Heterozygosity analysis of Bionda Piemontese and Bianca di Saluzzo chicken breeds by microsatellites markers: a preliminary study

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ABSTRACT: Conservation of genetic variability is one of the main goals in animal production science and the analysis of breeds genetic asset can supply objective basis for effective conservation programs and selection strategies. Bionda Piemontese (PIB) and Bianca di Saluzzo (SAB) chicken breeds originated in Piemonte region. Breeds conservation programmes started in 1999 in Verzuolo (CN) aiming to preserve the breeds and to improve their diffusion being particularly adapted to free range rearing systems thanks to their resistance. PIB and SAB are both suggested for traditional recipes and production (e.g. Morozzo capon) and are Slow Food presidia. A total of 76 birds were analysed: PIB (n=36), SAB (n=40). Genomic DNA was extracted from blood samples. All birds were genotyped at eight microsatellite loci. Each marker was subjected to PCR and the products were separated by electrophoresis in 4.2% denaturing polyacrylamide gels on ABI Prism 377 DNA Sequencer equipped with Genescan and Genotyper software. The results of this preliminary study highlight the genetic differences occurring between PIB and SAB populations

Key words: Bionda Piemontese, Bianca di Saluzzo, Heterozygosity, Chicken microsatellites.

INTRODUCTION – Poultry breeds with their differences in morphology, productive performances, physiological characteristics, behaviour and coping ability, display the effect of domestication and selection on red jungle fowl (*Gallus gallus*) (Ruane, 1999). Conservation of genetic variability is one of the main goals in animal production science and the results of breeds genetic investigation can supply objective basis for effective conservation programs and selection strategies (Weigend *et al.* , 2001; Hillel *et al.*, 2003; De Marchi *et al.*, 2006)**.** Rare poultry breeds and populations characterized by a limited size strictly depend on the maintenance of genetic differences (Wimmers, *et al.*, 2000, Bozzi *et al.*, 2006). Bionda Piemontese (PIB) and Bianca di Saluzzo known as Bianca of Cavour too (SAB) chicken breeds originated in Piemonte region in the west part of northern Italy with overlapping diffusion areas. In particular SAB breeding stocks are mainly present in the southern-west part of the Piemonte region around Cuneo town. Breeds conservation programmes started in 1999 in Verzuolo (CN) aiming to preserve the breeds and to improve their diffusion being particularly adapted to free range rearing systems thanks to their grazing ability, adaptability and resistence, and being suggested for traditional recipes and production (e.g. Morozzo capon). PIB and SAB are Slow Food presidia. These dual purpose breeds are well known for their tasty meat (http://www.asproavic.it; http://www.dsz.unito.it). Heterozygosity and genetic distances can be conveniently and effectively determined at DNA level using microsatellites markers.

MATERIAL AND METHODS – A total of 76 Subjects were collected in 2 breeding farms in CN province and analysed: PIB (n=36), SAB (n=40). DNA was extracted from blood samples using classic procedures. The birds were genotyped at eight microsatellite loci (ADL102, ADL158, ADL176, ADL181, ADL210, ADL267, ADL136, ADL 171) isolated from domestic chicken (*Gallus gallus*) (http://dad.fao.org.). Each marker was subjected to PCR amplifica-

tion using a PTC 200 Thermal Cycler (MJ Research ®) in single reactions in 10ml volumes with 25 ng of template DNA. The PCR products were separated by electrophoresis in 4.2% denaturing polyacrylamide gels on ABI Prism 377 DNA Sequencer equipped with Genescan and Genotyper softwares (Applied Biosystems). Allele frequencies, inbreeding coefficient $F_{IS} \equiv f$¶ and Hardy-Weinberg equilibrium deviation (P-value) at the eight microsatellite loci were calculated using the GENEPOP statistic package (Raymond, 1995). Heterozygosities, G_{ST} value and Factorial Correspondence Analysis (FCA) were calculated using the GENETIX program (Belkhir, 1996-2002).

RESULTS AND CONCLUSIONS – Analysed loci were polimorphic in both breeds ranging from 4 alleles (ADL 158 and ADL 181) to 13 alleles (ADL 136). Fifty-eight alleles have been found in SAB and 48 allelic variants in PIB genetic variability has been computed. In table 1 expected, unbiased and observed heterozygosity results are shown, genetic variability is underlined by the high average number of alleles per locus. Hillel *et al.* in 2003 calculated an average alleles number ranging between 1.8 and 6.5 (52 chicken popultions, 22 microsatellites). Inbreeding coefficients are shown in table 2, low inbreeding levels were recorded. Table 3 shows the unbiased coefficients of gene differentiation G_{ST} due to breed differences. The coefficient ranged from 0, 006 (ADL181) and 0,141 (ADL 210), with an overall value of 0,035. The 8 microsatellites explain 3,5% of the genetic difference between the two breeds. Figure 1 shows the spatial representation of the individuals as defined by the Factorial Correspondence Analysis, a clear breed-grouping trend is shown with only few subject out of breed cluster. The results of this preliminary study highlight the genetic differences occurring between PIB and SAB populations; these northern Italian breeds are important examples of traditional rural chickens particularly adapted to extensive production systems. Further studies are needed to investigate the genetic basis of productive and ethological traits able to typifie traditional poultry productions and to increase the commercial value of autochthonous breeds products.

Table 1. Heterozygosity values and average number of alleles per locus.

Breed	Heterozygosity			Average number of alleles/locus
	Expected (s.e.)	Unbiased (s.e.)	Observed (s.e.)	
SAB	0,763 (0,07)	0,771 (0,07)	0,724 (0,06)	7,250
BIP	0,661 (0,21)	0,671 (0,21)	0,600 (0,28)	6,385

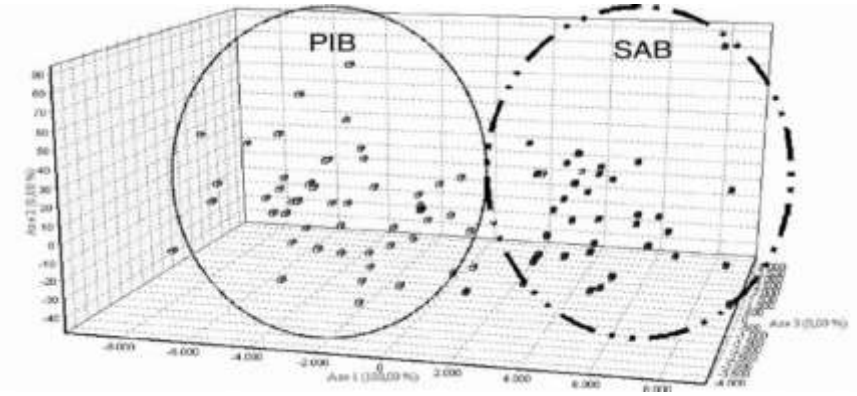
Table 2. PIB and SAB inbreeding values ($F_{IS} \equiv f$).

Loci	SAB	PIB
	$F_{IS} \equiv f$	$F_{IS} \equiv f$
ADL 102	0,2215	0,5879
ADL 158	-0,1243	0,2176
ADL 176	-0,0029	-0,166
ADL 181	-0,0242	-0,1365
ADL 210	0,1372	0,0986
ADL 267	0,0498	0,3077
ADL 136	0,164	0,0628
ADL 172	0,023	-0,0264
All	0,0613	0,1095

Table 3. H_S,H_T and G_{ST} values.

Locus	H_S	H_T	G_{ST}
ADL 102	0,745	0,788	0,055
ADL 158	0,508	0,523	0,030
ADL 176	0,773	0,784	0,014
ADL 181	0,740	0,745	0,006
ADL 210	0,500	0,582	0,141
ADL 267	0,806	0,818	0,014
ADL 136	0,846	0,861	0,018
ADL 172	0,779	0,802	0,029
Multilocus	0,712	0,738	0,035

Figure 1. Spatial representation of the individuals as defined by the Factorial Correspondence Analysis.



REFERENCES – **Belkhir,** K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 1996-2002. GENETIX 4.04, Logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, interactions CNRS UMR 5000, Université de Montpellier II, Montpellier (France). **Bozzi,** R., Franci, O., Forabosco, F., Pugliese, C., Crovetti, A., Filippini, F., 2006. Genetic variability in three Italian beef cattle breeds derived from pedigree information. Ital. J. Anim. Sci. 5:129-137. **De Marchi,** M., Dalvit, C., Targhetta, C., Cassandro, M., 2006. Assessing genetic diversity in indigenous Veneto chicken breeds using AFLP markers. Anim. Gen. 37:101-105. **Hillel,** J, Groenen, M.A.M., Tixier-Boichard, M., Korol, A., David, L., Kirzhner, V.M.,Burke, T., Barre-Dirie, A., Crooijmans, R.P.M.A., Elo, K., Feldman, L.W., Freidlin, P.J., Maki-Tanila, A., Oortwijn, M., Thomson, P., Vignal, A., Wimmers, K., Weigend, S., 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet. Sel. Evol. 35:533-557. **Raymond,** M., Rousset, F., 1995. GENEPOP version 3.1d.. J. Hered. 86:248-249. **Ruane,** J., 1999. A critical review of the value of the genetic distance studies in conservation of animal genetic resources J. Anim. Breed. Genet. 116:317-323. **Weigend,** S., Romanov, M.N., 2001. Current strategies for the assessment and evaluation of genetic diversity in chicken resources W. Poult. Sci. J. 57:275-288. **Wimmers,** K., Ponsuksili, S., Hardge, T., Valle-Zerate, A., Mathur, P.K., Horst, P., 2000. Genetic distinctness of African Asian and South American local chickens. Anim. Genet. 31:159-165. **WEB SITES**: http://www.asproavic.it ; http://www.dsz.unito.it; http://dad.fao.org.