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Genome-wide analysis of Collo Nudo Italiana and Millefiori Piemontese local chicken breeds: genetic variability and structure analysis in the context of Italian chicken biodiversity

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ABSTRACT

This study aimed to assess the genetic diversity and population structure of two Italian local chicken breeds, Millefiori Piemontese (PMP) and Collo Nudo Italiana (PCI), using the 600K Affymetrix SNP chip array, and to contextualise these breeds in the national biodiversity landscape based on 23 Italian local chicken breeds previously characterised. Minor allele frequency (MAF), expected heterozygosity (He), and observed heterozygosity (Ho) of PCI were 0.311 ± 0.170, 0.484 ± 0.107, and 0.469 ± 0.128, respectively. The inbreeding coefficient (F_{HOM}) was -0.001 ± 0.066 and the inbreeding coefficient calculated on runs of homozygosity (ROH) values (F_{ROH}) was 0.003 ± 0.004. The PMP had MAF, He, and Ho of 0.245 ± 0.201, 0.383 ± 0.112, and 0.428 ± 0.149, respectively, and exhibited higher F_{HOM} (0.149 ± 0.405) and F_{ROH} (0.016 ± 0.009) than PCI. The multi-dimensional scaling plot and neighbour-joining tree depicted clear genetic clustering for both the PCI and PMP breeds. The ROH analysis identified key genomic regions with significant gene annotations. In particular, four ROH islands in GGA4, GGA5, and GGA8 of PCI, and three ROH islands in GGA3 of PMP were detected. For PCI, ROH regions contained genes related to immune response and thermoregulation, such as *S1PR1* and *PLA2G4A*. For PMP, ROH regions included genes involved in muscle development and immune response, such as *RRM2*, *KLF11*, and *CPSF3*. This research provided insights into the genetic diversity and adaptive traits of PCI and PMP breeds, contributing to widen the understanding of Italian local chicken genetic resources.

HIGHLIGHTS

- Genetic diversity and population structure of Millefiori Piemontese (PMP) and Collo Nudo Italiana (PCI) local chicken breeds were assessed.
- Both the breeds showed low level of inbreeding and high level of heterozygosity.
- ROH islands highlighted the genetic adaptive traits of PCI to face thermoregulation, and of PMP in muscle development.

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Introduction

Chickens are the most common domestic livestock species worldwide. Due to their efficient feed utilisation, they have emerged as a prime candidate to meet the increasing global demand for protein. According to the Food and Agriculture Organisation of the United Nations, chickens accounted for approximately 35% of worldwide meat production in 2020 (FAO 2022). Between 1960 and 2010, the global chicken stock increased fivefold and the average carcase weight more than doubled, resulting in a roughly 12–fold increase in chicken meat (Zuidhof et al. 2014).

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This trend can largely be attributed to genetic selection that has primarily focused on productive traits. Unfortunately, indigenous chicken breeds have faced competition from commercial lines and have often been replaced by more productive genotypes (Mastrangelo et al. 2023). This process has led to pauperisation of the genetic diversity of poultry species and the predominance of commercial lines (Pym 2008). The preservation of genetic resources is therefore essential to address future demands in animal breeding, and local chicken populations stand out as crucial reservoirs of new genetic diversity for microevolution and environmental adaptability (Bettridge et al. 2018). It follows that obtaining information on animal genetic diversity is vital to developing strategies for their long-term management. In light of this conservation priority, the TuBAvI project has been established to preserve, enhance, and valorise the Italian local poultry biodiversity (https://www.pollitaliani.it/en/).

The Millefiori Piemontese (PMP) and Collo Nudo Italiana (PCI) are two Italian local chicken breeds. Specifically, the PMP is a dual-purpose breed originally spread in the Cuneo area of Piemonte region (Italy). It is characterised by a predominantly black ground colour with white spots that resemble the pattern of the Ancona breed, but with less regular markings and occasionally bearing traces of red. The breed has been at risk of extinction due to uncontrolled crossbreeding and the abandonment of rural activity (Newsletter R.A.R.E 2012). The University of Torino (Italy) has taken steps to recover the breed through identifying the remaining animals in the Piemonte region and implementing a marker assisted selection program. The PCI has been imported from Transylvania region of Romania, where animals are characterised by white mumps, yellow skin, green shanks, and white or slightly pink eggshell (Răşinar et al. 2023). Although it has been largely crossbred with local Italian chicken breeds during the last century, the morphological feature of naked neck is still present, with some animals exhibiting plumage localised in the front part of the naked neck (Mazzon 1932). The PCI is an excellent layer of large-sized eggs, with a comb remarkably resistant to cold temperatures.

In the present study a genome-wide analysis has been performed to assess the genetic diversity and population structure of PMP and PCI local chicken breeds, and to contextualise them in the framework of the national biodiversity landscape based on 23 Italian local chicken breeds previously characterised (Cendron et al. 2020). This is expected to provide useful information for the conservation of PMP and PCI.

Materials and methods

Samples collection and genotyping

Blood samples were collected from the ulnar vein of 48 PMP animals (20 females and 28 males) and 48 PCI animals (26 females and 22 males). The DNA extraction and genotyping were performed at Neogen (Ayr, Scotland) using a commercial kit and the 600K Affymetrix Axiom Chicken Genotyping Array, respectively, and results of genotyping were aligned using the *Gallus gallus* chicken assembly (GRCg6a), considering the 28 autosomes as in Cendron et al. (2021). The data were then merged with a dataset of 582 genotyped animals of 23 Italian local chicken breeds (from 20 to 24 animals per breed, males and females) and 4 commercial lines (from 9 to 13 animals per commercial line, males and females) from a previous project (Cendron et al. 2020). Those animals were genotyped through the same chip used for PMP and PCI.

The data were manipulated using PLINK software (Chang et al. 2015) to remove SNP with a call rate $<$95% and minor allele frequency (MAF) $<$5%, as well as animals with $>$10% of missing genotypes. After quality control, 673 animals (including 48 and 47 animals of PMP and PCI breeds, respectively) and 467,723 SNP remained for subsequent analyses. Average MAF, expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (F_{HOM}) were estimated for PMP and PCI.

Admixture and genetic relationships

The model-based clustering algorithm was performed in ADMIXTURE software to estimate the population structure, using K values ranging from 1 to 25 (Alexander et al. 2009). The most likely number of populations was estimated using the cross-validation procedure. The genetic relationships within and between breeds were assessed through genome-wide identity-by-state genetic distances, which were calculated using the 'cluster' command of PLINK (Chang et al. 2015). The genetic distances were then charted in a multidimensional scaling (MDS) plot, which represented the first two principal components (C1 and C2) using the 'mds-plot' command. Phylogenetic relationships between the breeds were analysed through Reynolds genetic distances, which were calculated using the *ape* package in the R software (Paradis and Schliep 2019). Neighbour networks were constructed

from the estimated genetic distances using the FIGTREE software (Huson and Bryant 2006). The graphical representations were visualised using R (Huson and Bryant 2014).

Runs of homozygosity

The following criteria were adopted in PLINK software (Chang et al. 2015) to define the runs of homozygosity (ROH) on the newly genotyped populations (PCI and PMP): (1) the minimum length was set to 1 Mb (–homozig-kb), (2) two missing SNP and up to one possible heterozygous genotype were allowed in the ROH (–homozyg-window-missing 2 and –homozyg-window-het 1), (3) the minimum number of SNP that formed the ROH was set to 100 (–homozyg-snp 100), (4) the minimum SNP density per ROH was set to one SNP every 100 kb (–homozyg-density 100), and (5) the maximum gap between consecutive homozygous SNP was 1000 kb (–homozyg-gap 1000) (Strillacci et al. 2018; Cendron et al. 2020; Moscarelli et al. 2021). The sliding windows method was used to identify shorter ROH and those located in regions with variable SNP density. This method scans the genome using sliding windows of 50–100 kb and applies a lower homozygosity threshold of 80% in regions with sparse SNP. It is particularly useful to detect ROH in regions with lower or uneven SNP coverage, allowing for the detection of shorter or fragmented ROH that might otherwise be missed. A consecutive runs method was applied using the R package *detectRUNS* (Biscarini et al. 2018). The following criteria were used to define ROH with the consecutive runs method in *detect*RUNS: (i) the minimum number of SNP required to define a segment as ROH was set to 20, (ii) the number of missing calls allowed within a ROH segment ranged from 0 to 4, (iii) the number of heterozygous calls allowed within a ROH segment varied from 0 to 2, (iv) the minimum length of ROH segments was set to 250 kb, and (v) the maximum gap between consecutive ROH segments was set to 1 Mb (Smaragdov 2023). The consecutive runs method was applied to detect long, high-confidence ROH in regions with high SNP density. This method identifies continuous stretches of homozygosity with a minimum length of 500 kb to 1 Mb and a homozygosity threshold of 90%, ensuring robust and reliable ROH calls. It was particularly effective in regions with a high density of SNPs, where uninterrupted homozygous genotypes are more likely to occur.

The total length of the genome covered by ROH was divided by the total chicken autosomal genome length covered by the SNP array to calculate the individual genomic inbreeding coefficient based on ROH (F_{ROH}), which was then reported as mean per breed. To identify the regions of high homozygosity, the top 0.999 percentile of SNP based on locus homozygosity was selected (Strillacci et al. 2018; Cendron et al. 2020). The GALLO package in R software was used to identify quantitative trait locus (QTL) regions that overlapped with the ROH island regions. QTL information was determined using the *Gallus gallus* genome annotation file (GRCg6a) from the QTL database (https://www.animalgenome.org/cgi-bin/QTLdb/GG/index). Genes mapping within these ROH islands were annotated using GRCg6a from the NCBI database (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000002315.5/). A QTL enrichment analysis was performed in GALLO package to test the significance of the QTL representativeness (Fonseca et al. 2020).

To investigate the biological functions and perform gene ontology analysis the webtool GOrilla was used (Eden et al. 2009). Finally, a literature search was carried out to investigate the biological functions and phenotypes associated with the annotated genes.

Results and discussion

Genetic diversity and population structure

The average MAF for PCI breed was 0.311 ± 0.170, which was similar to the MAF of commercial lines included in the full dataset of Cendron et al. (2020). The He and Ho of PCI averaged 0.484 ± 0.107 and 0.469 ± 0.128, respectively (Table 1). The small difference between He and Ho values, while seemingly minor, may indicate sufficient population substructure. This could be indicative of distinct genetic groups within the population, potentially resulting from factors such as limited gene flow, geographical barriers, or local adaptation. In addition, the standard deviations for He and Ho were relatively large, suggesting considerable variability in heterozygosity across different loci, which could reflect differences in mutation rates, selection pressures, or demographic history that influence genetic diversity at specific loci. Such variability is not uncommon in natural populations and can provide insight into the complex evolutionary processes shaping the genetic architecture of the species under investigation. Also, this variability could be partly attributed to the population substructure observed in the ADMIXTURE analysis (Figure 1). Indeed, at $K = 25$ a highly similar genetic background was observed between PCI and the Broiler Ross 708 commercial line (He = 0.324 ± 0.162; Ho = 0.369 ± 0.219),

Table 1. Means and standard deviation (SD) of genetic diversity indices[1] for Collo Nudo Italiana and Millefiori Piemontese local chicken breeds.

		MAF		He		Ho		F_{HOM}		F_{ROH}	
Breed	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Collo Nudo Italiana	47	0.311	0.170	0.484	0.107	0.469	0.128	−0.001	0.066	0.003	0.004
Millefiori Piemontese	48	0.245	0.201	0.383	0.112	0.428	0.149	0.149	0.405	0.016	0.009

[1]MAF: minor allele frequency; He: expected heterozygosity; Ho: observed heterozygosity; F_{HOM}: inbreeding coefficient; F_{ROH}: inbreeding coefficient calculated on runs of homozygosity (ROH) values.

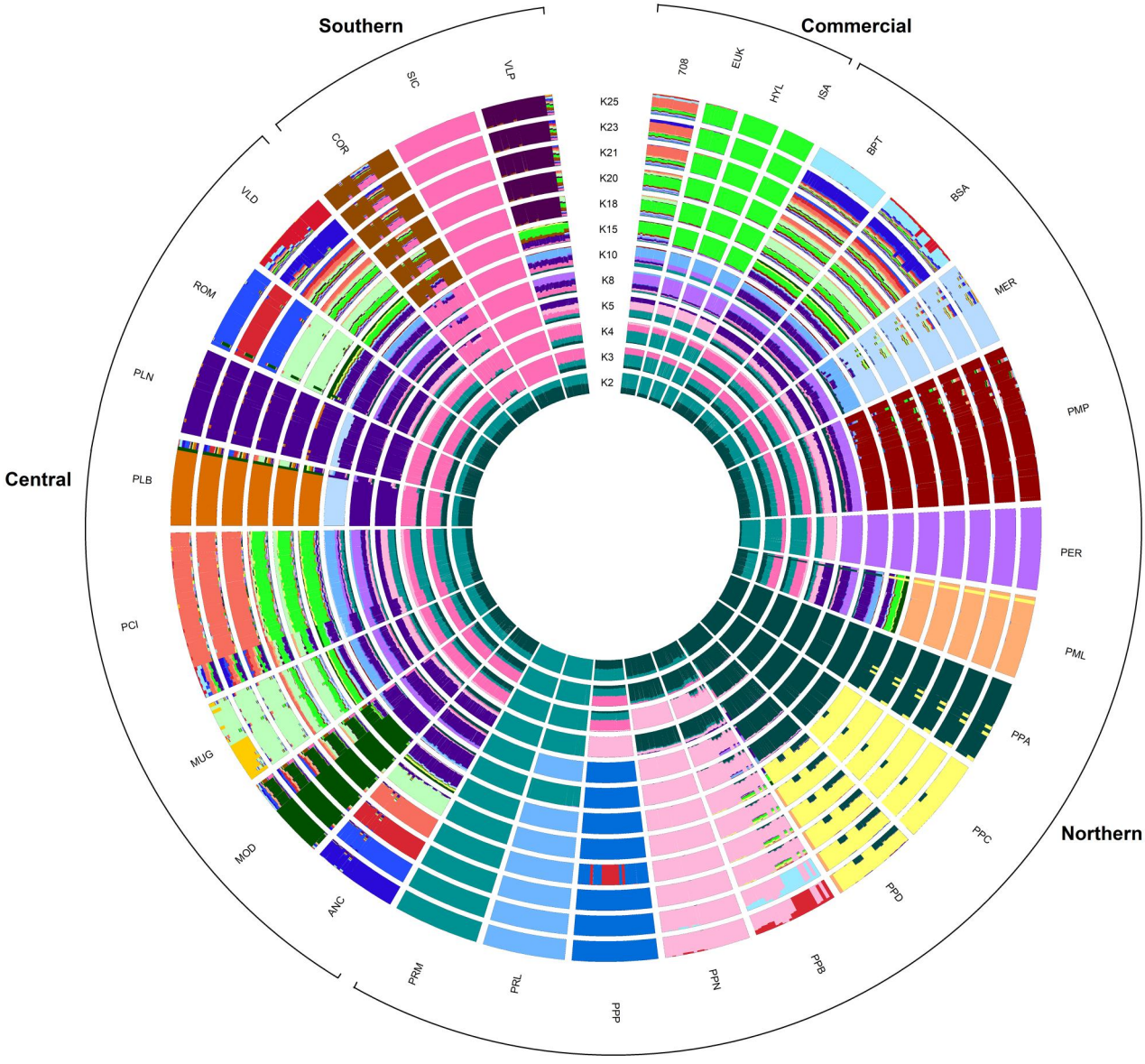

Figure 1. The admixture plot based on different number of assumed ancestors (K). The figure reports the Collo Nudo Italiana (PCI) and Millefiori Piemontese (PMP) along with the 23 local chicken breeds (ANC: Ancona; BSA: Bianca di Saluzzo; BPT: Bionda Piemontese; COR: Cornuta di Caltanissetta; PER: Ermellinata di Rovigo; PLB: Livorno Bianca; PLN: Livorno Nera; MER: Mericanel della Brianza; PML: Millefiori di Lonigo; MOD: Modenese; MUG: Mugellese; PPA: Padovana Argentata; PPC: Padovana Camosciata; PPD: Padovana Dorata; PPP: Pepoi; PPB: Polverara Bianca; PPN: Polverara Nera; PRL: Robusta Lionata; PRM: Robusta Maculata; ROM: Romagnola; SIC: Siciliana; VLD: Valdarnese; VLP: Valplatani) and 4 commercial lines (EUK: Eureka; ISA: ISA Brown; HYL: Hy-line white eggs; 708: Broiler Ross 708) from Cendron et al. (2020). Local chicken breeds are grouped according to the geographical distribution.

with an introgression in the genetic structure of some animals exhibiting partial background complementarity with Ancona (He = 0.274 ± 0.187; Ho = 0.263 ± 0.181), Livorno Nera (He = 0.231 ± 0.195; Ho = 0.233 ± 0.211), and Livorno Bianca (He = 0.218 ± 0.186; Ho = 0.205 ± 0.196) local breeds. The Broiler Ross 708 had

higher He and Ho compared to local breeds, except for the PCI, which had higher He and Ho than the Broiler Ross 708. This may be attributed to the larger population size in terms of both population and number of analysed animals in the present study.

The mean F_{HOM} and F_{ROH} of PCI were -0.001 ± 0.066 and 0.003 ± 0.004, respectively (Table 1), and were similar to values reported for commercial lines in Cendron et al. (2020), suggesting a population substructure (Zhi et al. 2023). Low F_{HOM} and F_{ROH} suggest that this breed might have experienced introgression from other breeds through either reconstitution or crossbreeding over the years. Consequently, it is uncertain whether PCI has retained its original genetic purity, indicating a decline in its conservation. This finding highlights the need for active conservation efforts. Indeed, as reported by Cendron et al. (2020) most Italian local breeds exhibit F_{HOM} and F_{ROH} from 0.40 to 0.60.

With regard to PMP, the MAF of 0.245 ± 0.201 (Table 1) was in line with results retrieved from the literature on indigenous chicken breeds worldwide (e.g. Malomane et al. 2019; Cendron et al. 2021; Meyermans et al. 2024; Xie et al. 2024). The He and Ho averaged 0.383 ± 0.112 and 0.428 ± 0.149, respectively (Table 1). These levels of heterozygosity were higher than those reported for the other Italian local chicken breeds, which averaged 0.237 (He) and 0.228 (Ho) (Cendron et al. 2020). This difference suggests a relatively higher genetic diversity in the population, which could be attributed to several factors. First, the PMP may have experienced different historical or evolutionary dynamics, such as larger effective population size, reduced inbreeding, or greater gene flow between subpopulations, all of which could contribute to increased heterozygosity. Additionally, the higher heterozygosity observed in this population may reflect effective conservation efforts or breeding programs that aim to maintain genetic diversity. Indeed, the PMP showed low inbreeding, as indicated by F_{HOM} (0.149 ± 0.405) and F_{ROH} (0.016 ± 0.009), which were much lower than those observed in the other Italian local chicken breeds (Cendron et al. 2020), thus remarking the correct management of PMP in terms of allele pools and genetic variability (Talebi et al. 2020). It is also worth noting that a low inbreeding coefficient in local breeds is not always positive. In the case of PCI, the low F_{ROH} could suggest recent crossbreeding with other breeds, resulting in higher genetic variability. Since this breed has only recently entered conservation, its genetic history is almost unclear. Typically, such low F_{HOM} and F_{ROH} are observed in commercial lines (Cendron et al. 2020), whereas ancient local breeds show much higher levels of F_{ROH}.

The MDS plot (Figure 2) depicts the good genetic identity of both the PCI and PMP breeds, as emphasised by their well-defined clustering pattern for all the animals, without any outlier. In Figure 2, the PMP was close to the Bionda Piemontese, which is a breed originating from the same geographical region (Piemonte). In addition, the PMP was closely related to Millefiori di Lonigo, a breed from Veneto region with similar plumage pattern (Perini et al. 2020). In the neighbour-joining tree the PMP breed is located near the cluster of Padovana and Polverara breeds (Figure 3), which suggests that some genotypes from Polverara and/or Padovana breeds might have been utilised in the past for the re-establishment of the breed, or that there was a common ancestor. The PCI breed is located within the main branch of central Italy breeds, along with Millefiori di Lonigo, Bionda Piemontese, and Bianca di Saluzzo, and the group of breeds of Central Italy (Ancona, Romagnola, Modenese, Livorno Bianca, and Livorno Nera). This highlighted the potential for genetic contamination of Livorno Nera and Livorno Bianca due to crossbreeding utilisation of PCI and corroborated the proximity of the PCI breed to the Central Italy breeds, particularly the Mugellese, Valdarnese, and Bianca di Saluzzo (Figure 2).

The analysis of the complete dataset using the ADMIXTURE software revealed both the population structure and the extent of admixture within the populations (Figure 1). The most likely *K* was 25, as most of the studied breeds showed their own genetic makeup. The PMP demonstrated a distinct genetic structure when compared to the other breeds, with all PMP animals having a unique structure and genetic identity. In contrast, the structure of animals of the PCI resembled that of the Broiler Ross 708 commercial line. While most PCI animals exhibited an unvarying genetic structure, some had a high level of mixed genetic background, similar to the predominant structure found in Broiler Ross 708. These results emphasised differences between sampled populations, and a variability in genetic structure due to the uncontrolled crossbreeding reported from the last century and still in use (Mazzon 1932). The crossbreeding of PCI with other genetic resources aligns with high He and Ho, and low inbreeding values. The structure of PCI subpopulations can skew results and does not allow for the establishment of a definitive and shared genetic foundation that accurately represents the PCI breed.

Figure 2. Multidimensional scaling plot of Collo Nudo Italiana (PCI) and Millefiori Piemontese (PMP) along with the 23 local chicken breeds (ANC: Ancona; BSA: Bianca di Saluzzo; BPT: Bionda Piemontese; COR: Cornuta di Caltanissetta; PER: Ermellinata di Rovigo; PLB: Livorno Bianca; PLN: Livorno Nera; MER: Mericanel della Brianza; PML: Millefiori di Lonigo; MOD: Modenese; MUG: Mugellese; PPA: Padovana Argentata; PPC: Padovana Camosciata; PPD: Padovana Dorata; PPP: Pepoi; PPB: Polverara Bianca; PPN: Polverara Nera; PRL: Robusta Lionata; PRM: Robusta Maculata; ROM: Romagnola; SIC: Siciliana; VLD: Valdarnese; VLP: Valplatani) and 4 commercial lines (EUK: Eureka; ISA: ISA Brown; HYL: Hy-line white eggs; 708: Broiler Ross 708) from Cendron et al. (2020).

ROH islands analysis

The ROH islands within the breeds were mapped in GGA3 for PMP and in GGA4, GGA5, GGA8 for PCI (Table 2 and Figure 4). Three ROH regions in the PMP breed were identified in GGA3 and were located within close proximity (94–100 Mb). A total of 55 genes for PCI and 44 genes for PMP were mapped within the ROH regions. The PCI genes within the GGA5 ROH region showed the only significantly enriched annotation cluster (Benjamini corrected p-value < 0.05) for processes of inorganic molecular entity transmembrane transporter activity (GO:0015318). The analysis of the GO enrichment showed that genes involved in this process are *Solute Carrier Family members (SLC17A6*, *SLC5A12*, *SLC6A5*) and *Anoctamin* (*ANO3*, *ANO5*). The annotation in ROH regions of PCI highlighted QTL related to productive performances such as body weight, average daily gain, and feed

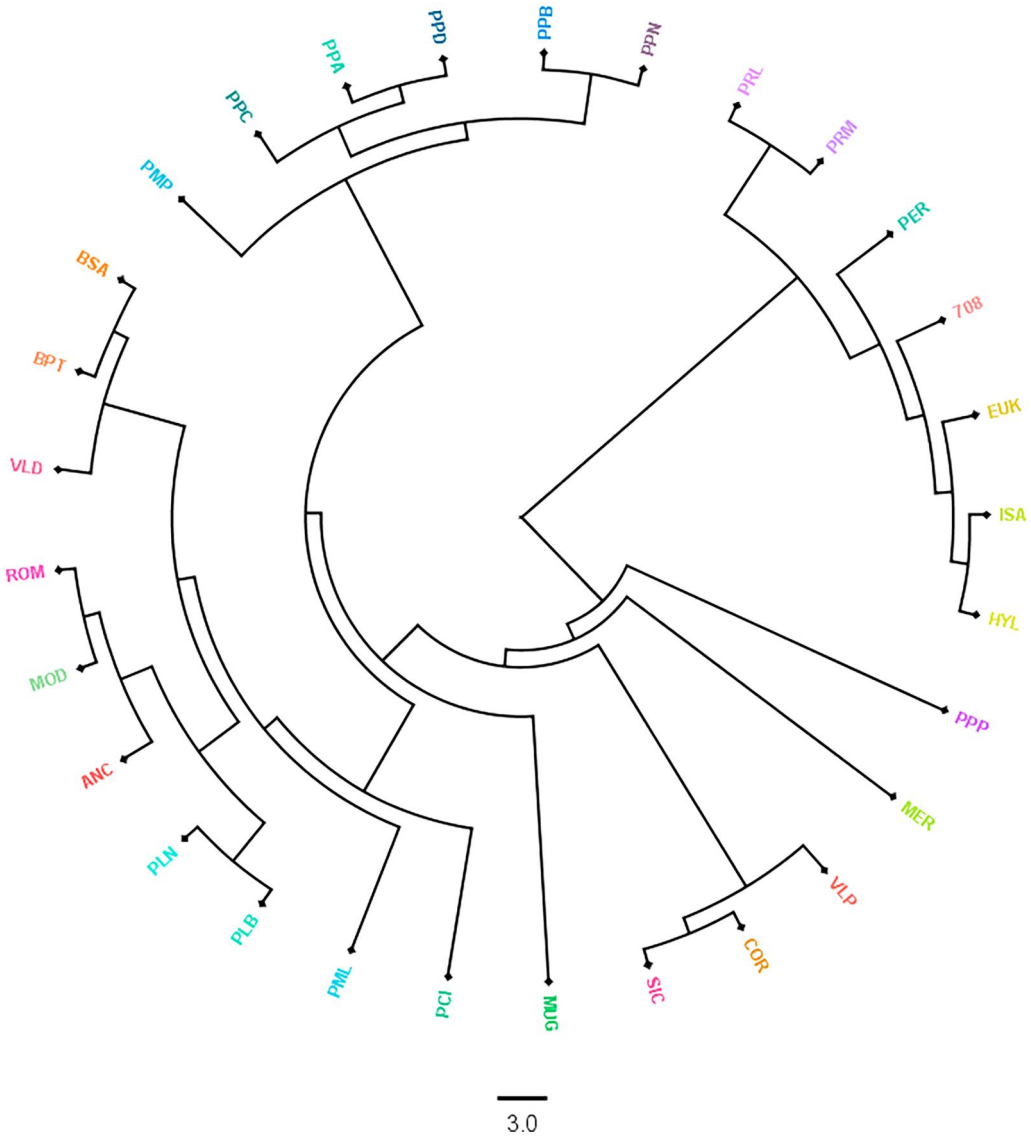

Figure 3. Neighbour-joining tree plot for Collo Nudo Italiana (PCI) and Millefiori Piemontese (PMP) along with the 23 local chicken breeds (ANC: Ancona; BSA: Bianca di Saluzzo; BPT: Bionda Piemontese; COR: Cornuta di Caltanissetta; PER: Ermellinata di Rovigo; PLB: Livorno Bianca; PLN: Livorno Nera; MER: Mericanel della Brianza; PML: Millefiori di Lonigo; MOD: Modenese; MUG: Mugellese; PPA: Padovana Argentata; PPC: Padovana Camosciata; PPD: Padovana Dorata; PPP: Pepoi; PPB: Polverara Bianca; PPN: Polverara Nera; PRL: Robusta Lionata; PRM: Robusta Maculata; ROM: Romagnola; SIC: Siciliana; VLD: Valdarnese; VLP: Valplatani) and 4 commercial lines (EUK: Eureka; ISA: ISA Brown; HYL: Hy-line white eggs; 708: Broiler Ross 708) from Cendron et al. (2020).

intake. Moreover, QTL related to fat, and pigmentation of feather and eggshell have been reported (Table 2). On the other hand, the PMP breed showed QTL mainly related to muscle development and average daily gain (Table 2). The enrichment analysis enforced the previous results, but also pointed out that important QTL related to claw and shank features were found around the ROH in GGA4 (Figure 5A). The QTL were previously described by Li et al. (2021) and Zhang et al. (2021).

In the PMP breed, genes related to physiological adaptation like the *Integrin Subunit Beta 1 Binding Protein 1* (*ITGB1BP1*) have been detected in GGA3. As the PMP is phenotypically similar to the Ancona breed with black background and irregular white pickling, it has been reported that the red colour could be randomly found in few animals. Indeed, in the ROH island the genes such as *ATPase H + Transporting V1 Subunit C2* (*ATP6V1C2*), *Protein Disulphide Isomerase Family A Member 6* (*PDIA6*), and *ITGB1BP1* have been reported for red plumage colour in Chinese ducks (Zhang et al. 2024). In the same ROH island two genes related to diseases resistance, the *Cleavage and Polyadenylation Specificity Factor 3 (CPSF3)* and the *Cytidine Monophosphate Kinase 2 (CMPK2)*. The *CPSF3* is an essential protein involved in the regulation of gene

Table 2. Annotated QTL and genes in runs of homozygosity (ROH) islands[1] for Collo Nudo Italiana and Millefiori Piemontese local chicken breeds.

Breed	Chr	Start	End	SNP	Length	Genes	QTL
Collo Nudo Italiana	4	70,895,731	71,679,196	265	783,465	*PCDH7*	Age at first egg Claw weight Shank length Claw percentage
	5	2,350,447	3,783,537	271	1,433,090	*PRMT3, LOC112532546, SLC6A5, NELL1* *MIR1775, LOC112532484, LOC107053350, ANO5, SLC17A6 LOC112532530, FANCF, GAS2* *SVIP, LOC112532481, ANO3, SLC5A12, FIBIN, BBOX1*	Body weight Heptadecanoic acid content
	8	10,358,029	11,892,267	281	1,534,238	*PLA2G4A, PTGS2* *PDC, C8H1orf27, TPR, PRG4, LOC112532878, HMCN1, IVNS1ABP, SWT1, TRMT1L, AMY1AP, AMY1A, RNPC3, COL11A1, MIR6561*	Abdominal fat weight Average daily gain Feed intake Drumstick and thigh muscle weight Abdominal fat percentage
	8	11,902,867	12,606,760	243	703,893	*OLFM3, S1PR1, MIR1610, DPH5, SLC30A7, EXTL2, CDC14A, VCAM1, RTCA, DBT, LRRC39, TRMT13, SASS6, MFSD14A, SLC35A3, LOC424473, AGL, FRRS1, PALMD, LOC107053968*	Feather pigmentation Eggshell cuticle coverage Abdominal fat weight
Millefiori Piemontese	3	94,927,984	96,999,305	989	2,071,321	*CMPK2, RSAD2, RNF144A, ID2, KIDINS220, MBOAT2, ASAP2* *ITGB1BP1, CPSF3, IAH1, ADAM17, YWHAQ, TAF1B, GRHL1, KLF11, RRM2, TRNAM-CAU, HPCAL1, MIR6655, ODC1, NOL10, ATP6V1C2, MIR1329, PDIA6*	Feather pigmentation Breast muscle weight Average daily gain Muscle fibre diameter Thigh muscle weight
	3	97,000,896	97,999,611	398	998,715	*KCNF1, LOC101750599, C2orf50, PQLC3 ROCK2, E2F6, GREB1, LOC112532134, LPIN1, TRIB2*	
	3	98,000,219	100,129,559	820	2,129,340	*FAM84A, NBAS, LOC107051693, DDX1, MYCN, LOC112532188, FAM49A, LOC107051696, RAD51AP2, VSNL1*	

[1]Chr: chromosome; Start: start bp of the ROH island; End: end bp of the ROH island; SNP: number of SNP mapped within the ROH island; Length: length in bp of the ROH island; Genes: genes mapped within the ROH island; QTL: QTL detected in the genomic region of the ROH island.

expression, and it has been reported to enhance chicken immunity and promote growth (Pan et al. 2024). Instead, the *CMPK2* gene plays a pivotal role in host immune response against Avian Influenza and Newcastle Disease infection (Li et al. 2022). Gene related to immune response in ROH island of PCI breed has been also identified. In particular, in GGA8 the gene *Sphingosine-1–Phosphate Receptor 1* (*S1PR1*) is involved in immune response to viral infections, especially in influenza infection (Jiang et al. 2017).

In the PMP breed, most of the genes mapped within the ROH islands are associated with muscle development. This is the case of *Ribonucleotide Reductase Regulatory Subunit M2* (*RRM2*), *MYCN Proto-Oncogene* (*MYCN*), and *KLF Transcription Factor 11* (*KLF11*) which have been found highly expressed in muscle in chicken promoting the proliferation of myoblast (Yin et al. 2023; Chen et al. 2024). The *Lipin 1* (*LPIN1*) gene is involved in meat production; in particular, it has been found over expressed in Bionda Piemontese breed when compared to a broiler commercial line (Perini et al. 2023). Indeed, the PMP has a role in meat production, and it can be categorised as a dual-purpose breed (Stoppani et al. 2024). The meat production attitude in PMP is also confirmed by enrichment QTL analysis (Figure 5). The feather pigmentation was the most valuable QTL in PMP due to the spotted plumage reported for this breed (Figure 5B) (Stoppani et al. 2024).

Focusing on ROH island in PCI breed, the region 5:2350447–3783537 has been already reported in other studies as putative candidate gene for human selection (Cendron et al. 2020; 2021; Tan et al. 2024). The ROH island in GGA8 in PCI points out the thermoregulation process, which is theoretically traceable to a naked neck breed as the PCI. Indeed, *Phospholipase A2 Group IVA* (*PLA2G4A*) and *Prostaglandin-Endoperoxide Synthase 2* (*PTGS2*) genes are able to limit the production of prostaglandins involved in temperature regulation (Zhao et al. 2022). The *Translocated Promoter Region* (*TPR*) and RNA *Binding Region Containing 3* (*RNPC3*) genes interact with *Heat Shock Protein* family *HSP70*, *HSP70*, and *HSP90*, and are required for regulation of protein folding and transport (Srikanth et al. 2019; Videla Rodriguez et al. 2024). Moreover, the *S1PR1, Amylase Alpha 1A* (*AMY1A*), and *Cell Division Cycle 14A* (*CDC14A*) genes mapped in GGA8:11902867–12606760 have been

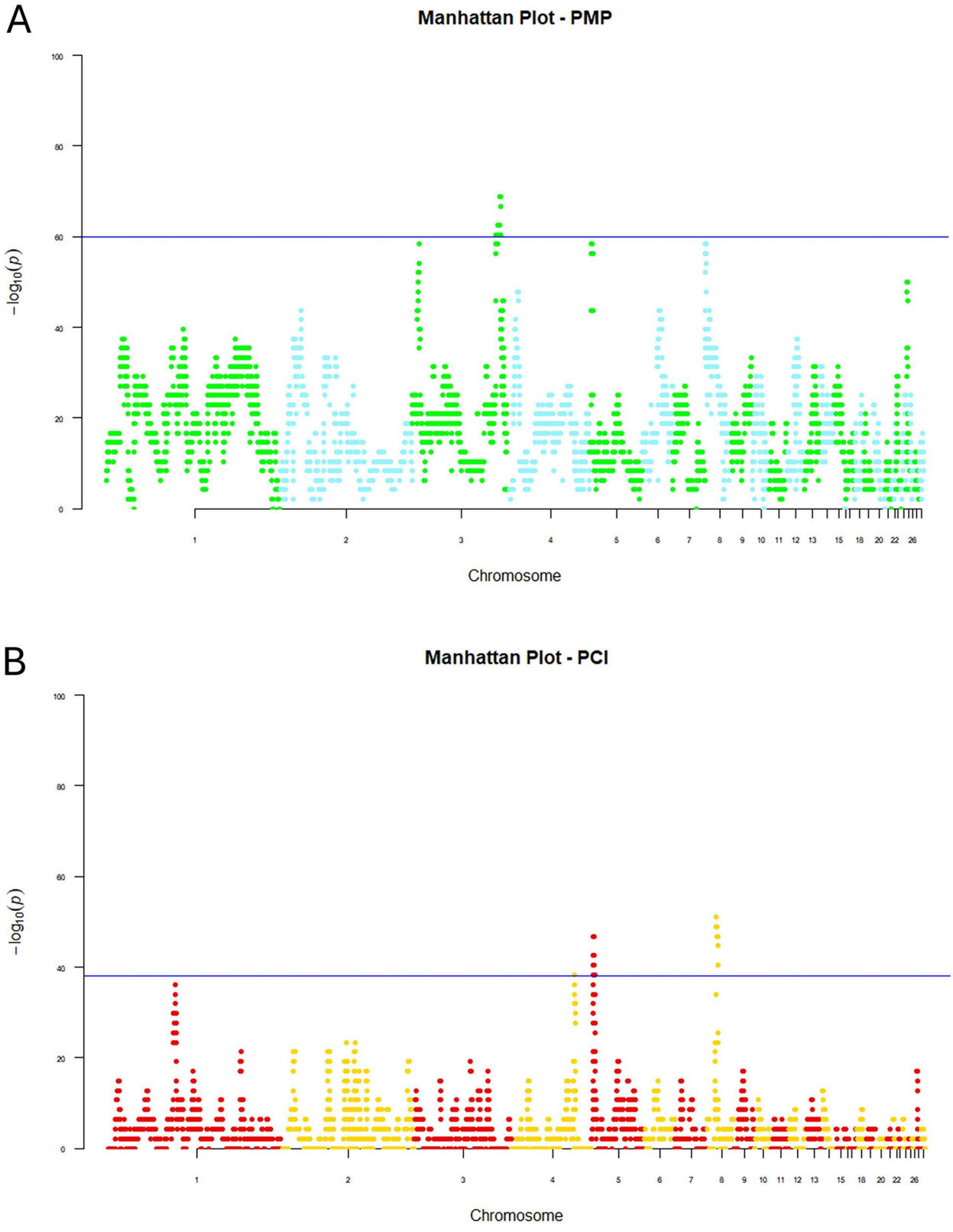

Figure 4. Manhattan plot of occurrences (%) of a SNP in runs of homozygosity (ROH) across breeds for (A) Millefiori Piemontese (PMP) and (B) Collo Nudo Italiana (PCI).

reported to be involved in disease resistance, heat tolerance, immune regulation, and behavioural traits (Xie et al. 2024). Furthermore, the *AMY1A* gene has been linked to carcase traits and feed intake efficiency in chickens (Zhang et al. 2021). Finally, *amylo-alpha-1, 6–glucosidase, 4–alpha-glucanotransferase* (*AGL*) gene is

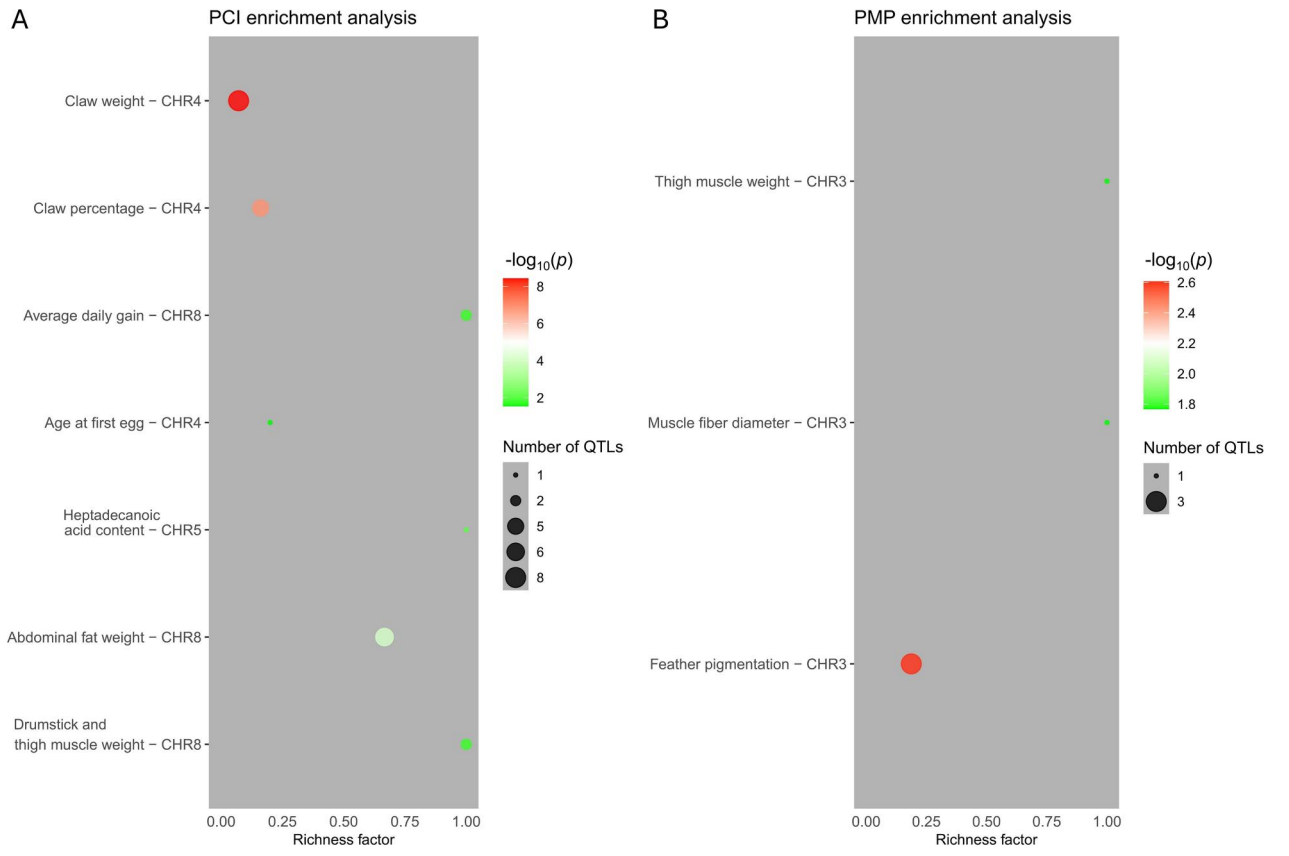

Figure 5. Top enriched traits identified in the QTL enrichment analysis for (A) Collo Nudo Italiana (PCI) and (B) Millefiori Piemontese (PMP) local chicken breeds. The area of the bubbles represents the number of observed QTL for that class and the colour represents the p-value scale (the deeper the red colour, the smaller the p-value). Additionally, the x-axis shows the richness factor for each QTL, representing the ratio of number of QTL and the expected number of that QTL.

involved in the regulation of vascular permeability, and plays a key role in angiogenesis and regulation of blood pressure (Zhao et al. 2022).

Conclusions

This study elucidated the genetic diversity and population structure of PCI and PMP Italian local chicken breeds through a high-density chip. Both breeds had high levels of heterozygosity and low inbreeding coefficients, reflecting their genetic diversity. Notably, the ROH analysis identified genomic regions associated with key adaptive traits, including muscle development and immune response in PMP, and thermoregulation and immune response in PCI. These findings highlight the distinct genetic identities of the breeds and their specific adaptive traits, which are important for conservation strategies. However, the PCI showed evidence of genetic introgression, likely due to past crossbreeding, leading to the emergence of subpopulations with mixed genetic backgrounds. This admixture is particularly visible in some PCI individuals, sharing genetic similarities with commercial lines. The presence of this subpopulation complicated the breed genetic landscape and suggests that conservation efforts must be vigilant to preserve the original genetic identity of the breed. In summary, the present study reinforces the value of local chicken breeds as reservoirs of genetic diversity and adaptive traits, which are crucial for the long-term sustainability of poultry production. The findings also emphasised the need for tailored conservation efforts, especially for PCI, where subpopulation structures may influence genetic management strategies.

Ethical approval

Ethical approval was not required for the current study. Blood samples were collected in compliance with the European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009) during routine health controls by the public veterinary service.

Disclosure statement

The authors declare that there are no conflicts of interest associated with the paper. The authors alone are responsible for the content and writing of this article.

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Data availability statement

None of the data were deposited in an official repository. The data that support the findings presented in this study are available from the corresponding author upon reasonable request.

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