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Estimation of genetic diversity over time in an *in-situ* marker assisted conservation scheme of local chicken breeds

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ABSTRACT - The aim of this research was to study the genetic variability of two local chicken breeds (Ermellinata di Rovigo and Robusta Maculata) involved in a conservation programme, over a period of three years. Samples were collected in 2002 and in 2005 and analysed using 15 microsatellite markers. Obtained results evidenced that Robusta Maculata was genetically less variable than Ermellinata di Rovigo even after three years of conservation activities. During the studied period the observed heterozigosity was maintained and the excess of homozygous animals found in both breeds in 2002 was strongly reduced in 2005. These findings suggested that the conservation activities helped to overcome the probable presence of population substructures and to limit inbreeding. Moreover, this study evidenced the usefulness of microsatellite markers to monitor genetic diversity in conservation programmes for animal breeds.

Key words: Genetic variability, Conservation programme, Chicken.

Introduction - In 2000, an *in-situ* marker assisted conservation scheme for Veneto local poultry breeds has started (De Marchi *et al.*, 2005a; Targhetta *et al.*, 2005; De Marchi *et al.*, 2006). The scheme involved five local chicken breeds evenly distributed in three conservation flocks and was based on breed maintenance and multiplication within their production system. Main objectives of the scheme were to allow the preservation of animal biodiversity and to support the development of marginal areas through the revaluation of these local breeds and their products (De Marchi *et al.*, 2005b). One of the provided conservation activities was the collection of individual blood samples from the animals involved in the programme in order to investigate them with molecular markers. In fact, the use of molecular markers for animal genotyping at microsatellite loci permits to collect information on breed genetic variability and inbreeding (Marletta *et al.*, 2006; Dalvit *et al.*, 2008) and both information are useful to organize and monitor conservation activities. Moreover, in chickens, where no pedigree information was available, the investigation of microsatellites was the only possibility to estimate and monitor inbreeding. The aim of this study was to estimate the genetic variability of two chicken breeds involved in the conservation scheme, Ermellinata di Rovigo (ER) and Robusta Maculata (RM), over a three year period. Genetic variability was measured, at the beginning of the programme (2002) and after three years of conservation activities (2005). The obtained results will be useful to evaluate the power of the conservation scheme to maintain genetic variability and to decrease the occurrence of high inbreeding in small populations.

Material and methods - The dataset was composed of 154 animals. Sixty-two chickens (28 ER and 34 RM) were chosen among the ones born in 2002 and 90 (45 ER and 45 RM) among the ones born in 2005. The chosen animals were evenly distributed among the 3 existing flocks and they were destined to produce the flock replacement for each breed. Individual whole blood samples were taken from the

wing vein onto a sterile collecting vacuum tube containing sodium citrate. Genomic DNA was isolated using a modified DNA purification kit (Gentra System PUREGENE DNA purification kit) and stored at -20°C until analyses were performed. Genomic DNA was amplified at 15 microsatellite loci, included in the list of recommended markers for chicken of the ISAG/FAO Standing Committee (MoDAD project, FAO, 2004). Multiplex reactions were set up at the following conditions: initial denaturation step of 30 s at 98°C, 35 cycles of 5 s at 98°C, 15 s at X°C and 30 s at 72°C, and a final extension of 7 min at 72°C, where X° is the annealing temperature for each multiplex. Details about the used protocol are available upon request. Molecular markers data were analysed using the software Genetix 4.05.2 (Belkhir *et al.*, 1996-2004) to calculate allele frequencies, to estimate the observed and expected heterozigosity, and to estimate the level of inbreeding (F_{IS}) in each breed. Test for deviation from Hardy-Weinberg equilibrium was performed by GENEPOP 3.4 (Raymond and Rousset, 1995).

Results and conclusions - The obtained results showed a different level of genetic diversity in the two studied breeds. Ermellinata di Rovigo exhibited higher heterozigosity both at the beginning of the conservation programme and after three years (Table 1). However, our results on genetic diversity are comparable with what found by Granevitze *et al.* (2007) in a study characterizing world chicken populations. In particular, these authors showed European chicken breeds to be less variable than Asiatic ones. In our study not all loci were polymorphic. We considered polymorphic only loci showing allele frequencies lower then 0.95 and, according to this classification, ER evidenced one monomorphic locus (LEI166), whereas RM three (ADL0268, MCW0037, and MCW0098). The presence of monomorphic loci was observed also by Granevitze *et al.* (2007) suggesting that choosing the FAO recommended microsatellites did not guarantee the loci to be multiallelic. However, all loci were retained in order to compare results with literature; in fact the analysis of polymorphic loci only, would result in an overestimation of breed heterozigosity estimates.

Table 1. Variation of the expected (exp.) and observed (obs.) heterozigosity (H) and of the inbreeding coefficient (F_{IS}) over a three year period in Ermellinata di Rovigo (ER) and Robusta Maculata (RM).

	Year 2002			Year 2005		
Breed	H exp.	H obs.	F_{IS}	H exp.	H obs.	F_{IS}
ER	0.535±0.192	0.395±0.207	0.280	0.418±0.187	0.400±0.240	0.044
RM	0.403±0.244	0.263±0.180	0.352	0.279±0.205	0.272±0.200	0.024

At the beginning of the conservation programme both breeds showed a highly significant ($P<0.001$) deviation from Hardy-Weinberg equilibrium; in fact in both of them 11 loci out of the 15 investigated were not in equilibrium. Moreover, both ER and RM highlighted high F_{IS}. Results agreed with the study carried out by Granevitze *et al.* (2007), which obtained similar estimates for most of the European chicken populations. The high F_{IS} estimates could be due to the presence of inbreeding or to the presence of substructures in both breeds. Both hypotheses were possible for ER and RM; in fact they are small populations and probably, before entering in the conservation programme, there was not exchange of genetic material among breeders rearing them, which could have caused population substructures. However, after three years of conservation activities, the F_{IS} was much lower and the observed heterozigosity was maintained in both breeds. This result was very important in fact it meant that conservation activities helped to decrease the level of inbreeding in both breeds and to overcome the probable population substructures. Moreover, F_{IS} estimates observed in 2005 were in agreement

with estimates found in other chicken breeds under conservation management (Granevitze *et al.*, 2007). In the three year period, males were rotated among the three conservation flocks and this activity certainly contributed to reduce the presence of possible subpopulations in the two breeds permitting an exchange of genetic material. In addition, male rotation helped to decrease the F_{IS} present in each flock at the beginning of the programme. However, deeper studies should be carried out to better understand how the population structure was at the beginning of the conservation programme. Nevertheless, Table 1 shows that the expected heterozigosity decreased during the three years in both ER and RM. Such result could be due to the loss of some alleles. In chickens in fact, it was not possible to implement an optimal within family selection, as a group of cocks was mated with a group of hens; so, maybe not all cocks had the same progeny leading to the loss of some rather rare alleles. Moreover, the conservation scheme also aimed to the selection of individuals on both morphological and productive basis. This selection, therefore, might be responsible of the loss of allelic diversity and subsequently of the expected heterozigosity. However, for the maintenance of breeds in a productive environment, such as the one of this project, productive traits cannot be ignored. The FAO as well, agreed that *in-situ* conservation should be preferred to the *ex-situ* one, as it maintains both animal genetic resources and particular productive environments. For this reason it is necessary to come to a compromise between the need to maintain a certain degree of variability and the allele loss necessarily caused by selection for productive traits. The use of a certain selection pressure for productive traits is indispensable to persuade farmers to rear local breeds, which will be otherwise forgotten in favour of broiler lines.

Concluding, this research showed the usefulness of molecular marker information for monitoring the genetic variability of breeds involved in conservation programmes. In the future, genotyping information could also be used to better organize matings and maybe to create a new selection index for local chicken breeds.

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