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Genetic similarities among Modenese, Romagnola and three Veneto chicken breeds

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RIASSUNTO – Similarità genetiche tra le razze di polli Modenese, Romagnola e tre razze Venete. *Le similarità genetiche tra cinque razze di polli locali italiane, originarie di diverse regioni, sono state calcolate tramite l'uso di marcatori molecolari di tipo AFLP. Le razze studiate sono la Modenese e la Romagnola native dell'Emilia-Romagna e la Padovana, la Pèpoi e la Robusta Maculata originarie del Veneto. L'analisi, condotta su un totale di 81 animali, ha permesso l'identificazione di 71 marcatori. I risultati ottenuti evidenziano una maggiore similarità genetica tra le due razze emiliane rispetto a quella ottenuta confrontandole con le tre razze venete e ne confermano quindi la diversa origine. Per ciascuna popolazione inoltre è stato calcolato il valore dell'eterozigosità attesa; la Romagnola e la Robusta Maculata presentano i valori più bassi (0,157) mentre quello più alto è detenuto dalla Padovana (0,188). Tali risultati si rivelano utili per studi filogenetici ma soprattutto come strumento per l'impostazione di programmi di conservazione al fine di preservare tale patrimonio genetico.*

KEY WORDS: genetic similarities, AFLP, indigenous chicken breeds.

INTRODUCTION – A recent Italian poultry study (Zanon *et al.*, 2001) showed, in the last decades, the presence of 90 poultry breeds (53 of them are chicken's), they represent a historical and cultural patrimony, and a genetic source of biodiversity. Despite their importance the situation of these breeds in the last years has become worrying as the 61% of the estimated breeds is now extinct, the 13.3% is endangered and the 16.7% is scarcely diffused (Zanon *et al.*, 2001). In this work five indigenous chicken breeds have been studied, two from the Emilia-Romagna region: the Modenese and the Romagnola, and three from the Veneto region: the Padovana, the Pèpoi and the Robusta Maculata. These indigenous breeds are limited diffused; in particular the Modenese, the Romagnola and the Padovana breed seem to be reared just in small farms. All these breeds are involved in regional conservation programs, in particular the Padovana, the Pèpoi and the Robusta Maculata are involved in a marker assisted conservation scheme (Cassandro *et al.*, 2004). The aim of this work was to describe the genetic relationship among these five chicken breeds and to define the population heterozygosity using AFLP molecular markers. These markers have already been an important tool to enrich existing genetic maps in plants, bacteria and less widely in animal genomes but they demonstrate to be advantageous also in studies regarding the genetic characterization of animal breeds (Crepaldi *et al.*, 2000, Óvilo *et al.*, 2000, Ajmone-Marsan *et al.*, 1997).

MATERIAL AND METHODS – Experiment consisted of 81 indigenous chickens: 11 from the Modenese (MOD), 13 from the Romagnola (ROM), 22 from the Padovana (PPD), 25 from the Pèpoi (PPP), 10 from the Robusta Maculata (PRM). Genomic DNA was extracted from whole blood through blood cells lysis and the nucle-

ic acid was subsequently precipitated with ammonium acetate. The protocol used is described in Barcaccia *et al.* (1998) and based on Vos *et al.* (1995) original protocol. The analysis of AFLP marker loci was based on the detection of genomic fragments cleaved by *EcoRI* and *TaqI* restriction enzymes, and subsequently amplified by PCR with three different primer combinations (E32/T35, E45/T32 and E45/T33). Bands were scored as dominant markers and listed in binary matrices of 1/0 values respectively for band presence and absence. A total of 71 polymorphic markers were single out. Values of expected heterozygosity (H), assuming Hardy-Weinberg equilibrium at all marker loci as proposed by Nei (1987) were estimated from allele frequency data and PIC (Polymorphism information content) values were calculated according to the equation proposed by Botstein *et al.* (1980). The 71 marker loci were exploited to estimate the Jaccard similarity matrix and define genetic similarities between all pairs of individuals (Jaccard, 1908). Finally a factorial analysis was performed using the software Genetix.

RESULTS AND CONCLUSIONS – The expected heterozygosity (H) and polymorphism information content (PIC) for the five indigenous breeds are shown in Table 1. All breeds showed low heterozygosity values (less than 0.2). The Padovana breed showed the highest level of heterozygosity (0.188) while the Romagnola and the Robusta Maculata breeds showed the lowest (0.157). This result is probably due to the small size of the populations (around 2000 animals for the Padovana breed, 1500 for the other Veneto breeds and only 250 for the Emilia-Romagna breeds) and to the limited areas of diffusion. The Padovana breed expected heterozygosity is comparable with the value reported by Hillel *et al.* (1999) in a study on the Mediterranean local chicken breeds using microsatellite markers and with the result obtained by De Marchi *et al.* (2003) using AFLP. The Padovana breed showed the highest PIC value (0.152) while the Robusta Maculata the lowest (0.126).

Table 1. Heterozygosity (H) and polymorphism information content (PIC) of AFLP markers.

Indigenous chicken breeds	H	PIC
Modenese	0.166	0.133
Romagnola	0.157	0.127
Padovana	0.188	0.152
Pepoi	0.171	0.136
Robusta Maculata	0.157	0.126

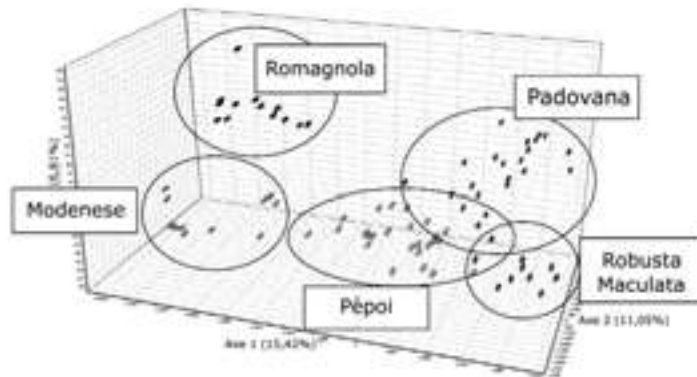
The Jaccard indexes (Table 2) showed that the genetic similarity between the Emilia-Romagna breeds (MOD and ROM) was higher (0.564) than the ones among them and the three Veneto breeds (PPD, PPP and PRM). The Romagnola breed showed higher genetic similarity with all Veneto breeds than the Modenese. The genetic similarity among the Veneto indigenous breeds ranged from 0.466 (between the Padovana and the Robusta Maculata breeds) to 0.509 (between the Padovana and Pepoi breeds). These results evidenced a lower similarity among Veneto breeds respect to the Emilia-Romagna ones and suggest a different origin of these breeds.

Table 2. Jaccard index of similarity between and within breeds (MOD = Modenese, ROM = Romagnola, PPD = Padovana, PPP = Pèpoi, PRM = Robusta Maculata).

Indigenous chicken breeds	MOD	ROM	PPD	PPP	PRM
MOD	0.678				
ROM	0.564	0.734			
PPD	0.429	0.492	0.633		
PPP	0.475	0.543	0.509	0.692	
PRM	0.364	0.463	0.466	0.508	0.680

In Figure 1 is shown a three-dimensional distribution of chickens obtained by factorial analysis performed by the software Genetix. The graphic showed a clear distinction between the Emilia-Romagna and the Veneto breeds, and a good grouping of the five indigenous breeds. On the whole the results seem to be promising to define and control the animal conservation program and to set a genetic traceability system of animal chicken products.

Figure 1. Three-dimensional distribution of the five indigenous breeds based on factorial analysis.



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