Genetic characterization of Collo Nudo Italiana and Millefiori Piemontese chicken breeds through high-density SNP genotyping

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Introduction

Preserving local genetic resources like Millefiori Piemontese (PMP) and Collo Nudo Italiana (PCI) chicken breeds is essential for future breeding needs. The PMP, a dual-purpose breed from the Piemonte region (Italy), is characterized by black and white spotted plumage and has faced extinction risks due to crossbreeding and abandonment of rural activity. The PCI, imported from Romania, features a distinctive naked neck and has been crossbred with local Italian breeds. **This study assessed the genetic diversity and population structure of PMP and PCI through genome-wide analysis, contextualizing these breeds within the national local chicken biodiversity landscape.**

Conclusions

- The PMP breed exhibited a unique genomic structure.
- The PCI breed showed evidence of subpopulation differentiation, likely due to uncontrolled crossbreeding.
- Both PCI and PMP had higher heterozygosity and lower inbreeding than other Italian local chicken breeds.
- The analysis of ROH islands revealed adaptive genetic traits of PCI for thermoregulation and of PMP for muscle development.
- Findings of this study are useful for the conservation of these breeds.

Materials and Methods

Samples Collection and Genotyping

- Blood samples collected from the ulnar vein of 48 PMP and 48 PCI chickens (males and females).
- DNA extracted using a commercial kit and genotyped through the 600K Affymetrix SNP chip array.
- Alignment with the *Gallus gallus* chicken assembly.
- Data merged with a dataset of 582 genotyped animals from 23 Italian local chicken breeds and 4 commercial lines, males and females [1].
- 673 animals and 467,723 SNP after quality control.

Admixture and Genetic Relationships

- Population structure estimated using the ADMIXTURE software.
- Genetic relationships assessed through genome-wide identity-by-state distances and visualized in a multidimensional scaling (MDS) plot.
- Phylogenetic relationships analysed using Reynolds genetic distances and neighbor networks.

Runs of Homozygosity

- Runs of Homozygosity (ROH) defined using specific criteria.
- Calculation of individual genomic inbreeding coefficient based on ROH.
- Identification of high homozygosity regions and annotation of genes within these regions.
- Identification of quantitative trait loci (QTL) in ROH islands.
- Analysis of gene ontology to investigate biological functions.

Results and Discussion

Genetic Diversity and Population Structure

- Average minor allele frequency for PCI similar to commercial lines [1] (Table 1).
- Expected and observed heterozygosity and inbreeding coefficients for PCI indicated population substructure [2] (Table 1).
- For PMP, minor allele frequency was consistent with indigenous breeds globally [3, 4, 5, 6] (Table 1).
- The PMP showed higher expected and observed heterozygosity and lower inbreeding than other Italian local breeds, suggesting effective management [7] (Table 1).
- The ADMIXTURE analysis revealed genetic similarities between PCI and the Broiler Ross 708 line, and some PCI animals showed genetic backgrounds similar to Livorno breeds (Figure 1).
- The MDS plot and neighbor-joining tree confirmed the genetic identity and relatedness of PMP to breeds from the same region and with similar plumage patterns [8] (Figure 2).

ROH Islands Analysis

- ROH islands identified in specific genomic regions for PMP and PCI.
- Mapped genes showed significant functions (Table 2).
- Genes in GG3, GGA5 and GGA8 were enriched for inorganic molecular transporter activity, muscle development, disease resistance, immune response and physiological adaptation.
- QTL were related to body weight, fat, and pigmentation [9, 10] (Table 2).

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

Breed	n	MAF Mean	MAF SD	He Mean	He SD	Ho Mean	Ho SD	F_{HOM} Mean	F_{HOM} SD	F_{ROH} Mean	F_{ROH} SD
Collo Nudo Italiana	47	0.311	0.170	0.655	0.117	0.673	0.140	-0.001	0.066	0.003	0.004
Millefiori Piemontese	48	0.245	0.201	0.566	0.120	0.601	0.120	0.149	0.405	0.016	0.009

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.

Table 2. Annotated QTL and genes in runs of homozygosity (ROH) islands 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	-
	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
	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	Breast muscle weight, Average daily gain, Muscle fiber 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	

Chr = chromosome; Start = start bp of the ROH island; End = end bp of ROH island; SNP = number of SNP mapped within a 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.
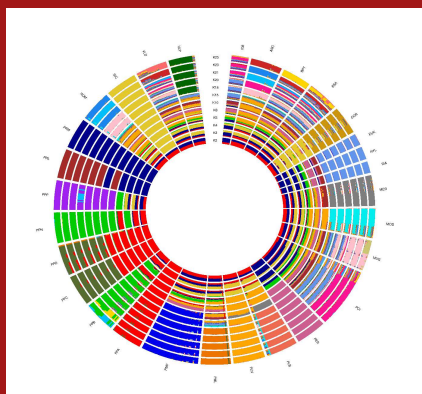

Figure 1. The admixture plot based on different number of assumed ancestors (K). The figure reports the Collo Nudo Italiana (PCI) and Millefiori Piemontese (PMP) local chicken breeds along with the 23 local chicken breeds and 4 commercial lines from [1].
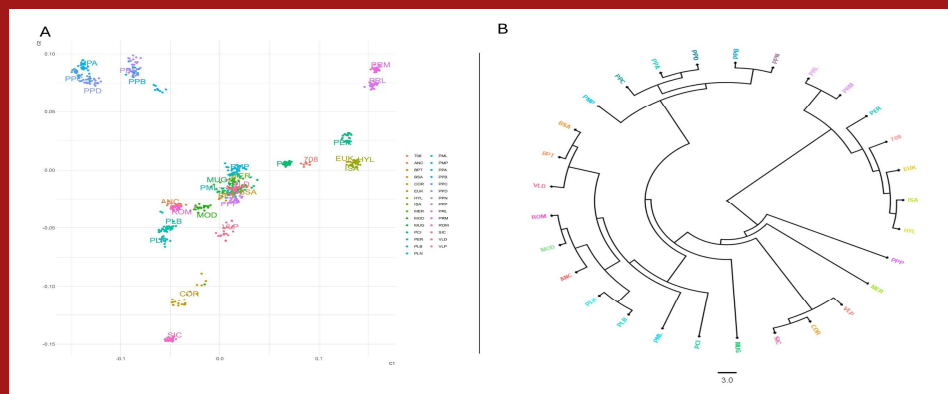

Figure 2. A) MDS plot and B) neighbour-joining tree using Collo Nudo Italiana (PCI) and Millefiori Piemontese (PMP) local chicken breeds along with the 23 local chicken breeds (Ancona - ANC, Bianca di Saluzzo - BSA, Bionda Piemontese - BPT, Cornuta di Caltanissetta - COR, Ermellinata di Rovigo - PER, Livorno Bianca - PLB, Livorno Nera - PLN, Mericanel della Brianza - MER, Millefiori di Lonigo - PML, Modenese - MOD, Mugellese - MUG, Padovana Argentata - PPA, Padovana Camosciata - PPC, Padovana Dorata - PPD, Pepoi - PPP, Polverara Bianca - PPB, Polverara Nera - PPN, Robusta Lionata - PRL, Robusta Maculata - PRM, Romagnola - ROM, Siciliana - SIC, Valdarnese - VLD, Valplatani - VLP) and 4 commercial lines (Eureka - EUK, ISA Brown – ISA, Hy-line white eggs - HYL, Broiler Ross 708 - 708) from [1].

References

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