The genetic variability of the Podolica cattle breed from the Gargano area. Preliminary results

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ABSTRACT

The Podolica cattle breed is autochthonous of Southern Italy and denoted by its particular rusticity. This study presents the preliminary results of the genetic characterization of the Podolica breed using DNA STR markers. A total of 20 microsatellite loci were analysed in 79 individuals reared in the Gargano area. Number of polymorphisms, allele frequencies, deviations from Hardy-Weinberg proportions, linkage disequilibrium between loci and genetic similarities between animals were calculated. The results showed a high deficiency of heterozygotes, the observed mean of heterozygosis being 0.449, whereas the expected mean was 0.766. Many markers showed also deviations from the Hardy-Weinberg proportions and significant linkage disequilibrium between loci. However the genetic similarity within the population was low (0.281) and the average number of alleles per locus was high (10), representing a high genetic variability. In order to explain these results, a stratification of the breed in sub-populations with a high interior genetic homogeneity but markedly differentiated one from each other could be hypothesized; this situation probably derived from non-random mating within each herd (consanguinity) and from the lack of exchange of genetic material between the herds. A further study is needed on a wider sample and extending the analysis to FAO-ISAG microsatellite panel in order to confirm this hypothesis. This could eventually provide the information necessary for the correct management of the reproductive schemes and for genomic traceability of meat production.

Key Words: Microsatellites, Cattle, Genetic variability, Polymorphism, Heterozygosis.

RIASSUNTO

LA VARIABILITÀ GENETICA DELLA RAZZA BOVINA PODOLICA GARGANICA. RISULTATI PRELIMINARI

Vengono riferiti i risultati di una indagine preliminare sulla variabilità genetica della razza bovina Podolica, caratterizzata da doti di rusticità e frugalità, condotta avvalendosi di metodologie molecolari. Sono stati analizzati 20 microsatelliti del DNA su un campione di 79 soggetti Podolici presenti sul territorio garganico. L'analisi statistica ha considerato i seguenti parametri: numero di alleli per locus, frequenze alleliche, deviazioni dalle proporzioni di Hardy-Weinberg, linkage disequilibrium *tra i loci e rassomiglianza genetica tra i soggetti indagati. I risultati mostrano una consistente deviazione dalle proporzioni di Hardy-Weinberg, associata a difetto di eterozigoti e linkage disequilibrium significativo per molti loci. L'eterozigosi media osservata è pari a 0,449, contro un'eterozigosi media attesa di 0,766. Si è tuttavia anche osservata una bassa rassomiglianza genetica (0,281) ed un numero medio di alleli per locus pari a 10 all'interno della popo-*

lazione studiata. Una possibile interpretazione dei risultati potrebbe considerare l'esistenza nella popolazione di sotto-popolazioni geneticamente omogenee al loro interno, ma ben differenziate le une dalle altre. Tale tendenza potrebbe derivare dalla presenza di legami di parentela tra i soggetti, causata da accoppiamenti non casuali all'interno delle singole aziende e dal mancato scambio di materiale genetico tra le aziende. In questo modo sarebbe possibile spiegare sia l' elevata variabilità genetica che l'eccesso di omozigoti. Un ulteriore studio condotto su un più ampio campione e l'ampliamento dei loci analizzati a favore del panel raccomandato FAO-ISAG potrà permettere di confermare queste ipotesi e di giungere alle opportune valutazioni per una più corretta gestione riproduttiva, nonché per la tracciabilità molecolare della produzione della carne.

Parole chiave: *Microsatelliti, Bovino, Variabilità genetica, Polimorfismo, Eterozigosità.*

Introduction

Over the last decade a lot of negative episodes, such as Bovine Spongiform Encephalopathy and dioxine scandal, affected the bovine meat industry in Europe. Consumers are consequently going to be increasingly careful about information regarding meat quality and its origin. In Italy there are many different local cattle breeds, but some of them are not yet genetically characterized. One of the most interesting is the Podolica breed, which is a typical breed well adapted to the difficult climatic conditions (Cianci, 1986) of the rural areas of Southern Italy and which produces high quality milk and meat. Unfortunately, in the past the Podolica cattle breed was crossed with other populations to improve productive performances. This practice resulted in a partial loss of original genetic variants, still not well known, due to the lack of checked genealogical information and to the availability of limited genotypic data (Bruzzone *et al.*, 2001, Moioli *et al.*, 2004). Nevertheless, in recent years breeders have been trying to restore the native population which better fits under local environmental conditions. This research addresses the genetic characterization of the Podolica cattle breed from the Gargano area using DNA microsatellites as genomic markers.

Material and methods

Animals

We investigated 79 Podolica subjects reared in Gargano area (Puglia, Southern Italy). Individuals belonged to seven different farms (denoted as A to G). Samples were selected in order to minimize relationships between animals, though this was particularly difficult due to the characteristics of the breeding system of Podolica cattle breed in the Gargano area and to the general lack of accurate genealogical information.

DNA extraction

DNA was extracted from 5 mL of peripheral blood samples. To each sample 45 mL of saline buffer (0.32 M-Sucrose, 10mM-Tris-HCl pH 7.5, 5 mM-$MgCl_2$, 1% Triton X-100) were added. The pellet was spun down at 3500 rpm for 30 min at 4°C and washed twice with 10 mL of 0.075 M-NaCl, 0.025 M-EDTA. The pellet was then suspended in 3 mL of 10 mM-Tris-HCl pH 8.0, 2 mM-EDTA, 100 μL of 10% SDS and 30 μL of Proteinase-K (10 mg/mL) was added. The resulting nuclear lysate was incubated at 65°C for one hour. After incubation 500 μL of 5 M-NaCl was added and the precipitated proteins were spun down at 3000 rpm for 10 min. The aqueous layer was recovered and DNA was precipitated by adding 6 mL of isopropanol. The resulting DNA helix was recovered and washed twice with 70% ethanol. DNA was then redissolved in 500 μL of TE buffer.

Microsatellite analysis

Information on the 20 microsatellites investigated is presented in Table 1. Four of them (BM1818, INRA032, ETH152, INRA063) belong to the cattle biodiversity panel (ISAG/FAO, 2004). The 20 microsatellites were amplified in 4 multiplex independent PCR reactions. The PCR amplifications were performed in a 50 μL reaction containing 20 ng of extracted DNA, 25 μL of Quiagen Multiplex PCR Master Mix containing 3 mM-$MgCl_2$ and 0.2 μM of each primer. The PCR reactions were carried out in a thermocycler (i-Cycler, Biorad) with the following conditions: preheating at 95°C for 15 min followed by 35 cycles of 94°C for 30 s, 58°C for 120 s and 72°C for 60 s. A final

Table 1. Information about the 20 analysed microsatellite loci.

Microsatellite	Chr	Locus	N. of alleles[1]	Size range (bp)[1]	References
BM143	6	D6S13	14	90-122	Kappes et al., 1997
BM1818	23	D23S21	5	256-270	Kappes et al., 1997
BM4311	6	D6S8	6	89-105	Kappes et al., 1997
BMS1678	14	BMS1678	8	116-134	Kappes et al., 1997
BMS1747	14	BMS1747	14	79-117	Kappes et al., 1997
BMS1782	24	BMS1782	6	70-86	Kappes et al., 1997
BMS518	6	BMS518	9	134-160	Kappes et al., 1997
BMS690	6	BMS690	12	129-159	Kappes et al., 1997
ETH131	21	D21S4	12	138-164	Steffen et al., 1993
ETH152	5	D5S2	12	163-203	Steffen et al., 1993
INRA006	3	D3S9	8	100-118	Vaiman et al., 1992
INRA032	11	D11S9	9	160-206	Vaiman et al., 1994
INRA050	15	D15S6	16	120-164	Vaiman et al., 1994
INRA053	7	D7S6	9	94-112	Vaiman et al., 1994
INRA063	18	D18S5	12	158-184	Vaiman et al., 1994
RBP3	28	RBP3	9	126-148	Kappes et al., 1997
TGLA227	18	D18S1	10	75-99	Georges et al., 1992
TGLA304	20	D20S10	8	81-99	Kappes et al., 1997
TGLA53	16	D16S3	12	151-183	Georges et al., 1992
URB011	29	D29S27	9	122-148	Kappes et al., 1997

[1] *Number of alleles and allele size referred to the Podolica sample analysed in the present study.*

extension at 60°C for 30 min was then performed. PCR amplifications were controlled by agarose gel electrophoresis. Genotyping was carried out using an Applied Biosystems 310 DNA sequencer. Data GeneScan Software (Perkin Elmer/ABI) and analysed with Genotyper 2.0 Software (Perkin Elmer/ABI).

Statistical analyses

Allelic frequencies were estimated by direct counting. The presence of null alleles for each locus was tested using MICRO-CHECKER version 2.2.1 (Van Oosterhout *et al.,* 2004). Exact tests for deviations from the Hardy-Weinberg equilibrium (HWE), heterozygote deficiency and pair-wise linkage disequilibrium among microsatellite loci were performed using the ARLEQUIN package (Schneider *et al.*, 2000). Wright's F-statistics was computed using FSTAT version 2.9.3 (Goudet, 2001).

Genetic similarities among the 79 animals were investigated by comparing individual multilocus genotype of each individual with each other (Ciampolini *et al.*, 1995). Genetic similarity is defined as P=A/2L, where P is the proportion of common alleles (A) in relation to the 2L possibilities (L= number of considered loci). The similarities between each pair of individuals were then averaged over the whole population. Genotype assignment test of individuals from different farms (A to F) was performed using the ARLEQUIN package in order to highlight possible genetic sub-structures in the total sample originating from farm membership.

Results and discussion

Allelic size and number of alleles per locus are presented in Table 1. The mean number of alleles per locus in the population was 10 (ranging from 5 to 16). Microsatellites INRA032, INRA063 showed a number of alleles higher than reported in litera-

Table 2. Observed (H_{obs}) and expected (H_{exp}) heterozygosity for each microsatellite in the total sample.

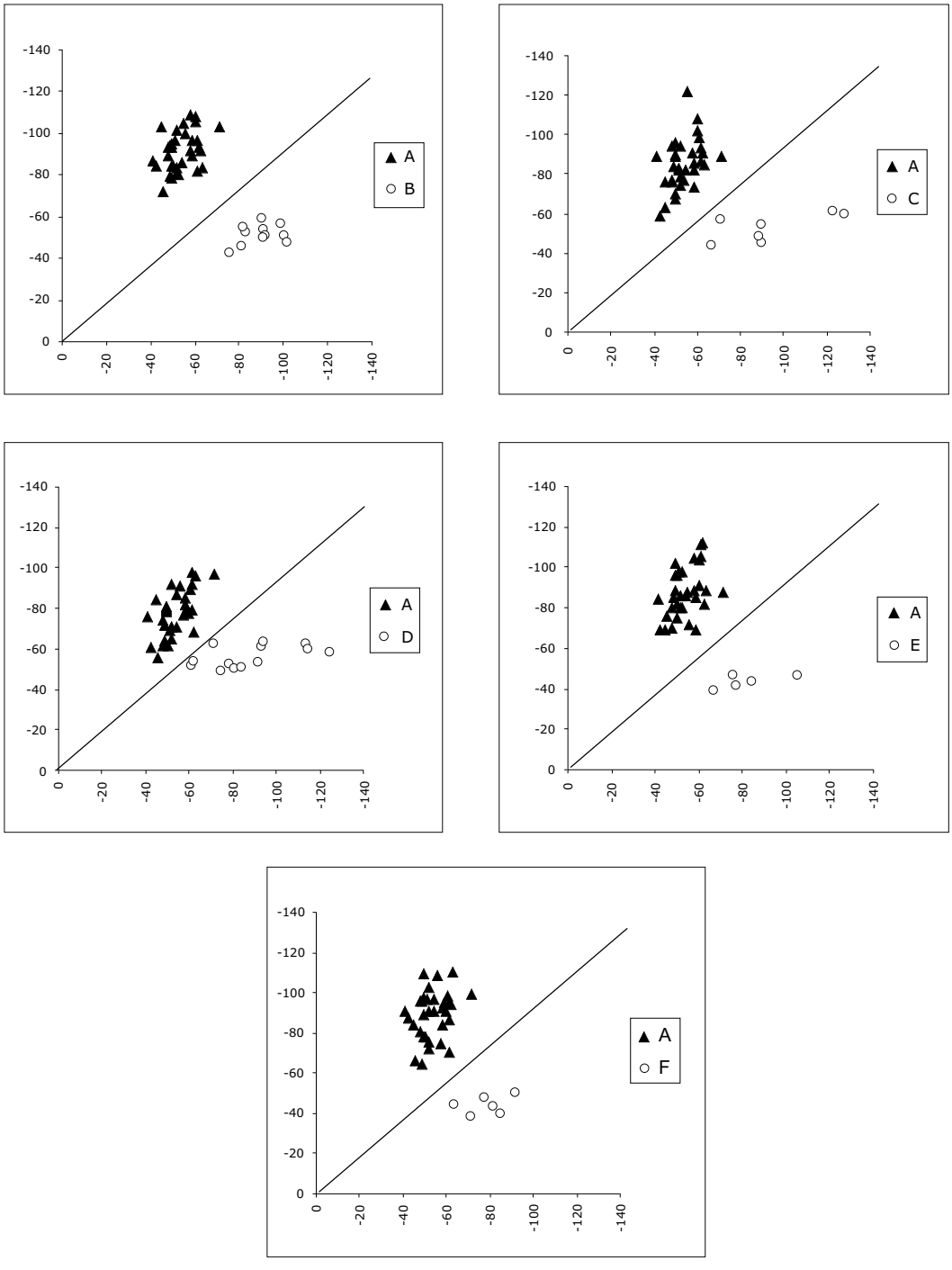
Microsatellite	H_{obs}	H_{exp}	*P*	*SD*[1]
BM143	0.350	0.844	<0.05*	<0.05*
BM1818	0.625	0.645	0.116	<0.05*
BM4311	0.600	0.695	0.343	<0.05*
BMS1678	0.362	0.778	<0.05*	<0.05*
BMS1747	0.362	0.879	<0.05*	<0.05*
BMS1782	0.225	0.671	<0.05*	<0.05*
BMS518	0.400	0.610	<0.05*	<0.05*
BMS690	0.325	0.742	<0.05*	<0.05*
ETH131	0.325	0.853	<0.05*	<0.05*
ETH152	0.562	0.856	<0.05*	<0.05*
INRA006	0.375	0.731	<0.05*	<0.05*
INRA032	0.550	0.837	<0.05*	<0.05*
INRA050	0.637	0.838	<0.05*	<0.05*
INRA053	0.675	0.781	<0.05*	<0.05*
INRA063	0.475	0.780	<0.05*	<0.05*
RBP3	0.337	0.529	<0.05*	<0.05*
TGLA227	0.637	0.847	<0.05*	<0.05*
TGLA304	0.225	0.797	<0.05*	<0.05*
TGLA53	0.425	0.862	<0.05*	<0.05*
URB011	0.512	0.753	<0.05*	<0.05*
average	0.449	0.766		

* *Significant P values*

[1] *SD = Standard Deviation*

ture (Ciampolini *et al.*, 1995; Ciampolini *et al.*, 2000; Bruzzone *et al.*, 2001), while ETH131, INRA006, INRA032, INRA053, TGLA53 showed a number of alleles lower than reported in literature (Ciampolini *et al.*, 2000; Moioli *et al.*, 2004). A remarkable proportion of all alleles (24.5%) was found in a single farm only. Observed heterozygosity averaged over loci was 0.449, whereas expected heterozygosity was 0.766 (Table 2). Only 2 microsatellites (BM1818, BM4311) were in Hardy-Weinberg equilibrium ($P < 0.05$); deviations from Hardy-Weinberg proportions were in all cases associated with heterozygote deficiency. Possible interpretations for these results are: *I)* inbreeding within subjects coming from the same farm, *II)* low exchange of genetic material between herds, *III)* segregation of non amplifying (null) alleles. The presence of null alleles for each locus was tested using MICRO-CHECKER; the software indicated the presence of possible null alleles for 18 out of 20 analysed loci ($P < 0.05$); such a high proportion could hardly be explained as a failure in PCR amplification due to indiscriminate mutations at priming sites. Therefore, results suggest that the phenomenon is rather due to a real genetic effect than to experimental artefacts. The high positive FIS value observed for the Podolica breed (0.411) compared to those highlighted for other breeds (Holstein Friesian, 0.030; Marchigiana 0.052; Chianina 0.056; Charolaise 0.080; Limousine, 0.115; unpublished data) supports the hypothesis of inbreeding within subjects coming from the same farm. Inbreeding could also be considered as a possible explanation for the significant linkage disequilibrium observed in this breed. In fact, as more than one marker was located at times on the

Figure 1. Genotype assignment of individuals coming from the Farm A against all the other possible farm origins. Symbols represent the log-likelihood that an individual coming from a given farm belongs to its true farm versus the log-likelihood that it belongs to the other farm.



same chromosome, we tested all possible pairs of loci for linkage disequilibrium. A total of 190 pairwise tests were carried out and 66 of them (> 34%) showed P values < 0.05; this proportion is significantly higher than expected by chance (χ^2 = 6.59 x 10^{-79}, 1 d. f., P < 0.05). Syntenic loci contributed only two significant contrasts out of 66, thus revealing the presence of highly significant gamete imbalance in the total sample.

Genetic similarity within the population was 0.281 (data not shown), representing a rather high genetic variation. We could suppose a subdivision of the breed in sub-populations genetically homogeneous, but markedly differentiated one from each other, probably resulting from non-random mating. This could explain such a high heterozygote deficiency and the low genetic similarity within the population.

To test this hypothesis we performed a genotypic assignment test of individuals coming from different farms. Results showed a clear stratification of the total sample into six separate sub-populations corresponding to subjects with a different farm origin. None of the tested animals was assigned to the wrong farm. As an example, Figure 1 shows the results of the assignment test performed on farm A versus all the other farms. The remarkably high proportion of correct assignment (100%) may be related to the significant presence of alleles private to individuals coming from each farm.

Conclusions

The high genetic variability found in the Podolica cattle breed, as compared with the variability of other cattle breeds, with similarity coefficients of 0.281 *vs.* 0.350 – 0.490 (Ciampolini *et al.*, 2001), is consistent with evidence that the population has undergone in the past the influx of other breeds, in order to improve its productive performances. Subsequently, the non-random mating practiced by breeders within each farm has probably been the cause of significant deviations from Hardy-Weinberg equilibrium and marked homogeneity within each herds. A further study is needed on a wider sample and extending the analysis to FAO-ISAG microsatellite panel in order to confirm this hypothesis. This could eventually provide the information necessary for the correct management of the reproductive schemes and for genomic traceability of meat production.

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