Genetic Traceability of Chicken Breeds

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SUMMARY

Aims of this study were to apply AFLP markers to assess the genetic diversity and to define a marker-assisted traceability system in local chicken breeds. Data were based on 107 cocks of three different local chicken breeds from Veneto region (Italy): Robusta (PRR: n=54), Pepoi (PPP: n=33) and Padovana (PPD: n=20). Chickens were individually identified at birth with wing tag and reared in four different herds using a free-range system. Genomic DNA was extracted from whole blood and AFLP analysis was performed according to the protocol described in Barcaccia et al. (1998). Values of expected heterozygosity (H) and polymorphism information content (PIC) at AFLP loci were calculated for each breed. Genetic similarities of all possible pairs of genotypes were estimates using a Jaccard index; the values obtained were subsequently used in a factorial analysis in order to define latent variables which explain the whole genetic similarity relation system between individuals. The average PIC index within breed was generally low: 24.1% for PRR, 23.6% for PPD and 17.2% for PPP. The average heterozygosities of the three breeds for all markers were 29.5% for PRR and PPD and 21.3% for PPP. In the majority of cases (from 90% to 100% of individuals within breed), marker-assisted traceability system used in this research correctly identified the breed of cocks. Hence, results are promising to identify biological tissue (meat, gamets, embryo, etc.) from these local chicken breeds. However, the method used in this study should be improved in terms of cost reduction for single sample, work effort, reproducibility and accuracy of results obtained.

KEY WORDS

local chicken breeds, genetic markers, AFLP, traceability.

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INTRODUCTION

The importance of traceability of animals and animal products has grown as food production and marketing have been removed from direct consumer control (McKean, 2001). Traceability can be related to product identity, origin of materials and parts, product processing history, and to the distribution and location of the product after delivery. Genetic traceability using deoxyribonucleic acid profiling is developing rapidly for domestic and wild populations (Ajmone-Marsan et al., 1997; Óvilo et al., 2000; Slate et al., 1998). The direct identification at DNA level is an effective tool to check and authenticate advanced identification system and it may be used with very high confidence to assign animals or products to their claimed breed (and thus origin) or to exclude them from it; it may be also used as evidence in court proceedings. Moreover, the possibility to verify and guarantee the origin of animals and animal products increases the value of quality certification, favouring the development of economically marginal areas through the revaluation of local and typical breeds and products leading to the conservation of local breeds and preserving biodiversity. Biodiversity is essential for the survival of organisms ad it is assuming greater importance in modern animal science with some high selected breeds (Notter, 1999). Amplified fragment length polymorphism (AFLP) (Vos et al. 1995; Ajmone-Marsan et al., 1997) is a DNA based technology that is now well established in plants and animals to study gene mapping and genetic diversity. Polymorphisms are reproducible and are displayed in the form of presence/absence of bands (biallelic markers); they are determined by single-nucleotide polymorphisms at the restriction sites, deletions and insertions (Jones et al. 1997) distributed all over the genome and allow estimations of relative distances between genomes of individual animals. In chicken breeds some studies have already been carried out to evaluate genetic diversity using AFLP (Knorr et al. 1999) and microsatellite (Zhou et al. 1999) molecular markers. Aims of this study were to use AFLP markers to assess genetic diversity and to define a marker-assisted traceability system for local chicken breeds.

MATERIAL AND METHODS

Data set analysed was based on 107 cocks of three different local chicken breeds from Veneto region: Robusta (PRR: n=54), Pepoi (PPP: n=33) and Padovana (PPD: n=20). Animals were individually identified at birth with a wing tag and were reared in four different herds on a free-range system. Genomic DNA was extracted from whole blood through white blood cells lysis and the nucleic 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 markers loci was based on the detection of EcoRI and TaqI (Ajmone-Marsan et al., 1997) genomic restriction fragments by PCR amplification with three different primer combinations (E32/T35, E45/T33, E45/T32) having three selective nucleotides (Table 1). Labelled restricted-selectively amplified DNA fragments separated on standard 4,75% polyacrylamide gels were visualized by autoradiogram (Biomax MR-1 film, Kodak) after 18 h exposure at –80°C using a hypercassette with intensifying screens (Amersham, Life Science, Uppsala, Sweden). Bands were scored as dominant markers and listed in binary matrices of 1/0 –values (band presence and absence, respectively). The three primer combinations generated 66 polymorphic markers with a size range of 60-650 bp. Values of expected heterozygosity (H) at AFLP loci were calculated for each breed assuming the populations in Hardy-Weinberg equilibrium at all marker loci as proposed by Nei (1987). Polymorphism information content (PIC) per marker was calculated according to the equation described by Botstein et al. (1980). Genetic similarities of all possible pairs of genotypes were estimated using a Jaccard index with a range from 0 to 1 (Ajmone-Marsan et al., 1997). A factorial analysis was performed using the PROC FACTOR of software SAS (1999) in order to define latent variables

Table 1. Adapters and primers used in AFLP analysis.

	Name	Sequenze
Adattatori EcoRI	Eco top strand	5'- CTCGTAGACTGCGTACC –3'
	Eco bottom strand	5'- AATTGGTACGCAGTCTAC -3'
Adattatori TaqI	Taq top strand	5'- GACGATGAGTCCTGAC -3'
	Taq bottom strand	5'- CGGTCAGGACTCAT -3'
Primer EcoRI	E01 (preamplificazione)	5'- GACTGCGTACCAATTC A -3'
	E32	5'- GACTGCGTACCAATTC AAC -3'
	E45	5'- GACTGCGTACCAATTC ATG -3'
Primer TaqI	T01 (pre-amplification)	5'- GATGAGTCCTGACCGA A -3'
	T32	5'- GATGAGTCCTGACCGA AAC -3'
	T33	5'- GATGAGTCCTGACCGA AAG -3'
	T35	5'- GATGAGTCCTGACCGA ACA -3'

which explain the whole genetic similarity relations system existing between individuals. As suggested by Fabbris (1990) the criterion used to define number of factors to extract was based on the threshold value of 65%. This threshold was obtained using the ratio between the total communality estimated by the three first factors (69.27) and the total number of animals considered (107 individuals) which is the sum of eigenvalues of the similarity Jaccard matrix. The varimax rotation method was used to guarantee the independence of factors. The assessment of each individual at the claimed breed was done using threshold values for each factor by average minus three times standard deviation of factorial weights within each breed.

RESULTS AND DISCUSSION

The sixty-six AFLP markers selected were tested and analysed using 107 DNA individual samples belonging to the three different local chicken breeds. Fifteen of these AFLP markers were found to be monomorphic in PPP chicken breed. Monomorphism was also detected in the PPD (4 markers) and in PRR (1 markers) chicken breeds. The averages of H and PIC values for each breed considered are reported in Table 2. The average PIC index per breed was generally low respect to values (44% for sire line and 46% for dam line) reported by Zhu et al. (2001), and, among breeds, slightly higher in PRR (24.1%) and PPD (23.6%) than in PPP (17.2%). The information about the level of polymorphism for the AFLP markers used in this study suggests the need to performe further research on marker with higher PIC values; this may allow to implement markers rapidly in the breeding program, to identify genotypes efficiently and to study association genetics.

The average heterozygosities of the three breeds for all markers were 29.5% for PRR and PPD, while for PPP was 21.3%. Comparable results were found by Hillel et al. (1999) in mediterranean traditional selected chicken breeds as Padovana (18%), Fayoumi (36%) and Light Brown Leghorn (38%). In Table 3 are shown the descriptive statistics of the three factors derived by the factorial analyses of Jaccard similarities between all pairs of animals among the three breeds. The first factor was strongly correlated with the PRR breed

Table 2. Heterozygosity (H) and polymorphism information content (PIC) of AFLP markers for Padova (PPD), Pepoi (PPP) and Robusta (PRR) chicken breeds.

Breed	Animals number	Markers number	H	PIC
PRR	54	66	0.295	0.241
PPP	33	66	0.213	0.172
PPD	20	66	0.295	0.236

Table 3. Descriptive statistics of the three factors for Padova (PPD), Pepoi (PPP) and Robusta (PRR) chicken breeds.

Breed	Mean	SD	Minimum	Maximum
PRR				
- Factor 1	0.713	0.083	0.487	0.824
- Factor 2	0.264	0.057	0.149	0.4068
- Factor 3	0.234	0.065	0.056	0.393
PPP				
- Factor 1	0.272	0.059	0.181	0.395
- Factor 2	0.737	0.097	0.389	0.856
- Factor 3	0.201	0.096	0.064	0.508
PPD				
- Factor 1	0.307	0.062	0.218	0.410
- Factor 2	0.263	0.135	0.078	0.566
- Factor 3	0.631	0.120	0.351	0.778

Table 4. Number of animals and percentage between bracket of each breed assigned to the Padova (PPD), Pepoi (PPP) and Robusta (PRR) chicken breeds using AFLP markers.

Individuals of breed	Breed assigned by AFLP markers		
	PPD	PPP	PRR
PRR	0	0	54 (100%)
PPP	2 (10%)	32 (97%)	0
PPD	18 (90%)	1 (3%)	0

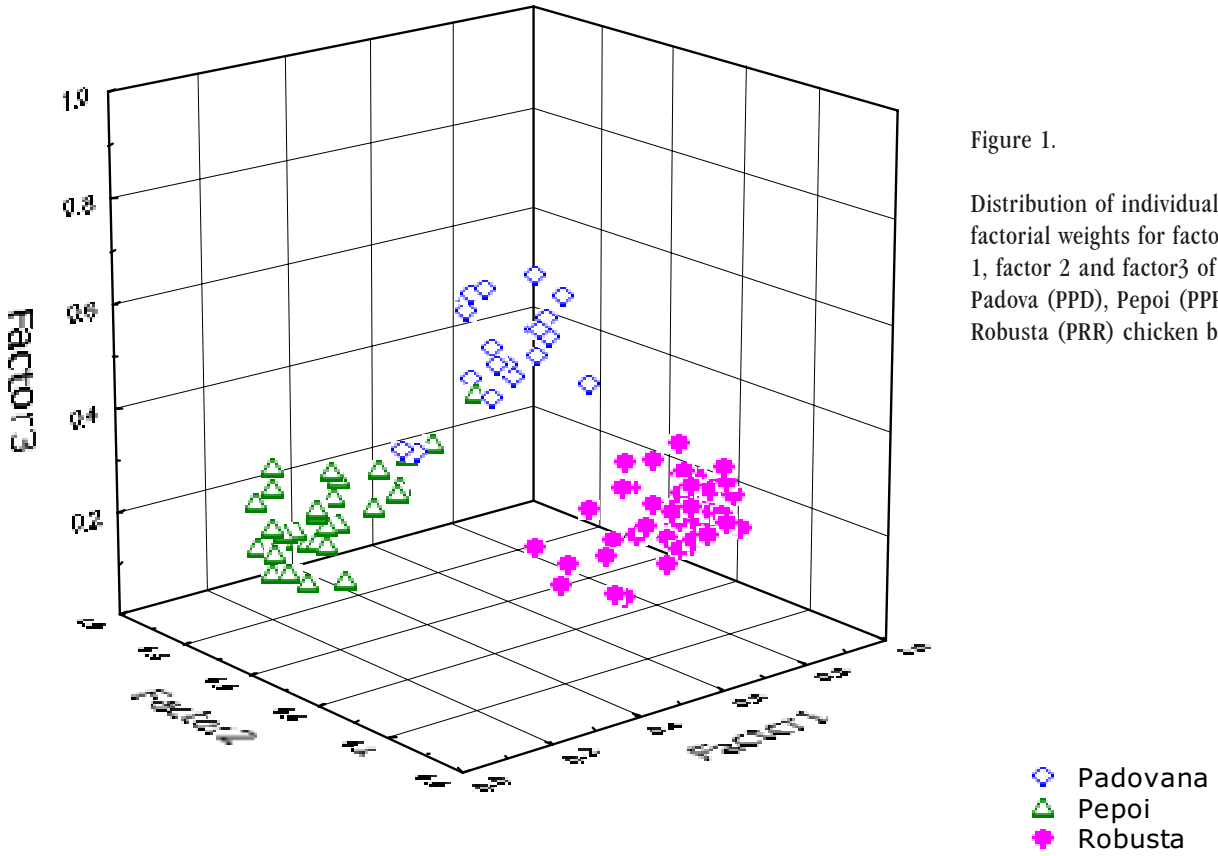

Figure 1.

Distribution of individual factorial weights for factor 1, factor 2 and factor3 of Padova (PPD), Pepoi (PPP) and Robusta (PRR) chicken breeds.

(0.713±0.083) while the second factor identified the PPP breed (0.737±0.097) and the third factor was associated to PPD breed (0.631±0.120). The single cocks in function of three different combination of factors are shown in Figure 1. Using these factors, in most cases (from 90% to 100% of individuals) animals were correctly identified in terms of original breed (Table 4). Three animals were miss-identified, two PPD were identified as PPP and one PPP was defined as a PPD cock. All PRR cocks were rightly identified. Hence, results of this research are promising in order to identify biological tissue (meat, gamets, embryo, etc.) from these three local chicken breeds.

CONCLUSION

Genetic diversity within breeds showed that PPP chicken breed is the least variable with H=21.3%, while PRR and PPD breeds are the most variable with an average H of 29.5%, overall 66 loci. The AFLP markers used in this study revealed moderate to low PIC values (17.2% to 24.1%) suggesting a low diversity. Results obtained in this study encourage the use of AFLP markers as molecular tags for local chicken breeds. This study is a preliminary but essential step towards a marker-assisted traceability method for Veneto local animal populations, that can guarantee an high degree of confidence to assign to animals or unidentified biological tissues and products (meat, gamets, embryo etc.) to their claimed breed. However, further studies should be done in order to estimate the optimum number of markers which is necessary to guarantee an high probability of discrimination among breeds. Moreover, the method used in this study should be improved in terms of costs reduction for single sample, effort and time need to obtain results.

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