Genetic characterization of local Italian breeds of chickens undergoing in situ conservation

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ABSTRACT The objectives of this study were to determine genetic variation and to analyze population structure of 6 Italian local chicken breeds involved in a conservation program. Twenty microsatellite markers were investigated in 337 birds belonging to 6 breeds: Ermellinata di Rovigo, Robusta Maculata, Robusta Lionata, Pépoi, Padovana, and Polverara; a commercial layer cross was used as reference. One hundred twelve alleles were detected in the overall population, with a mean number of 5.6 ± 2.1 alleles per locus. For the local breeds, the observed and expected heterozygosity ranged from a minimum of 0.240 to a maximum of 0.413 and from 0.243 to 0.463 for the Pépoi and Polverara breeds, respectively. Deviation from Hardy-Weinberg equilibrium was observed in 5 breeds and in the commercial cross. The overall population heterozygote deficiency was 0.427, the average inbreeding coefficient was 0.097, and the heterozygote deficiency due to breed subdivisions was 0.437. Reynolds' distances were used to draw an unrooted neighbor-joining tree, which topology gave information on the genetic origin of these breeds and confirmed their known history. The estimated molecular kinship within a breed ranged from 0.559 to 0.769, evidencing high coancestry. Structure analysis was performed to detect the presence of population substructures. Inferred clusters corresponded to the different breeds, without presence of admixture. The exception was the Polverara breed, for which a more complex genetic structure was found. The results supported the decision of safeguarding these breeds as an important reservoir of genetic diversity and confirmed the usefulness of microsatellite markers to characterize and to monitor genetic variability in local chicken breeds.

Key words: chicken breed, genetic diversity, microsatellite, population structure

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INTRODUCTION

In recent years, animal biodiversity management has become an important issue in the international scientific community because of changes in large-scale production systems (FAO, 2007). In North America, Europe, and China, about 50% of documented breeds are classified as extinct, critical, or endangered (Hammond, 1996) and local breeds have often been diluted by indiscriminate cross-breeding with imported stocks (FAO, 2007). The reduction in local poultry breeds due to replacement with cosmopolitan ones suggests a need for conservation of local genetic resources.

In the absence of comprehensive breed characterization data and documentation of the origin of breeding populations, molecular marker information may provide reliable estimates of genetic diversity within and between a given set of populations. It is useful to explore genetic diversity within and between breeds or populations to analyze genetic relationships and admixtures and to provide information on evolutionary relationships and parentage within populations. Moreover, for breeds undergoing conservation, molecular data should be integrated with other information (i.e., adaptative, productive, and reproductive performances; extinction probabilities) to guide decision makers.

In Italy, the interest in conservation of local poultry breeds was concretized in 2000 by the regional government with the "Conservazione e Valorizzazione delle Razze Avicole Venete" conservation program (De Marchi et al., 2005a). "Conservazione e Valorizzazione delle Razze Avicole Venete" is an in situ conservation program involving 12 breeds belonging to 4 poultry species (chicken, duck, helmeted guinea fowl, and turkey) maintained as distinct flocks distributed in the Veneto region of Italy. Molecular marker information was used to monitor genetic diversity of populations (Targhetta et al., 2005; De Marchi et al., 2006) and to valorize genetic resources using genetic traceability systems (Dalvit et al., 2007). Among molecular markers, microsatellites were preferred because they are well dis-

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persed in the genome and highly polymorphic (Cheng et al., 1995). They have been used in many countries to study the genetic relationships among local breeds (Takahashi et al., 1998; Hillel et al., 2003; Baumung et al., 2004; Muchadeyi et al., 2007; Dalvit et al., 2009), and their use allows meta-analysis and comparisons among independent research units.

The aim of this study was to analyze genetic diversity, genetic relationships, population structure, and molecular coancestry in the Italian local chicken breeds undergoing in situ conservation using microsatellite markers.

MATERIALS AND METHODS

Conservation Program

"Conservazione e Valorizzazione delle Razze Avicole Venete" is an in situ marker-assisted conservation program, initiated in 2000, that involves 3 organic flocks located in the plains, hills, and mountains of the Veneto region in the north of Italy (De Marchi et al., 2005a). Initially, the program involved 5 local chicken breeds: Ermellinata di Rovigo (**ER**), Pèpoi (**PP**), Robusta Lionata (**RL**), Robusta Maculata (**RM**), and Padovana (**PD**) with 2 different strains (they differed only in plumage color): Dorata (**PDd**) and Camosciata (**PDc**). In 2006, the Polverara breed (**PV**) with 2 different strains (they differed only in plumage color), Nera (**PVn**) and Bianca, was also included. The origin of these local breeds is documented in literature (De Marchi et al., 2005a, 2006) with the exception of PV that, until 1899, was confused with PD. As reported by De Marchi et al. (2005b), just in 1900, the PV and PD breeds were described separately, nevertheless in the last 30 yr, the PV was crossed with other breeds and so its features are not fully fixed.

Bird Sampling

A total of 337 birds were analyzed: ER (n = 45; 13 females and 32 males), PP (n = 45; 16 females and 29 males), RL (n = 43; 15 females and 28 males), RM (n = 45; 15 females and 30 males), PVn (n = 52; 20 females and 32 males), Polverara Bianca (n = 36; 14 females and 22 males), PDd (n = 24; 10 females and 14 males), PDc (n = 26; 9 females and 17 males), and a commercial brown layer cross (**BL**, n = 21, all females; Hubbard Golden Comet) was used as the reference population. Individuals from the local breeds were randomly selected from all conservation flocks. The population sizes of the local breeds are estimated as about 1,500 for ER, PP, RL, RM, PV, and about 2,000 for PD.

Whole blood samples were taken from the wing vein onto a sterile collecting vacuum tube (Vacutainer, BD, Milan, Italy) containing sodium citrate and citric acid and stored at 4°C. Genomic DNA was isolated from blood using a modified DNA purification kit (Gentra System Puregene DNA, Gentra System, Minneapolis, MN; Dalvit et al., 2008) and stored at −20°C until subsequent use as a template for PCR reaction.

Amplification and Genotyping of Microsatellite Markers

A set of 20 microsatellite markers, included in the list of recommended microsatellites for chicken analysis by the ISAG/FAO Standing Committee (MoDAD project, FAO Standing Committee, 2004), were used to amplify microsatellite regions in the genome (Table 1). The PCR primer pairs were synthesized and 5′ ends of the forward primers were fluorescently labeled with cy5 or cy5.5 dyes. The 20 microsatellites were individually amplified by a PX2 Thermo Hybaid thermal cycler (Thermo Hybaid, Ashford, UK) at the following conditions, the X temperature being the annealing temperature of each primer (National Center for Biotechnology Information): initial denaturation step of 10 min at 94°C, 35 cycles of 45 s at 94°C, 1 min at X°C and 1.5 min at 72°C, and a final extension of 10 min at 72°C. A reaction volume of 15 μL contained 25 ng of genomic DNA, 1.5 m*M* $MgCl_2$, 1.5 μL of Taq Buffer 1× (Sigma-Aldrich, Milan, Italy), 0.04 U of Taq Gold (Sigma, St. Louis, MO), 3 m*M* deoxynucleoside triphosphate, and 10 μ*M* of each primer. Amplified fragments were pooled in 4 multiplex and analysis was performed using an automated DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter, Brea, CA). Electropherogram processing was carried out using the CEQ 8000 software (Beckman Coulter). Alleles were scored according to PCR product size.

Statistical Analysis

Total number of alleles, average number of alleles per locus across breeds, allelic frequencies and expected ($\mathbf{H_E}$) and nonbiased observed heterozygosity ($\mathbf{H_O}$; i.e., observed heterozygosity corrected for bias due to sampling according to Nei, 1978) were estimated using Genetix software (Belkhir, 1996–2002). Exact tests for deviation from Hardy-Weinberg equilibrium (Guo and Thompson, 1992) were applied using the Markov chain Monte Carlo simulation (100 batches, 5,000 iterations per batch, and a dememorization number of 10,000) as implemented in Genepop version 3.4 (Raymond and Rousset, 1995). Polymorphism information content described by Botstein et al. (1980) is a general measure of how informative a marker is and was calculated using the Molkin software (Gutièrrez and Goyache, 2004). Wright's fixation indices (F_{IS}, F_{ST}, and F_{IT}), estimated according to Weir and Cockerham (1984), were calculated for the whole population using the FSTAT 2.9.3 software (Goudet, 1995) to quantify within- and between-breed partitioning variances. The F_{ST} distances among breeds were computed using MolKin (v. 3.0). Reynolds' distances ($\mathbf{D_R}$; Reynolds et al., 1983) were

Table 1. Microsatellite markers with corresponding fragment size, chromosomal location, average number of alleles, and polymorphism information content (PIC)

Locus	Fragment size (bp)	Chromosome	Number of alleles	PIC
ADL0268	104 to 119	1	6	0.702
ADL0278	102 to 121	8	6	0.648
LEI0094	251 to 283	4	7	0.604
LEI0166	251 to 261	3	3	0.592
MCW0014	166 to 189	6	6	0.415
MCW0020	183 to 189	1	4	0.701
MCW0037	151 to 159	3	5	0.554
MCW0078	134 to 150	5	7	0.534
MCW0081	143 to 155	5	7	0.620
MCW0098	255 to 257	4	2	0.233
MCW0103	268 to 272	3	2	0.320
MCW0104	190 to 228	13	10	0.546
MCW0111	98 to 106	1	4	0.607
MCW0123	112 to 134	14	7	0.584
MCW016	136 to 154	3	8	0.589
MCW0165	112 to 123	23	4	0.587
MCW0216	141 to 147	13	4	0.615
MCW0222	217 to 225	3	5	0.531
MCW0248	213 to 245	1	8	0.350
MCW0295	86 to 102	4	7	0.597
Mean			5.6	0.546

estimated using the PHYLIP 3.66 software package (Felsenstein, 2005). A consensus tree was reconstructed and tree robustness was evaluated by bootstrapping over loci (1,000 replicates). Neighbor-joining trees were plotted from D_R distances using TreeView (v. 1.6.6; Page, 2001).

Molecular coancestry coefficients within a breed and kinship distances between breeds were measured according to Caballero and Toro (2002) using MolKin 3.0 (Gutiérrez et al., 2005); to avoid bias, because of unequal sample sizes, 100 samples of 50 individuals per breed were generated with a bootstrap procedure. To set conservation priorities, MolKin 3.0 (Gutiérrez et al., 2005) was used to quantify the contribution of each analyzed population to the diversity of the whole data set using the method proposed by Caballero and Toro (2002). Because BL is not a Veneto local breed involved in the conservation scheme but has been used as reference population, its data were not included in the approach for setting conservation priorities. Kinship distance between breeds was simply computed averaging the corresponding values for all within- or between-breed pairs of individuals.

To study population structure and to detect the most likely number of clusters (**K**) in the data set, the software Structure version 2.2 (Pritchard et al., 2000) was used. The analysis involved an admixture model with correlated allelic frequencies (Pritchard et al., 2000; Dalvit et al., 2009; Granevitze et al., 2009). The application of the method included detection of the presence of population structure, identification of distinct genetic populations (K), assignment of individuals to populations, and identification of migrants and admixed individuals. To choose the appropriate number of inferred clusters to model the data, 2 to 14 inferred clusters were performed with 50 independent runs each. All analyses used a burn-in period of 50,000 iterations and then 300,000 iterations for data collection. The best number of clusters fitting the data was established by plotting the mean Ln Pr(X|K) over the 50 independent runs for each K, as suggested by Pritchard et al. (2000). The SIMCOEF procedure of the statistical package R (v. 2.6.0) was used to make a comparison of the 50 solutions, defining identical solutions with at least 95% similarity and considering the most frequent solutions as the most probable. The output obtained was used directly as input by the cluster visualization program Distruct (Rosenberg, 2004).

RESULTS AND DISCUSSION

Genetic Variability at Microsatellite Loci

Information about the variability of the investigated loci is shown in Table 1. All loci studied were polymorphic with 112 alleles detected and a mean of 5.6 ± 2.1 alleles per locus (Table 1). Polymorphism information content per marker ranged from 0.233 to 0.702, with an average of 0.54. According to Botstein et al. (1980), polymorphism information content at all loci analyzed were reasonably informative, with the exception of MCW0098. Within breeds, several loci were monomorphic: 4 for PP (MCW0295, MCW0123, MCW0222, and MCW0098), 4 for RL (MCW0078, MCW0014, ADL0278, and MCW0020), 4 for RM (MCW0104, MCW0037, MCW0098, and ADL0268), and 1 for PD (MCW0081) (data not show). This situation could be due to a rather high inbreeding or to the choice of the markers. The microsatellite investigated in the present study are included in the list of recommended microsatellites for chicken analysis by the ISAG/FAO Standing Committee (MoDAD project, FAO Standing Commit-

Table 2. Private alleles in base pairs (frequencies in parentheses) for brown layer (BL), Ermellinata di Rovigo (ER), Pépoi (PP), Robusta Lionata (RL), Polverara (PV), and Padovana (PD)

Locus	BL	ER	PP	RL	PV	PD
ADL268						119 (0.07)
ADL278				108 (0.01)[1]	102 (0.01)	
LEI94	279 (0.05)		259 (0.01)			271 (0.08)
MCW104	202 (0.05)		204 (0.02)		218 (0.1)[1]	
	210 (0.13)[1]		216 (0.04)			
	228 (0.05)					
MCW123		126 (0.01)			119 (0.18)[1]	
MCW14	168 (0.05)	176 (0.13)[1]			189 (0.01)	
	170 (0.03)					
MCW16					136 (0.02)	
MCW16	148 (0.41)[1]					
	152 (0.05)					
	154 (0.05)					
MCW165			123 (0.01)			
MCW222	217 (0.02)					
MCW248	213 (0.06)				230 (0.01)	
	227 (0.06)				245 (0.01)	
MCW295					102 (0.01)	
MCW37	151 (0.12)[1]					
MCW78	144 (0.05)		146 (0.02)			150 (0.01)
MCW81			147 (0.29)[1]			

[1]Alleles with frequency higher than 0.10.

tee, 2004) and should display at least 4 alleles per locus; however, this could be difficult to ensure for previously unanalyzed breeds.

Private alleles for the breeds analyzed are shown in Table 2. Thirty-four private alleles (30.3%) were found; 8 of these showed a frequency greater than 10%: 3 for BL, 2 for PV, and 1 for ER, PP, and RL, respectively. Taking into account that these local breeds came from a relatively close geographic area, the presence of private alleles was rather high considering that Tadano et al. (2007) detected just 15% of private alleles in their study of 12 chicken lines bred based on 5 breeds. Such differentiation can be explained considering the different origin and management practices that did not allow crossbreeding in the local Veneto breeds.

Breed Variability and Differentiation

The genetic variability of each breed was studied in terms of average number of alleles, H_E and H_O, and molecular coancestry as shown in Table 3. The average number of alleles per breed ranged from 2.17 (RM) to 3.80 (BL). These results were comparable to those reported by Tadano et al. (2007) and Bodzsar et al. (2009) for the Hungarian chicken breeds and lower than those reported by Muchadeyi et al. (2007) for several chicken ecotypes in Zimbabwe.

Values of H_O and H_E for the local breeds ranged from 0.240 (PP) to 0.413 (PVn) and from 0.243 (PP) to 0.463 (PVn), respectively, whereas values for BL were 0.622 and 0.559, respectively. The high number of monomorphic loci detected may explain the low number of heterozygotes. Values of H_O and H_E were similar to those reported for other European chicken breeds (Hillel et al., 2003; Granevitze et al., 2007). Concerning the PD breed studied by Hillel et al. (2003) and Granevitze et al. (2007), the H_O values of 0.170 and 0.360, respectively, were consistent with our 0.287 and 0.329 for PDc and PDd, respectively.

The low genetic diversity of European chicken breeds as reported by Mignon-Grasteau et al. (2005) may be a consequence of the loss of variability observed in all

Table 3. Number of analyzed samples, total number of alleles (TNA), expected (H_E) and observed (H_O) heterozygosity, and within-breed molecular coancestry (FIJ) for each breed analyzed

Breed[1]	Sample size	TNA	$H_E \pm$ SD	$H_O \pm$ SD	*P*-value	FIJ
BL	21	3.8	0.559 ± 0.141	0.622 ± 0.233	***	0.439
ER	45	3.1	0.420 ± 0.175	0.384 ± 0.248	***	0.573
PP	45	2.5	0.243 ± 0.239	0.240 ± 0.236	*	0.769
RL	43	2.4	0.367 ± 0.229	0.317 ± 0.264	***	0.657
RM	45	2.2	0.293 ± 0.225	0.292 ± 0.226	NS	0.721
PVb	36	3.0	0.436 ± 0.190	0.366 ± 0.201	***	0.577
PVn	52	3.5	0.463 ± 0.177	0.413 ± 0.170	***	0.559
PDc	26	2.3	0.305 ± 0.257	0.287 ± 0.271	NS	0.704
PDd	24	2.7	0.340 ± 0.199	0.329 ± 0.230	NS	0.689

[1]BL = brown layer; ER = Ermellinata di Rovigo; PP = Pépoi; RL = Robusta Lionata; RM = Robusta Maculata; PVn = Polverara Nera; PVb = Polverara Bianca; PDc = Padovana Camosciata; PDd = Padovana Dorata.

*$P < 0.05$; ***$P < 0.001$.

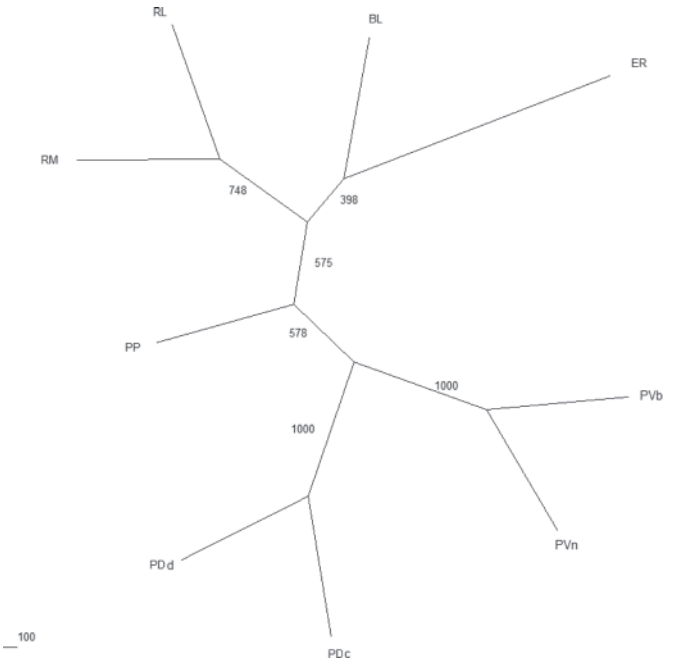

Figure 1. Representation of neighbor-joining Reynolds' genetic distance among brown layer cross (BL), Ermellinata di Rovigo (ER), Pépoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), Polverara Bianca (PVb), Polverara Nera (PVn), Padovana Camosciata (PDc), and Padovana Dorata (PDd), based on 1,000 replicates (numbers in nodes are percentage bootstrap values).

animal species outside their center of domestication. Moreover, for the Veneto local breeds, a founder effect, when the breeds were involved in the conservation scheme, could also be responsible for a loss of genetic variation.

The breeds showed considerable genetic differentiation; in fact, F_{IT} was equal to 0.427 (99% CI 0.427 to 0.533), the average F_{IS} value was 0.097 (99% CI 0.045 to 0.165), and F_{ST} was 0.437 (99% CI 0.371 to 0.498). Results underlined a high degree of breed differentiation according to values reported by Tadano et al. (2008) for native Japanese poultry breeds and in contrast with lower genetic differentiation values found in 8 Finnish chicken breeds by Vanhala et al. (1998).

Another way to measure within-breed diversity is the estimation of molecular coancestry, a measure of relatedness among individuals. Molecular coancestry estimates varied from 0.559 (PVn) to 0.769 (PP), as shown in Table 3. Although information about molecular coancestry in chicken breeds is lacking, those obtained by us seem high when compared with the Iberian pig (Fabuel et al., 2004) and Spanish and Italian horse breeds (Marletta et al., 2006).

The F_{ST} and kinship distances for the analyzed breeds are shown in Table 4. The F_{ST} values ranged from 0.035 (BL-PV) to 0.142 (RM-PP) and a close relationship among the tufted breeds (PD and PV) was highlighted. The kinship distances ranged from 0.262 (RL-RM) to 0.359 (PV-ER); the close relationships were expected and consistent with the origin of these breeds (De Marchi et al., 2005a,b).

The neighbor-joining tree constructed on D_R estimates is show in Figure 1. The common origin of RL and RM and PD and PV, already highlighted by kinship distances, seemed to be confirmed by tree topology; moreover, a clear distinction between PP and other chicken breeds was evidenced.

Results obtained with the Caballero and Toro (2002) approach to set up conservation priorities are illustrated in Table 5. The removal of one breed from the data set resulted in loss or gain of the total genetic diversity in the population which ranged from −4.23% to +1.34% when ER and PD were removed, respectively. The highest gain of between-breed diversity was found removing the PV breed (+3.48%); on the other hand, its removal resulted in a loss of the within-breed diversity (−6.78%). On the contrary, removal of PP gave a high contribution to the internal diversity (+3.41%) and a loss of between-breed diversity (−2.85%), resulting in a global modest gain of total genetic diversity (+0.56%). The high contribution to internal diversity due to PP extinction depended on its high inbreeding as evidenced by high molecular coancestry coefficients within a breed. Ignoring within-breed variability will favor inbred populations and populations with extreme allele frequencies (Glowatzki-Mullis et al., 2008). As previously mentioned, PV and PD are closely related breeds. This was confirmed by genetic distances, morphology, and known historic origin. The exclusion of 1 of these 2 breeds seemed to compromise poorly the total genetic diversity, but when both breeds were removed, there was a loss of genetic diversity in the whole population (−6.90%). This loss was mostly due to the among-breed diversity (−25.19%), and the extinction of PV and PD would result in a loss of the only 2 tufted breeds involved in the conservation program.

Table 4. Kinship distances (below diagonal) and F_{ST} distances (above diagonal) among brown layer (BL), Ermellinata di Rovigo (ER), Pépoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), Polverara (PV), and Padovana (PD)

Breed	BL	ER	PP	RL	RM	PV	PD
BL		0.067	0.098	0.073	0.075	0.035	0.070
ER	0.325		0.129	0.102	0.116	0.087	0.115
PP	0.330	0.340		0.125	0.142	0.087	0.111
RL	0.319	0.332	0.318		0.084	0.070	0.112
RM	0.298	0.327	0.318	0.262		0.099	0.110
PV	0.312	0.359	0.313	0.316	0.343		0.059
PD	0.315	0.347	0.290	0.328	0.298	0.285	

Table 5. Loss or gain of genetic diversity (GD, in %) in the population when one breed is removed according to the Caballero and Toro (2002) approach

Breed[1]	GD	Within breed	Between breed	Loss (−)/gain (+)
All breeds	0.597			
ER	0.575	−2.059	−2.183	−4.242
PP	0.603	+3.411	−2.846	+0.565
RL	0.598	−0.292	−0.110	−0.402
RM	0.598	+1.621	−2.027	−0.406
PV	0.580	−6.776	+3.481	−3.295
PD	0.608	+0.470	+0.873	+1.343
PD + PV	0.499	+8.281	−25.194	−16.913

[1]ER = Ermellinata di Rovigo; PP = Pépoi; RL = Robusta Lionata; RM = Robusta Maculata; PV = Polverara; PD = Padovana.

Population Structure

The structure of the breeds was analyzed using a Bayesian approach that inferred the number of clusters (K) present in the population, permitting detection of differences among breeds and hidden structures within breeds. The Ln Pr(X|K) increased sharply from K = 2 to K = 8 and reached a plateau without showing a significant decrease from K = 9 to K = 14 (data not show). The highest Ln Pr(X|K) over the 50 independent runs performed for each value of K was found at K = 10 identifying the most probable number of clusters in the population. Results of Structure analyses are shown in Figure 2 for K ranging from 2 to 10 and the most probable solutions are reported per each K. It can be noticed that a clear distinction among the 7 breeds was possible only considering K = 8 in correspondence to 7 distinct clusters. The RL and RM breeds were divided in 2 different populations only considering K = 8, whereas the PVn and PVn breeds exhibited a more complex structure with proportion of membership split into 2 or more clusters. These situations agree with the origin of these breeds mentioned previously and in particular the complex situation of PV seems to be linked to its recent involvement in the "Conservazione e Valorizzazione delle Razze Avicole Venete" project. Results obtained reflected that inbreeding and no gene flow during the last decades among these breeds has contributed to a strict breed differentiation. With the only exception of the PV breed, no structures within a flock were visible using the genetic structure analysis method. Similar population structures (low level of admixture) were observed for the Hungarian chicken breeds (Bodzsar et al., 2009), whereas the opposite situation was evidenced for Zimbabwean chickens by Muchadeyi et al. (2007). For the Veneto chickens, the deficit of

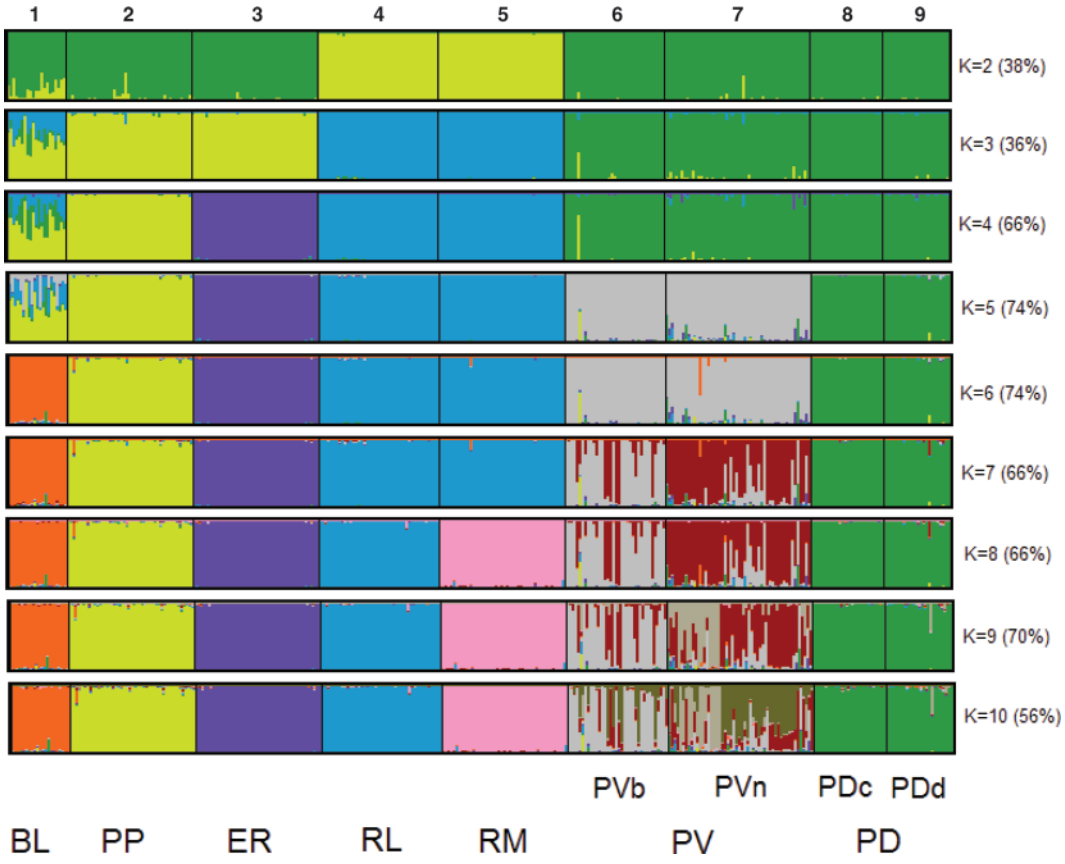

Figure 2. Graphical representation of the estimated membership fractions of individuals of the breeds analyzed in each of the K-inferred clusters, for K = 2 to K = 10. In parentheses, the percentage of identical solutions with 95% of similarity, only the most probable solutions for each most likely number of clusters (K) are shown. Brown layer cross (BL), Ermellinata di Rovigo (ER), Pépoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), Polverara (PV), Polverara Bianca (PVb), Polverara Nera (PVn), Padovana (PD), Padovana Camosciata (PDc), and Padovana Dorata (PDd).

heterozygotes seems to be caused mainly by inbreeding as evidenced by Structure analysis. This consideration is supported by the high molecular coancestry estimates within breeds.

Our results highlighted the high level of genetic diversity among the local chicken breeds. Regardless of the method used to analyze genetic differentiation (i.e., genetic distances, structure clustering), breeds were distinct, with no admixture, and homogeneous. The exception was PV, which presented complicated population substructures. The high level of genetic differentiation, clear distinction among breeds, and the low level of admixture are important factors that support the idea of conserving these breeds with unique genetic features. According to Ruane (1999), adaptive features, traits of scientific and economic interest, cultural-historical values, strong links to regional traditions, and ability to generate income from tourism justify conservation efforts and this is the case for Italian chicken genetic resources. For this reason, sampling for molecular analysis should be combined with surveying or monitoring of productive and phenotypic traits, or both, because molecular information alone cannot be used for conservation decisions. Finally, once decisions about conservation have been taken, molecular markers can be a useful tool to perform chicken characterization, to monitor conservation programs, and to design breeding programs.

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