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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’eterozogosi media osservata è pari a 0,449, contro l’eterozogosi 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-
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-MgCl₂, 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-MgCl₂ 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
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).

**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 literature.

### Table 1. Information about the 20 analysed microsatellite loci.

| Microsatellite | Chr | Locus | N. of alleles | Size range (bp) | References |
|---------------|-----|-------|---------------|----------------|------------|
| BM143         | 6   | D6S13 | 14            | 90-122         | Kappes et al., 1997 |
| BM1818        | 23  | D2S321| 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            | 200-164        | Vaiman et al., 1994 |
| INRA053       | 7   | D7S6  | 9             | 94-112         | Vaiman et al., 1994 |
| INRA063       | 18  | D18S5 | 12            | 158-214        | 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             | 122-149        | Kappes et al., 1997 |

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

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

| Microsatellite | \(H_{obs}\) | \(H_{exp}\) | \(P\)  | \(SD\)  |
|----------------|------|------|-----|-------|
| 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*|
| BMS5518        | 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
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 ($\chi^2 = 6.59 \times 10^{-79}$, 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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