Genetic variability in Valdarnese Bianca chicken breed using microsatellite markers

S.P. MARELLI[1*], M.G. STRILLACCI[1], V. FERRANTE[1], P. PIGNATTELLI[1], M. GUALTIERI[2], L. GUIDOBONO CAVALCHINI[1]

[1] Istituto di Zootecnica, Faculty of Veterinary Medicine, University of Milan, via Celoria 10, 20133 Milan, Italy;
[2] Dipartimento di Scienze Zootecniche, University of Florence, via delle Cascine 5, 54144 Florence, Italy.
***Corresponding author: Stefano.Marelli@unimi.it**

Poultry biodiversity is considered one of the most endangered genetic resource. The determination of within-populations genetic characterization is a basic step in the evaluation of local breeds as genetic resources. Microsatellites markers are a convenient and powerful tool in heterozygosity and genetic distances determination at DNA level. Information about traditional Italian chicken breeds characteristics is scarce and the investigation of their genetic make-up plays an important role in conservation programmes supplying objective selection supports. Valdarnese Bianca (VB) is a traditional white feathered breed from Tuscany, cock weight 3.1-3.5Kg, hen weight 2.5-3.0Kg. Eggshell: white, average egg weight: 50g, average number of egg per year: 135, hen age at first egg: 7 months, slaughter age for broiler: 120d or more. 83 VB birds from 10 different breeding-farms were analysed (4-13 birds/farm), 3 Livornese Bianca (LB) and 3 Golden Comet (GC) (commercial hybrid) samples were added in the analysis as control groups. Genomic DNA was extracted from blood samples using classic procedures. All birds were genotyped at 8 microsatellite loci (ADL102, ADL158, ADL176, ADL181, ADL210, ADL267, ADL136, ADL 171). PCR amplification: 10μl volumes with 25 ng of template DNA. 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 and deviation from Hardy-Weinberg equilibrium (P-value) at the eight microsatellite loci were calculated using the GENEPOP statistic package; in case of less than five alleles, an extract P-value, by the complete enumeration method was calculated, while for more than five alleles a Markov-Chain method was computed. The heterozygosities (H_O, H_E) were calculated by FSTAT. Factorial analysis three-dimensional distribution was carried out using the GENETIX program (12 populations). A total of 73 alleles was detected across the 8 loci. The lowest allele number was calculated in ADL 181 and ADL 210 (6 alleles). The highest allele number was calculated in ADL 136 (15 alleles). H_O ranged from 0.355 (ADL 210) to 0.654 (ADL 181).The test for H-W equilibrium revealed significant deviations ($P<0.05$); 5 farm-populations of VB were in equilibrium. Genetic differences within VB breeding stocks were detected.

Keywords: biodiversity; breeds conservation; Valdarnese Bianca; genetic variability; microsatellite markers

Introduction

The loss of genetic variability that characterizes productive species, with a very limited number of world wide spread commercial hybrids, leads to consider chicken biodiversity one of the most endangered genetic resources.

Valdarnese Bianca can be considered the only traditional Italian meat-type chicken breed, birds are white feathered with dark yellow shank and a single comb. The area where the breed developed is constituted by the valleys between Florence and Arezzo in Tuscany, central Italy. The cock and the hens weights range from 1.0 to 3.5Kg and from 2.5 to 3.0 Kg respectively. This breed is characterized by very slow juvenile feathering. Valdarnese Bianca egg is characterized by a white eggshell and an average egg weight of 50g, the average number of egg per year is 135 and hen age at first egg is 7 months. Broilers slaughter age is 120 days or more. Valdarnese Bianca meat is typically very tasty and compact. Due to their behavioural characteristics these birds are recommended for extensive rearing methods, ethological researches underline the reactivity of this breed.

Investigation of local chicken populations genetic make-up, performance and behavioural traits play an important role in conservation programmes, furthermore genetic assessment of within population genetic variability is a basic step to assess selection projects effectiveness.

Genetic distance and heterozygosity at DNA level can be effectively and conveniently investigated using microsatellite markers; objective selection supports can be supplied.

Materials and methods

83 VB birds from 10 different breeding-farms (4-13 birds/farm) were analysed 3 Livornese Bianca (White Leghorn Italian type) (LB) and 3 Golden Comet (commercial hybrid) (GC) samples were added as control groups. Genomic DNA was extracted from blood samples following standard protocols. All birds were genotyped at 8 microsatellites loci (ADL 102, ADL 158, ADL 176, ADL 181, ADL 210, ADL 267, ADL 136, ADL 171) isolated from domestic chicken. Each microsatellite marker was subjected to PCR amplification in 10 µl 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 and deviation from Hardy-Weinberg equilibrium (P-value) at the eight microsatellite loci were calculated using the GENEPOP statistic package; in case of less than five alleles, an exact P-value, by the complete enumeration method was calculated, while for more than five alleles a Markov-Chain method was computed. H_O and H_E heterozygosities were calculated using FSTAT software. Factorial analysis three-dimensional distribution was carried out using the GENETIX program (12 populations).

Results and discussion

A total of 73 alleles was detected across the 8 loci. The lowest allele number was calculated in ADL 181 and ADL 210 (6 alleles). The highest allele number was calculated in ADL 136 (15 alleles).

H_O ranged from 0.355 (ADL 210) to 0.654 (ADL 181) (Table1). ADL210 showed a low H_O due to the low number of detected alleles, otherwise ADL181 was able to reveal a balanced distribution of different alleles among the investigated populations.The calculated overall F_{ST} was 0.1692. The main part of genetic variability (0.8308) is related to differences between single birds. Breeds differences are present but very limited. In the analysed breeds a heterozygous subjects loss (0.1417) was recorded (F_{IS}). In the overall population the loss of heterozygous birds was recorded to be 0.2869 (F_{IT}) (Table1).

The test for H-W equilibrium revealed significant deviations ($P<0.05$); 5 farm-populations of VB were in equilibrium.

Table 1: Nei's estimation of Heterozigosity and Wright's fixation index F-statistics

marker	H_O	H_E	H_T	F_{IS}	F_{ST}	F_{IT}
ADL102	0.482	0.685	0.829	0.2593	0.1670	0.3830
ADL158	0.596	0.562	0.689	-0.0598	0.1729	0.1235
ADL176	0.636	0.737	0.826	0.1963	0.1095	0.2843
ADL181	0.654	0.660	0.761	0.0667	0.1344	0.1922
ADL210	0.355	0.480	0.671	0.1538	0.2699	0.3822
ADL267	0.638	0.805	0.889	0.1260	0.0981	0.2117
ADL136	0.427	0.599	0.871	0.2525	0.2443	0.4351
ADL171	0.557	0.608	0.764	0.0927	0.1677	0.2449
overall	0.543	0.642	0.788	0.1417	0.1692	0.2869

Genetic differences within VB breeding stocks were detected (Figure 1). The figure shows the three-dimensional distribution of the ten VB populations/breeding farms and the two control populations the commercial hybrid GC and the pure breed LB. The occurring distances between populations 1,2,4,6,7,9 grouped around GC and the other populations (8,3,5,10) are due to a very low number of alleles detected in these last ones.

VB populations seems to be clearly distant from LB control group.

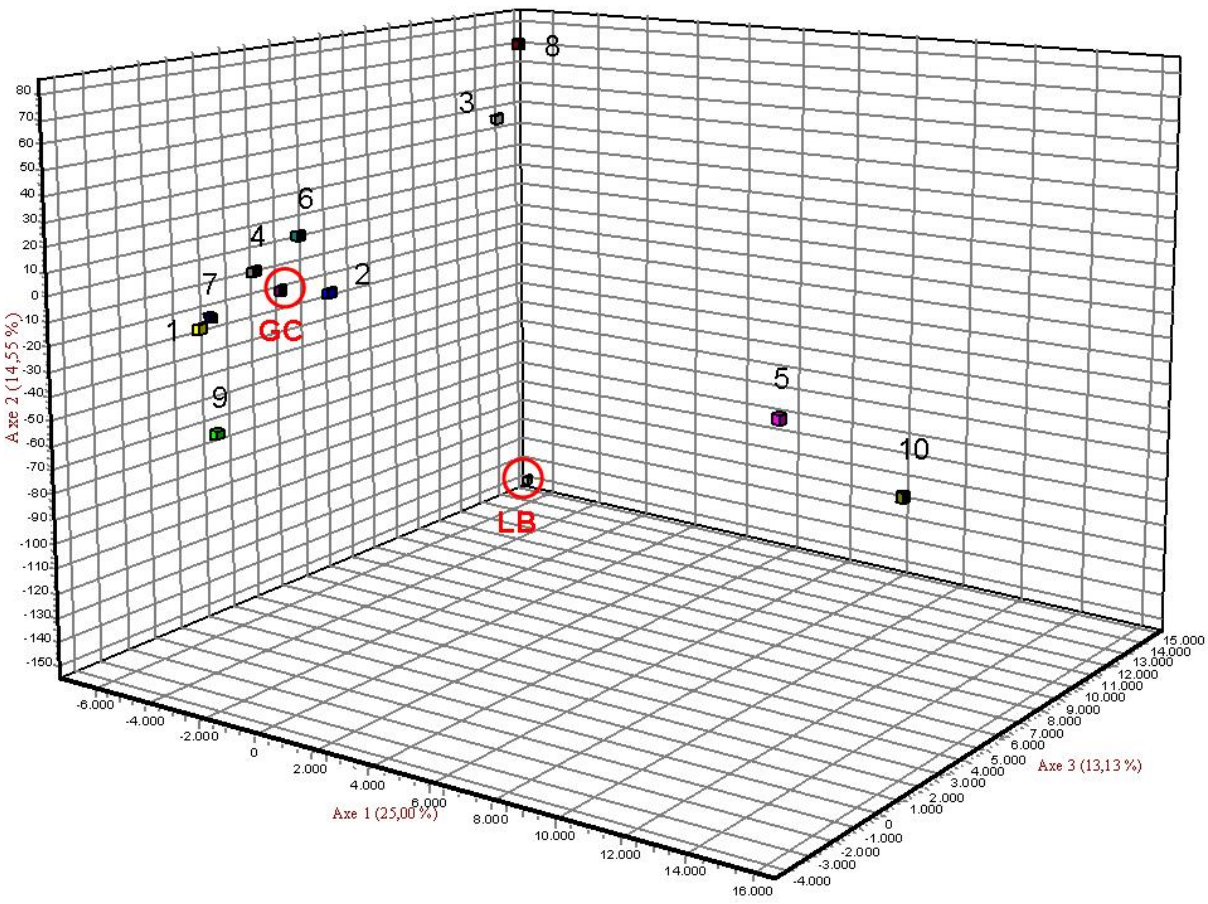

Figure 1: Three-dimensional distribution of the VB populations (1-10) and control groups (GC and LB)

The occurring genetic distances calculated vary between farms, thus selection programmes should take into consideration the opportunity to plan breeding strategies aimed to limit homozygosis and inbreeding maximizing performances.

References

DE MARCHI, M., DALVIT, TARGHETTA, C. and CASSANDRO, M. (2006) Assessing genetic diversity in indigenous Veneto chicken breeds using AFLP markers. *Anim.* Gen. **37**: 101-105

DE MARCHI, M., CASSANDRO, M., DALVIT, C. and RAMANZIN, M. (2004) Analysis of genetic relationships between various populations of domestic poultry breeds and commercial boiler line using AFLP markers. *Book of Abstract of the 55th Annual Meeting of the European Association for Animal Production, Bled, Slovenia.* 34.

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. and WEIGEND, S. (2003) Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools *Gen. Sel. Evol.* **35**: 533-557.

PIGNATTELLI, P. (2001) Il consumatore ha scelto la Valdarno bianca *Riv. Di Avicoltura* **1**: 12-18.

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. and 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. and HORST, P. (2000) Genetic distinctness of African Asian and South American local chickens *Animal Genetics* **31**: 159-165.

ZHOU, H. and LAMONT, S.J. (1999) Genetic characterization of biodiversity in highly inbreed chicken lines by microsatellites markers *Anim. Gen.* **30**, 256-264.

WEB SITES: http://germoplasma.arsia.toscana.it/Germo/Repertori/Animale.htm ; http://dad.fao.org/en/refer/library/guidelin/marker.pdf