Genetic characterization of indigenous poultry breeds of Veneto region

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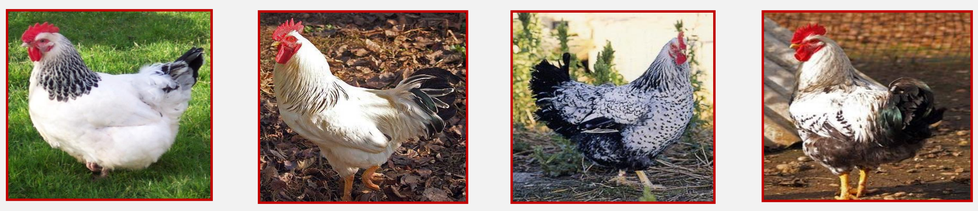
Aim

Aim of the present study is to conduct a genome-wide comparative study of eight indigenous Italian poultry breeds, all under a plan of conservation, to estimate their genetic variability and clarify their genetic relationships.

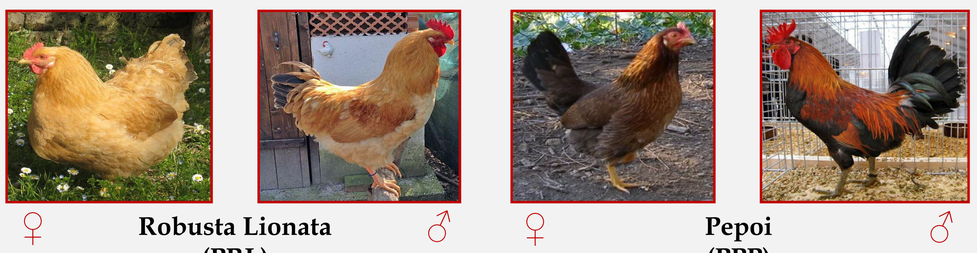
Conclusion

The information obtained from these studies represented a useful tool for monitoring the conservation activities and to verify the correct genetic management, which until now has been performed in the good way, leading each population to show its own genetic identity. Therefore, these results also represent a starting point for the valorization of local breeds as an important reservoir of genetic diversity.

Breeds



♀ **Ermellinata di Rovigo (PER)** ♂ ♀ **Robusta Maculata (PRM)** ♂



♀ **Robusta Lionata (PRL)** ♂ ♀ **Pepoi (PPP)** ♂

♀ **Padovana Dorata (PPD)** ♂ ♀ **Millefiori di Lonigo (PML)** ♂

♀ **Polverara Bianca (PPB)** ♂ ♀ **Polverara Nera (PPN)** ♂

Discussion

The results showed that most breeds formed non-overlapping clusters and were clearly separate populations. According to their common origin, PRL and PRM breeds grouped together in the same cluster, as well as PPB and PPN. PPD is closed to the two Polverara breeds due to the same evolutionary history (Mazzon, 1934), as PRL and PRM, that are characterized by common ancestors (Arduin, 2014). Consistent with the MDS plot, the Neighbor-Net graph showed that the two Robusta breeds originated from the same branch and displayed a very close relationship. The shortest branch was observed for PPB, whereas the longest one was found for PPP. This last breed showed the highest mean value of inbreeding, followed by PRM, whereas PML showed the lowest one. Controlling molecular inbreeding would restrict inbreeding depression, and therefore the risk of extinction. The information generated in this study has important implications from economic and scientific perspectives and highlights the necessity to implement a genomic-driven conservation program for these local breeds.

Results

Table 1. Genetic diversity indexes for the analyzed Italian local chicken populations.
Number of individuals per population (N), Observed (Ho) and expected (He) heterozygosity, average minor allele frequency (MAF), inbreeding coefficient (F_{HOM}). Standard Deviation was considered for each value.

Razza	Code	N	MAF	SD	H_o	SD	H_e	SD	F_{HOM}	SD
Ermellinata di Rovigo	*PER*	22	0.296	± 0.307	0.207	± 0.193	0.228	± 0.198	0.439	± 0.049
Millefiori di Lonigo	*PML*	20	0.295	± 0.248	0.308	± 0.208	0.293	± 0.181	0.165	± 0.062
Polverara Bianca	*PPB*	17	0.262	± 0.254	0.225	± 0.183	0.258	± 0.186	0.391	± 0.06
Padovana Dorata	*PPD*	22	0.267	± 0.274	0.227	± 0.195	0.241	± 0.186	0.385	± 0.082
Polverara Nera	*PPN*	20	0.253	± 0.283	0.205	± 0.196	0.218	± 0.193	0.443	± 0.064
Pepoi	*PPP*	15	0.283	± 0.342	0.162	± 0.201	0.172	± 0.198	0.562	± 0.038
Robusta Lionata	*PRL*	18	0.288	± 0.33	0.19	± 0.206	0.192	± 0.197	0.486	± 0.038
Robusta Maculata	*PRM*	18	0.286	± 0.343	0.166	± 0.199	0.173	± 0.196	0.558	± 0.026

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

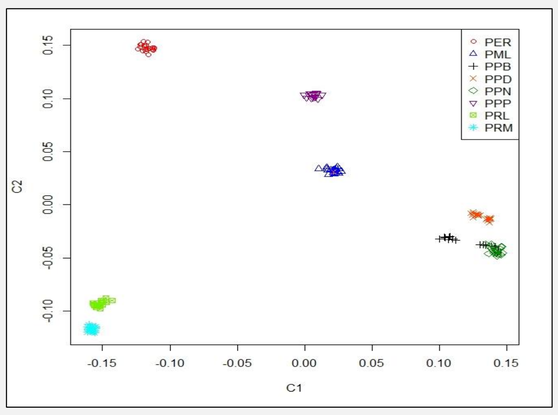


Figure 2. A neighbor-joining tree based on the Reynold's genetic distance for the Italian local chicken breeds.

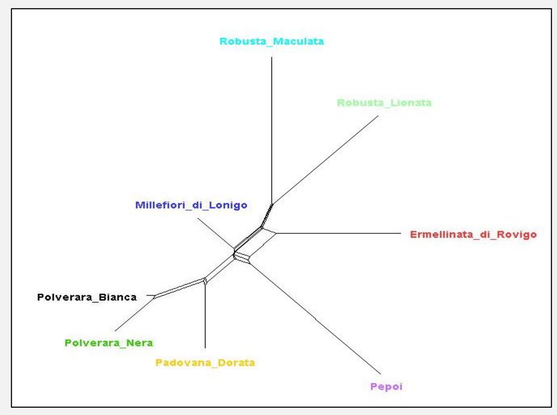


Figure 3. Box plot of the inbreeding coefficients inferred from runs of homozygosity (F_{ROH}) for each chicken breed. For a full definition of breeds see Table 1.

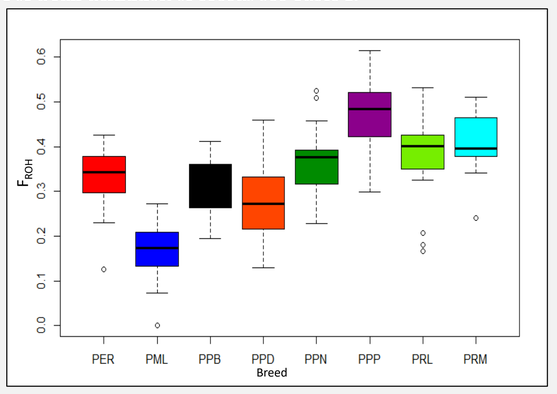
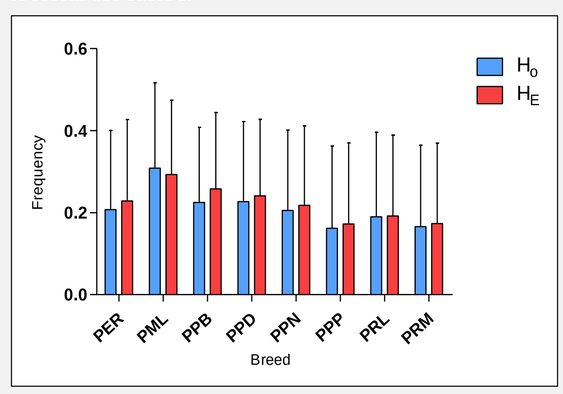


Figure 4. Comparison among Observed and Expected Heterozygosity ± SD of chicken breeds. For a full definition of breeds see Table 1.



Introduction

During the last century, erosion of livestock genetic resources was observed as the result of massive replacement of low-productivity local breeds with highly productive ones. However, the local breeds are the result of particular adaptation to a singular, sometimes harsh environment. Therefore, the conservation and monitoring of the genetic diversity of these local breeds are fundamental to meet future breeding needs, especially in the context of global climate change. An investigation of genomic variation within a breed is an important prerequisite to maintain its integrity and to ensure appropriate conservation. Italians have a long history of poultry breeding and still raise several local breeds. Smaller-scale studies carried out at regional level on these local breeds reported that they are genetically distinct, but showed low levels of genetic diversity (Marelli et al., 2006; Tadano et al., 2007; Zanetti et al., 2010; Strillacci et al., 2017).

Materials and methods

Samples and genotyping

A total of 152 samples (17 to 22 per breed) from 8 different local chicken populations were sampled. All the blood samples were collected from brachial veins of chickens by standard venipuncture. 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, distributed across the genome with an average spacing of 1.7 Kb, respectively. The Gallus_gallus-5.0 chicken assembly was used in this study as reference genome.

Genetic diversity

Preliminary filters were applied: (i) SNPs with a call rate <95% and (ii) minor allele frequency 5% and (iii) animals with more than 10% of missing genotypes were removed. File editing was carried out using PLINK 1.9 (Chang et al., 2015). Observed (H_o) and expected (H_e) heterozygosity, the genomic inbreeding, which is based on the difference between the observed and expected numbers of homozygous genotypes (F_{HOM}), and average MAF (≥ 0.05) were estimated by PLINK 1.9 (Chang et al., 2015).

Genetic distance

Genome-wide identity- by-state genetic distances between breeds were calculated using the cluster command in PLINK 1.9 and visualized in a multidimensional scaling (MDS) plot. Reynolds genetic distances were estimated and used to construct a Neighbor networks using SPLITSTREE (Huson and Bryant, 2006).

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 ROH: (i) the minimum length was set to 1Mb, (ii) two missing SNPs and up to one possible heterozygous genotype was allowed in the ROH, (iii) the minimum number of SNPs that constituted the ROH was set to 100, (iv) the minimum SNP density per ROH was set to one SNP every 100 kb and (v) the maximum gap between consecutive homozygous SNPs was 1000 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).

References

- Arduin M. (2014) Polli di razza. Robusta Maculata. Veneto Agricoltura;
- Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience, 4(1), 7;
- Huson, D. H., & Bryant, D. (2005). Application of phylogenetic networks in evolutionary studies. Molecular biology and evolution, 23(2), 254-267;
- Marelli, S. P., Strillacci, M. G., Ferrante, V., Pignattelli, P., Gualtieri, M., & Guidobono Cavalchini, L. (2006). Genetic variability of Valdarnese Bianca Chicken breed using microsatellite markers. World's Poultry Science Journal, 62, 207-208;
- Mazzon, I. (1932). Pollicoltura padovana. Soc. Coop. Tipografica Ed., Padova, Italy;
- Tadano, R., Sekino, M., Nishibori, M., & Tsudzuki, M. (2007). Microsatellite marker analysis for the genetic relationships among Japanese long-tailed chicken breeds. Poultry science, 86(3), 460-469;
- Zanetti, E., De Marchi, M., Dalvit, C., & Cassandro, M. (2010). Genetic characterization of local Italian breeds of chickens undergoing in situ conservation. Poultry Science, 89(3), 420-427;
- Strillacci, M. G., Cozzi, M. C., Gorla, E., Mosca, F., Schiavini, F., Román-Ponce, S. I., ... & Bagnato, A. (2017). Genomic and genetic variability of six chicken populations using single nucleotide polymorphism and copy number variants as markers. Animal, 11(5), 737-745.

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