ABSTRACT

Genetic diversity in two ancient indigenous chicken breeds of the Veneto region was assessed using Amplified Fragment Length Polymorphism (AFLP) markers. A total of 63 individuals were analysed using three selected AFLP primer combinations that produced 66 clear polymorphisms. The breeds analyzed were the Padovana and the Polverara (two ancient breeds) and a reference broiler line. The expected heterozygosity (Het) did not differ significantly among breeds. The variability at AFLP loci was largely maintained across breeds, as indicated by the coefficient of genetic differentiation (Gst) value. The lowest genetic distance is found between the Padovana and Polverara breeds suggesting that they could be genetically close.

Key words: AFLP, Indigenous breed, Chicken, Genetic diversity

Introduction

The Padovana and Polverara chicken breeds are phenotypically very similar and their origins are very old; it is thought that the Polverara breed developed by a cross between the Padovana and other local Veneto chicken breeds. It is historically documented that the Padovana breed was introduced to Italy from Poland in 1300 by a Padova noble, Giovanni D’ondi dell’Orologio. The Padovana breed was described for the first time in the Ornitologiae book of Ulisse Aldrovandi (1600) but until 1899 it was still confused with the Polverara breed. Trevisani (1900) and Pascal (1905) were the first authors to describe separately the Padovana and Polverara breeds. The peculiarity of these breeds is the absence of crest that is substitute by a tuft of feathers. The Padovana breed tuft is more pronounced than the Polverara one because the first is caused by a skull hernia. The Padovana breed produces a meat of particular quality (De Marchi et al., 2005), like the Polverara breed, providing a typical product interesting in some local market. Moreover these breeds can be very important biodiversity sources. Indeed biodiversity is essential for the survival of species and populations, and it is assuming greater importance in modern animal science because of an expanding global emphasis on only a few highly selected breeds (Notter, 1999). The aim of this study was to investigate the genetic diversity in the Padovana and Polverara breeds using Amplified Fragment Length Polymorphism (AFLP). AFLP is a multilocus marker technique and employed in several studies to investigate genetic variability at the molecular level also in chicken breeds (Plastow et al., 2003).

Material and methods

Sixty three blood samples were collected from
unrelated male chickens, 15 from a private broiler breeding and 48 belonging to two indigenous chicken breeds: Padovana (22) and Polverara (26) from Co.Va. (De Marchi et al., 2005) project nuclei. The reference breed is a commercial line selected for meat production. Genomic DNA was extracted from whole blood through cells lyses and subsequently precipitated with ammonium acetate. AFLP analysis was carried out following the protocol described in Barcaccia et al., (1999) modified for the use of TaqI endonucleases. TaqI is recommended in mammals (Ajmone-Marsan et al., 1997) and also in poultry (Mock et al., 2002). Sixty three samples were individually assayed with three primer combinations that were tested for the analysis of poultry species and breeds (Ajmone-Marsan et al., 1997). AFLP markers were visually evaluated and scored as dominant markers. To estimate the information content of AFLP markers the following indices were calculated: the number of polymorphisms identified per primer combination within and across breeds, an assay efficiency index (Ai) of the information carried by AFLP markers per analysis (corresponding to a primer combination) as described by Ajmone et al., (1997), average expected heterozygosity (Het) within breeds calculated assuming the population at Hardy-Weinberg equilibrium. Indexes of total (Ht) and within population (Hs) gene diversity were calculated according to Nei (1973), the Gst index (Nei, 1973) was also defined. The following genetic distances between breeds were calculated: standard genetic distance (Nei, 1972), Chord distance (Cavalli-Sforza and Edwards, 1967) and Reynolds distance (1983). The computations were performed by the use of appropriated software (Popgene, Dispan, Phylip).

**Results and conclusions**

The three primer combinations assayed revealed 66 clear AFLP polymorphisms, with an average of 22±2.6 markers per primer pair and ranging from 21 to 25 (Table 1). The total number of polymorphisms observed within breed ranged from 34 (broiler) to 43 (Padovana).

Only one breed specific marker was detected in the broiler line. Across breeds, the Ai was 32.4, indicating that an average of 32.4 effective alleles (mean: 1.47 alleles per locus) were identified per primer pair (Table 2).

The across breed Ai value of these chicken breeds was lower than that calculated by Ajmone-Marsan (1997, 2001) for other local species. The total and within population gene diversity were respectively 0.291 and 0.188, the Gst value showed that the 35.4% of total variation is accounted by the

| Breed  | Padovana (n = 22) | Polverara (n = 26) | Broiler (n = 15) | Across (n = 63) |
|--------|------------------|-------------------|-----------------|----------------|
| E32/T35| 13               | 13                | 10              | 20             |
| E45/T32| 18               | 17                | 11              | 25             |
| E45/T33| 12               | 15                | 13              | 21             |
| Total  | 43               | 45                | 34              | 66             |
| Mean ± SD | 14.3 ± 3.2    | 15 ± 2            | 11.3 ±1.5       | 22 ± 2.6       |

**Table 2.** Mean and standard error (SE) of expected heterozygosity (Het), average number of effective alleles per locus (ne) and assay efficiency index (Ai) calculated within and across chicken breeds.

| Breed   | Het (n = 66) (mean ± SE) | Ne (mean ± SE) | Ai     |
|---------|--------------------------|---------------|-------|
| Padovana| 0.20 ± 0.02              | 1.32 ± 0.33   | 28.9  |
| Polverara| 0.21 ± 0.02              | 1.34 ± 0.34   | 29.5  |
| Boiler  | 0.17 ± 0.02              | 1.27 ± 0.36   | 26.8  |
| Across breeds | -                  | 1.47 ± 0.31   | 32.4  |
across breed component. The Gst value reported by Ajmone-Marsan (2001) for the Italian goat populations was lower (0.11) than the value reported in this study. The Polverara breed showed the highest Het index (0.21), followed by the Padovana (0.20) and the broiler line (0.17). The average expected heterozigosity of broiler line was not statistically different from heterozigosity value calculated for the other two indigenous breeds. The Padovana and Polverara breeds showed the lowest genetic distance (Table 3), evidencing to be similar breeds.

The use of AFLP molecular markers permitted a preliminary characterisation of the indigenous chicken breeds of Padova area important for their valorisation, and suggested a genetic similarity between the Padovana and Polverara breeds as reported in historical documents. This study indicates that AFLP is a fast and reliable method to analyse the genome and identify genetic polymorphisms. These markers could be useful for fingerprinting and to estimate genetic relations among individuals and breeds, thus to design marker assisted conservation programmes.

The authors wish to thank Maristella Baruchello, Pelà Antonio & C. for broiler sample blood and the Polverara chicken association.

This research was funded by Veneto Agricultural Agency.

REFERENCES

AJMONE-MARSAN P., NEGRINI R., CREPALDI P., MILANESI E., GIORNI C., VALENTINI A., CICOUGNA M., 2001. Assessing genetic diversity in Italy goat populations using AFLP™ markers. Anim. Genet. 32:281-8.

AJMONE-MARSAN P., VALENTINI A., CASSANDRO M., VECCHIOTTO-ANTALDI G., BERTONI G., KUIPER M., 1997. AFLP™ markers for DNA fingerprinting in cattle. Anim. Genet. 28:418-26.

ALDROVANDI U., 1600. Ornithologiae, XIV:193, Bologna, 310-311. Baraccia G., Albertini E., Falcinelli M., 1999 AFLP fingerprinting in Pelargonium peltatum: Its development and potential in cultivar identification. J. Hort. Sci. & Biotecn. 74 (2):243-50.

CAVALLI-SFORZA L. L., EDWARDS A. W. F., 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32:550-70.

DE MARCHI M., CASSANDRO M., LUNARDI, SEIGEL P.B., 2005. Carcass Characteristics and Qualitative Meat Traits of the Padovana Breed of Chicken. Int. J. Poult. Sci. 4:233-8.

DE MARCHI M., CASSANDRO M., TARGHETTA C., BARUCHELLO M., NOTTER D.R., 2005. Conservation of poultry genetic resource in the Veneto region of Italy. Anim. Genet. Res. (In press).

DISPAOTA T., 1993. Institute of Molecular Evolutionary Genetics, Pennsylvania State University.

FLESENSTEIN J., 2004. PHYLP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

MOCK, K.E., THEIMIER, T.C., RHODES JR, O.E., GREENBERG, D.L., KEIM, P., 2002. Genetic variation across the historical range of the wild turkey (Meleagris Gallopago). Mol. Ecol. 11:643-57.

NEI M., 1972. Genetic distance between populations. Am. Nat. 106:283-92.

NEI M., 1973. Analysis of gene diversity in subdivided population. Proceedings of the National Academy Science USA 70:3321-3.

NOTTER D. R., 1999. The importance of genetic diversity in livestock populations of the future. J. Anim. Sci. 77:61-9.

PASCAL T., 1905. Le razze della gallina domestica. C.e Roux e Viarego, 98-111.

TREVISANI G., 1900. Pollicoltura. C e Ulrico Hoepli, 32-35.

PLASTOW G., SIEGENS M., BAGGA M., BROWN B., HEUVEN H., PELEMAN J., 2003. Utilization of AFLP for genetic distance analysis in pigs. Arch. Zootec. 52:157-64.

REYNOLDS J., 1983. Estimation of the coancestry coefficient basis for a short-term genetic distance. Genetics 105:767-9.

YEH F.C., YANG R-C., BOYLE, TIMOTHY B.J., YE Z-H., MAO J. X., 1997. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.