GENETICS AND MOLECULAR BIOLOGY

Genome-wide analysis reveals the patterns of genetic diversity and population structure of 8 Italian local chicken breeds

F. Cendron,[*,1] S. Mastrangelo,[†] M. Tolone,[†] F. Perini,[‡] E. Lasagna,[‡] and M. Cassandro[*]

*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, 35020 Legnaro, PD, Italy; [†]Department of Agricultural, Food and Forest Sciences, University of Palermo 90128 Palermo, PA, Italy; and [‡]Department of Agricultural, Food and Environmental Sciences, University of Perugia 06121 Perugia, PG, Italy

ABSTRACT The aim of this study was to conduct a genome-wide comparative analysis of 8 local Italian chicken breeds (Ermellinata di Rovigo, Millefiori di Lonigo [**PML**], Polverara Bianca, Polverara Nera, Padovana, Pepoi [**PPP**], Robusta Lionata, and Robusta Maculata), all under a conservation plan, to understand their genetic diversity and population structure. A total of 152 animals were analyzed using the Affymetrix Axiom 600 K Chicken Genotyping Array. The levels of genetic diversity were highest and lowest in PML and PPP, respectively. The results of genomic inbreeding based on runs of homozygosity (**ROH**; F_{ROH}) showed marked differences among breeds and ranged from 0.161 (PML) to 0.478 (PPP). Furthermore, in all breeds, short ROH (<4 Mb in length) were more frequent than long segments. Patterns of genetic differentiation, model-based clustering, and neighbor networks showed that most breeds formed nonoverlapping clusters and were clearly separate populations. The 2 Polverara breeds shared a similar genetic background and showed the lowest genetic differentiation in comparison with purebred lines; the local populations showed separated groups. PPP and PML were closer to the group of the purebred broiler lines (BRSA, BRSB, BRDA, and BRDB). Six genomic regions are presented as hotspots of autozygosity among the Italian chicken breeds, with candidate genes involved in multiple morphological phenotypes as breast muscle, muscle dry matter content, and body weight. This study is the first exhaustive genome-wide analysis of the diversity of these Italian local chickens from Veneto region. We conclude that breeds have conserved authentic genetic patterns. The results are of significant importance because they will help design and implement conservation strategies. In fact, the conservation of these breeds may also have positive impacts on the local economy, niche traditional markets, and offering a source of high-quality products to consumers. In this context, genomic information may play a crucial role in the management of local breeds.

Key words: genetic diversity, population structure, local poultry breed, SNP marker, runs of homozygosity

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INTRODUCTION

During the last century, decline of genetic resources was observed as the result of massive replacement of low-productivity local breeds with highly productive ones. Therefore, in the international scientific community, animal biodiversity management has become an important issue, and there is increasing interest in recovering and preserving local breeds (Ruane, 1999; Caballero et al., 2010).

Chicken breeding for meat and egg production is a widespread activity in the Italian countryside, but it is supported only by few genetically unified commercial breeds. These animals have a limited genetic variation, related to the productive traits, concerning specialized management and controlled environment (Biscarini et al., 2015). Data provided by the Domestic Animal Diversity Information System of the Food and Agriculture Organization of the United Nations (DAD-IS 2018, FAO) highlight the risk status of farm animals in our territory. The last census carried out on the poultry population in Italy reported that almost about 60% of the 90 historical known breeds should be considered extinct, while 13% are threated, 17% poorly spread, and only 9% widespread (Zanon and Sabbioni, 2001). It is noteworthy that a growing interest for the conservation of local breeds has developed during the last years; indeed,

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[1]Corresponding author: filippo.cendron@unipd.it

some institutions promoted several recovery programs with the aim to preserve their genetic diversity (Zanetti et al., 2011). The conservation of local breeds from extinction allows conserving the traits of adaptability, required in future environmental and production conditions, to promote animal adaptation. In this manner, these breeds could be perceived as better components for crossbreeding to generate more resistant commercial lines (Soglia et al., 2020).

In the Veneto region of Italy, there are several poultry breeds included in conservation plans as their biodiversity is important as a genetic resource. The most important breeds are Padovana, Polverara, Robusta, Millefiori di Lonigo, Pepoi, and Ermellinata di Rovigo (De Marchi et al., 2005a, 2005b; Cassandro et al., 2015). Padovana is an ancient breed with uncertain origin; however, it is known that it was introduced in Italy from Poland and it is present in 5 different colors: black, white, gold, silver, and buff (FAO, 2004). Polverara is a very old breed, developed by a cross between Padovana and other local Veneto chicken breeds (De Marchi et al., 2005a). The Robusta chicken breed was developed from crosses between Tawny Orpingtons and White Americans and was selected to provide eggs and meat and to exhibit 2 different colors of the plumage (tawny color and white-black spotted) (De Marchi et al., 2005b). Ermellinata di Rovigo was developed in the last 60 yr from crosses between the Sussex and Rhode Island breeds. This breed is characterized by white plumage with dark pens, helmsman, and cape (De Marchi et al., 2005b). The Pepoi breed is very small (size and population number) and is present in the North-West of Italy. The chickens have a clear brown plumage that changes to a gilded color; however, this breed was not officially recognized by the Italian Federation of Poultry because the characteristics of Pepoi are too variable (De Marchi et al., 2003). The most widespread breed of the Veneto region is Millefiori di Lonigo. It is characterized by a particular color of the feathers, "millefiori," and its origin is strictly related to the north-east area of Italy (Spalona et al., 2007).

Today, conservation plans of these local breeds are supported by previous studies based on microsatellite markers (Cassandro et al., 2015; Carcò et al., 2018). With the availability of high-throughput affordable genotyping techniques, fine genome-wide analysis of the genetic structure and relationships in livestock populations has become possible. These technologies have opened new perspectives to livestock genetics, as part of both the genomic selection revolution in livestock industry and the introduction of genomic approaches in conservation programs for small and endangered populations (e.g., Mastrangelo et al., 2018) including chicken (Elbeltagy et al., 2016; Chen et al., 2019; Zhang et al., 2020).

The aim of this study was to conduct a genome-wide comparative analysis of 8 local Italian chicken breeds, to investigate the patterns of genetic diversity and population structure, and to verify the effectiveness of a regional conservation program.

MATERIALS AND METHODS

Samples and Genotyping

A total of 152 samples (17–22 per breed) belonging to 8 different local chicken breeds (Ermellinata di Rovigo, Millefiori di Lonigo, Polverara Bianca, Polverara Nera, Padovana, Pepoi, Robusta Lionata, and Robusta Maculata) were sampled (Supplementary Table 1). All the blood samples were collected from brachial veins by standard venipuncture with Vacutainer tubes containing EDTA as an anticoagulant. Blood sample collection was conducted as part of routine health screening by qualified veterinarians following guidelines established by the Institutional Animal Care and Use Committee. DNA extraction and genotyping were performed at Neogen (Ayr, Scotland) using a commercial kit and the Affymetrix Axiom 600 K Chicken Genotyping Array, containing 580,961 SNPs, respectively. The Gallus_gallus-5.0 chicken assembly was used in this study as a reference genome (Warren et al., 2017). Only markers positioned on chromosomes (**Chr**) from 1 to 28 were used. Moreover, the following filtering parameters were adopted to exclude certain loci and animals and to generate the pruned input file: 1) SNPs with a call rate <95%, 2) minor allele frequency <5%, and 3) animals with >10% of missing genotypes were excluded. File editing was carried out using PLINK 1.9 (Purcell et al., 2007).

Genetic Diversity Indices

PLINK 1.9 (Chang et al., 2015) was used to estimate the observed heterozygosity (**Ho**) and expected heterozygosity, the genomic inbreeding, which is based on the difference between the observed and expected numbers of homozygous genotypes (F_{HOM}). Minor allele frequency (**MAF**) boxplot, median, and first and third interquartile were estimated through R (R Development Core Team, 2017).

Genetic Relationship and Admixture

To examine pairwise genetic relationships within and between the breeds, genome-wide identity-by-state genetic distances were calculated using the *cluster* command in PLINK 1.9 (Chang et al., 2015). The genetic distances were visualized in a multidimensional scaling (**MDS**) plot that represented the first 2 components identified with the *mds-plot* command. In addition, in order to place the 8 local chicken breeds in a wider context and to investigate more finely their relationships, we also performed a separate MDS analysis by combining our genotyping data with those of 12 commercial purebred lines (4 white egg layers, 4 brown egg layers, and 4 broilers) included in the Synergistic Plant and Animal Breeding (SYNBREED) project (Malomane et al., 2019).

Population structure was inferred by applying the model-based clustering algorithm implemented in Admixture from $K = 2$ to $K = 7$ (Alexander and Lange, 2011). The BITE R package was used to

graphically represent the results (Milanesi et al., 2017). The most likely number of populations was estimated with the cross-validation procedure.

Phylogenetic relationships among breeds were also explored using Reynolds genetic distances estimated with Arlequin v. 3.5.2.2 software (Excoffier and Lischer, 2010). Neighbor networks were constructed from the estimated genetic distances using SPLITSTREE (Huson and Bryant, 2005). Arlequin v. 3.5.2.2 software was also used to estimate population relatedness using pairwise estimates of F_{ST} among all breeds. Graphical representation was obtained using the statistical R software.

Runs of Homozygosity

Runs of homozygosity were estimated for each animal using PLINK 1.9 (Chang et al., 2015). The following criteria were used to define the runs of homozygosity (**ROH**): 1) the minimum length was set to 1 Mb, 2) 2 missing SNPs and up to 1 possible heterozygous genotype were allowed in the ROH, 3) the minimum number of SNPs that constituted the ROH was set to 100, 4) the minimum SNP density per ROH was set to 1 SNP every 100 kb, and 5) the maximum gap between consecutive homozygous SNPs was 1,000 kb. To estimate individual genomic inbreeding coefficients using the ROH data (F_{ROH}), the length of the genome covered by ROH was divided by the total chicken autosomal genome length covered by the SNP array (944,270 kb).

Each ROH was categorized based on its physical length as follows: <2 , 2 to <4, 4 to <8, 8 to < 16, and ≥ 16 Mb. For each of the ROH length categories, the mean sum of ROH per breed was calculated by summing all ROH values per animal in that category and averaging this per breed.

The percentage of SNP residing within a ROH was estimated by counting the number of times that each SNP appeared in a ROH and by dividing that number by the total number of animals (152). To identify the genomic regions of "high homozygosity," also called ROH islands, the top 0.999 SNPs of the percentile distribution of the locus homozygosity range were selected. After downloading the list of chicken autosome Gallus_gallus-5.0 from the Ensembl database (http://www.ensembl.org), annotation of gene mapping within the ROH island was also conducted. The Chicken Quantitative Trait Loci (**QTL**) Database (https://www.animalgenome.org/cgi-bin/QTLdb/GG/index) was interrogated for the presence of QTLs in the ROH islands. To investigate the biological function and the phenotypes that are known to be affected by each annotated gene, we conducted a comprehensive search in the available literature. The genes were further analyzed with the Panther Classification System (Mi et al., 2013) to identify significant ($P < 0.05$) gene ontology terms.

RESULTS

After filtering, the final number of animals and SNPs retained for analyses were 152 and 449,837, respectively. All animals had high-quality genotyping and were therefore included in the analysis.

Genetic Diversity Indices Within Breeds

Results of the genetic diversity indices are reported in Table 1. Ho and expected heterozygosity ranged from 0.162 ± 0.200 (Pepoi) to 0.308 ± 0.208 (Millefiori di Lonigo) and from 0.172 ± 0.187 (Pepoi) to 0.293 ± 0.181 (Millefiori di Lonigo), respectively. The distribution of MAF values was approximately uniform over the genome in all breeds and ranged from 0.128 ± 0.160 in Robusta Maculata and 0.129 ± 0.162 in Pepoi, to 0.220 ± 0.160 in Millefiori di Lonigo. The median, and first and third interquartile of MAF are reported in Supplementary Figure 1. The highest average F_{HOM} was obtained for the Pepoi (0.562 ± 0.038) and Robusta Maculata (0.558 ± 0.026) breeds, whereas the lowest F_{HOM} was identified for the Millefiori di Lonigo breed (0.165 ± 0.062).

Genetic Relationship and Admixture

We used an MDS plot of the pairwise identity-by-state distances in order to identify the genetic relationship among the Italian local chicken breeds (Figure 1). The results showed that most breeds formed nonoverlapping clusters and were clearly separate populations; in particular, this was unequivocal for Ermellinata di Rovigo, Millefiori di Lonigo, and Pepoi. The first dimension (C1) distinguished the 2 Robusta (Lionata and Maculata) and the Ermellinata di Rovigo from the other chicken breeds. Polverara Bianca and Nera breeds could be identified as separate clusters, except for some of the Polverara Bianca chickens that fell within the Polverara Nera animals. Padovana Dorata breed was positioned close to Polverara breeds. Moreover, 1 animal classified as Padovana Dorata was positioned within the cluster of Millefiori di Lonigo.

To investigate the genetic relationships between the 8 local chicken breeds and other breeds, we performed an MDS analysis using a combined dataset that included 498,322 SNPs and 385 individuals (Supplementary Figure 2). As reported earlier, in a wider context, the local populations showed separated groups. Among the local breeds, Pepoi and Millefiori di Lonigo were closer to the group of the purebred broiler lines (BRSA, BRSB, BRDA, and BRDB).

The degree of genetic differentiation between pairs of breeds is shown in Supplementary Table 4. These results agreed with the MDS plot. The F_{ST} values ranged from 0.114 (Polverara Bianca and Nera) to 0.565 (Pepoi and Robusta Maculata). Moderate genetic differentiation was observed between all other breeds. Again, the genetic differentiation was limited between the 2 Polverara breeds (Supplementary Table 2).

Results from within population substructure through Admixture analysis (Figure 2), considering a range of 2 through 12 potential clusters (K), pointed out that the best-fitting number of populations present in the total

Table 1. Genetic diversity indices for the analyzed Italian local chicken breeds.

Breed	Acronym	N	Ho± SD	He± SD	MAF± SD	F_{HOM}± SD
Ermellinata di Rovigo	PER	22	0.207 ± 0.193	0.228 ± 0.198	0.171 ± 0.166	0.439 ± 0.049
Millefiori di Lonigo	PML	20	0.308 ± 0.208	0.293 ± 0.181	0.220 ± 0.160	0.165 ± 0.062
Polverara Bianca	PPB	17	0.225 ± 0.183	0.258 ± 0.186	0.191 ± 0.160	0.391 ± 0.060
Padovana Dorata	PPD	22	0.227 ± 0.195	0.241 ± 0.186	0.177 ± 0.160	0.385 ± 0.082
Polverara Nera	PPN	20	0.205 ± 0.196	0.218 ± 0.193	0.161 ± 0.162	0.443 ± 0.064
Pepoi	PPP	15	0.162 ± 0.200	0.172 ± 0.198	0.129 ± 0.162	0.562 ± 0.038
Robusta Lionata	PRL	18	0.189 ± 0.206	0.192 ± 0.197	0.143 ± 0.163	0.486 ± 0.038
Robusta Maculata	PRM	18	0.166 ± 0.198	0.173 ± 0.196	0.128 ± 0.160	0.558 ± 0.026

Abbreviations: F_{HOM}, inbreeding coefficient; He, expected heterozygosity; Ho, observed heterozygosity; MAF, average minor allele frequency; N, number of individuals per breed.

sample was $K = 7$. In agreement with the results of C1 in the MDS plot, the inferred breed structure for $K = 2$ separated the Ermellinata di Rovigo and the 2 Robusta breeds (red color) from the others. When K increased from 3 to 7, the breeds were progressively assigned to specific clusters and showed a well-defined genetic identity: Ermellinata di Rovigo at $K = 3$, Pepoi at $K = 4$, Padovana Dorata at $K = 5$. At $K = 6$, the 2 Robusta breeds were further split into separate clusters. In contrast, the 2 Polverara breeds shared a similar genetic background. In fact, admixture between these breeds was detectable at $K = 7$.

To provide additional insight into the relationships among the chicken breeds, we constructed a Neighbor-Net graph based on Reynolds genetic distances (Figure 3) between pairs of breeds. Consistent with the MDS plot and Admixture analyses, the Neighbor-Net graph showed some clear clusters and relationships between breeds, notably the Polverara Bianca and Nera and Padovana Dorata. The graph also depicted that the 2 Robusta breeds originated from the same branch and displayed a very close relationship. The shortest branch was observed for Polverara Bianca, whereas the longest one was found for Pepoi.

Runs of Homozygosity

Individual genomic inbreeding was also evaluated using ROH data. Pepoi breed showed the highest mean value of inbreeding (F_{ROH} = 0.478), followed by

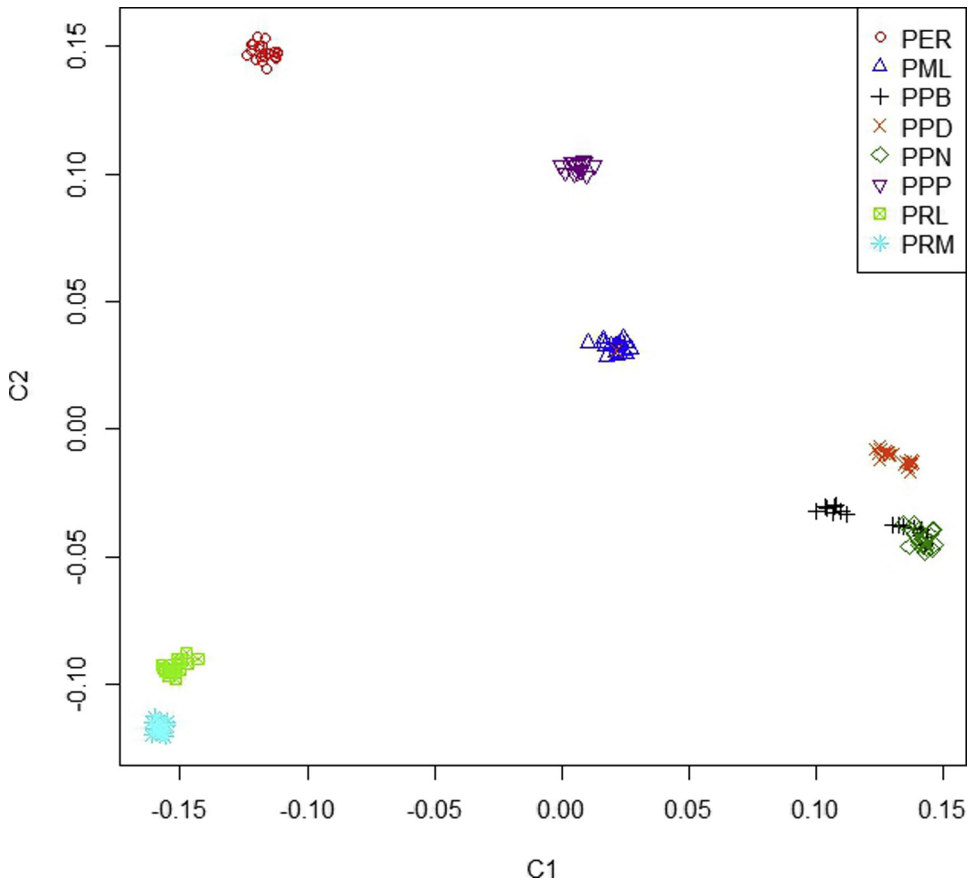

Figure 1. Genetic relationships among the Italian local chicken breeds defined through multidimensional scaling analysis. For a full definition of breeds, see Table 1.

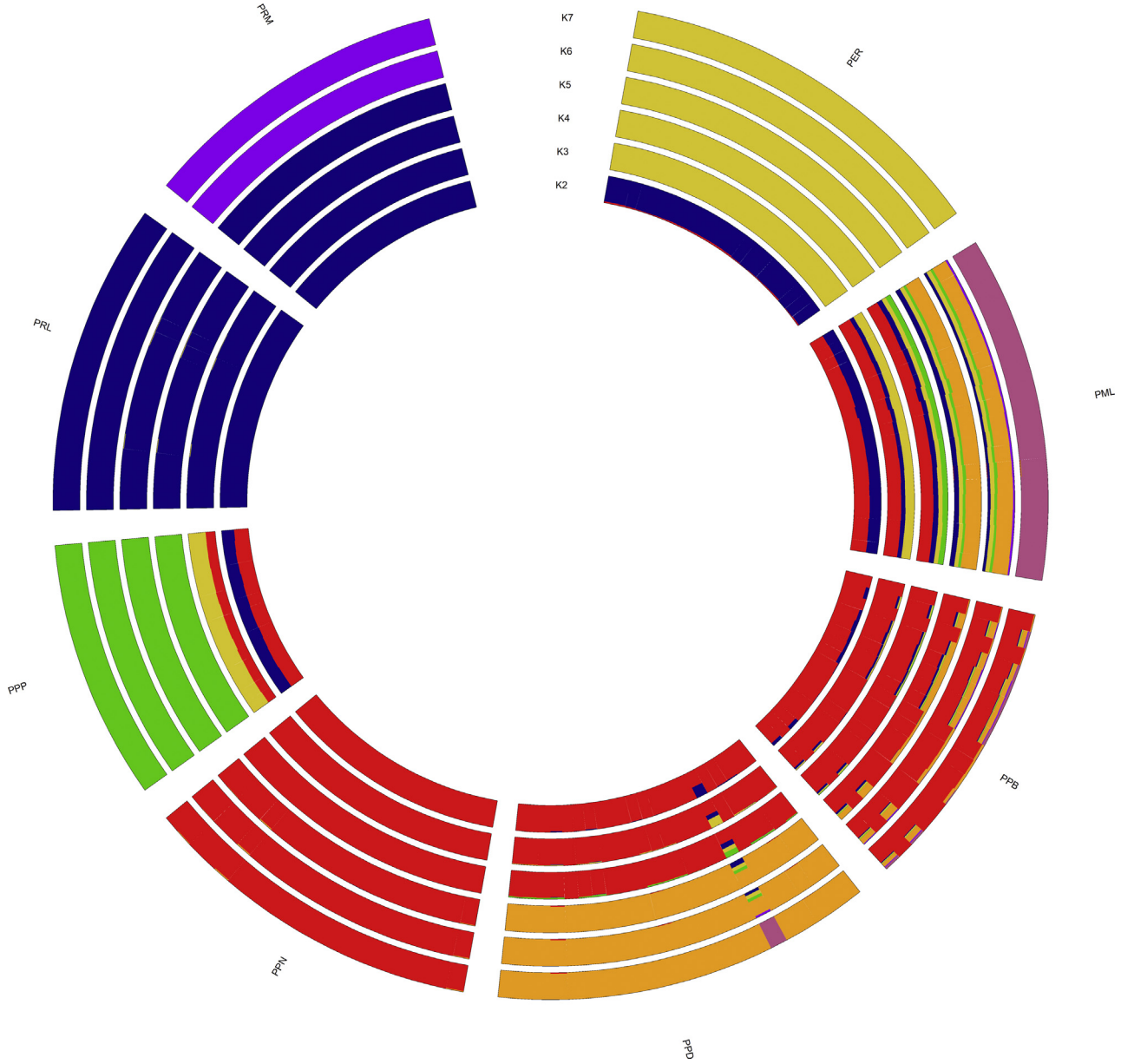

Figure 2. Model-based clustering of the Italian local chicken breeds analyzed in each of the inferred clusters (K), from $K = 2$ to $K = 7$. For a full definition of breeds, see Table 1.

Robusta Maculata ($F_{ROH} = 0.413$), whereas Millefiori di Lonigo showed the lowest value ($F_{ROH} = 0.161$) (Figure 4 and Table 2).

A total of 19,548 ROH segments >1 Mb were detected. The mean number of ROH per individual within breeds ranged from 55.45 (Millefiori di Lonigo) to 172.27 (Robusta Maculata). Each ROH segment was categorized based on its physical length into 5 categories, and the mean sum of ROH per breed was calculated (Figure 5). The results showed that, for all breeds, the majority of ROH segments were <4 Mb in length. Polverara Nera had a larger mean portion of their genome (11.4 Mb) covered in longer ROH (>16 Mb).

To identify the genomic regions that were most commonly associated with ROH in all the breeds, the top 0.999 SNPs of the percentile distribution of the locus homozygosity range was chosen as an indication of possible ROH islands in the genome (Figure 6). Table 3 provided the Chr position, the start and the end of these genomic regions. A total of 6 regions were identified: 2 regions on Chr 1 (141.58–141.63 and 141.92–142.51 Mb), 1 region on Chr 4 (41.00–41.12 Mb), 1 region on Chr 5 (2.09–3.51 Mb), and 2 regions on Chr 11 (3.34–3.38 and 3.59–3.76 Mb). On Chr 1 several "located on chromosome" genes were identified with unknown function. Some of these were also present within the ROH islands of Chr 4, 5, and 11. The other regions contained few annotated genes, some of which were reported to be involved in multiple morphological phenotypes as breast muscle, muscle dry matter content, and body weight, important from the production perspective. Several QTLs have been reported on these genomic regions in chicken. As reported in Table 3, the most representative QTLs were associated with muscle

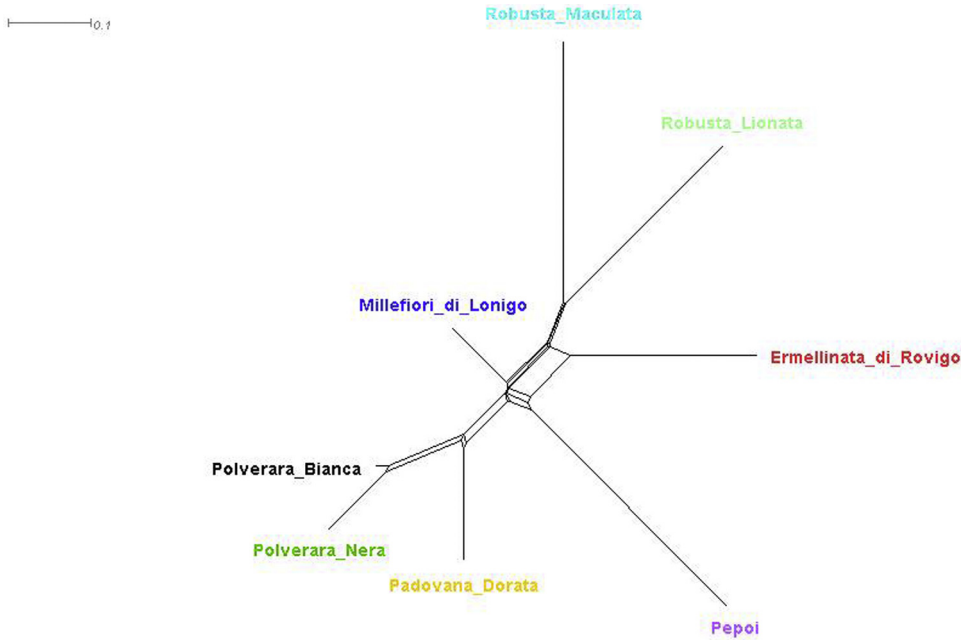

Figure 3. A neighbor-joining tree based on Reynold's genetic distance for the Italian local chicken breeds.

dry matter content, breast muscle pH, ileum weight, body weight, and feed intake, pointing out the importance of these regions in body conformation and structure. Gene ontology study was performed through 3 domains: cellular component, molecular function, and biological process for all the genes identified within the ROH islands (Supplementary Table 3).

DISCUSSION

Improving our knowledge about within-breed diversity and the population structure in livestock species is fundamental for improving selection plans and breeds, understanding environmental adaptation, and implementing conservation programs (Bortoluzzi et al., 2018; Mastrangelo et al., 2018; Malomane et al., 2019). While most efforts are dedicated to studying cosmopolitan breeds, there is a growing interest in the genetics of local breeds; these are important genetic resources for their potential to contribute to solving problems in agriculture related to environmental change (Fleming et al., 2016; Soglia et al., 2020). This study investigates for the first time the genome-wide structure of 8 Italian local chicken breeds using high-density genome-wide SNPs. These breeds are part of TuBAvI project (Protection of biodiversity in Italian poultry breeds) aimed to study and characterize Italian local poultry breeds. In fact, assessing the genetic diversity and understanding the relationships

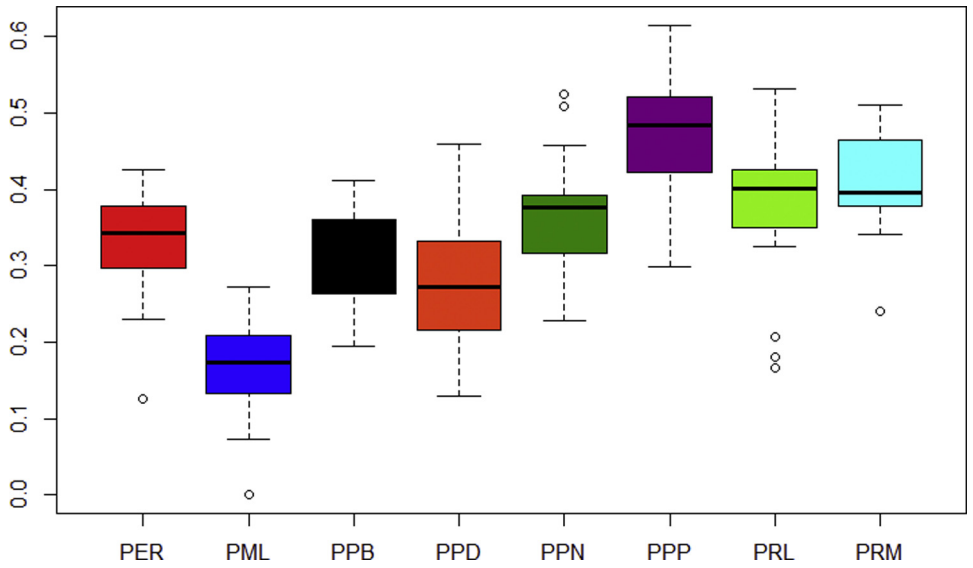

Figure 4. Box plot of the inbreeding coefficients inferred from runs of homozygosity (F_{ROH}) for each chicken breed. Abbreviation: ROH, runs of homozygosity.

Table 2. Descriptive statistics for ROH for the analyzed Italian local chicken breeds.

Breed	F_{ROH} ± SD	MN_{ROH} ± SD
Ermellinata di Rovigo	0.322 ± 0.068	140.54 ± 22.79
Millefiori di Lonigo	0.161 ± 0.064	55.45 ± 18.16
Polverara Bianca	0.321 ± 0.061	120.52 ± 24.25
Padovana Dorata	0.249 ± 0.06	107.22 ± 25.08
Polverara Nera	0.376 ± 0.079	132.10 ± 19.28
Pepoi	0.478 ± 0.077	170.00 ± 17.23
Robusta Lionata	0.359 ± 0.091	146.94 ± 24.19
Robusta Maculata	0.413 ± 0.073	172.27 ± 16.22

Abbreviations: F_{ROH}, mean ROH-based inbreeding coefficient with SD; MN_{ROH}, mean number of ROH per individual and per breed; ROH, runs of homozygosity.

and population structure among breeds are necessary steps to verify the effectiveness of a conservation program.

Genetic diversity indices and genomic inbreeding, which were estimated using different approaches and which are key parameters in the genetic management of populations, were used to determine the levels of genetic variability within the breeds. The levels of genetic diversity were lowest in Pepoi breed. This could have resulted from population bottlenecks caused by the reduced demographic size for this breed. The average MAF values agree with the range reported in a study on Dutch chicken (Table1) (Bortoluzzi et al., 2018). On the contrary, the average Ho values differed from the results of a previous study based on microsatellite markers (Zanetti et al., 2010), in which the authors reported higher values for these breeds. However, as expected, results for SNP were lower than those for microsatellites (Viale et al., 2017). Indeed, single-loci SNP analyses presented loss of information due to the biallelic nature of the markers, as compared to the multi-allelic microsatellites having larger numbers of alleles per locus, and hence higher frequency of heterozygotes. Although the results were obtained by using different markers, the difference could be also due to an increase in inbreeding linked to a reduction in the number of individuals within the breeds, during the years. Certainly, the low values of heterozygosity in Pepoi and Robusta breeds, compared with the others, underline the difficulty to preserve the biodiversity in these breeds; thus special attention must be paid for the conservation of these breeds. Indeed, specific genetic conservation programs could be applied to these breeds through appropriate mating plans with the aim of increasing genetic variability, controlling inbreeding, and developing in situ and ex situ conservation schemes (Zanetti et al., 2010).

The comparison (Supplementary Figure 2) among the local Italian breeds and the purebred lines underlines a weak genetic relationship among the breeds. Furthermore, Ermellinata di Rovigo, Pepoi, and Millefiori di Lonigo breeds were closer to the 4 commercial hybrids (BRSA, BRSB, BRDA, and BRDB). This aspect highlights the possibility of a genetic commercial origin of the 3 local breeds that could be investigated in future studies. Nevertheless, it is also known that some local breeds of Veneto regions were restored by commercial pure lines (De Marchi et al., 2005b).

To understand the genetic relationship and the population structure, we carried out an MDS and Admixture analysis, and calculated Reynold's genetic distances and the pairwise estimates of F_{ST} for the Italian local chicken breeds (Figures 1 and 3 and Supplementary Table 2).

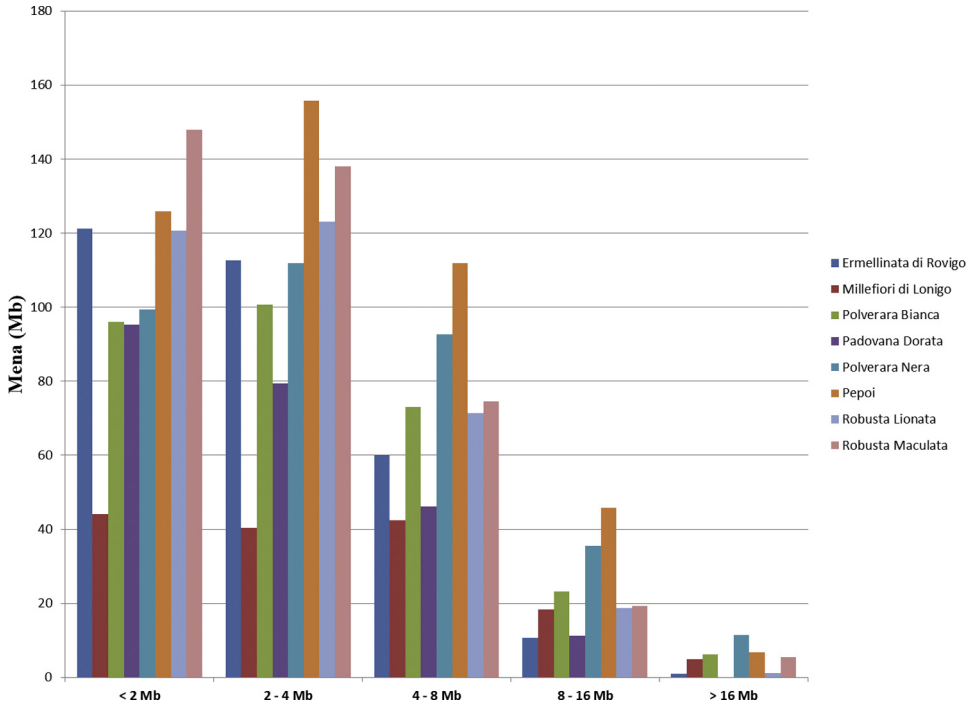

Figure 5. Classification of ROH in 5 categories according to size (from <2 to more than 16 Mb) (x-axis) and mean sum of ROH in Mb (y-axis) within each ROH length category per breed. Abbreviation: ROH, runs of homozygosity.

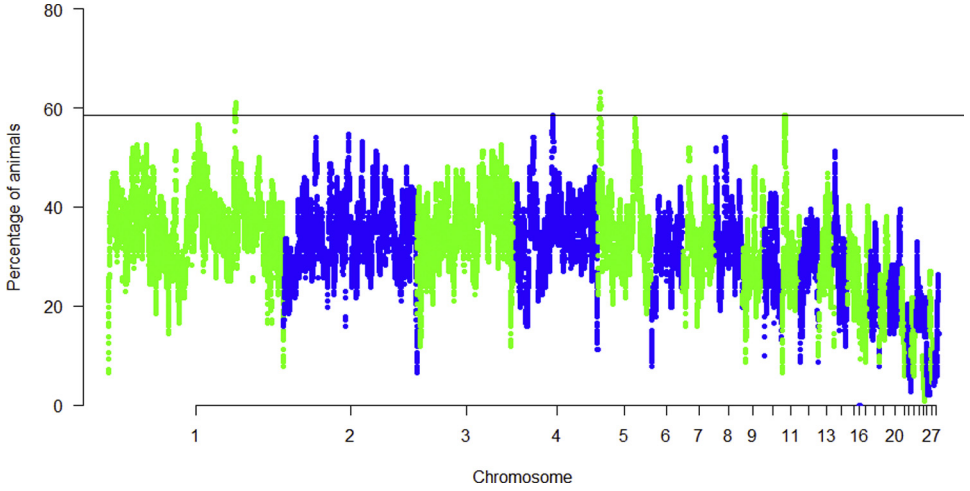

Figure 6. Manhattan plot of the incidence of each SNP in the runs of homozygosity among the Italian local chicken breeds.

The results obtained by the different approaches largely agree with the breeding history of the chicken breeds under investigation (Zanetti et al., 2010). For instance, the close genetic relationships among the Padovana and the 2 Polverara breeds are as expected, due to the contribution of Padovana to the origin of Polverara (De Marchi et al., 2005b). In fact, Polverara is a very old breed, developed by a cross between Padovana and other local Veneto chicken breeds (De Marchi et al., 2005a). Other examples are Ermellinata di Rovigo and the 2 Robusta breeds, which at $K = 2$ shared a substantial proportion of their ancestry, due to their common Anglo-American derivation (Baruchello and Cassandro, 2012). The results also indicate the 2 Robusta breeds to be the most distant group, and emphasized clear genetic differences compared to the other local chicken breeds considered in this study. In fact, the MDS clearly separated the 2 breeds from the other breeds, and this was in agreement with Neighbor-Net, F_{ST}, and model-based clustering (from $K = 3$). The results are consistent with the genetic origins of the Robusta breeds, because their ancestors were from outside of Italy: Tawny Orpingtons and White American, which are completely different from the other breeds (Arduin, 2014).

Genome-wide SNPs are particularly suitable for detecting genomic regions with reduced heterozygosity (Bortoluzzi et al., 2018). Currently, ROH-based F estimates (F_{ROH}) are considered one of the most powerful approaches to detect inbreeding (Strillacci et al., 2017; Bertolini et al., 2018; Marchesi et al., 2018). The length and genomic location of ROH are related to several aspects of information about the demographic history of the poultry species (Fleming et al., 2016; Strillacci et al., 2018; Zhang et al., 2020). Analysis of ROH may be useful for conservation programs, since animals with high levels of F_{ROH}, as observed in Pepoi breed, can be excluded or assigned a lower priority for mating purposes in endangered populations, to minimize the loss in genetic diversity and increase genetic diversity. This analysis supports the genetic diversity estimates, emphasizing that historical inbreeding had an impact on the genome of the poultry breeds of Veneto region. The analysis of ROH highlights the importance of novel marker-based information to prevent future loss of diversity. The prevalence of long ROHs across local chicken breeds is consistent with the limits to effective genetic management resulting from the absence of pedigree data and breed registry (Bortoluzzi et al., 2018). Interestingly,

Table 3. Genomic regions of extended homozygosity (ROH islands) identified in the Italian local chicken breeds.

GGA	No of SNPs	Start	End	Length (bp)	Genes	QTL
1	17	14,15,83,475	14,16,39,278	55,803	*LOC107050425*	Breast muscle pH QTL (157157)
1	144	141921,559	14,25,17,751	5,96,192	*LOC107051457*, *LOC101748187*	Muscle dry matter content QTL (24,459) Muscle dry matter content QTL (24,460) Muscle dry matter content QTL (24,461) Muscle dry matter content QTL (24,462) Breast muscle pH QTL (157157)
4	39	4,10,07,013	4,11,28,124	1,21,111	*TENM3*, *LOC101748815*	Ileum weight QTL (96,634)
5	261	20,90,157	35,19,023	14,28,866	*PRMT3*, *NELL1*, *SLC6A5*, *MIR1775*, *LOC107053351*, *LOC107053350*, *ANO5*, *SLC17A6*, *FANCF*, *GAS2*, *SVIP*, *ANO3*, *SLC5A12*, *FIBIN*, *BBOX1*, *LOC107053349*, *LOC107053348*	Body weight (28 d) QTL (95,416) Body weight (28 d) QTL (95,415)
11	16	33,44,808	33,89,428	44,620	*ESRP2*	Feed intake QTL (64,558)
11	71	35,96,573	37,60,321	1,63,748	*SLC12A4*, *LOC107054268*, *LOC101752262*, *SLC6A2*, *LPCAT2*	Feed intake QTL (64,559)

Abbreviations: GGA, *Gallus gallus chromosome*; ROH, runs of homozygosity; QTL, quantitative trait loci.

the low abundance of long ROH in Ermellinata di Rovigo, Padovana Dorata, and Robusta Lionata reflects proper breed management and a higher effective population size compared to the other breeds. In fact, the relatively low proportion of genomes covered by homozygous segments supports effective genetic management, which is meant to pursue a conservation program allowing recessive deleterious alleles to be purged with inbreeding.

Shared ROHs among populations identify genomic regions under selection in which a reduced haplotype variability produces ROH islands. In chicken, ROH islands have been used to identify genomic regions and genes with potential roles in defining breed-specific traits and adaptations to different production systems (e.g., Elbeltagy et al., 2016; Strillacci et al., 2018; Zhang et al., 2020).

We found that some SNPs in ROH islands occur in regions with uncharacterized genes (i.e., gene located on chromosome). This may reflect selection acting on uncharacterized regulatory regions or simply the fixation of noncoding DNA by genetic drift due to the absence of any selection (Qanbari et al., 2011). On the contrary, a number of prominent genes are located within the ROH islands. Among these genes, several are worth mentioning because they showed associations with several specific traits related to livestock. On Chr 4 (41.00–41.12 Mb) *Teneurin transmembrane protein 3* gene is identified; it encodes for a transmembrane glycoprotein involved in developing visual and nervous systems in chicken (Rubin et al., 1999). The analysis of ROH islands inside Chr 5 (at 2.09–351 Mb) identified several genes that seem to be involved in morphological traits. The first was *Anoctamin 5* gene involved in the development of muscle tissue and estrogen production in mice (Sun et al., 2014). Other candidate genes are *NEL-like protein 1*, an important growth factor linked to bone tissue formation and skeleton integrity, typically expressed in the commercial broiler for high growth rate and meat production (Elferink et al., 2012); *SLC65* (*metal ion SLC transporters*) family gene has been identified. These genes are involved in embryogenesis events, in the regulation of digestive enzyme activity, and in the development of the digestive intestine (Li et al., 2008) in poultry species. Besides, *BBOX1* (*gamma-butyrobetaine hydroxylase 1*) gene has been also found in the ROH island of Chr 5, that seems to regulate feed efficiency; indeed, in high-growth commercial chickens, this gene is overexpressed (Lee et al., 2015). Inside the last region identified on Chr 11 (at 3.34–3.38 Mb), an important gene called *Epithelial Splicing Regulatory Protein 2* is present; this is important because its activity is associated with biological processes of daily gain trait (Zhang et al., 2012).

CONCLUSIONS

This study is the first exhaustive genome-wide analysis of the diversity of 8 Italian local chicken breeds. The results obtained by the different approaches largely agreed with the breeding history of the studied breeds. The breeds have preserved distinctive characteristics, probably due to differences in genetic origin, environment, genetic isolation, and inbreeding. The results for Pepoi and Robusta breeds reiterate that their genetic variability is lower than the other studied breeds. On the contrary, the genetic structure of Millefiori di Lonigo seems to be more stable compared with the other local breeds. The results are of significant importance because they will help design and implement conservation strategies. Moreover, the levels of differentiation are an important factor that support the work of the conservation plans; indeed, analysis of genetic diversity can promote the development of targeted mating plans to protect the most endangered breeds.

Genetic distances confirmed the history of these breeds highlighting that, over the years, their genetic identity was maintained and the genetic heritage remained preserved. However, it is necessary to consider a compromise between the need to maintain a specific degree of variability and the loss of alleles due to selection for productive traits. This aspect is critical because the use of a certain pressure for productive traits is important to persuade farmers to rear the indigenous breeds, which will be otherwise forgotten, promoting broiler lines.

The information obtained from these studies represented a useful tool for supervising conservation activities and to verify the correct genetic management. Moreover, these results represent a starting point for the valorization of local breeds as an important reservoir of genetic diversity and mark the ancient and recent inbreeding story of the genome of the local breeds of Veneto region. Minor relatedness and low inbreeding are essential for small, local breeds to maintain the native genetic diversity and good inbreeding management for the progeny as well as to preserve biodiversity.

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DISCLOSURES

Authors declare no conflict of interest.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.10.023.

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