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Variation of genetic diversity over time in local Italian chicken breeds undergoing conservation

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ABSTRACT

Aim of this study was to measure the variation of different genetic diversity measures in five Italian local chicken breeds over a four years period of conservation. A total of 413 individuals were chosen for the analysis among the animals born in 2002 (a) and in 2006 (b) and genotyped for 20 microsatellite markers. In the animals sampled after four years of conservation activities the loss of alleles occurred, but only for those which had lower frequencies, with a mean number of alleles equal to 7.75±3.08 in the year 2002 and 4.64±1.60 in the year 2006. During the four years of conservation from 2002 to 2006, all breeds showed a not significant decrease of observed heterozygosis, ranging from 2.8% for Robusta Maculata to a maximum of 8.4% for Padovana. Expected heterozygosis showed the same trend, decreasing of 1% for Robusta Lionata to a maximum of 15% for Robusta Maculata. A decrease in the inbreeding coefficient occurred from 2002 to 2006 for Pépoi, Ermellinata di Rovigo and Robusta Maculata breeds, probably due to the unleashing of within breed genetic structures attributable to the rotation of males among the conservation flocks. On the other hand, an increment of the same index was noticed for the Robusta Lionata and Padovana breeds, for which an increase of inbreeding is probably due to the annual selection of individuals for morphological and productive traits. Genetic structure analysis revealed that the best number of populations fitting the dataset was five. Individuals were so assigned to one of five groups, approximately corresponding to the breed of origin. This result shows how no evident genetic structures were detectable within breed, both for 2002 and 2006 individuals. However, an increase in the proportion of membership for each breed has occurred in the year 2006 compared to the data obtained in the year 2002. Results obtained are very useful for planning new strategies for the conservation scheme, including the choice of the animals, a more efficient organization of matings and for the creation of a new selection index based on the maintenance of the existing genetic variation.
(Keywords: conservation, local chicken breeds, genetic diversity, microsatellites)

INTRODUCTION

Animal biodiversity conservation and management has become an important issue for the international scientific community because of changes in large-scale production systems (*FAO*, 2007). Therefore, to get information and provide reliable estimation of the genetic diversity within and between a given set of populations the use of molecular markers gained importance, especially in absence of comprehensive breed characterization data and documentation about the origin of breeding populations

(*Jordana et al.*, 2001; *Dalvit et al.*, 2008; *Glowatzki- Mullis et al.*, 2008). Studies about genetic diversity within and between breeds or populations are hence useful to unfold genetic relationships and admixtures and to provide data on evolutionary relationships and parentage within populations (*Hillel et al.*, 2003; *Baumung et al.*, 2004). In the poultry sector a reduction in the number of the local breeds occurred, mainly due to the replacement with cosmopolitan poultry breeds and with highly productive crosses, suggesting an urgent need for conservation of these endangered genetic resources.

In the Veneto region of Italy, ten years ago, local poultry breeds have been put through a governmental *in-situ* marker assisted conservation scheme called "Conservazione e Valorizzazione delle Razze Avicole Venete" (*De Marchi et al.*, 2006). This conservation scheme is based on breed maintenance and multiplication within their production system. Five local chicken breeds were involved, reared in three conservation flocks. The main objectives were to enable the preservation of animal biodiversity and to support the development of marginal areas of the region through the revaluation of these local breeds and their products (*De Marchi et al.*, 2005b). The animals belonging to the conservation programme were investigated and genotyped by the analysis of molecular markers at microsatellite loci. Among all molecular markers, microsatellites were preferred because they are well dispersed 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 (*Baumung et al.*, 2004; *Muchadeyi et al.*, 2007; *Dalvit et al.*, 2009), and they are suitable for monitoring genetic variability and inbreeding of local chicken breeds involved in the project, so to organize and monitor the conservation activities (*Marletta et al.*, 2006; *Dalvit et al.*, 2008).

Aim of this study was to measure the variation of different genetic diversity measures in five Italian local chicken breeds over a four years period of conservation.

MATERIALS AND METHODS

The 413 blood samples used for the analysis were chosen among the animals born in 2002 (a) and the ones born in 2006 (b). The breeds involved are five: Ermellinata di Rovigo (ER=45 (a), 45 (b)), Pèpoi (PP=41(a), 45 (b)), Robusta Lionata (RL=26 (a), 43 (b)), Robusta Maculata (RM=45 (a), 45 (b)), and Padovana (PD=28 (a), 50 (b)). Genomic DNA was extracted from blood samples using a modified DNA purification kit (Gentra System PUREGENE DNA purification kit). The DNA was used as a template for the PCR reactions. A total of 20 microsatellite loci were investigated (*Table 1*). They are included in the list of recommended markers for chicken of the ISAG/FAO Standing Committee (*MoDAD project, FAO*, 2004). Amplification was performed in multiplex and singleplex reactions at the following conditions: initial denaturation step of 30 s at 98 °C, 40 cycles of 7 s at 98 °C, 15 s at X °C and 20 s at 72 °C, the final extension of 7 min at 72 °C, X° being the annealing temperature for each multiplex and singleplex (available from the National Center for Biotechnology Information). All the details regarding the used protocols are available upon request. Four different poolings were used to analyze the amplified fragments with an automated DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter, Brea, CA) and the electropherograms were processed with the CEQ 8000 software (Beckman Coulter).

The calculation of the allele frequencies, the estimation of the observed and expected heterozygosity, and the inbreeding coefficient (FIS) were calculated with MolKin (v. 3.0). Test for deviation from Hardy-Weinberg equilibrium was performed by GENEPOP 3.4 (*Raymond and Rousset*, 1995). Within breed significant differences for

mean number of alleles, observed and expected heterozygosity values calculated for the two years were tested by using PROC UNIVARIATE of *SAS* (1997). Population structure was inferred by using the software STRUCTURE v. 2.2 (*Pritchard et al.*, 2000). For the analysis a burn-in period of 25.000 iterations and 250.000 repetitions were set, with K ranging from 1 to 15. The best number of clusters fitting the data was established by plotting the mean Ln Pr(X|K) for each K., as suggested by *Pritchard et al.* (2000).

RESULTS AND DISCUSSION

Information about the genetic variability of the twenty investigated loci are listed in *Table 1*. The total number of alleles found was 182 with a mean of 9.1±3.67 and ranging from 3 to 16, for Mcw98 and Mcw104, respectively. The polymorphism information content (PIC) ranged from 0.357 to 0.837, with the minimum value for Mcw103 and the maximum value for the locus Mcw81, with an average of 0.644. Comparing the PIC of all our microsatellite loci used, remarkable differences were not highlighted. In the animals sampled after four years of conservation activities an highly significant ($P<0.001$) loss of alleles occurred, but only for those which had lower frequencies, with a mean number of alleles equal to 7.75±3.08 in the year 2002 and 4.64±1.60 in the year 2006. For the loci Mcw104, Mcw111 and Mcw16 a loss of 9, 9 and 6 alleles respectively was measured. For this reason the PIC value doesn't differ comparing the two years with a mean of 0.614 and 0.578, for 2002 and 2006 respectively. Causes of this situation are attributable to the impossibility to implement an optimal within family selection, as a group of cocks was mated with a group of hens, so probably not all cocks had the same progeny leading to the loss of some rare alleles. Moreover, the additional selection of individuals on both morphological and productive basis, that is a normal practice for the maintenance of the genetic resources in their productive environment, is also responsible for the loss of alleles.

Wright's fixation indices (FIS, FST, and FIT) for the overall population were 0.096, 0.473 and 0.524 respectively, underlining a high degree of breed differentiation. The genetic variability was studied in terms of Expected (Hexp.) and observed (Hobs.) heterozygosity and inbreeding coefficient (FIS) for each breed analyzed (*Table 2*). In general, heterozigosity estimates were low if compared to other studies (*Muchadeyi et al.*, 2007), but consistent with the data previously reported by *Zanetti et al.* (2010) about these local breeds and similar to those reported by other authors about European pure breeds (*Hillel et al.*, 2003; *Granevitze et al.*, 2007; *Dalvit et al.*, 2009). During the four years of conservation from 2002 to 2006, all breeds showed a decrease of Hobs, ranging from 2.8% for RM to a maximum of 8.4% for PD. Expected heterozygosis showed the same trend, decreasing of 1% for RL to a maximum of 15% for RM. Anyway, within all breeds, significant differences between 2002 and 2006 subjects were not detected for both observed and expected heterozygosity, evidencing that the decrease in heterozygosity values is mainly attributable to the sampling and has a minor impact for the studied breeds. A decrease in the inbreeding coefficient (FIS) occurred from 2002 to 2006 for PP, ER and RM breeds, probably due to the unleashing of within breed genetic structures attributable to the rotation of males among the conservation flocks. On the other hand, an increment of the same index was noticed for the RL and PD breeds, for which an increase of inbreeding is probably due to the annual selection of individuals for morphological and productive traits. New strategy has been adopted recently to contain inbreeding for these two breeds: backcross with the breeds of origin for RL and the

introduction of new unrelated individuals for the PD could succeed in reestablishing contained levels of inbreeding. All the studied breeds showed a persistent significant deviation from Hardy-Weinberg equilibrium at the beginning of the conservation due to a significant excess of homozigotes within breeds, that is maintained after four years, with the only exception of RM that in 2006 reached the equilibrium. The genetic 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. *Table 3* describes the proportion of membership of the five local chicken breeds in each of the 5 clusters in the two years (2002 and 2006). The analysis revealed that the best number of populations fitting the dataset was five. Individuals were so assigned to one of five groups, approximately corresponding to the breed of origin. This result shows how no evident genetic structures were detectable within breed, both for 2002 and 2006 individuals. However, an increase in the proportion of membership for each breed has occurred in the year 2006 compared to the data obtained in the year 2002. So within breed individuals in 2006 appear more homogeneous producing clearer, more distinctive and separated groups.

Table 1

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

Locus	Frag.size (bp)	Chromo-some	Total N. All.	Total PIC	N All. (a)	N. All. (b)	PIC (a)	PIC (b)
Mcw78	134 to 150	5	10	0.659	10	6	0.750	0.571
Mcw104	190 to 228	13	16	0.701	15	6	0.637	0.640
Mcw123	112 to 134	14	12	0.771	6	6	0.561	0.617
Mcw81	143 to 155	5	16	0.837	9	7	0.698	0.718
Mcw14	166 to 189	6	11	0.732	8	3	0.665	0.525
Mcw248	213 to 245	1	10	0.658	8	4	0.371	0.463
Lei94	251 to 283	4	12	0.561	9	7	0.552	0.600
Mcw111	98 to 106	1	13	0.647	13	4	0.699	0.658
Mcw216	141 to 147	13	5	0.547	5	4	0.571	0.602
Mcw222	217 to 225	3	6	0.566	6	4	0.615	0.562
Mcw37	151 to 159	3	5	0.445	4	4	0.493	0.446
Mcw98	255 to 257	4	3	0.428	3	2	0.243	0.166
Adl278	102 to 121	8	8	0.650	6	7	0.682	0.682
Lei166	251 to 261	3	6	0.743	5	3	0.744	0.647
Mcw103	268 to 272	3	4	0.357	4	2	0.479	0.317
Mcw16	136 to 154	3	10	0.713	10	4	0.770	0.675
Mcw165	112 to 123	23	7	0.637	6	4	0.601	0.588
Mcw20	183 to 189	1	9	0.706	9	4	0.659	0.735
Adl268	104 to 119	1	9	0.794	9	6	0.748	0.717
Mcw295	86 to 102	4	10	0.726	10	6	0.744	0.632
Mean			9.10	0.644	7.75	4.65	0.614	0.578

a: sampled in 2002; b: sampled in 2006

Table 2

Expected (Hexp.) and observed (Hobs) heterozygosity, inbreeding coefficient (FIS) for each breed sampled in 2002 (a) and 2006 (b)

Razze	PP[a] ± SD	PP[b] ± SD	RL[a] ± SD	RL[b] ± SD	ER[a] ± SD	ER[b] ± SD	RM[a] ± SD	RM[b] ± SD	PD[a] ± SD	PD[b] ± SD
Hexp.	0.322 ±0.225	0.228 ±0.236	0.364 ±0.205	0.355 ±0.228	0.553 ±0.179	0.414 ±0.174	0.439 ±0.234	0.289 ±0.222	0.407 ±0.207	0.334 ±0.208
Hobs.	0.278 ±0.254	0.227 ±0.236	0.363 ±0.264	0.311 ±0.264	0.441 ±0.241	0.384 ±0.247	0.320 ±0.262	0.292 ±0.226	0.391 ±0.236	0.306 ±0.225
FIS	0.131	-0.002	0.013	0.121	0.199	0.051	0.281	-0.003	0.042	0.081
P-value	***	*	***	***	***	***	***	N.S.	***	***

ER: Ermellinata di Rovigo; PP: Pépoi; RL: Robusta Lionata; RM: Robusta Maculata; PD: Padovana; SD: standard deviation; [a] 2002; [b] 2006; * P<0.05; *** P<0.001; N.S.: not significant

Table 3

Proportion of membership of the local breeds in each of the 5 clusters

	Clusters									
	2002					2006				
Breed	1	2	3	4	5	1	2	3	4	5
ER	0.007	0.965	0.020	0.002	0.006	0.004	0.982	0.005	0.004	0.005
PP	0.003	0.004	0.006	0.003	0.985	0.005	0.002	0.002	0.003	0.988
RL	0.004	0.039	0.940	0.003	0.013	0.008	0.002	0.985	0.003	0.002
RM	0.961	0.006	0.003	0.026	0.003	0.985	0.002	0.008	0.002	0.003
PD	0.011	0.004	0.006	0.965	0.013	0.003	0.006	0.003	0.985	0.003

CONCLUSIONS

In conclusion, this research shows the usefulness of molecular markers for monitoring the genetic variability of breeds involved in a conservation scheme. Results obtained are very useful for planning new strategies for the conservation scheme, including the choice of the animals, a more efficient organization of matings and for the creation of a new selection index based on the maintenance of the existing genetic variation. Besides, in order to guarantee higher levels of variability, others approaches should be considered such as sperm cryo-conservation techniques coupled with artificial insemination.

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