.); anna.perna@unibas.it (A.P.) andrea.rando@unibas.it (A.R.).

* Correspondence: paola.digregorio@unibas.it

Simple Summary: The recovery of Nero Lucano (NL) pig in the Basilicata region (Southern Italy) started in 2001 with the collaboration of several public authorities with the aim to prevent its extinction. This recovery should be pursued for the sustainability of the production system, based on pigs reared outdoors with a reduced environmental footprint, and for the improvement of the breeders income, determined by the strong appreciation of the quality of the obtained cured products. Both tasks are relevant for the “Farm to Fork Strategy” of the European Union. According to a previous study, this breed was characterized by a very low level of genetic variability. In this paper, we refer on variations of the genetic structure of NL pigs reared in a single herd after a time period of ten years corresponding to at least three generations.

Abstract: The recovery of Nero Lucano (NL) pig in the Basilicata region (Southern Italy) started in 2001 with the collaboration of several public authorities in order to preserve native breeds that can play a significant economic role both for the remarkable ability to adapt to difficult environments and for the value of typical products of the area of origin. In this study, by using the Illumina Porcine SNP60 BeadChip, we compared the genetic structures of NL pigs reared in a single farm in two different periods separated by a time interval corresponding to at least three generations. Results showed an increase of the percentage of polymorphic loci, a decrease of the inbreeding coefficient calculated according to ROH genome coverage (F_{ROH}), a reduction in the number of ROH longer than 16 Mb and an increase of ROH with a length between 2–4 Mb highlighting a picture of improved genetic variability. In addition, ROH islands analysis in the two groups allowed to identify five conserved regions (SSC 1, 4, 8, 14 and 15) containing 52 genes that, after GO-term enrichment, can be grouped in four superclusters of biological processes (inflammatory response, system development, regulation of cellular component organization and glycerolipid metabolism). Only the conserved ROH island on SSC14 contains markers which, according to the literature, are associated with QTLs affecting thoracic vertebra number, teat number, gestation length, age at puberty and mean platelet volume.

Keywords: Nero Lucano pig; Southern Italy; inbreeding coefficient (F_{ROH}); runs of homozygosity (ROH) islands

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date

Revised: date

Accepted: date

Published: date



Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Farms rearing Nero Lucano (NL) pigs, a typical autochthonous black pig, are characterized by a very low level of environmental impact [1] since animals are raised outdoors in the Basilicata region (Southern Italy) and are able to exploit marginal areas and feed resources available in the environment. The sustainability of the production chain and the specific organoleptic properties of the NL pig cured products are particularly appreciated by customers and, therefore, economically advantageous for breeders. As a consequence, the enhancement and protection of this breed is useful for the conservation

of biodiversity and for the reduction of environmental and climate footprint. These matters are goals of the “Farm To Fork Strategy” of the European Union [2] aiming to obtain a sustainable and affordable food chain effective for consumers, producers, climate and environment.

Starting from 2001, the NL pig breed is under intervention for the recovery from extinction thanks to public authorities (the Basilicata Region, the University of Basilicata, the Regional Breeders Association, the Comunità Montana Medio Basento). However, it still suffers all the problems (for example, slow growth speed and low number of newborns per delivery) [3, 4] determined by the inbreeding associated with the low number of sires and dams typical of “small” populations. In a recent article [5], we analyzed the genotypes at the 61,565 SNPs of the Illumina Porcine SNP60 BeadChip of about the 70% of sires and dams born from 2004 to 2014 of the NL pig population, in order to obtain a first picture of its genetic structure. As expected for a population recovered starting from only 6 subjects, the analyzed individuals were characterized by high levels of inbreeding coefficients (both F_{MOL} , calculated by referring to allelic frequencies, and F_{ROH} , calculated according to the ROH extension), low effective population size and long generation intervals. These results depicted a population still at risk and the need for actions to avoid an excessive inbreeding coefficient increase.

At the end of 2021 we received from the owners of a farm, whose NL pigs were analyzed ten years ago, other blood samples collected by the veterinary service during the normal activities of animal controls. As a consequence, we had the opportunity to make comparisons between two groups of NL pigs, reared in the same farm and separated by a time period of about ten years (i.e.; at least three generations), in order to obtain a picture of the evolution of their genetic structure by using ROH approach.

2. Materials and Methods

2.1. Animals

Animal blood samples were obtained from 76 Nero Lucano pigs reared in a single farm where, about ten years ago, 66 samples of the same breed had already been collected.

2.1. DNA Analyses

DNA samples were genotyped with the Illumina Porcine SNP60 BeadChip v2. The data quality control, accomplished by using PLINK v.1.9 [6], determined the removal of 3 samples due to genotyping rate lower than 95% and 2548 SNPs due to a call rate lower than 95%. Hardy–Weinberg equilibrium was calculated by considering only polymorphic loci located on the 18 autosomal chromosomes. The runs of homozygosity (ROH) were obtained by defining a sliding ‘window’ of 50 SNPs, a maximum of one heterozygote and one missing call were allowed in the ‘window’, with at least 50 SNPs per ‘window’. Individual inbreeding values based on ROH (F_{ROH}) were calculated as $F_{\text{ROH}} = \Sigma L_{\text{ROH}}/L$, where ΣL_{ROH} is the total ROH length per individual and L is the autosomal genome length (2265.77 Mb, according to Sscrofa 11.1 Genome Assembly).

Gene location was accomplished by referring to NCBI Sus scrofa Annotation Release 106 (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Sus_scrofa/106/, accessed on 30 December 2022).

Gene Ontology (GO) enrichment analysis was performed by using DAVID Knowledgebase v2022q4 [7, 8] (<https://david.ncifcrf.gov/home.jsp>, accessed on 15 February 2023) and protein–protein interaction by using STRING 11.5 software [9] (<https://string-db.org/>, accessed on 15 February 2023). REVIGO software was used to reduce and visualize GO terms [10] (<http://revigo.irb.hr/>, accessed on 15 February 2023).

Graphic plots were obtained by using R software [11]

3. Results

The variation of the genetic structure of Nero Lucano pig in a time period corresponding to at least three generations intervals [5] was analyzed by comparing 66 samples, NL-A, collected about 10 years ago and 73 samples, NL-B, collected in 2021 in the same farm.

The comparison of the chromosomal distribution according to the Minimum Allele Frequencies (MAF) of the SNPs in the two groups (Figure S1) showed that the percentage of the polymorphic SNPs increased from 67% to 83% (about 24%) in about ten years (Figure 1). Markers alignment between the two groups allowed to identify 9600 SNPs that were monomorphic in NL-A but polymorphic in NL-B. These results could be explained by introgression events or by the use of NL sires and dams from other farms.

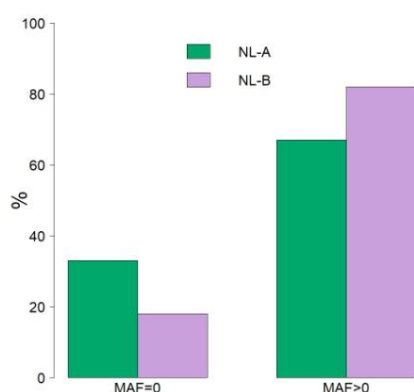


Figure 1. Comparison of the SNPs distribution according to the Minimum Allele Frequencies (MAF) in the two Nero Lucano pig groups.

The analysis of genotype distributions accomplished only for the SNPs located in the 18 autosomal chromosomes showed that in NL-B pigs the 6.32% of SNPs were not in Hardy-Weinberg equilibrium and in 65.48% of cases the disequilibrium was determined by an excess of homozygotes. The corresponding values in NL-A pigs were: 3.43% and 46.61%, respectively.

The ROH analysis of the 73 NL-B pigs allowed to identify 3690 total ROH, covering 28.11% of the 18 autosomal chromosomes, whereas in the 66 NL-A pigs the number of total ROH was 3626, covering 38.63% of the autosomal genome (Table 1).

Table 1. Features of ROH clustered according to length in NL-A and NL-B pigs.

ROH class	NL-A			NL-B		
	N. ROH	N. SNPs (mean ± SD)	% genome coverage	N. ROH	N. SNPs (mean ± SD)	% genome coverage
<2Mb	260	57.30±7.67	0.29	236	59.82±8.71	0.25
2-4Mb	897	85.67±22.93	1.73	1052	84.14±20.18	1.85
4-8Mb	859	169.26±46.37	3.24	913	161.81±41.76	3.16
8-16Mb	707	329.64±76.12	5.40	741	321.53±72.55	5.07
>16Mb	903	1180.92±783.82	27.97	748	1002.98±636.18	17.78
Total	3626		38.63	3690		28.11

In addition, the mean ROH number was 50.55±10.97 per pig in NL-B and 54.94±7.66 in NL-A with a total ROH length per pig spanning from a minimum of 38.09 Mb to a maximum of 1260.11 Mb (mean 636.80±219.04 Mb) in NL-B and from a minimum of 236.82 Mb to a maximum of 1366.99 Mb (mean 875.1±198.49 Mb) in NL-A. As a consequence, the mean ROH length per pig was 12.47±3.69 Mb in NL-B and 15.90±3.10 Mb in

NL-A. In both groups, the distribution of ROH among the size classes was, with the exception of those with a length lower than 2 Mb, balanced. As shown in Table 1, the ROH number and percentage of genome coverage showed the greatest increase for class 2–4 Mb and the greatest decrease for class >16 Mb in NL-B pigs.

Individual inbreeding values based on ROH extension (F_{ROH}) spanned from a minimum of 0.02 to a maximum of 0.56 with a mean value of 0.28 ± 0.10 in NL-B, whereas in NL-A the corresponding values were: 0.10, 0.60 and 0.39 ± 0.09 (Figure 2). As a consequence, the F_{ROH} mean value decreased of 28% in about ten years.

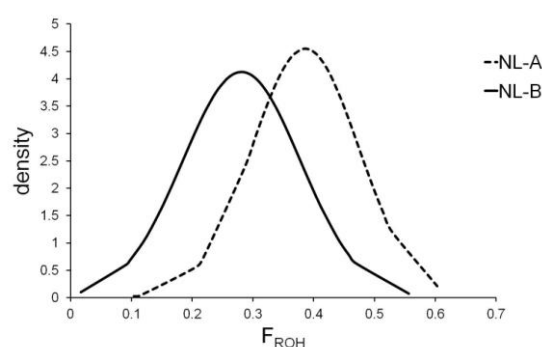


Figure 2. Distribution of the individual F_{ROH} (inbreeding coefficient based on Runs of Homozygosity) values in two groups of Nero Lucano pigs.

The core ROH, defined as the consensus regions determined by overlapping of individual ROH [6], were 587 in NL-B and 494 in NL-A (Figure S2, Table S1). ROH islands were obtained by considering an uninterrupted stretch of at least three SNPs both located in a core ROH and exceeding 99% of the standardized distribution [12] (Figure 3, Table S1). By using this approach, 24 and 19 ROH islands were identified in NL-B and NL-A, respectively (Table S1).

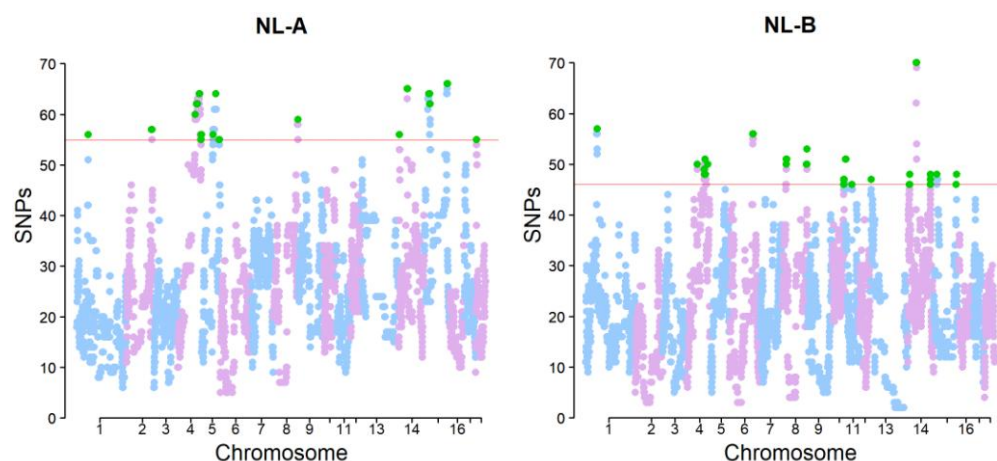


Figure 3. Manhattan plot of SNPs distribution in Runs of Homozygosity in NL-A and NL-B pig autosomal chromosomes. The red lines, different per each group, indicated the threshold of the top 1% of the observations. Green dots represent SNPs located in ROH islands.

The higher number of ROH islands observed in NL-B is, however, associated with a lower total extension (NL-B 22.79Mb versus NL-A 29.94Mb) (Table S2). As shown in Table 2, only in 5 ROH islands, located in chromosomes 1, 4, 8, 14, and 15, a partial or total overlap can be observed between the two groups.

Table 2. Features (extension, number of SNPs and genes) of ROH islands conserved between NL-A and NL-B pigs.

SSC	NL-A		NL-B		N. genes
	from-to	N. SNPs	from-to	N. SNPs	
1	61286411-63123772	52	61286411-63012075	47	1
4	96094167-96682751	19	96094167-96243742	8	7
8	135852700-136018155	4	135852700-136018155	4	2
14	46176964-48062822	60	46176964-47999414	58	39
15	126097384-126642052	17	126097384-126642052	17	3

According to Sus scrofa 11.1 Genome Assembly, the 5 overlapping regions contain 52 genes (Table S3). Gene ontology (GO) analysis using DAVID Knowledgebase v2022q4 showed that 26 genes were significantly involved in 15 biological processes whose GO terms were grouped in four superclusters (inflammatory response, system development, regulation of cellular component organization and glycerolipid metabolism) by using REVIGO software (Figure S3, Figure 4).

Table 3. Genes associated in biological processes after Gene Ontology (GO) analysis by using DAVID software.

GO term	Biological Process	Genes
GO:0006954	inflammatory response	S100A12, S100A8, S100A9, OSM, PLA2G3, RHBDD3, LIF, LIMK2, NF2, S100A8, S100A9, THOC5, AP1B1, CUL3,
GO:0048731	system development	GAL3ST1, GAS2L1, INPP5J, KREMEN1, NEFH, PLA2G3, RHBDD3, SELENOM, ZNRF3
GO:0051128	regulation of cellular component organization	LIF, LIMK2, MORC2, NF2, S100A9, CUL3, GAS2L1, HNRNPD, INPP5J, KREMEN1, MTMR3, PLA2G3,
GO:0033043	regulation of organelle organization	LIF, LIMK2, MORC2, NF2, CUL3, GAS2L1, HNRNPD, MTMR3,
GO:0009914	hormone transport	LIF, OSM, PLA2G3, SELENOM,
GO:0051046	regulation of secretion	LIF, S100A8, OSM, PLA2G3, RHBDD3,
GO:0046903	secretion	LIF, S100A8, OSM, PLA2G3, RHBDD3, SELENOM,
GO:0050729	positive regulation of inflammatory response	S100A8, OSM, PLA2G3
GO:0031345	negative regulation of cell projection organization	LIMK2, INPP5J, KREMEN1,
GO:0023061	signal release	LIF, OSM, PLA2G3, SELENOM,
GO:0051247	positive regulation of protein metabolic process	LIF, LIMK2, S100A8, TBC1D10A, CUL3, HNRNPD, OSM, RHBDD3,
GO:0010648	negative regulation of cell communication	LIF, NF2, CUL3, CASTOR1, KREMEN1, OSM, ZNRF3,
GO:0023057	negative regulation of signaling	LIF, NF2, CUL3, CASTOR1, KREMEN1, OSM, ZNRF3,
GO:0048232	male gamete generation	LIMK2, GAL3ST1, OSBP2, PLA2G3,
GO:0046486	glycerolipid metabolic process	GAL3ST1, INPP5J, MTMR3, PLA2G3,

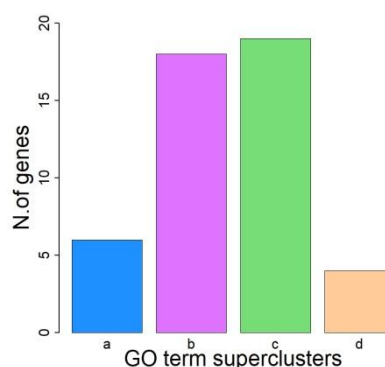


Figure 4. GO terms grouped in four superclusters by using REVIGO software: (a) inflammatory response, (b) system development, (c) regulation of cellular component organization (d) glycerolipid metabolism.

Furthermore, 3 genes (S100A7, S100A8, S100A9) are involved in the IL-17 signaling pathway that is engaged in several immune regulatory functions such as: response to injury, physiological stress, and infection [13].

Analysis of the 52 genes with STRING 11.5 software evidenced more protein-protein interactions than expected (26 edges rather than 4, PPI enrichment p-value $8.5e-13$) (Figure S4). As a consequence, some of the proteins coded by these genes are at least partially biologically connected. However, only part of the biological processes identified by DAVID software found a correspondence with protein-protein interactions highlighted by STRING software.

According to the Pig QTL database (version Sus scrofa 11.1 Genome Assembly), only the conserved ROH island on chromosome 14 contained six QTLs affecting thoracic vertebra number (#64660 and #64771) [14], teat number (#126628) [15], gestation length (#173177) [16], mean platelet volume (#37844) [17], and age at puberty (#22109) [18] (Table S3). Each of the six SNPs located at the peak of association with the above-mentioned QTLs were characterized by a MAF=0 in both NL-A and NL-B pigs.

4. Discussion

In this work we compared the results of Illumina PorcineSNP60 BeadChip genotyping accomplished on two groups of Nero Lucano pigs, NL-A and NL-B, reared in the same herd and sampled in two time periods separated by about ten years corresponding to at least three generations.

Analysis of the results showed that in this period the number of polymorphic loci increased of 23%. The origin of such increase could be explained either by the use of sires and dams belonging to other NL herds or by introgression events from other black Southern Italy pig populations. The first hypothesis is supported by the typical morphological traits characterizing the NL-B individuals, whereas the latter one by the observation that only 8036, out of the 9600 SNPs polymorphic in NL-B and monomorphic in NL-A, were also polymorphic in the pigs analyzed by Valluzzi et al. (2021). Of course, it is not possible to exclude that the polymorphism at the remaining 1564 SNPs could derive from the 30% of the NL pig population not analyzed ten years ago.

The ROH analyses in the two groups showed that mean ROH number, mean total ROH length and mean ROH length per pig were characterized by decreased values in NL-B. The distribution of the ROH per classes was consistent with these results, showing a reduction of ROH longer than 16 Mb and an increase in the number of ROH with a length between 2-4 Mb in NL-B. All the above-mentioned results were responsible for the strong decrease in ten years, from 0.39 to 0.28, of the inbreeding coefficient based on ROH genome coverage (F_{ROH}).

ROH islands are stretches of homozygous genomic sequences characterized by a very high frequency in a population. According to several authors the high frequency of these regions should be determined by the presence of genes under strong selection [19, 20]. We identified five ROH islands conserved between NL-A and NL-B groups. As a consequence, these regions, preserved for at least three generations, are much probably associated with effects on the adaptive selection of the Nero Lucano pig.

The 52 genes identified in these five regions are involved in biological processes affecting immune response, reproduction and production traits. For example, three of the calcium ion binding proteins belonging to the S100 family (S100A7, S100A8, S100A9) are involved in the IL-17 signaling pathway which is important in the immunity to pathogens or contribute to the pathogenesis of inflammatory diseases [13]. Furthermore, S100A6 is involved in the response to virus infection causing the Porcine Reproductive and Respiratory Syndrome (PRRS), one of the most economically significant swine infectious diseases [21, 22]; PPGRP-S has a role in the immunity against intestinal microorganisms [23]; RHBDD3 suppresses, in mouse, the production of IL6 preventing the development of autoimmune diseases [24]; PLA2G3 affects maturation and function of mast cells, key players in the inflammatory response [25]. The conservation of regions containing genes affecting immune response is probably due to the need for an efficient immunological system in animals living in semi-wild conditions, such as NL pigs.

As far as pig reproduction traits are concerned, MORC2 gene prevents apoptosis of granulosa cells [26], whereas SMTN, coding for a structural protein found exclusively in contractile smooth muscle cells, is associated with ovarian follicle growth and development [27]. Furthermore, both OSBP2 and LIMK2 are involved in spermatogenesis [28]. In mouse, the defective gene MORC2b is responsible for male and female sterility [29] and mice deficient in OSBP2 produce a severely reduced number of spermatozoa that show very low motility and no fertilizing ability in vitro [30]. Similar results were observed for mice with a disrupted LIMK2 gene in which progression of spermatogenesis is strongly affected [31] and for humans where mutations at the heterozygous state in LIMK2 were observed in infertile males [32]. Finally, a LIF gene polymorphism (rs3463076786: C/T) is associated with the number of stillborn in pig [33].

In the Chinese Anqing six-end-white pig, SELENOM has been identified as a candidate gene affecting body weight [28]. This gene is highly expressed in the brain and may be involved in neurodegenerative disorders. Transgenic mice with targeted deletion of this gene are characterized by obesity suggesting a possible role of SELENOM in the regulation of body weight and energy metabolism [34]. In addition, DOCK10 is a candidate gene associated with intramuscular fat [35] and EWSR1 is associated with meat quality traits, in particular meat colour [36]. The PLA2G3 gene belongs to the phospholipase A2 family that in pig, as in other vertebrates, is involved in lipid metabolism [37]. In man, KREMEN1 and ZNRF3 are involved in body fat distribution [38].

The conserved five ROH islands were also analyzed for the presence of QTLs and only the one on chromosome 14 contained markers associated with effects on thoracic vertebra number, teat number, gestation length, mean platelet volume and age at puberty. Unfortunately, these markers are monomorphic in all of the genotyped NL pigs, therefore cannot be used to analyze the variation of these traits in this breed. However, the conserved ROH island located on chromosome 14 could have had a key role in pig domestication since it was identified also in other breeds. In fact, the upstream region containing the AP1B1, EWSR1, KREMEN1, NEFH, THOC5, ZNRF3 genes (see table S3) was identified in Russian Large White pigs as a signature of selection associated with QTLs affecting reproduction and production traits [39]. Whereas, the downstream region containing MORC2, SMTN, INPP5J, PLA2G3 and RNF185 genes (see table S3), was identified by Li et al. [40] as a signature of selection in Chinese pigs. This region is associated with a QTL affecting intramuscular fat linoleic acid content [41, 42] that is positively correlated with pork flavor [43]. As a consequence, it is plausible that this ROH

island could also play an important role in the quality of NL pig cured meat products which are particularly appreciated for their organoleptic properties.

5. Conclusions

The analyses of SNP polymorphisms in two groups of NL pigs reared in the same herd and sampled in two time periods separated by at least three generations allowed to highlight the efforts of the breeder in order to increase the genetic variability of this autochthonous population. Thanks to this activity, a strong reduction of the inbreeding coefficient based on ROH genome coverage (FROH) was observed. Of course, it would be better if a single managing structure could plan and control the activities of all breeders rearing NL pigs in order to obtain an affordable and long-lasting recovery of this population. Comparison of the two groups allowed to identify 5 conserved ROH islands containing 52 genes related to immune response and some reproductive and productive traits. Further analyses of such genes could be useful in the characterization and preservation of the breed.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1. Chromosomal distribution according to the Minimum Allele Frequencies (MAF) of the SNPs in the two groups, NL-A and NL-B, of Nero Lucano pigs (0= non-defined chromosome position, 23=X chromosome, 24= Y chromosome, 25= XY ψ -autosomal region). Figure S2. Chromosomal distribution of core ROH (bars), and minimum and maximum frequencies of core ROH (lines) in the two groups, NL-A and NL-B, of Nero Lucano pigs. Dots indicate ROH islands. Figure S3. “Revigo Treemap” of Gene Ontology terms for genes located in the five ROH islands conserved between NL-A and NL-B pigs. Figure S4. Network of protein–protein interaction (PPI) analysis carried out on genes located in the five ROH islands conserved between NL-A and NL-B pigs (nodes represent proteins). Table S1. Chromosomal distribution of the number of core ROH and ROH islands in the two groups, NL-A and NL-B, of Nero Lucano pigs. Table S2. Features of the ROH islands in the two groups, NL-A and NL-B, of Nero Lucano pigs (total overlaps are highlighted in yellow, partial overlaps are in red). Table S3. Genes and QTLs mapped in the five ROH islands conserved between NL-A and NL-B pigs..

Author Contributions: Conceptualization and Methodology, P.D.G. and A.R.; Investigation, P.D.G.; Project administration, A.P.; Funding acquisition, A.R. A.P.; Formal analysis, P.D.G.; Writing—Original Draft Preparation, P.D.G.; Writing—Review and Editing, P.D.G., A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by PSR of the Basilicata Region 2014–2020, Misura 10, sottomisura 10.2. “Conservazione e uso sostenibile delle risorse genetiche in agricoltura” (Conservation and sustainable use of genetic resources in agriculture), project “(TGA)—Standardizzazione, stabilizzazione e valorizzazione dei tipi genetici autoctoni suini, ovicaprini ed equini” (TGA—Standardization, stabilization and enhancement of native pig, sheep, goat and equine genetic types); CUP C36C18000160006.

Institutional Review Board Statement: This research does not fall within Directive 63/210 of the European Parliament and of the Council on the protection of animals used for experimental purposes (transposed into Italian law by Legislative Decree 26/2014) and, thus, it does not require any authorization from the National competent Authorities (Protocol code: OpBA 07_2023_UNIBAS).

Data Availability Statement: The data analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We thank the Basilicata Region Breeders Association (Associazione Regionale Allevatori, Basilicata) for the kind collaboration.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hilborn, R.; Banobi, J.; Hall, S.J.; Pucylowski, T.; Walsworth, T.E. The environmental cost of animal source foods. *Front. Ecol. Environ.* 2018, 16, 329–335. <https://doi.org/10.1002/fee.1822>

2. European Commission. Farm to Fork Strategy: for a fair, healthy and environmentally-friendly food system. COM/2020/381 final. Date of document: 20/05/2020. https://ec.europa.eu/commission/presscorner/detail/en/fs_20_908
3. Farkas, J.; Curik, I.; Csato, L.; Csornyei, Z.; Baumung, R.; Nagy, I. Bayesian inference of inbreeding effects on litter size and gestation length in Hungarian Landrace and Hungarian Large White pigs. *Livest. Sci.* 2007, 112, 109–114. <https://doi.org/10.1016/j.livsci.2007.01.160>
4. Zhang, Y.; Zhuo, Y.; Ning, C.; Zhou, L.; Liu, J.F. Estimate of inbreeding depression on growth and reproductive traits in a Large White pig population. *G3* 2022, 12, jkac118. <https://doi.org/10.1093/g3journal/jkac118>
5. Valluzzi, C.; Rando, A.; Macciotta, N.P.P.; Gaspa, G.; Di Gregorio, P. The Nero Lucano Pig Breed: Recovery and Variability. *Animals* 2021, 11, 1331. <https://doi.org/10.3390/ani11051331>
6. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M.A.R.; Bender, D.; Maller, J.; Sklar, P.; de Bakker, P.I.W.; Daly, M.J.; et al. PLINK: A toolset for whole-genome association and population-based linkage analysis. *Am. J. Hum. Genet.* 2007, 81, 559–575. <https://doi.org/10.1086/519795>
7. Huang, D.W.; Sherman, B.T.; Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nat. Protoc.* 2009, 4, 44–57. <https://doi.org/10.1038/nprot.2008.211>
8. Sherman, B.T.; Hao, M.; Qiu, J.; Jiao, X.; Baseler, M.W.; Lane, H.C.; Imamichi, T.; Chang, W. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res.* 2022, 50, W216–W221. <https://doi.org/10.1093/nar/gkac194>
9. Szklarczyk, D.; Gable, A.L.; Nastou, K.C.; Lyon, D.; Kirsch, R.; Pyysalo, S.; Doncheva, N.T.; Legeay, M.; Fang, T.; Bork, P.; Jensen, L.J.; von Mering, C. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 2021, 49, D605–D612. <https://doi.org/10.1093/nar/gkaa1074>
10. Supek, F.; Bosnjak, M.; Skunca, N.; Smuc, T. REVIGO summarizes and visualizes long lists of Gene Ontology terms. *PLoS ONE* 2011, 6, e21800. <https://doi.org/10.1371/journal.pone.0021800>
11. R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
12. Szmatoła, T.; Gurgul, A.; Ropka-Molik, K.; Jasielczuk, I.; Ząbek, T.; Bugno-Poniewierska, M. Characteristics of runs of homozygosity in selected cattle breeds maintained in Poland. *Livest. Sci.* 2016, 188, 72–80. <https://doi.org/10.1016/j.livsci.2016.04.006>
13. McGeachy, M.J.; Cua, D.J.; Gaffen, S.L. The IL-17 family of cytokines in health and disease. *Immunity* 2019, 50, 892–906. <https://doi.org/10.1016/j.immuni.2019.03.021>
14. Rohrer, G.A.; Nonneman, D.J.; Wiedmann, R.T.; Schneider, J.F. A study of vertebra number in pigs confirms the association of vertnin and reveals additional QTL. *BMC Genet.* 2015, 16, 129; DOI: <http://dx.doi.org/10.1186/s12863-015-0286-9>
15. Rohrer, G.A.; Nonneman, D.J. Genetic analysis of teat number in pigs reveals some developmental pathways independent of vertebra number and several loci which only affect a specific side. *Genet. Sel. Evol.* 2017, 49, 4. <https://doi.org/10.1186/s12711-016-0282-1>
16. See, G.M.; Trenhaile-Grannemann, M.D.; Spangler, M.L.; Ciobanu, D.C.; Mote, B.E. A genome-wide association study for gestation length in swine. *Anim. Genet.* 2019, 50, 539–542. <https://doi.org/10.1111/age.12822>
17. Wang, J.Y.; Luo, Y.R.; Fu, W.X.; Lu, X.; Zhou, J.P.; Ding, X.D.; Liu, J.F.; Zhang, Q. Genome-wide association studies for hematological traits in swine. *Anim. Genet.* 2013, 44, 34–43. <https://doi.org/10.1111/j.1365-2052.2012.02366.x>
18. Nonneman, D.; Lents, C.; Rohrer, G.; Rempel, L.; Vallet, J. Genome-wide association with delayed puberty in swine. *Anim. Genet.* 2014, 45, 130–132. <https://doi.org/10.1111/age.12087>
19. Curik, I.; Ferencakovic, M.; Sölkner, J. Inbreeding and runs of homozygosity: a possible solution to an old problem. *Livest. Sci.* 2014, 166, 26–34. <http://dx.doi.org/10.1016/j.livsci.2014.05.034>
20. Toro-Ospina, A.M.; Herrera Rios, A.C.; Pimenta Schettini, G.; Vallejo Aristizabal, V.H.; Bizarria dos Santos, W.; Zapata, C.A.; Ortiz Morea, E.G. Identification of Runs of Homozygosity Islands and Genomic Estimated Inbreeding Values in Caqueteño Creole Cattle (Colombia). *Genes* 2022, 13, 1232. <https://doi.org/10.3390/genes13071232>
21. Zhou, X.; Wang, P.; Michal, J.J.; Wang, Y.; Zhao, J.; Jiang, Z.; Liu, B. Molecular characterization of the porcine S100A6 gene and analysis of its expression in pigs infected with highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV). *J. Appl. Genet.* 2015, 56, 355–363. <https://doi.org/10.1007/s13353-014-0260-7>
22. Raymond, P.; Bellehumeur, C.; Nagarajan, M.; Longtin, D.; Ferland, A.; Muller, P.; Bissonnette, R.; Simard, C. Porcine reproductive and respiratory syndrome virus (PRRSV) in pig meat. *Can. J. Vet. Res.* 2017, 81, 162–170.
23. Ueda, W.; Tohno, M.; Shimazu, T.; Fujie, H.; Aso, H.; Kawai, Y.; Numasaki, M.; Saito, T.; Kitazawa, H. Molecular cloning, tissue expression, and subcellular localization of porcine peptidoglycan recognition proteins 3 and 4. *Vet. Immunol. Immunopathol.* 2011, 143, 148–154. <https://doi.org/10.1016/j.vetimm.2011.05.026>
24. Liu, J.; Han, C.; Xie, B.; Wu, Y.; Liu, S.; Chen, K.; Xia, M.; Zhang, Y.; Song, L.; Li, Z.; Zhang, T.; Ma, F.; Wang, Q.; Wang, J.; Deng, K.; Zhuang, Y.; Wu, X.; Yu, Y.; Xu, T.; Cao, X. Rhd3 controls autoimmunity by suppressing the production of IL-6 by dendritic cells via K27-linked ubiquitination of the regulator NEMO. *Nat. Immunol.* 2014, 15, 612–622. <https://doi.org/10.1038/ni.2898>

25. Starkl, P.; Marichal, T.; Galli, S.J. PLA2G3 promotes mast cell maturation and function. *Nat. Immunol.* 2013, 14, 527–529. <https://doi.org/10.1038/ni.2612>
26. Liu, J.; Qi, N.; Xing, W.; Li, M.; Qian, Y.; Luo, G.; Yu, S. The TGF- β /SMAD signaling pathway prevents follicular atresia by upregulating MORC2. *Int. J. Mol. Sci.* 2022, 23, 10657. <https://doi.org/10.3390/ijms231810657>
27. Bonnet, A.; Le Cao, K.A.; Sancristobal, M.; Benne, F.; Robert-Granie, C.; Law-So, G.; Fabre, S.; Besse, P.; De Billy, E.; Quesnel, H.; Hatey, F.; Tosser-Klopp, G. In vivo gene expression in granulosa cells during pig terminal follicular development. *Reproduction* 2008, 136, 211–224. <https://doi.org/10.1530/REP-07-0312>
28. Zhang, W.; Yang, M.; Zhou, M.; Wang, Y.; Wu, X.; Zhang, X.; Ding, Y.; Zhao, G.; Yin, Z.; Wang, C. Identification of signatures of selection by whole-genome resequencing of a Chinese native pig. *Front. Genet.* 2020, 11, 566255. <https://doi.org/10.3389/fgene.2020.566255>
29. Shi, B.; Xue, J.; Zhou, J.; Kasowitz, S.D.; Zhang, Y.; Liang, G.; Guan, Y.; Shi, Q.; Liu, M.; Sha, J.; Huang, X.; Wang, P.J. MORC2B is essential for meiotic progression and fertility. *PLoS Genet.* 2018, 14, e1007175. <https://doi.org/10.1371/journal.pgen.1007175>
30. Udagawa, O.; Ito, C.; Ogonuki, N.; Sato, H.; Lee, S.; Tripvanuntakul, P.; Ichi, I.; Uchida, Y.; Nishimura, T.; Murakami, M.; Ogura, A.; Inoue, T.; Toshimori, K.; Arai, H. Oligo-astheno-teratozoospermia in mice lacking ORP4, a sterol-binding protein in the OSBP-related protein family. *Genes Cells.* 2014, 19, 13–27. <https://doi.org/10.1111/gtc.12105>
31. Takahashi, H.; Koshimizu, U.; Miyazaki, J.; Nakamura, T. Impaired spermatogenic ability of testicular germ cells in mice deficient in the LIM-kinase 2 gene. *Dev. Biol.* 2002, 241, 259–72. <https://doi.org/10.1006/dbio.2001.0512>
32. Kuzmin, A.; Jarvi, K.; Lo, K.; Spencer, L.; Chow, G.Y.; Macleod, G.; Wang, Q.; Varmuza, S. Identification of potentially damaging amino acid substitutions leading to human male infertility. *Biol. Reprod.* 2009, 81, 319–326. <https://doi.org/10.1095/biolreprod.109.076000>
33. Leonova, M.A.; Getmantseva, L.V.; Vasilenko, V.N.; Klimenko, A.I.; Usatov, A.V.; Yu, B.S.; Yu, K.A.; Shirockova, N.V. Leukemia Inhibitory Factor (LIF) Gene Polymorphism and its Impact on Reproductive Traits of Pigs. *American Journal of Animal and Veterinary Sciences.* 2015, 10, 212–216. <https://doi.org/10.3844/ajavsp.2015.212.216>
34. Pitts, M.W.; Reeves, M.A.; Hashimoto, A.C.; Ogawa, A.; Kremer, P.; Seale, L.A.; Berry, M.J. Deletion of selenoprotein M leads to obesity without cognitive deficits. *J. Biol. Chem.* 2013, 288, 26121–26134. <https://doi.org/10.1074/jbc.M113.471235>
35. Li, H.; Xu, C.; Meng, F.; Yao, Z.; Fan, Z.; Yang, Y.; Meng, X.; Zhan, Y.; Sun, Y.; Ma, F.; Yang, J.; Yang, M.; Yang, J.; Wu, Z.; Cai, G.; Zheng, E. Genome-Wide Association studies for flesh color and intramuscular fat in (Duroc Landrace Large White) crossbred commercial pigs. *Genes* 2022, 13, 2131. <https://doi.org/10.3390/genes13112131>
36. Li, X.; Kim, S.W.; Do, K.T.; Ha, Y.K.; Lee, Y.M.; Yoon, S.H.; Kim, H.B.; Kim, J.J.; Choi, B.H.; Kim, K.S. Analyses of porcine public SNPs in coding-gene regions by re-sequencing and phenotypic association studies. *Mol. Biol. Rep.* 2011, 38, 3805–3820. <https://doi.org/10.1007/s11033-010-0496-1>
37. Huang, Q.; Wu, Y.; Qin, C.; He, W.; Wei, X. Phylogenetic and structural analysis of the phospholipase A2 gene family in vertebrates. *Int. J. Mol. Med.* 2015, 35, 587–596. <https://doi.org/10.3892/ijmm.2014.2047>
38. Heid, I.M.; Jackson, A.U.; Randall, J.C.; Winkler, T.W.; Qi, L.; Steinthorsdottir, V.; Thorleifsson, G.; Zillikens, M.C.; Speliotes, E.K.; Mägi, R.; et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat. Genet.* 2010, 42, 949–960. <https://doi.org/10.1038/ng.685> Erratum in: *Nat. Genet.* 2011, 43, 1164.
39. Bakoev, S.; Getmantseva, L.; Kostyunina, O.; Bakoev, N.; Prytkov, Y.; Usatov, A.; Tatarinova, T.V. Genome-wide analysis of genetic diversity and artificial selection in Large White pigs in Russia. *PeerJ.* 2021, 9, e11595. <https://doi.org/10.7717/peerj.11595>
40. Li, X.; Yang, S.; Dong, K.; Tang, Z.; Li, K.; Fan, B.; Wang, Z.; Liu, B. Identification of positive selection signatures in pigs by comparing linkage disequilibrium variances. *Anim. Genet.* 2017, 48, 600–605. <https://doi.org/10.1111/age.12574>
41. Uemoto, Y.; Nakano, H.; Kikuchi, T.; Sato, S.; Ishida, M.; Shibata, T.; Kadowaki, H.; Kobayashi, E.; Suzuki, K. Fine mapping of porcine SSC14 QTL and SCD gene effects on fatty acid composition and melting point of fat in a Duroc purebred population. *Anim. Genet.* 2012, 43, 225–228. <https://doi.org/10.1111/j.1365-2052.2011.02236.x>
42. Munoz, M.; Rodríguez, M.C.; Alves, E.; Folch, J.M.; Ibanez-Escriche, N.; Silio, L.; Fernández, A.I. Genome-wide analysis of porcine backfat and intramuscular fat fatty acid composition using high-density genotyping and expression data. *BMC Genomics* 2013, 14, 845. <https://doi.org/10.1186/1471-2164-14-845>
43. Cameron, N.D.; Enser, M.; Nute, G.R.; Whittington, F.M.; Penman, J.C.; Fiskén, A.C.; Perry, A.M.; Wood, J.D. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. *Meat Sci.* 2000, 55, 187–195. [https://doi.org/10.1016/s0309-1740\(99\)00142-4](https://doi.org/10.1016/s0309-1740(99)00142-4)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.