ISSN 1330-7142
UDK = 636.5:636.084.5

GENETIC CHARACTERISATION OF ITALIAN CHICKEN BREEDS USING A PANEL OF TWENTY MICROSATELLITE MARKERS

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Original scientific paper

SUMMARY

Genetic relationships among four Veneto native breeds of chickens were studied on the basis of microsatellites polymorphisms. A total of 190 DNA samples (45 Robusta Lionata, 43 Robusta Maculata, 45 Ermellinata di Rovigo, 45 Pèpoi) and a commercial broiler line (12 Golden Comet) were genotyped at 20 microsatellite loci. The average number of alleles per locus was 5 and the expected heterozygosity resulted lower for the local breeds than for the commercial broiler line used as reference. The inbreeding coefficient showed a deficit of heterozygotes, highest for the Robusta Lionata breed. Nei's standard genetic distances corrected for bias due to sampling of individuals (Da), based on allele frequencies, and Reynolds distances ($D_{Reynolds}$) were calculated among breeds. The Robusta Lionata and Robusta Maculata resulted very similar approving the same genetic origin. A Neighbor-Joining tree drawn from $D_{Reynolds}$ distances clustered three groups, one including the Robusta Lionata and Robusta Maculata breeds, the second one formed by the Ermellinata di Rovigo and the Golden Comet commercial line and the third by the Pèpoi. The results showed the genetic differences occurring between the local chicken breeds.

Key-words: chicken, microsatellites, genetic distances, biodiversity, conservation.

INTRODUCTION

The dramatic size contraction of local poultry breeds due to replacement with cosmopolite improved breeds showed the need for native genetic resources conservation. Rare poultry breeds and population characterised by a limited size strictly depend on the maintenance of genetic differences (Wimmers et al., 2000). Conservation of genetic variability is of great importance in animal science; the analysis of breeds genetic structure can supply the basis for effective conservation programs. Since 2000, in the Veneto, region of Italy, a total of 12 local poultry breeds derived from four different species (chicken, duck, helmeted guinea fowl and turkey) have been available as genetic resources and involved in an *in situ* marker assisted conservation scheme (Cassandro et al., 2004).

In the present study four of these Veneto chicken breeds, Robusta Lionata (PRL), Robusta Maculata (PRM), Ermellinata di Rovigo (PER) and Pèpoi (PPP), were analysed using the commercial broiler line Golden Comet (PBR) as a reference. Robusta Lionata and Robusta Maculata are medium-heavy dual purpose breeds, selected in 1965 in the "Stazione Sperimentale di Pollicoltura" of Rovigo, by crossing Orpington with White America and probably other unspecified breeds. The Ermellinata di Rovigo breed was developed in 1959 crossing Sussex and Rhode Islands to obtain a valuable meat breed also useful for eggs production. The Pèpoi is a small size breed originated in the north-western part of the Veneto and Friuli regions of Italy. It appears to have a good attitude to the extensive production systems and is particularly appreciated for its meat (Veneto Agricoltura, 2004).

The application of molecular biology techniques helps avoiding the risk of compromising genetic variability of conservation programs of small populations. Until 2005 the genotyping of the individual animals for marker assisted conservation scheme was carried out using the AFLP technique (De Marchi et al., 2006). Afterward, microsatellites have been applied because these molecular markers

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are well dispersed in the genome and highly polymorphic (Cheng et al., 1995); their application to characterise chicken breeds is relatively recent but it has been used in many countries to study the genetic relationships among native breeds (Takahashi et al., 1998; Hillel et al., 2003).Aim of this study was to define the genetic relationships among four local chicken breeds: Robusta Lionata, Robusta Maculata, Ermellinata di Rovigo, Pèpoi and a commercial broiler line, the Golden Comet, using microsatellite DNA polymorphisms as markers.

MATERIAL AND METHODS

Individual blood samples 190 belonging to four local breeds of Veneto region, Robusta Lionata (PRL, 43 individuals), Robusta Maculata (PRM, 45 individuals), Ermellinata di Rovigo (PER, 45), Pèpoi (PPP, 45) and the Golden Comet commercial broiler line (PBR, 12) were randomly collected within breed in three different herds. Twenty sets of primers (Table 1), included in the lists of recommended primers for chicken analysis suggested by the FAO organisation (FAO, 2004), were chosen on the basis of their position in the chicken genome. The PCR primer pairs were synthesized and 5' ends of the forwards primers were fluorescently labelled. Chicken genomic DNA used as a template for PCR reaction was isolated from blood using a modified DNA purification kit (Gentra System PUREGENE DNA). The 20 microsatellites (STR) were individually analyzed by a PX2 Thermohybaid thermal cycler at the following conditions, the X temperature being the annealing t° of each primer (NCBI): initial denaturation step of 10 min at 94°C, 35 cycles of 45 s at 94°C, 1 min at X°C and 1.5 min at 72°C and a final extension of 10 min at 72°C. A reaction volume of 15 µl contained 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 1.5 µl of Taq Buffer 1X, 0.04 U Taq Gold (Sigma), 3mM dNTPs and 10 µM of each primer. Analysis of fragments was performed using an automated DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter) and a computer software (CEQ 8000 Beckman Coulter). Alleles were designated according to PCR product size whereas allelic frequencies were estimated. Values of observed, non biased (i.e. observed heterozygosity corrected for bias due to sampling) and expected heterozygosity, F_{IS} values (Weir and Cockerham, 1984) and genetic distances among breeds, calculated according to Nei (1978), were determined using the Genetix software (Belkhir, 1996-2002). Reynolds distances ($D_{Reynolds}$) (Reynolds et al., 1983) were calculated using the Phylip 3.66 software package (Felsenstein, 2005). A χ^2 test was performed to evaluate significant differences between observed and expected heterozygosity (H) values using the Genepop software (Raymond, 1995). A factorial correspondence analysis was carried out using the software Genetix, in order to define latent variables which explain the whole genetic similarity relation system existing among individuals.

RESULTS AND DISCUSSION

All twenty microsatellites examined approved to be polymorphic, a total of 100 alleles were detected and the average number of alleles per locus was 5 (Table 1).

Table 1. Polymerase chain reaction primers for microsatellite markers, chromosomes involved (Chr.), alleles detected and minimum and maximum fragments length

Loci	Chr.	Alleles	Length	Loci	Chr.	Alleles	Length
MCW0295	4	5	86-98	MCW0222	3	5	217-225
MCW0078	5	6	134-146	MCW0037	3	5	151-159
MCW0104	13	7	190-216	MCW0098	4	2	255-257
MCW0123	14	6	112-134	ADL0278	8	6	108-122
MCW0081	5	6	143-155	LEI0166	3	3	251-261
MCW0014	6	6	166-181	ADL0268	1	5	104-117
MCW0248	1	4	215-223	MCW0016	3	8	138-155
LEI0094	4	6	259-283	MCW0165	23	4	112-123
MCW0111	1	4	98-106	MCW0020	1	4	183-189
MCW0216	13	4	141-145	MCW0103	3	4	268-273

Expected and observed H values are reported in Table 2. These parameters are important because the conservation program aims to increase the genetic variability within and between breeds. The broiler

line showed the highest value of expected and observed heterozygosity (0.5580 and 0.6777, respectively). The PRL, PER and PPP showed a significant deficit of heterozygotes, deviating from Hardy-Weinberg equilibrium. The PBR, as expected from a commercial hybrid, showed a significant excess of heterozygotes. All the local breeds showed evidenced low H values if compared to those reported by other authors regarding other indigenous breeds (Zhang et al., 2002), but similar to those reported by Hillel et al. (2003) about standardized breeds selected on morphology (European breeds).

Table 2. Average values of expected (H exp), non biased (H nb), observed (H obs) heterozygosity and inbreeding coefficient (F_{IS})

Genetic type	H exp	H nb	H obs	P	F_{IS}
Robusta Lionata	0.3666	0.3712	0.3223	***	0.1233
Robusta Maculata	0.3062	0.3098	0.3074	n.s.	0.0106
Ermellinata di Rovigo	0.4143	0.4202	0.3836	***	0.0615
Pèpoi	0.2304	0.2334	0.2294	*	0.0198
Broiler	0.5580	0.5830	0.6777	***	-0.1768

*** = P<0.001; * = P<0.05; n.s. = not significant

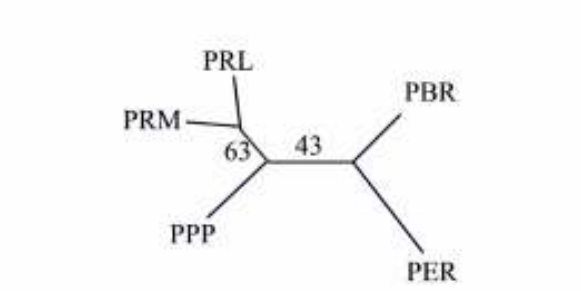
F_{IS} value, which indicates the degree of departure from random mating, was particularly high in Robusta Lionata (0.1233) compared to other breeds, indicating heterozygosity deficiency. It might be a result of a bottleneck effect, since its population size decreased drastically (few hundreds) before the beginning of the conservation project.
Nei's standard genetic distance (Da), corrected for bias, due to sampling of individuals, and Reynolds distance estimates ($D_{Reynolds}$) are reported in Table 3. Reynolds distance (Reynolds, 1983) were used to estimate pairwise genetic distances between the breed. This measure is recommended by Eding and Laval (1999) for populations with short divergence time. Calculating both distances, PRL and PRM breeds were closer (0.388 and 0.392) than the other breeds and the broiler line individuals. This result is in agreement with the known genetic origin of these two breeds, approving that the use of microsatellite markers for the study of genetic biodiversity is accurate and reliable.

Table 3. Distance matrices estimated by Da (above diagonal) and $D_{Reynolds}$ (below diagonal) distances

	PBR	PRL	PRM	PER	PPP
PBR		0.442	0.385	0.457	0.565
PRL	0.311		0.388	0.646	0.623
PRM	0.319	0.392		0.697	0.728
PER	0.298	0.434	0.479		0.852
PPP	0.428	0.534	0.596	0.561	

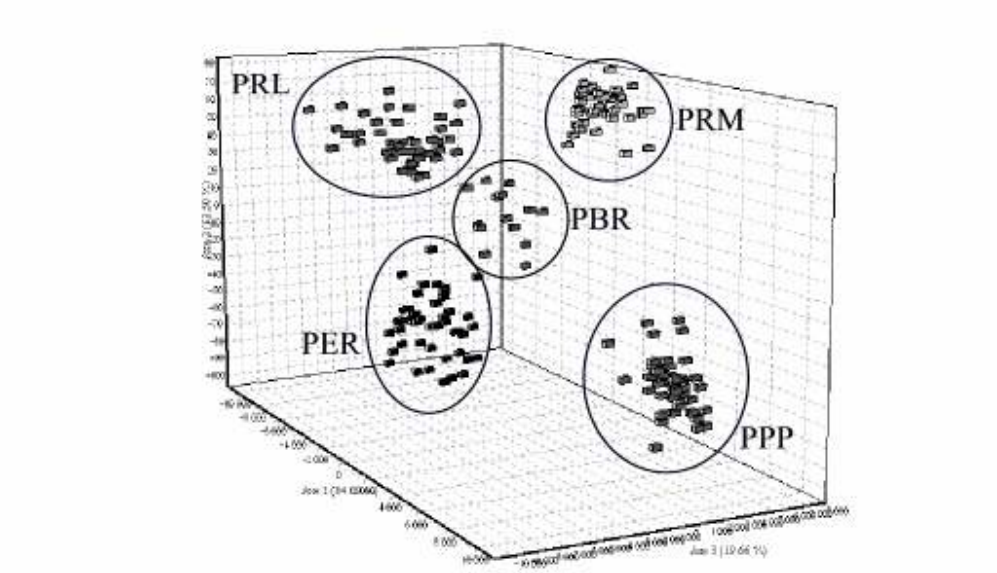
The Neighbor-Joining tree in Figure 1 was drawn from $D_{Reynolds}$ distance matrix obtained analysing the molecular markers. For the validation of the tree topology, 1000 bootstraps resampling were performed. The dendrogram clearly clustered two groups (supported by a bootstrap value of 63%): one includes PRL and PRM, the other one the remaining three breeds. In this second group PPP forms a separate cluster but with lower bootstraps scores (43%).

Figure 1. Neighbor-Joining tree drawn from $D_{Reynolds}$ distance estimated by microsatellite markers (1000 bootstraps resampling)



The factorial correspondence analysis defined three main factors (Figure 2). The first one explained the 34% of total variance, the second one 24% while the third one 20%. On the whole, this analysis reported a clear breed grouping trend and a good distinction among breeds.

Figure 2. Distribution of individual factorial weights for factor 1, factor 2 and factor 3 of broiler (PBR), Robusta Lionata (PRL), Robusta Maculata (PRM), Ermellinata di Rovigo (PER) and Pèpoi (PPP) chicken breeds



CONCLUSION

Microsatellite markers permitted the genetic characterisation of the four indigenous breeds of chickens. The optimum use of such information can help to preserve allelic diversity and the existing genetic variation. The obtained results seem to be promising to define and control the ongoing animal genetic resources conservation program. The microsatellites panel adopted for this study could also be useful for genetic traceability purposes. Tracing the breed of origin of animal products represents an opportunity for the promotion of local genetic resources with benefits for local economy, breed valorisation and sustainable conservation of biodiversity.

The authors wish to thank Martina Gervaso and Maristella Baruchello for their support. This research was funded by the "Veneto Agricoltura" regional agency.

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(Received on 16 June 2007; accepted on 3 July 2007)