Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tjas20

Genetic characterisation of a recovered Italian chicken breed: the Millefiori Piemontese

Nadia Stoppani, Eleonora Erika Cappone, Dominga Soglia, Margherita Profiti, Sandra Maione, Achille Schiavone & Stefano Sartore

To cite this article: Nadia Stoppani, Eleonora Erika Cappone, Dominga Soglia, Margherita Profiti, Sandra Maione, Achille Schiavone & Stefano Sartore (2024) Genetic characterisation of a recovered Italian chicken breed: the Millefiori Piemontese, Italian Journal of Animal Science, 23:1, 1456-1468, DOI: 10.1080/1828051X.2024.2408469

To link to this article: https://doi.org/10.1080/1828051X.2024.2408469

Published online: 30 Sep 2024.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2024, VOL. 23, NO. 1, 1456–1468
https://doi.org/10.1080/1828051X.2024.2408469

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Genetic characterisation of a recovered Italian chicken breed: the Millefiori Piemontese

Nadia Stoppani, Eleonora Erika Cappone, Dominga Soglia, Margherita Profiti, Sandra Maione, Achille Schiavone and Stefano Sartore

Department of Veterinary Sciences, University of Torino, Grugliasco, Italy

ABSTRACT

The recent rediscovering of the Millefiori Piemontese breed, previously considered as extinct, has led to its genetic characterisation: establishing the basis for its recovery and preservation. This study describes the morpho-biometric traits and compares the genetic variability of the Millefiori Piemontese breed with that of other local chicken breeds using 26 microsatellite markers. A subset of 14 markers was used to compare the genetic variation of the Millefiori Piemontese breed with that of two other Piedmontese chicken breeds (Bionda Piemontese and Bianca di Saluzzo) as well as 17 Italian and 2 commercial hybrids, whose genetic variability has already been investigated. The present study confirmed the sexual dimorphism and assessed the genetic variability of the Millefiori Piemontese in terms of number of alleles/locus (Na = 4), the effective number of alleles (Nea = 3), observed (Ho = 0.56) and expected heterozygosity (He = 0.53), self-coancestry (I_B = 0.65), potential extinction risk (ERI = 2), and its contribution to the Italian poultry biodiversity (GD_T = −0.60). The results indicate that, despite its small population size (Ne = 56), the Millefiori Piemontese population exhibits significant genetic diversity, making it a valuable resource for breeding programs focused on preserving the breed and safeguarding its biodiversity. This study is the first to investigate the genetic variability of the Millefiori Piemontese breed and compare it with other local poultry breeds. The findings highlight the genetic uniqueness of the Millefiori breed and its significant contribution to the biodiversity of chickens in Piedmont and Italy, emphasising the importance of its conservation.

HIGHLIGHTS

- The Millefiori Piemontese is an Italian local breed, whose population has drastically decreased due to the spread of commercial hybrids, bordering on extinction; today a little group of individuals has been identified in 5 small farms.
- Microsatellite markers were used to evaluate the genetic variability and the contribution to poultry biodiversity.
- Millefiori Piemontese showed a high degree of genetic variability useful for adapting to new environmental conditions.
- Millefiori Piemontese breed makes a positive contribution to overall Italian genetic diversity.

ARTICLE HISTORY
Received 3 June 2024
Revised 19 September 2024
Accepted 19 September 2024

KEYWORDS
Genetic variability; microsatellite markers; small population; local chicken breed

Introduction

Worldwide, both agriculture and livestock management systems are expected to undergo dramatic modifications over the coming decades, due to the ongoing climate changes. Farming systems need to become more sustainable and adaptable to new conditions (FAO 2017). In particular, free-range systems and local sources will provide important strategies for coping with future challenges (IPCC 2007).

A key factor within this context is the genetic variability preservation (Notter 1999).

The population size of local breeds has been further diminished by the industry's preference for more productive hybrids (Castillo et al. 2021). Local chicken breeds, irrespective of their current population size, are likely to provide an important reservoir of genetic diversity (Hoffman 2010).

Despite this, local breeds have retained a relatively high number of private alleles that are lacking in the commercial hybrids (Muir et al. 2008). Many of these breeds are now considered at risk of extinction (Granevitze et al. 2007; Dávila et al. 2009; Abebe et al. 2015; Palinkas-Bodzsar et al. 2020).

CONTACT Dominga Soglia dominga.soglia@unito.it

Genetic investigations of such breeds show that some have maintained a high level of genetic variability, whereas others have experienced significant declines (Granevitze et al. 2007; Berthouly et al. 2008; Bortoluzzi et al. 2018).

In Italy, a remarkable number of local breeds still exist, each exhibiting different levels of genetic variability (Zanon and Sabbioni 2001; Soglia et al. 2021; Polli Italiani 2024). According to the Domestic Animal Diversity Information System of the Food and Agriculture Organisation of the United Nations (DAD-IS 2018, FAO), approximately 60% of the 90 historically known Italian breeds are extinct, 13% are threatened, 17% are poorly spread, and only 9% have a widespread distribution (Zanon and Sabbioni 2001).

Encouraging alternative poultry farmers to rear autochthonous breeds could be beneficial, given the niche market for native breed products and increasing consumer awareness and demand for such alternative products (Franzoni et al. 2021).

Promoting the use of native breeds in alternative farming systems can also help safeguard Italy's poultry genetic resources. Following a surge in interest for local chicken breeds conservation, numerous recovery programs have been established in Italy, with the primary goal of preserving genetic diversity in these breeds (Zanetti et al. 2011; Cendron et al. 2020).

In the Piedmont region (northwest Italy), in addition to the local breeds Bionda Piemontese (BP) and Bianca di Saluzzo (BS), which have already been studied, the Italian Ministry of Agriculture, Food Sovereignty and Forestry has recognised an additional local chicken population: the Millefiori Piemontese (MP).

MP is a local, dual-purpose breed widespread in the south of Piedmont. These chickens were traditionally raised on farms and sold at country markets. They are characterised by black plumage speckled with white, although red spots are allowed. It is a chicken with rather large shapes and a stocky body, reminiscent of the Mediterranean chickens. The ears are red or red with white markings. The skin is yellow, the beak yellow shaded black. The crest, less developed than in other Piedmontese breeds, is simple and sometimes reclining on one side in females in deposition. The hens could reach the weight of 2.3–3.0 kg, with males being heavier (3.5–4 kg). The shape is similar to the BP (Zanon and Bigi 2022; https://www.pollitaliani.it/en/; https://archiviostoricoavicoltura.blogspot.com/).

Until the 1960s, MP was widespread in the Cuneo province (southwest Piedmont, Italy). However, due to uncontrolled crossbreeding and industrialisation, the population size gradually decreased. Few individuals were sporadically reported in the countryside until the 1990s, when the breed has officially been considered as extinct (Castillo et al. 2021; https://www.pollitaliani.it/en/; https://archiviostoricoavicoltura.blogspot.com/).

Recently, thanks to the passion of a discrete number of breeders, five small farms hosting MP breed have been found in the Piedmont Region.

Since 2023, the MP breed has been included in the TUBAVI project, which is dedicated to safeguarding, conserving, and valorising Italian poultry genetic resources (https://www.pollitaliani.it/en/; Castillo et al. 2021): the breed has been hosted at the Avian Conservation Centre for Valorisation of Local Genetic Resources (CoVaGEN), of the University of Turin (Italy) (44°50′58″ N and 7°43′13″ E).

Genetic characterisation of a population, together with its phenotypic description, is an important tool for defining a breed's characteristic and assessing its genetic diversity (Ajmone-Marsan et al. 2023).

Genetic diversity analysis and relatedness in local breeds are mainly assessed using DNA markers such as microsatellites, mitochondrial DNA, copy number variation, and single nucleotide polymorphism (SNP). While SNPs have been widely used in genetic researches, microsatellites markers offer several advantages, as they are comparatively cheap to genotype and provide more genetic information for the population per marker than SNPs (Roh et al. 2020).

Microsatellite markers are particularly valuable because they are codominant (the heterozygotes can be distinguished from homozygotes), they are locus-specific in nature, highly polymorphic and hypervariable, with a higher mutation rate than other markers, and easy to sample preparation. Their ease of sample preparation and high variability make them the preferred choice for studying genetic diversity (Boettcher et al. 2010; Abdul-Muneer 2014), especially in relation to local chicken breeds (Hillel et al. 2003; Tadano et al. 2013).

The aim of this investigation was to describe the morpho-biometric and the genetic characteristics of MP and assess its diversity in comparison to other Italian breeds. To achieve this, a total of 26 microsatellite markers were used to characterise the genetic profile of MP breed and asses its contribution to poultry biodiversity.

The genetic markers were selected based on previous research on Piedmontese chicken breeds (Sartore et al. 2016; Soglia et al. 2020); a subset of 14 microsatellites was used to investigate genetic diversity across 17 local Italian breeds (Soglia et al. 2021).

MP genetic variability, including intra-genetic distance, kinship, total and effective number of alleles,

private alleles, heterozygosity, and extinction risk index (ERI, Soglia et al. 2021) were evaluated. In addition, MP's contribution to the overall Piedmontese (MP, BP and BS breeds) and to Italian poultry biodiversity was assessed.

The results presented in this work represent the first morphological and molecular data on MP available in the literature.

Materials and methods

Samples collection and microsatellites genotyping

A group of 53 adult chickens (17 males and 36 females), phenotypically corresponding to the historical and anecdotical description of MP breed, were identified in five small farms located in the south-west area of the Piedmont Region. For each farm, the farmer selected the birds used for reproduction, thus considered the best in terms of morpho-biometric traits (Table 1).

Farm 1, located in Turin province, is genetically isolated from the other sampled farms. In contrast, the other farms, all situated in Cuneo province, occasionally exchange roosters, though not systematically. Breeders typically mate one or two females with a single male.

The morpho-biometric traits were collected according to the FAO guidelines (FAO 2012) and were namely the body weight (BW), the wingspan (WS) (measured as the distance between the two terminal phalanges), the body length (BL) (from the tip of the beak to the pygostyle), the chest circumference (CC), the tarsus length (TL), and the tarsus circumference (TC).

Blood samples were collected from all 53 individuals, in compliance with the European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009]. All blood samples (about 2 mL) were collected during routine health controls from ulnar veins, stored in Vacutainer tubes containing EDTA as an anticoagulant, then immediately frozen at −20 °C pending DNA analysis.

DNA was extracted using the NucleoSpinR Blood QuickPure kit (Macherey-Nagel, Düren, Germany). Genetic characterisation was performed using 26 microsatellite markers (Set1): ***ADL0112, ADL0268,*** *ADL0278,* ***LEI0094,*** *LEI0166, LEI0192, LEI0228, LEI0258,* ***MCW0014,*** *MCW0016,* ***MCW0165****, MCW0020,* ***MCW0206, MCW0222, MCW0248,*** *MCW0037,* ***MCW0067, MCW0081,*** *MCW0104,* ***MCW0111****,* ***MCW0183, MCW0216,*** *MCW0034, MCW0069 MCW0078* and ***PAX7*** (in bold the subset of the14 marker, Set2).

Multiplex polymerase chain reaction (PCR) and amplicon processing were carried out according to Sartore et al. (2014) using the same primers.

Multiplex PCR amplification for marker genotyping was carried out in 10 mL reactions at the following final concentrations: 1X buffer Qiagen (Hilden, Germany), 0.4 mM dNTPs, and 0.05 mM HotStart Taq Qiagen.

The following thermocycling conditions were used: an initial denaturation step of 15 min at 95 °C, 31 cycles of 30 s at 95 °C, 1 min at the annealing temperature specific to each multiplex PCR, 1 min at 72 °C, and a final extension time of 7 min at 72 °C.

The analysis of fragments was performed using the automated DNA Genetic Analyser SeqStudio (Thermo Fisher Scientific) and the computer software GeneMapper 4.0 (Applied Biosystems). An error assay was performed by replicating the genotyping on 10% of animal samples selected at random (Pompanon et al. 2005).

Genetic variability analysis

The genetic data from the Set1 analysis was used to investigate genetic variability within MP population.

Genetic variability analysis was performed as previously described by Soglia et al. (2021) using GenAlEx v6.50 (Peakall and Smouse 2012) and MolKin3.0 (Gutiérrez et al. 2005).

The following data were calculated using GenAlex:

Na = number of alleles; *Nea* = effective number of alleles = 1/(Sum pi^2) [where Sum pi^2 is the sum of the squares of population allele frequencies]; *Ho* = observed heterozygosity = number of heterozygotes/*Na*; *He* = expected heterozygosity = 1–Sum pi^2; *HWE* = Hardy-Weinberg Equilibrium; *H-ind* = individual heterozygosis; *Fis* = Fixation Index =1-(Ho/He); *Pa* = number of private alleles.

Using Molkin3.0 the following data were calculated:

K_{BW} = within breed kinship; K_{BB} = between breeds kinship; *PSA* = proportion of shared alleles; I_B = breed

Table 1. Millefiori Piemontese's morpho-biometric traits. The parameters are shown as mean ± standard error.

Sex	BW (kg)	CC (cm)	BL (cm)	TL (cm)	TC (cm)	WS (cm)
Male	3.32 ± 0.11	39.60 ± 0.38	52.70 ± 0.65	10.70 ± 0.23	6.50 ± 0.13	61.20 ± 0.92
Female	2.32 ± 0.78	37.50 ± 0.89	47.20 ± 0.74	8.60 ± 0.18	4.60 ± 0.16	52.50 ± 1.33
p value	<0.001	0.034	<0.001	<0.001	<0.001	<0.001

BW: body weight; CC: chest circumference; BL: body length; TL: tarsus length, TC: tarsus circumference; WS: wingspan.

inbreeding; Fi = the coefficient of inbreeding of an individual = $2s_i - 1$ [where s_i (self-coancestry) is the molecular coancestry of an individual *i* with itself]; *GD* = genetic diversity; GD_W = genetic diversity within breed; GD_B = genetic diversity between breed; GD_T = total contribution of the breed to the genetic diversity of the entire population = $GD_W + GD_B$ (Caballero and Toro 2002 as described in Soglia et al. 2021).

Comparisons between populations were carried out by analysis of molecular variance (AMOVA) and principal component analysis (PCoA) was used to calculate scores and produce scatter plots of the different breeds and groups.

MLNe 2.1.0.0 software (Wang 2022) was used to estimate the effective population size (Ne) and immigration rate (m) on genetic data.

Biodiversity contribution and breed risk extinction

To evaluate the contribution of MP to the Italian poultry biodiversity, a dataset of 817 bird samples (Set2), comprising 17 Italian chicken breeds (Soglia et al. 2021) and two commercial lines, Ross 708 broilers ($n = 61$ individuals) and ISA Brown laying lines ($n = 60$ individuals), previously analysed by Soglia et al. (2017), was used.

Genetic diversity has been defined in terms of molecular coancestry distances (Meuwissen et al. 2001), global diversity (GD) and breed diversity, which are computed by averaging the corresponding values for all the within- and between-breed pairs of individuals.

The contribution of a breed to overall population genetic diversity is assessed considering the change in the genetic diversity (GD) value after removing a single breed from the dataset: within-breed genetic diversity (GD_W), the mean contribution to the genetic diversity between different breeds (GD_B) and the total contribution of the breed to the genetic diversity of the entire population (GD_T, which is the sum of GD_W and GD_B) was evaluated.

To evaluate the genetic extinction risk of MP, the extinction risk index (ERI) as described by Soglia et al. (2021), was calculated. The ERI takes in account several factors: the fixation index (Fis), the breed-inbreeding value (I_B), the coefficient of inbreeding of an individual (Fi), within-breed kindship (K_{BW}), and the proportion of shared alleles within-breed (PSA).

Results

Figure 1 reports the phenotypic traits of both males and females of the MP breed.

The morpho-biometric traits (Table 1) are always statistically different between males and females, showing highest values in males.

Population genetic diversity

Table 2 shows the results of the genetic diversity analysis for MP, both for the entire population and separated by individual farm. The frequency-based

Figure 1. Millefiori Piemontese breed. Example of a male (in the left) and a female (in the right) of Millefiori Piemontese breed rediscovered from one of the five farms of the present study.

Table 2. Millefiori Piemontese's genetic variability. Genetic variability in the Millefiori Piemontese (MP) population and separated for each farm. The parameters are shown as mean ± standard error.

Pop	N	Na	Nea	Ho	He	Fis	Pa(Pa%)	IPa(IPa%)
MP	53	3.77 ± 0.26	2.47 ± 0.17	0.56 ± 0.05	0.53 ± 0.04	−0.02 ± 0.05	17(0.17)	6 (0.11)
Farm 1	3	1.92 ± 0.15	1.66 ± 0.12	0.44 ± 0.07	0.34 ± 0.04	−0.24 ± 0.10	10(0.10)	3(1.0)
Farm 2	10	2.69 ± 0.21	2.17 ± 0.17	0.52 ± 0.06	0.48 ± 0.04	−0.07 ± 0.06	0	0
Farm 3	8	2.85 ± 0.24	2.18 ± 0.16	0.58 ± 0.05	0.50 ± 0.04	−0.17 ± 0.06	0	0
Farm 4	10	3.35 ± 0.23	2.46 ± 0.17	0.56 ± 0.05	0.53 ± 0.04	−0.03 ± 0.04	7(0.07)	3(0.30)
Farm 5	22	3.00 ± 0.24	2.29 ± 0.18	0.58 ± 0.06	0.50 ± 0.04	−0.11 ± 0.07	0	0

N, sample size; *Na*, mean allele number per locus; *Nea*, effective allele number; *Ho*, observed heterozygosity; *He*, expected heterozygosity and *Fis*, the fixation index; *Pa,* the number of private alleles and its percentage (Pa%); *IPa*, the number of individuals with private alleles and its percentage (IPa%).

statistical analyses revealed a total number of 98 alleles. Of these, 17 were private alleles, detected in birds from farms 1 and 4 only. The average number of alleles per locus (Na) was 3.77, and the effective number of alleles (Nea) was 2.47. Observed heterozygosity (Ho) and expected heterozygosity (He) were 0.56 and 0.53, respectively, and the mean fixation index of the loci (Fis) was negative for all farms (Table 2).

The individual heterozygosity (H-ind) of all the investigated MP subjects revealed a high level of variability (mean 0.58) (Figure 2, whole dataset). The highest average H-ind value (0.60) was observed in farm 3 and farm 5; while the bird with the highest H-ind value (0.80) was from farm 4 (Figure 2).

The effective population size (Ne) of MP, estimated as single isolated population, was 56; while assuming an infinite source is 54, with $m = 1$.

The PCoA plot explained 96% (PC1) and 4% (PC2) of the total variance. PC1 indicated a clear separation of farm 1 from all the others, while PC2 indicated a clear separation of farm 3 (Figure 3).

Piedmont biodiversity contribution

The biodiversity of the three Piedmontese chicken breeds was analysed by comparing the genetic variability of MP with that of the other two Piedmontese local breeds (72 BP; 64 BS), using Set2 microsatellite markers.

Table 3 reveals a total of 139 alleles among the three breeds, 71 of which were private alleles. For MP, BP and BS, Na was 4.21, 6.5 and 6.71, respectively, whereas Nea was 2.70, 3.40 and 3.57, respectively. The values of Ho for the three breeds were 0.59, 0.68 and 0.69, respectively, and very similar values were revealed for He (0.59, 0.69 and 0.68).

PCoA plot explained 68.64% (PC1) and 31.36% (PC2) of the total variance. Individuals of MP clustered in the far right of the graph, while BS and BP clustered in the top left and bottom left, respectively (Figure 4). The analysis of molecular variance (AMOVA) showed a variance of 23% between populations (p value = 0.001).

The outcome of the analyses of molecular kinship, estimated as the average within-breed kinship (K_{BW}), between-breed kinship (K_{BB}) and as the proportion of shared alleles (PSA), revealed a higher level of inbreeding in MP (PSA = 0.54; $K_{BW} = 0.40$) compared with that of BP and BS (Table 4).

The results of MP extinction impact on Piedmont GD are reported in Table 5. The GD_T value was negative (−1.59), indicating its positive contribution to genetic diversity.

Contribution of MP to the genetic diversity in Italian breeds

The overall contribution of MP to the genetic diversity of all Italian breeds had a negative value (−0.60) (Table 6), revealing this breed to make a positive contribution to overall Italian genetic diversity. By contrast, breeds with a positive value, such as Mericanel della Brianza (1.50), Livorno Bianca (1.00) and Livorno Nera (0.57), contribute little to Italian poultry genetic biodiversity.

In the PCoA plot, MP clustered close to the two commercial lines (ISA and BR) in the top right of the graphic, far from the other Italian breeds, which were all clustered together (Figure 5). The first three principal components (PC) explained only 39% of the total variance: 17% (PC1) 12% (PC2) and 10% (PC3).

The AMOVA revealed 47% variation among the population, while intra-population variation was 53% (p value = 0.001).

The values of within-breed kinship (K_{BW}) for the 20 breeds considered were similar to those published by Soglia et al. (2021), ranging from 0.31 (in BP) to 0.78 (in Livorno Bianca). The values of between-breed molecular kinship (K_{BB}) were altered, since this study considered three additional breeds, and ranged between 0.22 (Robusta Lionata and ISA Brown) and 0.32 (Livorno Bianca). MP had a K_{BW} of 0.42, a K_{BB} of 0.25 and a PSA of 0.54. The overall contribution of the

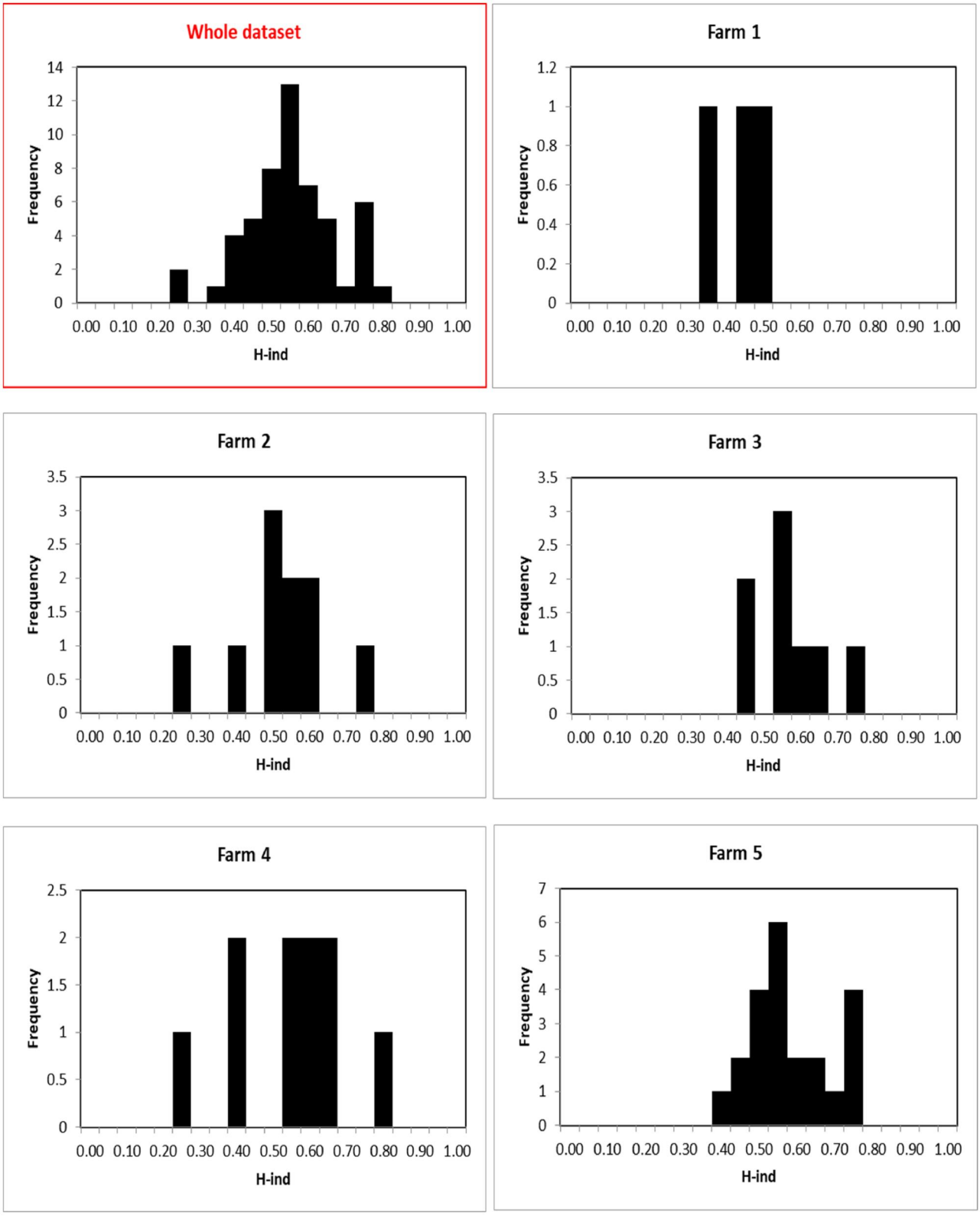

Figure 2. MP frequency distribution for individual heterozigosity (H-ind). Graphical output of individual heterozygosity value (H-ind).

individual breeds analysed ranged from −1.32 (BP) to 1.5 (Mericanel della Brianza). The value for MP was −0.60.

In addition, the analysis of the extinction risk showed ERI = 2.07 for MP (Table 6). Values less than 2.0 indicate a low genetic risk of extinction, values

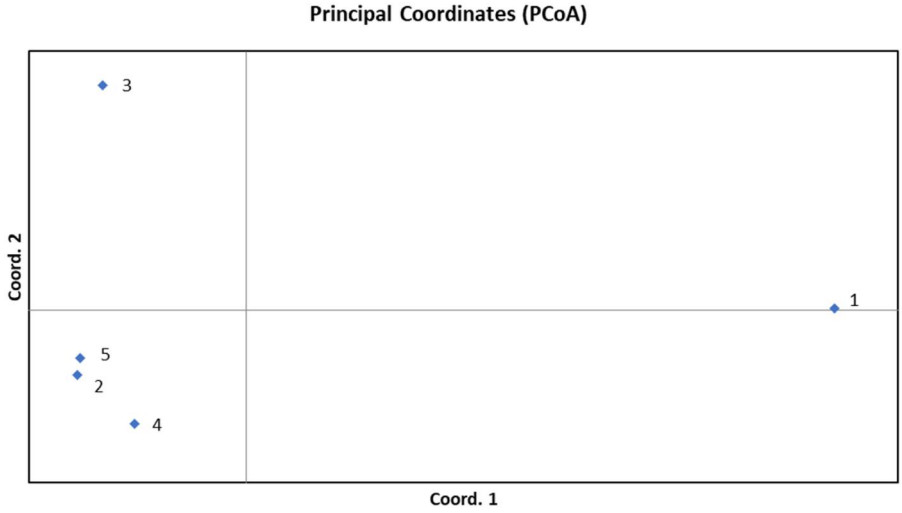

Figure 3. Analysis of principal coordinates (PCoA) between the five different farm of Millefiori Piemontese (MP) breed.

Table 3. Piemontese breeds genetic variability parameters. The parameters were evaluated for each breed (BP, Bionda Piemontese; BS, Bianca di Saluzzo, and MP, Millefiori Piemontese) and shown as mean ± standard errors (SE).

Breed	Ns	PIC	%P	Na	Rt	Nea	Ho	He	Fis	P	I_B	Pa	IPa
BP	72	0.52	100	6.50	4.89	3.40	0.68	0.69	−0.01	0.0009	0.65	31	59
SE				± 0.61		±0.23	± 0.05	± 0.02	± 0.02			(0.22)	(0.82)
BS	64	0.54	100	6.71	5.46	3.57	0.69	0.68	−0.03	0.0046	0.65	31	62
SE				± 0.62		0.41	± 0.03	± 0.03	± 0.02			(0.22)	(0.97)
MP	53	0.40	100	4.21	3.71	2.70	0.59	0.59	−0.02	0.0000	0.70	9	51
SE				±0.37		±0.23	± 0.06	±0.04	±0.03			(0.06)	(0.96)

Ns, sample size; *PIC*, polymorphic information content; *%P*, percentage of polymorphic loci; *Na*, mean allele number per loci; *Rt*, normalised allele size; *Ne*, effective allele number (1/sum pi^2); *Ho*, observed heterozygosity (number of hets/N); *He*, expected heterozygosity (1-sum pi^2); *Fis*, fixation index (1-(Ho/He)); *P*, *p* value for Global Hardy Weinberg test, and I_B, breed inbreeding; *Pa,* the number of private alleles and their percentage (Pa%); *IPa*, the number of individuals with private alleles and their percentage (PaI%).

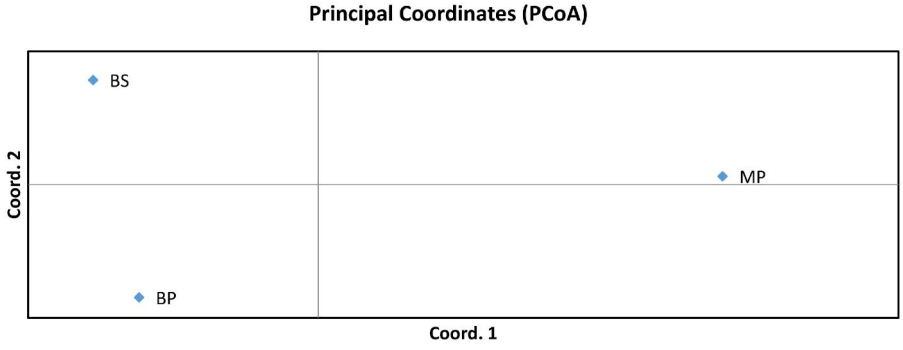

Figure 4. Analysis of principal coordinates (PCoA) between three Piemontese chicken breeds. Millefiori Piemontese (MP); Bianca di Saluzzo (PS); Bionda Piemontese (BP).

from 2.0 to 2.5 are considered to be at a medium risk, while breeds with values higher than 2.5 are considered to be at high risk of extinction.

Discussion

This study presents the first morpho-biometric and molecular investigation of the genetic variability of the MP breed and provides an initial comparison of its genetic variability and structure. Genetic characterisation of a breed is a useful tool for recognising new animal genetic resources to evaluate its risk status and planning the right management strategies for its conservation (Groeneveld et al. 2010)

The conservation of local animal genetic resources is a key objective of the EU, given their value as cultural heritage and international public goods. These resources are also crucial for ensuring the sustainable contribution of livestock farming for global food security and nutrition. In fact, the first Strategic Priority

Table 4. Molecular kinship. The table shows the molecular kinship in BP, Bionda Piemontese; BS, Bianca di Saluzzo and MP, Millefiori Piemontese breeds. Molecular kinship was estimated as the average within-breed kinship (K_{BW}), between-breed kinship (K_{BB}) and as the proportion of shared alleles (PSA).

Breed	K_{BW}	K_{BB}	PSA
BP	0.23	0.31	0.45
BS	0.23	0.33	0.46
MP	0.22	0.41	0.54

K_{BW}, molecular kinship within-breed; K_{BB} molecular kinship between-breed *PSA* proportion of shared alleles.

Table 5. Impact of each breed on Piedmontese biodiversity. The table shows the genetic diversity in three Piedmontese chicken breeds: Bionda Piemontese (BP), Bianca di Saluzzo (BS) and Millefiori Piemontese (MP). GD is the difference in the genetic diversity value once the breed is removed from the overall population; GD_W is the percentage of lost within-breed diversity; GD_B is the percentage of lost between-breed diversity. The global breed extinction impact on genetic diversity is calculated as $GD_T = GD_W + GD_B$.

Breed	GD	GD_W	GD_B	GD_T
BP	0.70	−3.15	−0.63	−3.77
BS	0.71	−0.98	−1.05	−2.03
MP	0.71	3.60	−5.19	−1.59

GD, genetic diversity; *GD_w*, genetic diversity within-breed; *GD_B*, genetic diversity between-breed; *GD_T*, total genetic diversity.

Table 6. Molecular kinship and impact of each breed on Italian biodiversity. The table shows the genetic diversity in 20 Italian chicken breeds. GD is the difference in the genetic diversity value once the breed is removed from the overall population; GD_W is the percentage of lost within-breed diversity; GD_B is the percentage of lost between-breed diversity. The global breed extinction impact on genetic diversity is calculated as $GD_T = GD_W + GD_B$.

Breed	K_{MP}	GD	GD_W	GD_B	GD_T	ERI
AN	0.26	0.73	−0.20	0.17	−0.02	2.48
BP	0.23	0.72	−2.11	1.14	−0.98	1.75
BR	0.22	0.72	−0.65	0.03	−0.62	2.07
BS	0.24	0.72	−1.74	1.41	−0.33	1.75
ER	0.21	0.73	0.49	−0.70	−0.21	2.94
ISA	0.22	0.72	−1.12	0.23	−0.89	1.77
LB	0.25	0.73	2.10	−1.19	0.90	3.44
LN	0.24	0.73	1.23	−0.72	0.52	2.61
MB	0.27	0.74	2.47	−1.09	1.37	2.81
ML	0.30	0.73	−0.14	0.19	0.06	2.25
MUG	0.25	0.73	−0.07	0.22	0.15	2.43
PD	0.23	0.73	0.03	0.02	0.06	2.68
PP	0.22	0.73	0.58	−0.59	−0.01	2.98
PV	0.24	0.73	0.05	−0.03	0.02	2.70
RL	0.26	0.72	0.40	−0.78	−0.38	2.85
RM	0.28	0.72	0.68	−0.96	−0.27	3.16
ROM	0.30	0.73	0.09	0.18	0.28	2.62
SIC	0.18	0.73	1.00	−0.97	0.04	2.90
VAL	0.27	0.72	−0.69	0.35	−0.35	1.96
MP	0.42	0.72	−0.72	0.27	−0.45	2.07

K_{MP}, molecular kinship Between Italian breed and MP; *GD*, genetic diversity; *GD_w*, genetic diversity within-breed; *GD_B*, genetic diversity between-breed; *GD_T*, total genetic diversity; *ERI*, extinction risk index.
AN, Ancona; BP, Bionda Piemontese, BR Ross 708 broilers; BS, Bianca di Saluzzo; ER, Ermellinata; ISA, Isabrown; LB, Livorno Bianca; LN, Livorno Nera; MB, Mercianel della Brianza; ML, Millefiori di Lonigo; MUG, Mugellese; PD, Padovana; PP, Pèpoi; PV, Polverara; RL, Robusta Lionata; RM, Robusta Maculata; ROM, Romagnola; SIC, Siciliana; VAL, Valdarno; MP, Millefiori Piemontese.

Area of the Global Plan of Actions is the characterisation, inventory and monitoring of trends and associated risks of animal genetic resources (Ajmone-Marsan et al. 2023).

The morpho-biometric traits reveal sexual dimorphism in MP, with males showing the highest value for BW, BL, CC, TL, and TC in line with others Italian local chicken breed (https://www.pollitaliani.it/en/).

In this study, 53 subjects (17 males and 36 females), phenotypically compatible with the MP breed, were genotyped using microsatellite markers. The number of subjects analysed in the present study is limited for precise population statistics, as obtaining a larger sample size was not feasible in a population at risk status, such as MP (Bortoluzzi et al. 2018). Indeed, the effective population size evaluated in this study is equal to 56 individuals.

The genetic characterisation of MP reveals that the selected markers were reliable and informative for this population analysis. In fact, a high number of alleles was found (total of 98 alleles).

Although the allelic richness (Na = 3.8) observed in the MP breed is lower than that of other Piedmontese breeds, it is higher than that reported in other Italian breeds.

In Soglia et al. (2021), it was reported that 11 Italian breeds – Ermellinata di Rovigo, Livorno Bianca, Livorno Nera, Mericanel della Brianza, Millefiori di Lonigo, Padovana, Pepoi, Robusta Lionata, Robusta Maculata, Romagnola and Siciliana – exhibited lower Na (values ranging from 1.86 to 3.50) than MP.

Since allelic richness is a fundamental indicator of genetic diversity within populations. (Caballero and García-Dorado 2013), the results highlight how the MP breed has maintained higher genetic variability compared to other Italian breeds

However, the effective number of alleles per locus (Nea = 2.5) is lower, reflecting the presence of low frequency alleles that can be lost with the genetic drift. Empirical studies demonstrate that even with a high number of observed alleles, Nea can remain low, indicating a genetic history of variability loss that must be considered in conservation strategies (Caballero and García-Dorado 2013).

Therefore, in a small population like MP, genetic drift can significantly impact genetic diversity, this breed should be managed with a careful mating plan to preserve its genetic variability.

Another important parameter for assessing the genetic variability is heterozygosis (Frankham et al. 2010; Ajmone-Marsan et al. 2023). The genetic variability of

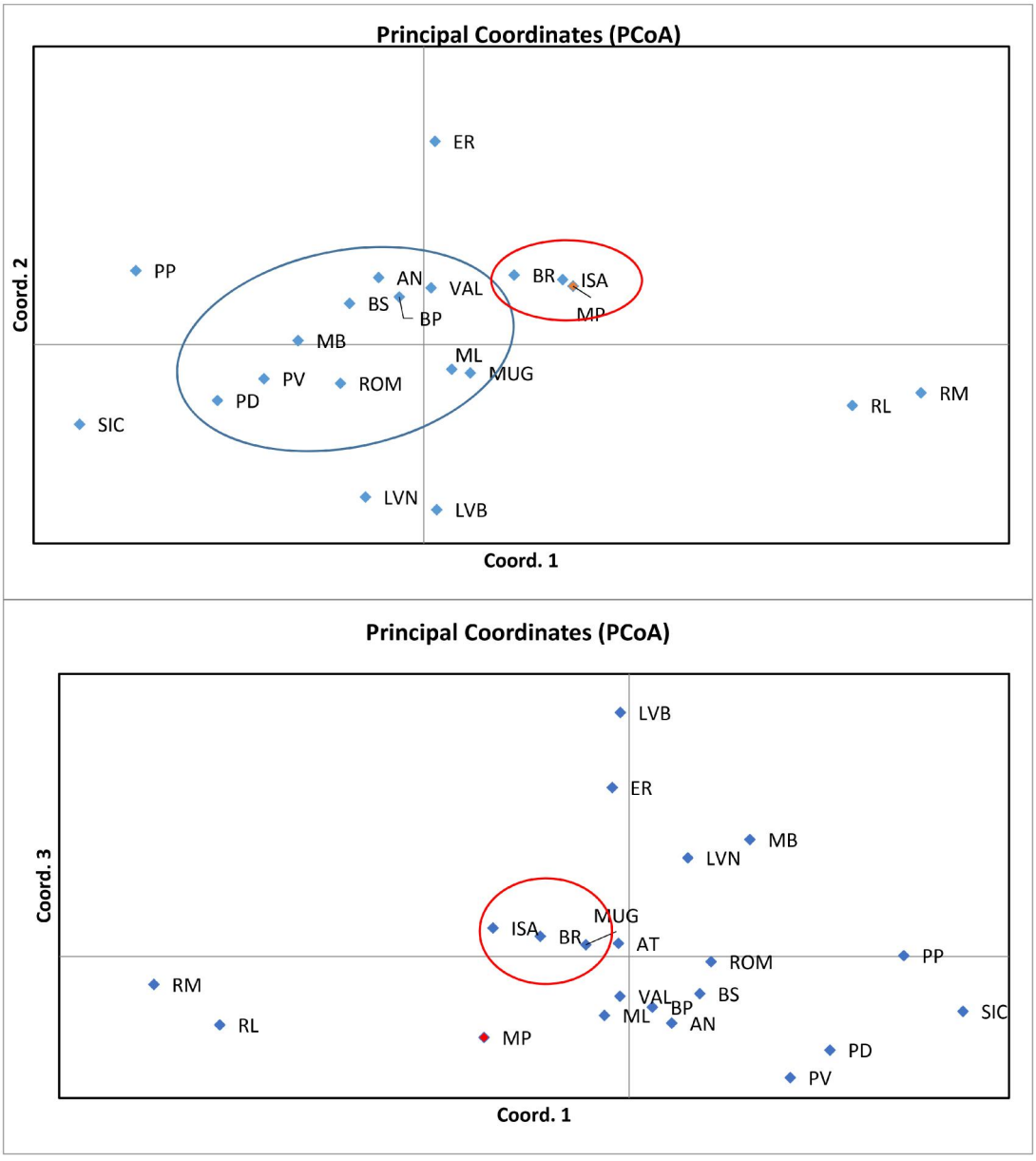

Figure 5. Analysis of principal coordinates (PCoA) between the 20 populations explained with the first 3 coordinates.
AN, Ancona; BP, Bionda Piemontese; BR, Ross 708 broiler; BS, Bianca di Saluzzo; ER, Ermellinata di Rovigo; ISA, Isabrown; LB, Livorno Bianca; LN, Livorno Nera; MB, Mericanel della Brianza; ML, Millefiori di Lonigo; MG, Mugellese; PD, Padovana; PP, Pepoi; PV, Polverara; RL, Robusta Lionata; RM, Robusta Maculata; RO, Romagnola; SC, Siciliana; VL, Valdarnese; MP, Millefiori Piemontese.

MP, with an observed heterozygosity (Ho) of 0.59, is relatively lower compared to other Piedmontese breeds, such as BP (Ho = 0.68) and BS (Ho = 0.69). However, MP showed greater variability compared to other Italian breeds using the same markers. The observed heterozygosity of broiler and 14 local breeds was lower than that of MP, with values ranging from 0.17 to 0.58. These local breeds included Ancona, Ermellinata di Rovigo, Livorno Bianca, Livorno Nera, Mericanel della Brianza, Millefiori di Lonigo, Mugellese, Padovana, Pepoi, Polverara, Robusta Lionata, Robusta Maculata, Romagnola and Siciliana (Soglia et al. 2021; Soglia et al. 2017).

When extending the comparison to include other Mediterranean chicken breeds from Spain, Serbia, Albania and Malta, which were similarly evaluated using microsatellite markers, MP demonstrates higher genetic variability than several of these breeds: Pita Pinta Asturiana (Ho = 0.42), Gallina Valenciana de Chulilla (Ho = 0.44), Gallina de Sobrarbe (Ho = 0.37), Sureña (Ho = 0.52), Combatiente Español (Ho = 0.35) and Extremeña Azul (Ho = 0.49) from Spain; Albanian Population (Ho = 0.52)

from Albania; Somborska Crested (Ho = 0.53) and Svrljig Hen (Ho = 0.52) from Serbia; and Maltese Black (Ho = 0.35) from Malta (Ceccobelli et al. 2015).

Heterozygosity evaluation also highlights how the MP breed exhibits higher genetic variability compared to other local breeds; the maintenance of relatively high genetic variability in the small MP population may be attributed to the management practices involving small mating groups (one male and 1–2 females), as reported by the breeders; this results in a higher male-to-female ratio compared to other chicken breeds, which typically have a sex ratio of 1:10.

Genetic variability, or genetic diversity, is crucial for the conservation of breeds, especially those at risk of extinction. In fact, this diversity allows populations to adapt to changing environmental conditions, resist diseases, and maintain overall health and viability (Frankham et al. 2010). For this reason, it is essential to develop conservation strategies to preserve the genetic variability of the MP breed. MP, with high genetic variability, possess a broader range of traits that can be selected for survival under different conditions. In addition, research has shown that populations with high Ho are more resilient to climate-induced changes in their habitats (Frankham et al. 2010).

Another aspect not to be underestimated in the genetic evaluation of a breed is the degree of inbreeding. MP showed a higher level of inbreeding ($I_B = 0.70$ and $K_{BW} = 0.40$) compared to other Piedmontese chicken breeds, BP and BS ($I_B = 0.65$ and $K_{BW} = 0.33$–0.31, respectively). However, the inbreeding levels of MP were still lower than those observed in other Italian breeds, except for the Siciliana breed which has a similar I_B of 0.69 and Valdarnese breed which has a K_{BW} of 0.35 (as reported in Soglia et al. 2021).

Higher levels of inbreeding can lead to inbreeding depression, characterised by reduced fitness, increased prevalence of genetic disorders, and a decrease in overall genetic diversity. Understanding and mitigating the impacts of inbreeding are crucial for the effective conservation of breeds, particularly those with small population sizes (Frankham et al. 2010).

Elevated rates of inbreeding can result from the fragmentation of the population into small farms that do not implement a systematic exchange of breeding individuals. In fact, PCoA reveals a distinct population structure: farm 1 is separated from the others, probably due to the fact that it is the only one that does not exchange birds with the other farms considered in the present study. This genetic structure of MP is further highlighted by the high number of private alleles (17 in total), with ten found exclusively in farm 1 and seven in farm 4. This might have led to genetic drift due to the small number of individuals in each farm or to management practices.

Based on the analysis of the five farms, the evaluated parameters are useful for developing mating schemes among farm, to preserve the genetic variability of this breed, with particular attention to the genetic uniqueness of the populations in farm 1 and 4 (Caballero and García-Dorado 2013); facilitating exchanges between breeders from farms 1 and 3 and those from other farms would be beneficial for enhancing genetic variability and reducing consanguinity.

Additionally, the combination of high genetic variability and high levels of consanguinity suggests the presence of distinct genetic lines within the breed. Consequently, conducting a census of the farms would be beneficial to determine the effective population size, genetic structure, and genetic variability: data essential for an accurate management and conservation.

The analysis of PCoA also highlighted the genetic differences between MP and other Piedmontese breeds, identifying 9 private alleles unique to MP. This analysis proved the MP contribution to Piedmont's poultry biodiversity, with an impact of 5% on the variability between breeds ($GD_B = -5.19$). However, the overall contribution of MP to the total variability was only 1.6% ($GD_T = -1.59$), due to the high kinship within population, which affects 3.6% of the within-breed genetic diversity (GDw).

The MP, within the context of Italian poultry variability, contributes positively to the genetic diversity with a 45% impact. This is lower than the contribution of BP (98%), BR (62%), and ISA (89). MP also contributes 72% to within-population variability, but shows −27% contribution to inter-population variability.

PCoA analysis also highlights the genetic uniqueness of the MP breed. The PCoA plot (Figure 5) showed that MP clustered distinctly from other Italian breeds. However, MP is positioned close to the two hybrid lines (ISA and BR) along the first two components, while, using the third genetic components, it was highlighted the differences between these 3 populations. This might suggest that a crossbreeding was performed in the past to preserve the local breed or a shared genetic origin among these breeds.

In addition, the calculated molecular kinship (K_{BB}) between MP and the hybrid line (BR and ISA) revealed a 22% probability that two alleles of a gene are identical by descendent, which is consistent with the average K_{BB} values observed between MP and other Italian breeds.

This observation ruled out the possibility that the sampled MP individuals could be hybrids (MP with ISA or

BR). However, it does not exclude the possibility of past crossbreeding between these populations. Nonetheless, a comprehensive genomic analysis with different genetic markers, as SNP, is essential to reconstruct both MP local and non-local ancestry and fully understand its genetic background (Ajmone-Marsan et al. 2023).

The analysis of the genetic extinction risk for MP showed a value ranging from 2.0 to 2.5, placing it at medium risk of extinction, together with Ancona, Mugellese and Millefiori di Lonigo and broiler (Table 6, ERI). This could be because, even if MP presented a high rate of inbreeding, the breed has a high genetic variability.

However, MP must be considered at risk of extinction due to its very little population sizes. According to FAO, a breed is categorised as 'critical' if the overall population size is less than or equal to 120 (Ajmone-Marsan et al. 2023).

Conclusions

This study presents the first morpho-biometric and molecular investigation of the genetic variability of the Millefiori Piemontese (MP) breed and provides an initial comparison of MP with other local poultry breeds, both Italian and International. The results underscore the genetic uniqueness of MP and highlight its contribution to the Italian chicken biodiversity, emphasising the conservation priority of this breed.

Despite its small population size, the MP exhibits a high degree of genetic variability, which is valuable for breeding programs aimed at preserving the breed and enhancing Italian poultry biodiversity. However, further detailed genetic analyses are necessary to better understand the extent of introgression and the origins of the current MP population.

In addition, future efforts should include a comprehensive census plan to identify and recover new genetic lines within this population.

Funding

This study was carried out within the Agritech National Research Centre and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

ORCID

Dominga Soglia http://orcid.org/0000-0002-4285-3795

Data availability statement

Data will be made available from the corresponding author dominga.soglia@unito.it upon reasonable request.

Disclosure statement

No potential conflicts of interest are reported by the authors.

References

Abdul-Muneer PM. 2014. Application of microsatellite markers in conservation genetics and fisheries management: recent advances in population structure analysis and conservation strategies. Genet Res Int. 2014:691759. doi: 10.1155/2014/691759.

Abebe AS, Mikko S, Johansson AM. 2015. Genetic diversity of five local Swedish chicken breeds detected by microsatellite markers. PLoS One. 10(4):e0120580. doi: 10.1371/journal.pone.0120580.

Ajmone-Marsan, P., Colli, L., Ginja, C, Kantanen J. & Lenstra, J.A., eds. 2023. Genomic characterization of animal genetic resources. In: FAO animal production and health guidelines no. 32. Rome: FAO. doi: 10.4060/cc3079en.

Berthouly C, Bed'Hom B, Tixier-Boichard M, Chen CF, Lee YP, Laloë D, Legros H, Verrier E, Rognon X. 2008. Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. Anim Genet. 39(2):121–129. doi: 10.1111/j.1365-2052.2008.01703.x.

Boettcher PJ, Tixier-Boichard M, Toro MA, Simianer H, Eding H, Gandini G, Joost S, Garcia D, Colli L, Ajmone-Marsan P, GLOBALDIV Consortium. 2010. Objectives criteria and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. Anim Genet. 41 Suppl 1(s1):64–77. doi: 10.1111/j.1365-2052.2010.02050.x.

Bortoluzzi C, Crooijmans RPMA, Bosse M, Hiemstra SJ, Groenen MAM, Megens H-J. 2018. The effects of recent changes in breeding preferences on maintaining traditional Dutch chicken genomic diversity. Heredity (Edinb). 121(6):564–578. doi: 10.1038/s41437-018-0072-3.

Caballero A, García-Dorado A. 2013. Allelic diversity and its implications for the rate of adaptation. Genetics. 195(4): 1373–1384. doi: 10.1534/genetics.113.158410.

Caballero A, Toro MA. 2002. Analysis of genetic diversity for the management of conserved subdivided populations. Conserv Genet. 3:289–299.

Castillo A, Gariglio M, Franzoni A, Soglia D, Sartore S, Buccioni A, Mannelli F, Cassandro M, Cendron F, Castellini C, et al. 2021. Overview of native chicken breeds in Italy: conservation status and rearing systems in use. Animals (Basel). 11(2):490. doi: 10.3390/ani11020490.

Ceccobelli S, Di Lorenzo P, Lancioni H, Monteagudo Ibáñez LV, Tejedor MT, Castellini C, Landi V, Martínez Martínez A, Delgado Bermejo JV, Vega Pla JL, et al. 2015. Genetic diversity and phylogeographic structure of sixteen Mediterranean chicken breeds assessed with microsatellites and mitochondrial DNA. Livest Sci. 175:27–36. doi: 10.1016/j.livsci.2015.03.003.

Cendron F, Perini F, Mastrangelo S, Tolone M, Criscione A, Bordonaro S, Iaffaldano N, Castellini C, Marzoni M, Buccioni A, et al. 2020. Genome-wide SNP analysis reveals the population structure and the conservation status of 23 Italian chicken breeds. Animals (Basel). 10(8):1441. doi: 10.3390/ani10081441.

Dávila SG, Gil MG, Resino-Talaván P, Campo JL. 2009. Evaluation of diversity between different Spanish chicken breeds, a tester line, and a White Leghorn population based on microsatellite markers. Poult Sci. 88(12):2518–2525. doi: 10.3382/ps.2009-00347.

FAO. 2012. Phenotypic characterization of animal genetic resources. In: FAO animal production and health guidelines no. 11. Rome, Italy: FAO.

FAO. 2017. The future of food and agriculture – trends and challenges. Rome.

Frankham R, Ballou JD, Briscoe DA. 2010. Introduction to conservation genetics. Cambridge, UK: Cambridge University Press.

Franzoni A, Gariglio M, Castillo A, Soglia D, Sartore S, Buccioni A, Mannelli F, Cassandro M, Cendron F, Castellini C, et al. 2021. Overview of native chicken breeds in Italy: small scale production and marketing. Animals (Basel). 11(3):629. doi: 10.3390/ani11030629.

Granevitze Z, Hillel J, Chen GH, Cuc NTK, Feldman M, Eding H, Weigend S. 2007. Genetic diversity within chicken populations from different continents and management histories. Anim Genet. 38(6):576–583. doi: 10.1111/j.1365-2052.2007.01650.x.

Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B, Pilling D, Negrini R, Finlay EK, Jianlin H, Groeneveld E, GLOBALDIV Consortium., et al. 2010. Genetic diversity in farm animals–a review. Anim Genet. 41 Suppl 1(s1):6–31. doi: 10.1111/j.1365-2052.2010.02038.x.

Gutiérrez JP, Royo LJ, Alvarez I, Goyache F. 2005. MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. J Hered. 96(6):718–721. doi: 10.1093/jhered/esi118.

Hillel J, Groenen MA, Tixier-Boichard M, Korol AB, David L, Kirzhner VM, Burke T, Barre-Dirie A, Crooijmans RPMA, Elo K, et al. 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet Sel Evol. 35(5):533–557. doi: 10.1186/1297-9686-35-6-533.

Hoffman AJ. 2010. Climate change as a cultural and behavioral issue: addressing barriers and implementing solutions. Organ Dyn. 39(4):295–305. doi: 10.2139/ssrn.2933572.

IPCC. 2007. Climate Change 2007: synthesis Report. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. Pachauri, R.K and Reisinger, A., editors. Geneva, Switzerland: IPCC; p. 104.

Meuwissen TH, Hayes BJ, Goddard ME. 2001. Prediction of the total genetic value using genome-wide dense marker maps. Genetics. 157(4):1819–1829. doi: 10.1093/genetics/157.4.1819.

Muir WM, Wong GKS, Zhang Y, Wang J, Groenen MAM, Crooijmans RPMA, Megens HJ, Zhang H, Okimoto R, Vereijken A, et al. 2008. Genome-wide assessment of worldwide chicken SNP genetic diversity indicates significant absence of rare alleles in commercial breeds. Proc Natl Acad Sci USA. 105(45):17312–17317. doi: 10.1073/pnas.0806569105.

Notter DR. 1999. The importance of genetic diversity in livestock populations of the future. J Anim Sci. 77(1):61–69. doi: 10.2527/1999.77161x.

Palinkas-Bodzsar N, Sztan N, Molnar T, Hidas A. 2020. Gene conservation of six Hungarian local chicken breeds maintained in small populations over time. PLoS One. 15(9): e0238849. doi: 10.1371/journal.pone.0238849.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 28(19):2537–2539. doi: 10.1093/bioinformatics/bts460.

Polli Italiani. 2024. Conservation of biodiversity in Italian poultry breeds, TuBAvI (2017-2020) and TuBAvI-2 (2021-2024). Italia: Department of Veterinary Medicine and Animal Science. [accessed 2024 May 28]. https://www.pollitaliani.it/en//.

Pompanon F, Bonin A, Bellemain E, Taberlet P. 2005. Genotyping errors: causes, consequences and solutions. Nat Rev Genet. 6(11):847–859. doi: 10.1038/nrg1707.

Roh H-J, Kim S-C, Cho C-Y, Lee J, Jeon D, Kim D-K, Kim K-W, Afrin F, Ko Y-G, Lee J-H, et al. 2020. Estimating genetic diversity and population structure of 22 chicken breeds in Asia using microsatellite markers. Asian-Australas J Anim Sci. 33(12):1896–1904). doi: 10.5713/ajas.19.0958.

Sartore S, Sacchi P, Soglia D, Maione S, Schiavone A, De Marco M, Ceccobelli S, Lasagna E, Rasero R. 2016. Genetic variability of two Italian indigenous chicken breeds inferred from microsatellite marker analysis. Br Poult Sci. 57(4):435–443. doi: 10.1080/00071668.2016.1187714.

Sartore S, Soglia D, Maione S, Sacchi P, De Marco M, Schiavone A, Sponza S, Dalmasso A, Bottero MT, Pattono D, et al. 2014. Genetic traceability of two local chicken populations, Bianca di Saluzzo and Bionda Piemontese, versus some current commercial lines. Ital J Agronomy. 9(4):176–181. doi: 10.4081/ija.2014.605.

Soglia D, Sacchi P, Sartore S, Maione S, Schiavone A, De Marco M, Bottero MT, Dalmasso A, Pattono D, Rasero R. 2017. Distinguishing industrial meat from that of indigenous chickens with molecular markers. Poult Sci. 96(8): 2552–2561. doi: 10.3382/ps/pex077.

Soglia D, Sartore S, Lasagna E, Castellini C, Cendron F, Perini F, Cassandro M, Marzoni M, Iaffaldano N, Buccioni A, et al. 2021. Genetic diversity of 17 autochthonous Italian chicken breeds and their extinction risk status. Front Genet. 12:715656. doi: 10.3389/fgene.2021.715656.

Soglia D, Sartore S, Maione S, Schiavone A, Dabbou S, Nery J, Zaniboni L, Marelli S, Sacchi P, Rasero R. 2020. Growth performance analysis of two Italian slow-growing chicken breeds: Bianca di Saluzzo and Bionda Piemontese. Animals (Basel). 10(6):969. doi: 10.3390/ani10060969.

Tadano R, Nagasaka N, Goto N, Rikimaru K, Tsudzuki M. 2013. Genetic characterization and conservation priorities of chicken lines. Poult Sci. 92(11):2860–2865. doi: 10.3382/ps.2013-03343.

Wang J. 2022. MLNe: simulating and estimating effective size and migration rate from temporal changes in allele frequencies. J Hered. 113(5):563–567. doi: 10.1093/jhered/esac039.

Zanetti E, De Marchi M, Abbadi M, Cassandro M. 2011. Variation of genetic diversity over time in local Italian

chicken breeds undergoing in situ conservation. Poult Sci. 90(10):2195–2201. doi: 10.3382/ps.2011-01527.

Zanon A, Bigi D. 2022. Atlante delle razze avicunicole autoctone: polli, Tacchini, Faraone, Anatre, Oche, Colombi, Quaglie, Conigli allevati in Italia. Edagricole, editor; p. 84–86.

Zanon A, Sabbioni A. 2001. Identificazione e salvaguardia genetica delle razze avicole italiane. [Identification and genetic protection of Italian poultry breeds]. Annali Della Facoltà Di Medicina Veterinaria Di Parma. 21:117–134. (in Italian).