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Morphological and genetic characterization of 13 Italian local chicken breeds

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According to census accomplished in 2001, only 9% of Italian poultry breeds are still widespread. This project aims to describe morphological variability and genetic background among 13 Italian autochthonous chicken breeds, 10 from Northern Italy and 3 from Central Italy. An updated biometrical measurement protocol was established starting from phenotypic characterization guidelines released by FAO. Six traits were registered on each animal: live body weight (LBW), body length (BL), shank length (SL), shank width (SW), breast width (BW), and wingspan (WS). Moreover, all breeds were genotyped using the Affymetrix 600 K Chicken SNP Array, in order to evaluate genetic variability and population structure. Means of BL and BW showed low variability among breeds, with the lowest value in Pepoi (BL = 32.29 ± 1.57 cm and BW = 28.92 ± 1.93 cm), and maximum in Robusta Lionata for BL (46.79 ± 1.66 cm) and in Robusta Maculata for BW (42.33 ± 3.60 cm). On the contrary, average LBW varied notably among breeds, with the highest value for Robusta Maculata (♂ 4,221.7 ± 450.6 g; ♀ 2,831.7 ± 253.2 g) and the lowest values for Modenese males (1,695.0 ± 128.1 g) and Pepoi females (1,293.3 ± 219.2 g). The lowest observed heterozygosity (Ho) and expected heterozygosity (He) were obtained for Padovana Argentata (Ho: 0.151 ± 0.198; He: 0.146 ± 0.185) and the highest for Millefiori di Lonigo (Ho: 0.293 ± 0.199; He: 0.291 ± 0.178). Furthermore, multidimensional scaling plot showed clear genetic identity for each breed, with clusters formed according to geographical and historical origin of the breeds, which were confirmed in neighbor networks. In conclusion, local breeds have conserved authentic genetic patterns and these results can help improve conservation strategies.

Keywords: safeguard, SNP, genomic, morphological trait, poultry, Italy

1 Introduction

The poultry sector is characterized by a constant process of specialization of breeding genetic resources, commercial hybrid lines, and breeding systems. This process, which began in the second half of the last century, has brought undisputed improvements in production performance, but also a progressive erosion of genetic variability and adaptability in livestock animals (Fulton, 2006). Indeed, the sequencing of chicken genome has shown that commercial lines have lost 90% of the alleles present in the native breeds, which represent, on the contrary, a fundamental resource of biodiversity (Muir, 2008). The native breeds are the result of a long process of domestication and adaptation to the natural environment typical of a particular ecosystem; moreover, they represent a socio-economic, cultural and ecological value (FAO, 2018). According to a census carried out in 2001, about 61% of the 90 historically known Italian poultry breeds must be considered extinct and only 9% are still widespread. In general, the size of the indigenous populations has drastically reduced over the years with a consequent increase in inbreeding and reduced performance (Cerolini et al., 2010). Consequently, the urgency to collect new information and organize updated databases with regards to indigenous genetic resources in poultry farming is evident. Furthermore, genetic characterization

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studies can be an important tool for the management of populations that do not provide for the registration of kinship, as occurs in most cases in poultry farming. Moreover, it is important to collect an exhaustive dataset of phenotypic traits from local chicken breeds, in order to verify the stability of somatic changes of the animals in conservation management.

In this study we provide an overview of genetic population structure and main phenotype morphometric features of 10 local chicken breeds from Northen Italy (Ermellinata di Rovigo [PER], Millefiori di Lonigo [PML], Padovana Argentata [PPA], Padovana Camosciata [PPC], Padovana Dorata [PPD], Pepoi [PPP], Polverara Bianca [PPB], Polverara Nera [PPN], Robusta Lionata [PRL], Robusta Maculata [PRM]), and 3 breeds from Central Italy (Ancona [ANC], Modenese [MOD], and Romagnola [ROM]).

The MOD and the ROM are two breeds from Emilia-Romagna region, used in the areas of origin as dual purpose breeds to meet family needs for both egg and meat supply and not for commercial purpose (Sabbioni et al., 2006). The MOD breed is characterized by the presence of medium-large animals with golden or blue golden wheat plumage (Mazzon, 1932). During the past century, the breed was crossed with the ancestral Italian Leghorn (black red), to improve egg production (Zanon et al., 2006). The ROM breed is characterized by a somewhat varied plumage as it results from the few pictures of the past century, but the silver, the grey, the golden red, the white and the partridge are common (Zanon et al., 2006). The ROM fowl is particularly typified by a "wild" behaviour that pushes it, having the possibility, to pass the night on the trees, rather than in the hen house. Because of its geographic localization in areas not far from areas voted to the avicultural breeding of intensive type, the ROM fowl in the past century underwent numerous crosses and it was gradually replaced by more precocious and productive breeds (Zanon et al., 2006). The ANC breed was obtained from crosses with Leghorn, so its morphological and productive traits are similar. The main difference is the colour of the plumage, which is black with evenly white-tipped feathers (Mugnai et al., 2009).

In the Veneto region of Italy, there are several poultry breeds undergoing conservation plans. The Padovana is an ancient breed introduced in Italy from Poland and it is present in 5 different colors: black, white, gold, silver and buff (Cassandro et al., 2015). The Polverara is an old breed, resulting from a cross between Padovana and other local chicken breeds (De Marchi et al., 2005a). Robusta chicken breeds have been developed by crosses between Tawny Orpingtons and White Americans and have been selected to provide eggs and meat and to exhibit two different colors of the plumage (tawny color and white-black spotted) (De Marchi et al., 2005b). The PER was established in the last 60 years from the Sussex and Rhode Island breeds. Dark pens, helmsman, and cape are typically recognized in its phenotype (De Marchi et al., 2005b). The PPP is the only breed in dataset of this study to be a dwarf breed and not officially recognized by the Italian Federation of Poultry because of wide plumage variability (De Marchi et al., 2003). The most spread breed of the Veneto region is the PML, which is characterized by a multi-color plumage, and its origin is strictly related to the North-Est area of Italy (Spalona et al., 2007).

2 Material and methods

2.1 Morphological measurement protocol

An updated biometrical measurement protocol was established starting from phenotypic characterization guidelines released by FAO (2012). A total of 312 animals, 24 chickens (♂/♀) for each of the above mentioned 13 local breeds, were selected. Six traits, namely live body weight (LBW), body length (BL), shank length (SL), shank width (SW), breast width (BW), and wingspan (WS) were collected for each animal. Means and standard deviations of the traits were calculated using R software (R Development Core Team, 2017).

2.2 Samples and genotyping

A total of 312 samples (24 per breed) belonging to the 13 local chicken breeds were sampled. Blood samples were collected from brachial veins by standard venipuncture with Vacutainers® tubes containing EDTA as an anticoagulant. Sample collection was conducted as part of routine health screen by qualified veterinarians following guidelines established by Institutional Animal Care and Use Committee (IACUC, USA). 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. The Gallus_gallus-5.0 chicken assembly was used in this study as reference genome (Warren et al., 2017). Only markers positioned on chromosome from 1 to 28 were used. Moreover, the following filtering parameters were adopted to exclude certain loci and animals and to prune the

dataset. In particular, SNPs with a call rate <95%, minor allele frequency <5%, and animals with more than 10% of missing genotypes were removed. The editing was carried out using PLINK 1.9 (Chang et al., 2015). After filtering, the number of SNPs in the dataset was 474,412.

2.3 Genetic diversity indices

PLINK 1.9 software (Chang et al., 2015) was used to estimate observed heterozygosity (Ho) and expected heterozygosity (He), the genomic inbreeding, which is based on the difference between the observed and expected number of homozygous genotypes (F_{HOM}), and average minor allele frequency (≥ 0.05).

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 two components identified with the *mds-plot* command of PLINK 1.9.

Phylogenetic relationships among breeds were analyzed through Reynolds genetic distances by using the package *ape* of R software (Paradis et al., 2004). Neighbor networks were constructed from the estimated genetic distances using *FigTree* software (Huson et al., 2006). Graphical representation was visualized using R software (R Development Core Team, 2017).

3 Results and discussion

3.1 Morphometric comparison

For morphometric data, 24 adult animals per breed divided between males and females were measured. Sexual dimorphism within each breed was evident (Table 1). Means of BL and BW showed low variability among breeds, with the lowest values for PPP (BL = 32.29 ± 1.57 cm and BW = 28.92 ± 1.93 cm), and maximum for PRL for BL (46.79 ± 1.66 cm) and PRM for BW (42.33 ± 3.60 cm). On the contrary, WS and especially LBW showed great variability among breeds. Indeed, the highest LBW was obtained for PRM (♂ 4,221.7 ± 450.6 g; ♀ 2,831.7 ± 253.2 g) and the lowest for MOD males (♂ 1,695.0 ± 128.1 g) and PPP females (♀ 1,293.3 ± 219.2 g). Consistent with LBW data, WS exhibited the highest values for PRM males and the lowest for ROM females. Phenotypic data for shank traits showed similarities to previous data. Indeed, PRM and PRL males showed maximum values both for SL and SW, while the minimum was observed for MOD and ROM females, respectively. Data distinctly demonstrated how three breeds form Northern region (PRM, PRL, PER) are bigger for all morphometric traits. These results could be explained by the history of these 3 breeds: all of them are partially derived from old commercial lines designated for meat production (De Marchi et al., 2005b). Breeds from central regions are smaller than breeds from Northern region (except for dwarf breed PPP) for several traits. Among them, ANC showed higher values than MOD and ROM, and similar values to Padovana and Polverara breeds. Data from the 2 latter breeds are consistent with data from other similar autochthonous breeds as Livorno Bianca and Nera (Franzoni et al., 2018).

3.2 Genetic diversity within breeds

Improving the knowledge about the genetic structure of local livestock populations is fundamental to enhance the efficient use of local breeds and implement conservation programs. The use of high-throughput genotyping arrays has deeply facilitated the study of genetic structure of local breeds, which are generally understudied. The present study characterized and compared several Italian local poultry breeds from different locations through high-density genome-wide SNPs. This great collection of genetic variability, together with the high-resolution characterization of genomes allow to improve the knowledge about the local breeds. For these reasons, the patterns of genetic differentiation, diversity and population structure were investigated

Minor allele frequency pointed out a small divergence among breeds, with a minimum in PPC (0.238 ± 0.303) and a maximum in PER (0.309 ± 0.321; Table 2). Values of minor allele frequency remarked good conservation of the local breeds as a good genetic purity and low variation of the loci (Zanetti et al., 2010, Strillacci et al., 2017), and they were consistent with those reported by Bortoluzzi et al. (2018) for Dutch chickens.

The Ho and He differed from those of previous studies for breeds of Northern Italy, as well as for ANC, MOD and ROM breeds (Zanetti et al., 2010; Bianchi et al., 2011). In this study, the lowest value was observed for PPA (Ho: 0.151 ± 0.198; He: 0.146 ± 0.185) and the highest for PML (Ho: 0.293 ± 0.199; He: 0.291 ± 0.178). Only these two breeds, together with ROM, showed Ho values higher than He.

Observed results indicate severe effects of small population size, which agrees with DAD-IS database about risk status of these 3 breeds. However, based on He and Ho, all breeds seem to have good genetic variability. In SYNBREED project database, the wild and less selected African, South American and some local Asian and European breeds had average Ho greater than 0.225 (Malomane et al., 2019). The average Ho reported in the present study was 0.211 (data not shown), due to the low values of PPA, PPC, and PPP, reported as fancy breeds. Indeed, the selection practices to meet the European breed standards may also have had a huge impact on the reduction of genetic diversity within the fancy breeds. These standards are very strict, and breeders aim at an almost "perfect" phenotype through the mating of very close relatives. Moreover, PRL and PRM showed low Ho values, probably due to their genetic background linked to a crossing with commercial line, as already described. To underline what has just been reported, PPA, PPC, PPP, PRL and PRM exhibited the highest F_{HOM}. Finally, the F_{HOM} was lower in chickens of Central Italy and PML breed (0.202 ± 0.08).

Table 1 Mean and standard deviation (SD) of morphometic traits for each chicken breed and sex (F = female; M = male)

Breed	Sex	Live body weight (g)		Body length (cm)		Shank length (cm)		Shank width (cm)		Breast width (cm)		Wingspan (cm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ancona	F	1,970.0	204.1	37.86	1.75	6.91	0.90	3.97	0.35	34.78	3.88	39.76	3.43
	M	2,580.8	222.1	42.07	1.35	8.40	1.30	5.10	0.23	38.50	2.35	44.83	2.79
Modenese	F	1,630.0	249.7	33.39	2.10	6.35	0.48	3.82	0.29	32.79	2.15	36.54	3.01
	M	1,695.0	128.1	37.05	2.03	7.38	0.63	4.37	0.47	34.40	2.99	38.70	1.75
Ermellinata di Rovigo	F	2,322.5	152.5	40.08	1.94	9.13	0.61	4.79	0.26	34.88	1.65	45.96	1.29
	M	3,436.7	216.2	45.75	1.36	11.00	0.37	5.96	0.14	39.08	1.38	53.54	0.66
Millefiori di Lonigo	F	1,990.0	125.7	37.42	1.26	8.63	0.31	4.13	0.23	32.00	1.13	43.88	1.58
	M	2,820.0	322.4	43.42	1.46	10.71	0.54	5.29	0.33	36.50	2.15	50.46	1.51
Padovana Argenta	F	2,126.6	178.8	36.95	1.47	7.95	0.36	3.95	0.22	31.95	1.39	40.80	0.89
	M	2,600.0	150.5	40.50	1.00	9.75	0.50	5.00	0.00	34.50	1.00	49.50	0.58
Polverara Bianca	F	1,675.0	261.3	34.79	1.47	8.29	0.72	3.96	0.26	29.67	2.33	34.71	9.92
	M	2,253.3	306.0	39.46	2.54	10.00	0.37	4.88	0.23	33.63	1.71	44.42	2.61
Padovana Camosciata	F	2,105.8	181.2	36.46	2.12	8.67	0.44	4.00	0.00	32.38	2.13	40.75	4.10
	M	2,715.0	176.9	41.29	1.84	10.25	0.50	4.83	0.33	35.08	2.57	47.83	2.25
Padovana Dorata	F	1,893.3	192.5	36.08	1.66	8.54	0.66	3.68	0.35	31.17	2.31	42.17	3.22
	M	2,790.8	226.1	42.92	1.66	10.29	0.33	4.88	0.31	35.00	2.41	50.92	2.18
Polverara Nera	F	1,530.3	474.0	36.46	1.45	8.50	0.37	3.96	0.14	31.13	1.60	41.17	2.31
	M	2,187.5	209.7	40.67	1.66	10.08	0.47	4.83	0.25	34.50	1.62	46.46	1.94
Pepoi	F	1,293.3	219.2	32.29	1.57	7.54	0.40	3.75	0.34	28.92	1.93	37.21	1.75
	M	1,860.0	182.1	36.92	1.33	9.38	0.53	4.63	0.38	33.42	3.18	43.04	1.78
Robusta Lionata	F	2,753.3	378.0	41.46	2.44	9.29	0.69	4.75	0.26	36.92	2.91	46.71	1.74
	M	3,702.5	1,024.6	46.79	1.66	11.38	1.07	6.17	0.25	41.29	3.94	53.42	1.61
Robusta Maculata	F	2,831.7	253.2	38.79	2.23	9.54	0.75	4.75	0.26	37.29	3.51	46.21	1.80
	M	4,221.7	450.6	45.63	2.14	11.50	0.80	6.00	0.30	42.33	3.60	54.08	1.40
Romagnola	F	1,432.5	211.4	35.32	1.64	7.67	0.67	3.61	0.34	33.02	2.18	32.91	1.96
	M	1,932.5	137.7	42.45	1.15	9.36	0.22	4.23	0.24	37.81	0.37	39.20	0.32

3.3 Genetic distance among breeds

Figure 1 depicts the population stratification of all sampled chickens. Multidimensional scaling plots pointed out different clusters: the first in the left bottom part of the plot grouped PER and the two Robusta breeds; this explains the strong correlation between these breeds, which shared the highest values for morphometric traits. In the top right quarter PPP and PML were unexpectedly grouped, and the 3 breeds from Central Italy overlapped together. The overlapping could be due to geographic relationship among ANC, MOD, and ROM. The right bottom quarter showed 2 overlapped clusters related to each other: PPN and PPB on one hand, and all Padovana varieties (Argentata, Camosciata, and Dorata) on the other hand.

Table 2 Number of animals (*n*) and descriptive statistics (mean and standard deviation, SD) of minor allele frequency (MAF), expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (F_{HOM}) per breed

Breed	*n*	MAF		He		Ho		F_{HOM}	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ancona	24	0.267	0.242	0.274	0.187	0.263	0.181	0.284	0.100
Modenese	24	0.273	0.252	0.270	0.181	0.260	0.197	0.296	0.083
Ermellinata di Rovigo	23	0.309	0.321	0.220	0.198	0.199	0.192	0.459	0.044
Millefiori di Lonigo	23	0.281	0.238	0.291	0.178	0.293	0.199	0.202	0.080
Padovana Argentata	24	0.241	0.331	0.146	0.185	0.151	0.198	0.588	0.098
Polverara Bianca	24	0.260	0.261	0.248	0.187	0.216	0.179	0.411	0.052
Padovana Camosciata	24	0.238	0.303	0.179	0.193	0.169	0.191	0.538	0.095
Padovana Dorata	24	0.247	0.264	0.232	0.187	0.219	0.194	0.404	0.081
Polverara Nera	24	0.257	0.290	0.213	0.194	0.201	0.193	0.454	0.062
Pepoi	24	0.277	0.341	0.168	0.196	0.154	0.191	0.579	0.039
Robusta Lionata	23	0.305	0.345	0.185	0.195	0.181	0.199	0.508	0.039
Robusta Maculata	24	0.304	0.358	0.167	0.193	0.157	0.190	0.572	0.032
Romagnola	24	0.271	0.241	0.278	0.182	0.281	0.197	0.235	0.091

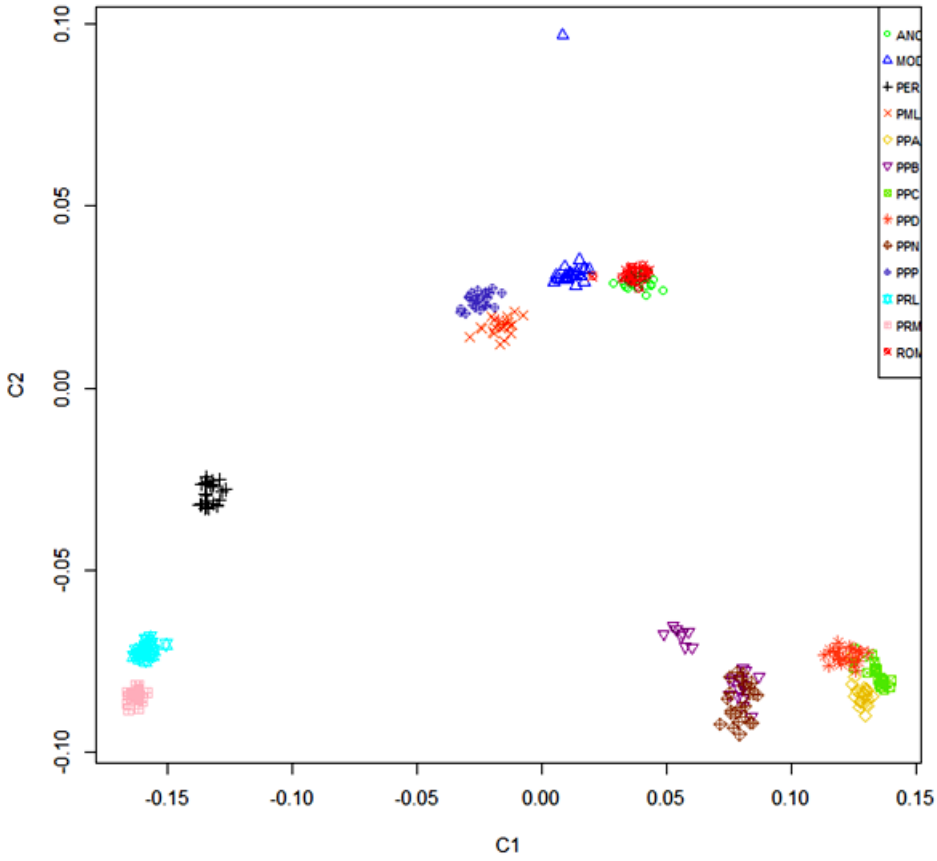

Figure 1 Multidimensional scaling plot of genetic populations distance by item. Ancona [ANC], Ermellinata di Rovigo [PER], Millefiori di Lonigo [PML], Modenese [MOD], Padovana Argentata [PPA], Padovana Camosciata [PPC], Padovana Dorata [PPD], Pepoi [PPP], Polverara Bianca [PPB], Polverara Nera [PPN], Robusta Lionata [PRL], Robusta Maculata [PRM], Romagnola [ROM]

To provide additional information in support of previous results, the relationships among the chicken breeds were deepened through a neighbor-net graph based on Reynolds genetic distances (Figure 2). The phylogenetic tree showed the differences obtained through multidimensional scaling and reinforced what has already been observed. Indeed, it is easy to find the same clusters of multidimensional scaling plot: the greatest size chickens, the 3 breeds from Central Italy and the 2 subgroups formed by Polverara and Padovana breeds. Figure 2 clarifies the great genetic distance that exists between PML and PPP. The orientation of the clusters could match with what has been found for morphometric data. Indeed, every breed in the same neighbor tree's group shares similar morphological conformation and features.

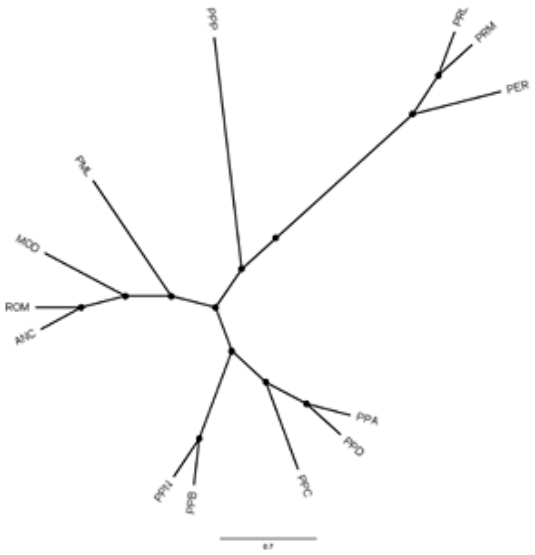

Figure 2 Neighbor-network of Reynolds' distances

4 Conclusions

The results showed the existence of genetic variability and low inbreeding in almost all breeds. Population structure and genetic distances showed a clear separation among the breeds with some particular clusters related to the region of origin. This study helps evaluate the genetic background of some Italian local chicken breeds, in order to develop new conservation programs, and to design novel selection schemes. Finally, this work also allows us to carry out an available genetic traceability method according to the excellent ability to distinguish between the various breeds/populations.

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