Journal of Heredity 2004:95(3):250–256
DOI: 10.1093/jhered/esh032

Genetic Diversity between Piedmontese, Maremmana, and Podolica Cattle Breeds

B. Moioli, F. Napolitano, and G. Catillo

From the Istituto Sperimentale per la Zootecnia, via Salaria 31, 00016 Monterotondo, Italy.

Address correspondence to Bianca Moioli at the address above, or e-mail: bianca.moioli@isz.it.

One hundred twenty animals of three native Italian cattle breeds, Piedmontese, Maremmana, and Podolica, were genetically characterized at 21 microsatellite loci located on 13 chromosomes. Allele numbers ranged from 3 to 19; average gene diversity ranged from 0.206 to 0.878 (average 0.738). The breed that preserved the highest genetic variability was the Podolica, where the chosen markers show the highest gene diversity (0.741) and the highest heterozygosity (0.155). The lowest inbreeding rate (0.102) was registered by the Piedmontese. Genetic distances were 0.069 (Piedmontese versus Maremmana), 0.050 (Piedmontese versus Podolica), and 0.041 (Maremmana versus Podolica) and reflect the different phylogenetic origins of the breeds: Maremmana and Podolica originated from the Grey Steppe cattle group, while Piedmontese belongs to the Northern Italy Lowland cattle group. Observed heterozygosity was not significantly different from expected in any of the breeds, which is an indication that they maintain a random mating structure. The probabilistic assignment of all sampled individuals to three theoretical populations, on the basis of allele frequencies, indicated that 82% of Piedmontese, 66% of Maremmana, and 33% of Podolica can be assigned to the appropriate breed with a probability higher than 90%. This result very well reflects the impact of the selection activity on the breed genetic structure. The chosen microsatellites proved to be a good tool for describing the correct reality of the analyzed populations, but they are not sufficient to discriminate between breeds.

The genetic characterization of three native Italian cattle breeds—Piedmontese, Maremmana, and Podolica—was performed with the use of microsatellites. These breeds were very popular and widespread in Italy before World War II, as dual- or triple-purpose breeds, but registered a consistent reduction in their numbers afterwards due to three major factors: mechanization in agriculture; urbanization; and competition from high-yielding breeds. Their numbers of 640,000, 288,000, and 630,000, respectively (Rognoni and Pagnacco 1983), were reduced by 55%, 90%, and 80%, respectively (FAO 2002).

The Piedmontese belongs to the cattle breeds of the Northern Italy Lowland group, the ancestral origin of which is referred to *Bos brachyceros* and to a mixing of *B. brachyceros* and *Bos primigenius* (Baker and Manwell 1980). Typical of a triple-purpose breed, it was selected in the 1970s for improvement of milk production, through milk performance recording of productivity, while maintaining beef characteristics. In the 1980s, the Breed Society decided to give up milk recording activity and modified the breeding goal to improve beef traits only. The particular characteristic of the Piedmontese cattle breed is in fact muscular hypertrophy, better known as the "double muscle factor." Milk production of the breed is, however, still more than sufficient to suckle the calf, and several farmers still milk their cows and process milk into typical cheeses. Selection for improving beef characteristics has been regular and intense, and has taken advantage of artificial insemination, which is widespread in this breed.

The Maremmana and Podolica breeds belong to the Grey Steppe group of cattle, the ancestral origin of which is referred to *B. primigenius* (Baker and Manwell 1980). Typical rustic breeds used mainly for draught, the Podolica used to be milked as well, while the Maremmana has never been milked. In the 1970s, they were included in the Italian Beef Breeds Society (ANABIC), but no performance testing was carried out until the mid-1980s, when two performance testing stations for young bulls were created. Both breeds are now officially performance tested and selected for improvement of growth and beef characteristics; however, it is believed that selection has not modified the genetic structure of either of them, because artificial insemination is used very little, and because the Podolica farmers milk their cows and are supposed to perform their own selection of females on the basis of milk yield.

The goal of this study was to investigate and compare the genetic diversity of the three native breeds; although their population size is far from making them "critical" or

"endangered" (FAO 2000), the very consistent decline, which affected the Maremmana, caused the Breed Society to search for tools providing scientific awareness of the genetic structure of the breed that could be taken into account to guide conservation programs with objective criteria. Moreover, because the main breeding goal for the three breeds is meat production, a further opportunity for their relaunch is to provide genetic markers that allow individual traceability of slaughtered animals. At the moment, also in this context, microsatellites are the markers of choice, considering that none of these breeds, up to now, was widely genotyped at microsatellite loci.

Materials and Methods

Animals for genotyping were chosen in order to ensure that they were a representative sample of each breed, covering all geographical areas in which the breeds are reared and sampling as many herds as possible. The Piedmontese sample consisted of 37 animals from 13 herds located in six different geographical areas (Torino, Cuneo, Novara, Vercelli, Savona, and Alessandria); the Maremmana sample consisted of 38 animals from 12 different herds located in four different geographical areas (Grosseto, Viterbo, Roma, and Latina); the Podolica sample consisted of 45 animals from 17 different herds in three different geographical areas (Potenza, Matera, and Foggia). In order to avoid the random occurrence of mutations within each breed, age differences between sampled animals of the same breed was a maximum of 24 months.

DNA extracted from frozen blood with the GENOMIX Extraction Kit (Talent, Trieste, Italy) was amplified at the following 21 microsatellite loci—*BMC1013*, *CSSM33*, *CSSM36*, *CSSM38*, *CSSM43*, *CSSM47*, *CSSM60*, *CSSM70*, *ETH03*, *ETH10*, *ETH121*, *ETH225*, *TGLA44*, *TGLA53*, *TGLA110*, *TGLA122*, *TGLA126*, *TGLA226*, *TGLA337*, *TGLA431*, and *SPS115*—with polymerase chain reaction (PCR) using primer sequences as suggested in the literature (Moore et al. 1995; Solinas Toldo et al. 1993; National Centre for Biotechnology Information–GenBank). Chromosome assignment of the chosen microsatellites (McGraw et al. 1997; Moore et al. 1994; Solinas Toldo et al. 1993) indicates that they are located on 13 different chromosomes. For all microsatellites, allele size was determined, on all samples, with a Perkin Elmer ABI Prism 310 Genetic Analyzer, using the Genescan software (Perkin Elmer), which detects different alleles through comparing sizes with standard DNA sizes (Tamra, Perkin Elmer).

Two approaches were used in analyzing the genotyping results. The first was to calculate all common estimators of genetic variability (Nei 1973; Wright 1943, 1951) between and within breeds; the second approach was to pretend to ignore the breed of each sample, and to make groups of the animals on the basis of the most common allele recurrence in each group in order to maximize allelic similarities within each group, maximizing allelic differences between groups.

The analyses of the genetic variation of each breed and locus were performed using the FSTAT computer program (Goudet 1995). This program calculates the average gene diversity at each locus according to Nei's formula (Nei 1973), observed and expected heterozygosity at each locus and overall (Nei 1978), and Weir and Cockerman (1984) estimators of Wright's fixation indices (F_{IT}, F_{ST}, and F_{IS}) of genetic differentiation.

The effects contributing to increasing average gene diversity were estimated by regressing the average gene diversity at each locus on the number of alleles at that locus (Barker et al. 1997) and on the difference between the total number of alleles and the average number of shared alleles; in both regression analyses, allele frequency was also included, as a fixed effect, with the following three classes: (a) alleles at highest frequency are different for the three breeds; (b) allele at highest frequency is the same for two breeds; (c) allele at highest frequency is the same for the three breeds (SAS version 6; SAS Institute 1989).

Paired t tests were performed (SAS version 6; SAS Institute 1989) to determine if there were significant differences between expected and observed heterozygosities within each population, at each locus, and overall. The probabilistic assignment of the animals to different populations on the basis of allele frequencies was performed through the program Structure (Pritchard et al. 2000), which implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. The percentage of animals of each breed admitted to the appropriate cluster at different probability levels was determined by direct counting.

For the Piedmontese breed, the values of genetic merit for three traits—growth, beef conformation, and breed characteristics—were available for all genotyped animals; therefore differences in the indexes of animals with different probabilities of belonging to the Piedmontese cluster were compared with SAS version 6 (SAS Institute 1989).

Results

In Tables 1 and 2, allele size, allele frequencies, and the number of total and shared alleles for each of the 21 microsatellite loci are reported. The number of alleles per locus varied from 3 (*BMC1013*) to 19 (*TGLA122*). The mean number of alleles per locus is 7.3, 7.5, and 8.5, respectively, for the Piedmontese, Maremmana, and Podolica samples.

Only at two loci (*BMC1013* and *CSSM70*) were all detected alleles found in the three breeds. At six loci (*CSSM33*, *CSSM47*, *CSSM60*, *TGLA431*, *TGLA110*, *SPS115*) the same alleles are at the highest frequency for the three breeds. At five loci (*CSSM36*, *ETH10*, *TGLA53*, *TGLA122*, *TGLA226*) Maremmana and Podolica showed the highest frequencies at the same alleles; at six loci (*CSSM43*, *ETH121*, *ETH225*, *TGLA44*, *TGLA126*, *TGLA377*) Maremmana showed the highest allele frequency at the same loci as the Piedmontese, while the Podolica and the Piedmontese have highest allele frequencies at only two loci (*CSSM38* and *CSSM70*). At loci *BMC1013* and *ETH03*, the three breeds registered the highest allele frequency at

Table 1. Detected allele size and allele frequencies (%) in the breeds Piedmontese (PD), Maremmana (MA), and Podolica (PO)

Locus/allele size (bp)	Allele frequency %			Locus/allele size (bp)	Allele frequency %			Locus/allele size (bp)	Allele frequency %		
	PD	MA	PO		PD	MA	PO		PD	MA	PO
BMC1013				*CSSM47*				*ETH10*			
212	24.3	48.7	21.1	138	—	6.6	—	215	1.3	—	1.1
216	25.7	19.7	41.1	140	—	4.0	—	217	1.3	—	16.7
218	50.0	31.6	37.8	142	—	4.0	5.6	219	48.7	10.5	28.9
CSSM33				146	—	11.8	2.2	221	28.4	51.3	33.3
153	9.5	2.1	4.5	148	73.0	27.6	36.7	223	12.2	27.6	15.6
155	23.0	12.5	34.1	150	12.2	25.0	15.6	225	8.1	5.3	4.4
157	1.3	10.4	—	152	6.8	—	5.6	227	—	5.3	—
159	12.2	12.5	13.7	156	—	4.0	9.0	*ETH121*			
161	48.6	62.5	47.7	158	5.4	2.6	12.0	176	13.5	2.7	1.1
163	5.4	—	—	164	1.3	6.6	5.6	178	5.4	2.7	7.8
CSSM36				166	1.3	1.2	3.3	182	—	—	1.1
161	2.8	16.2	12.2	170	—	6.6	—	186	—	5.4	—
163	45.8	9.7	11.0	172	—	—	4.4	188	13.5	—	—
167	—	2.7	1.2	*CSSM60*				190	—	2.7	2.2
169	2.8	—	1.2	86	—	—	1.1	192	2.7	4.0	10.0
171	—	14.9	6.1	88	1.3	—	2.2	194	5.4	1.3	4.4
173	13.9	45.9	39.0	90	13.5	12.5	14.4	198	—	8.1	1.1
175	2.8	4.0	4.9	92	4.0	1.3	1.1	200	5.4	2.7	2.2
177	11.0	1.2	2.4	94	8.2	1.3	6.7	202	2.7	2.7	4.4
179	16.8	2.7	15.9	96	5.4	1.3	8.9	204	21.6	20.6	38.0
181	4.1	—	6.1	98	6.8	1.3	12.2	206	4.0	4.0	2.2
193	—	2.7	—	100	56.8	55.9	41.2	208	24.5	33.6	23.3
CSSM38				102	4.0	2.8	1.1	210	1.3	9.5	2.2
156	—	2.6	7.8	104	—	23.6	2.2	*ETH225*			
158	48.7	27.6	20.0	112	—	—	3.3	134	21.6	11.8	21.1
160	4.0	1.3	7.8	114	—	—	5.6	136	8.1	1.3	—
166	5.4	1.3	1.1	*CSSM70*				138	17.6	33.0	22.2
172	—	3.9	1.1	127	1.4	6.6	9.1	140	5.4	6.6	—
174	1.3	—	3.3	129	34.7	25.0	18.2	142	13.5	—	26.7
176	9.5	1.3	18.9	131	40.3	30.3	37.5	144	32.4	35.5	22.2
178	2.7	—	11.1	133	22.2	32.9	27.3	146	1.4	—	—
180	1.3	11.8	6.7	135	1.4	5.2	7.9	148	—	11.8	6.7
182	21.7	33.1	11.1	*ETH03*				158	—	—	1.1
184	5.4	6.6	7.8	106	1.3	—	1.2	*TGLA44*			
196	—	1.3	2.2	112	—	4.0	5.1	142	—	1.3	—
190	—	9.2	1.1	114	44.0	16.2	34.6	144	5.6	1.3	1.1
CSSM43				116	6.8	12.2	6.4	146	—	—	1.1
246	—	1.3	1.1	118	2.6	1.3	—	156	—	2.6	3.4
248	1.3	—	3.3	120	—	5.4	—	158	—	5.3	11.4
250	21.6	21.0	33.3	122	22.0	8.1	36.1	160	—	2.6	—
252	43.2	29.0	30.0	124	22.0	8.1	15.4	162	75.0	33.0	9.1
254	18.9	18.4	17.8	126	1.3	44.7	1.2	164	8.3	14.5	21.6
256	1.3	10.5	6.7					166	7.0	19.7	37.5
258	8.1	2.6	4.4					168	—	14.5	6.8
260	4.3	13.2	—					170	—	1.3	5.7
262	1.3	4.0	3.4					174	2.8	—	2.3
								176	1.3	3.9	—
TGLA53				*TGLA126*				*SPS115*			
146	4.0	5.3	—	114	—	—	2.2	243	26.6	70.0	35.2
148	5.4	3.9	2.2	116	25.0	10.0	29.0	245	3.0	—	9.3
154	15.1	31.8	25.7	118	25.0	34.3	23.3	247	26.6	2.5	18.7
156	—	—	2.2	120	—	1.4	7.8	249	18.7	10.0	21.0
158	10.8	7.9	1.1	122	8.3	24.3	22.2	251	11.0	—	5.2
160	2.7	3.9	6.7	124	19.5	22.9	14.4	253	6.3	5.0	1.6
162	23.0	15.8	7.8	126	22.2	7.1	1.1	255	6.3	7.5	7.4
164	21.6	10.5	12.2	*TGLA226*				257	1.5	5.0	1.6
166	2.7	2.6	4.4	130	—	14.9	1.1				
170	2.7	3.9	4.4	136	1.3	—	—				
172	4.0	3.9	14.5	142	25.7	25.7	18.6				
174	—	—	1.1	144	16.2	42.0	52.3				

Table 1. Continued

Locus/allele size (bp)	Allele frequency %			Locus/allele size (bp)	Allele frequency %			Locus/allele size (bp)	Allele frequency %		
	PD	MA	PO		PD	MA	PO		PD	MA	PO
176	5.4	1.3	11.1	146	29.8	4.0	21.0				
178	1.3	9.2	3.3	148	18.9	13.4	7.0				
180	—	—	2.2	150	8.1	—	—				
182	—	—	1.1	*TGLA377*							
184	1.3	—	—	98	28.4	26.3	27.4				
TGLA110				100	—	2.6	3.2				
168	89.2	92.1	85.6	102	50.0	42.1	21.0				
170	9.5	7.9	7.8	104	15.0	15.8	30.7				
174	1.3	—	4.4	106	5.3	5.2	6.4				
176	—	—	2.2	108	—	6.7	9.7				
TGLA122				110	1.3	1.3	1.6				
132	—	5.5	1.1	*TGLA431*							
136	1.3	5.5	3.5	132	15.0	16.4	11.0				
138	18.9	48.0	45.7	134	4.0	13.5	5.6				
140	—	—	3.5	136	6.8	—	1.0				
142	4.0	12.5	3.5	138	63.7	50.0	57.1				
144	10.8	—	1.1	140	4.0	13.5	2.2				
146	24.3	4.2	17.4	142	1.3	4.0	1.0				
148	14.9	5.6	10.5	148	1.3	—	2.2				
152	10.8	—	—	150	—	—	4.4				
156	7.1	—	4.6	152	1.3	—	—				
158	2.7	—	2.3	154	1.3	1.3	2.2				
160	—	—	2.3	156	—	—	3.3				
166	—	2.7	1.1	158	—	1.3	5.6				
168	1.3	2.7	—	162	1.3	—	4.4				
170	—	1.3	—								
172	1.3	—	1.1								
174	—	—	2.3								
176	1.3	5.6	—								
178	1.3	6.4	—								

three different alleles. Allele frequencies of microsatellite SPS115 in the Podolica breed showed exactly the same trend as in Bruzzone et al. (2001); they recently characterized Podolica animals from different herds than those from which our animals were sampled, using 17 microsatellites different from those chosen for the present work except SPS115.

Average gene diversity (Table 2) over all loci was highest in the Podolica breed (0.741), while for individual loci it ranged from 0.206 (*TGLA110*) to 0.878 (*TGLA53*). Across loci, average gene diversity increases with an increasing number of alleles (regression coefficient = 0.016 ± 0.006; $P = .01$), and if higher, are the differences in allele frequencies between breeds ($P = .02$). Moreover, average gene diversity increases with increasing differences between total and shared alleles (regression coefficient = 0.008 ± 0.003; $P = .03$), and if higher are the differences in allele frequencies between breeds ($P = .03$).

The values of heterozygosity, both observed and expected (Table 3), have the highest values in the Podolica breed. Observed heterozygosity is always less than the expected, although not significantly, when averaged over all loci. When the differences between observed and expected heterozygosity were analyzed at each locus, each breed showed, at only one locus, significant differences at a probability level less than 0.20. These loci are *ETH10* (Piedmontese), *CSSM70* (Maremmana), and *CSSM33* (Podolica).

The mean estimate for F_{ST} (differentiation between the three breeds) was 0.060 ± 0.011. Genetic distances were as follows: 0.069 (Piedmontese versus Maremmana), 0.050 (Piedmontese versus Podolica), and 0.041 (Maremmana versus Podolica). Results of the assignment of animals to each of the three clusters (Structure software) indicate that 82% of Piedmontese, 66% of Maremmana, and 33% of Podolica can be assigned to the appropriate breed with a probability higher than 90% (Table 4).

The Podolica animals again show the highest genetic variability: only 15 of them (33%) can be assigned to their breed with a probability higher than 90%, while 38% can be assigned at a probability between 70% and 90%, and 9% at a probability between 60% and 70%; however, the 33% that best fit in the Podolica cluster belong to 10 different herds (out of 17) of all three geographical areas, including one herd that is not registered in the official Herd Book (i.e., which is not supposed to carry out the selection activity following the directives of the Breed Society). The 20% that were genetically found to be the least Podolica (probability less than 60%; nine animals) belong to six herds, and in each of these herds there are also animals falling in the most typical 33%. In four of these nine animals, the alleles of the Maremmana breed prevail, while in five the alleles of the Piedmontese breed prevail. Three of the animals in which the Maremmana alleles prevail belong to the same herd.

Table 2. Number of microsatellite alleles at each locus in the three breeds, number of shared alleles, and average gene diversity within populations

	Number of alleles				Shared alleles by				Average gene diversity			
Locus	Total	PD	MA	PO	PD-MA	PD-PO	MA-PO	PD-MA-PO	Total	PD	MA	PO
BMC1013	3	3	3	3	3	3	3	3	0.664	0.634	0.633	0.651
CSSM33	6	6	5	4	5	4	4	4	0.656	0.693	0.580	0.658
CSSM36	11	8	8	10	5	9	8	6	0.811	0.736	0.741	0.796
CSSM38	13	9	11	13	7	9	11	7	0.833	0.713	0.796	0.885
CSSM43	9	8	8	8	7	7	7	6	0.779	0.730	0.817	0.769
CSSM47	13	6	11	10	5	6	8	5	0.755	0.451	0.840	0.816
CSSM60	12	8	8	12	7	8	7	7	0.711	0.651	0.628	0.787
CSSM70	5	5	5	5	5	5	5	5	0.728	0.678	0.745	0.749
ETH03	9	7	8	7	6	6	6	5	0.800	0.715	0.759	0.731
ETH10	7	6	5	6	4	6	4	4	0.736	0.675	0.653	0.760
ETH121	15	11	13	13	10	10	12	10	0.837	0.858	0.830	0.788
ETH225	9	7	6	6	6	4	4	4	0.798	0.802	0.744	0.791
TGLA44	13	6	11	10	5	5	8	4	0.784	0.430	0.817	0.794
TGLA53	17	13	12	15	12	11	11	10	0.878	0.866	0.852	0.879
TGLA110	4	3	2	4	2	3	2	2	0.206	0.198	0.148	0.263
TGLA122	19	13	11	14	8	9	7	5	0.816	0.864	0.752	0.755
TGLA126	7	5	6	7	5	4	6	4	0.803	0.793	0.768	0.794
TGLA226	7	6	5	5	4	4	5	4	0.755	0.788	0.727	0.652
TGLA377	7	5	7	7	5	5	7	5	0.734	0.652	0.732	0.786
TGLA431	13	10	7	12	6	9	6	6	0.649	0.575	0.696	0.660
SPS115	8	8	6	8	6	8	6	6	0.767	0.818	0.501	0.796
Mean/locus	9.8	7.3	7.5	8.5	5.9	6.4	6.5	5.3	0.738	0.682	0.703	0.741
SD	4.3	2.8	3.0	3.6	2.2	2.4	2.6	2.0	0.136	0.163	0.156	0.128

PD, Piedmontese; MA, Maremmana; PO, Podolica; SD, standard deviation.

Regarding the Maremmana breed, the 25 animals (66%) that can be assigned to their breed with a probability higher than 90% represent all sampled herds (12); moreover, 26% can be assigned to their breed at a probability between 70% and 90%. The three animals that were found to be the least Maremmana could be assigned to the Podolica breed with probabilities ranging from 30% to 70%.

Of the 37 Piedmontese animals, 30 (82%) can be assigned to their breed with a probability higher than 90%, while 14% can be assigned at a probability between 70% and 90%. In this breed the selection activity and the breeding goals are well established, artificial insemination is a common practice, and only the approved males issued from the progeny testing station can be used as breeding bulls. For the Piedmontese breed, the values for the genetic merits for beef conformation, growth, and global morphology traits of the top 7 bulls (i.e., those assigned to the breed with a probability of 97%) were compared with the corresponding average indices of the remaining 30 bulls. No significant difference between them was evident (Table 5); only for the global morphology index does a slightly significant difference appear, where the most typical seven have an index of 105.8 ± 4.9 versus an index of 101.8 ± 7.6 for the others ($P = .19$).

Discussion

The results of this analysis reflect very well the reality of the considered breeds, the husbandry systems through which they are managed, as well as the impact of the selection activity on their genetic structure: the more intensive and effective the selection system, the more animals can be correctly assigned to the cluster of their own breed. For none of the examined breeds, however, are the values of the estimators of genetic variability—inbreeding, average gene diversity, observed and expected heterozygosities—considered low, indicating that the decline in numbers has not yet produced an important loss of variability and that the breeds maintain a random mating structure.

The breed that preserved the highest genetic variability is the Podolica: in this breed in fact, the chosen markers show the highest gene diversity (0.741) and the highest heterozygosity (0.155). The inbreeding rate (0.106) is lower than in the Maremmana (0.138) and only slightly higher than in the Piedmontese (0.102). Moreover, although only 33% of the Podolica animals could be assigned to the cluster of the correct breed with a probability higher than 90%, in this group of most typical animals we find the only herd that is not registered in the Herd Book. Apparently, therefore, not much difference exists for the Podolica between the Herd Book animals and the others. Three considerations support our results: (1) the genetic improvement activity performed by the Breed Society through the progeny testing station started recently, and only five trials were performed; (2) artificial insemination is not very widespread, therefore the proven bulls issued from the performance stations are mainly used by one farmer only; and (3) the only recorded traits in the performance station (on-farm recording is not performed)

Table 3. Sample size, number of alleles per locus, and heterozygosities (standard deviations in parentheses) averaged over 21 microsatellites

Breed	Sample size	Mean heterozygosity	
		Observed	Expected
Piedmontese	37	0.148 (0.083)	0.163 (0.083)
Maremmana	38	0.140 (0.072)	0.165 (0.080)
Podolica	45	0.155 (0.082)	0.176 (0.080)
Average	40	0.148	0.168

Table 5. Least square means (standard deviations in parenthesis) and probabilities (*P*) of the differences of the genetic merit for beef conformation, growth, and morphology between the Piedmontese bulls fitting in the breed cluster at a probability > 97% (G1) and the others (G2)

Trait	Least square means		*P*
	G1	G2	
Beef conformation	102.0 (8.4)	98.8 (7.3)	0.31
Growth	103.8 (7.1)	101.0 (8.0)	0.39
Morphology	105.8 (4.9)	101.8 (7.6)	0.19

are growth and beef conformation, while most farmers milk their cows and are therefore interested in milk yield and persistency of lactation. In fact, although yield is on averagely only 1300 kg/year, all milk is processed into a typical cheese called "Caciocavallo Podolico," which has market prices similar to the most expensive Italian cheeses. It is evident therefore that the farmers perform on their own the selection of the females on the basis of milk yield.

The Maremmana breed (present population size of less than 30,000 head) is the one that has mainly suffered from the decline in numbers: it showed the highest inbreeding rate and the lowest average gene diversity and heterozygosities. Regarding the impact on the genetic variability of the performed selection activity, this breed has several aspects in common with Podolica; although it is genetically more defined, and therefore less variable (66% of the analyzed animals fit in the Maremmana cluster with a probability higher than 90%), the common aspects of the Podolica consist of the genetic improvement system, which is carried out in performance stations for males only, with growth and beef conformation being the breeding goals, as in the Podolica. However, because the breed is mainly used as a maternal rustic breed for crossbreeding with specialized beef breeds (Chianina, Marchigiana, Piedmontese, Charolaise, Limousine), the farmers keep their own purebred females for replacement on the basis of fitness in the environment where they live. The variability of the environment (lowland, hill, or mountain) and of the feeding systems (grazing only or grazing plus hay and concentrates) makes each farmer select females of different types, contributing to maintaining the variability of the breed.

The Piedmontese breed is genetically the best defined: 82% of the analyzed animals fit in the appropriate cluster with a probability higher than 90%. The reasons are evident: (1) the selection activity has a longer tradition; it started at the beginning of the 1970s and has been intensified in the past 20 years through the activity of one performance station; (2) artificial insemination is widespread. It is interesting to note that while the animals that best fit in the Piedmontese cluster are not different from the others in the genetic merit of the important selection traits (growth and beef conformation), a small difference was detected in the index for global morphology, where those who best fit in the correct cluster are also those with the best global morphology. The breed characteristics, in fact, play an important role in the selection of native breeds in Italy, where the farmers want to maintain some peculiar features in the look of the animals for the sake of tradition and culture.

Table 4. Number and percentage of animals that can be assigned to their breed at different probability levels

Probability level (%)	Piedmontese		Maremmana		Podolica	
	N	%	*N*	%	*N*	%
>90	30	82	25	66	15	33
70–90	5	14	10	26	17	38
60–70	1	2	—	—	4	9
<60	1	2	3	8	9	20
Total	37	100	38	100	45	100

Genetic distances between pairs of breeds, considered to be moderate (Hartl 1980), reflect what is indicated by phylogenetic analyses in cattle from the literature. The Maremmana and Podolica breeds have a common historical origin (Grey Steppe group) and show the shortest distance (0.041) between them. Rognoni and Pagnacco (1983), calculating the genetic distances between the same breeds from the variability of 13 protein coding loci, found that Podolica and Maremmana are less distant from each other than from Piedmontese, and the distances of each of them from the Piedmontese are similar. On the contrary, we found that the Piedmontese is less distant (0.050) from the Podolica than from the Maremmana (0.069). The number and type of loci, as well as the time gap of 20 years between the two analyses, might be responsible for this difference.

The number (21) and variability (average gene diversity = 0.738) of the chosen microsatellites can be considered good tools to describe cattle populations. It is evident that gene diversity is an optimal indicator of how good a marker is in describing the genetic variability of livestock: it takes into account both the number of alleles (the more, the better) and the differences in allele frequencies between breeds. In this case, microsatellite BMC1013, with only three alleles, each of them being the most frequent in a different breed, is a better indicator than TGLA110 (4 alleles, with one of them being the most frequent in all breeds), and it is as powerful as TGLA431 (13 alleles, most of which are at the same frequency in all breeds).

The microsatellites that were employed here are not sufficient to definitely discriminate between breeds. It is true that some alleles were not found in one or two breeds, but such alleles were also those that, when present, had the

lowest frequency. Moreover, although no exchange of breeding animals or semen between the considered breeds was performed, we have found, on the basis of allele recurrence, that a few animals could be assigned to their breed at probability levels lower than 60%.

It is possible to conclude that although the considered microsatellites do not allow discrimination between breeds, those markers where the three breeds registered consistent differences in the frequency of the same alleles, as well as those markers where the observed heterozygosity is lower than expected, could be usefully employed in analyses of association to quantitative trait loci. In fact, it was noted that at microsatellites *ETH10* (Piedmontese), *CSSM70* (Maremmana), and *CSSM33* (Podolica), differences between observed and expected heterozygosities are higher than at the other loci and tend to be statistically significant. Because these loci are not the same in the three breeds, it is believed that these differences reflect the results of some selection activity and it is suggested that we need to enlarge the characterization at these loci to many more individuals in order to provide evidence of the genes that might differentiate the productions of different breeds.

Acknowledgments

We thank Drs. Paolo Canestrari, Vittorio Faroppa, Gerardo Giuratrabocchetti, Roberto Giomini, and Augusto Calbi of the Herd-Book Societies who provided all blood samples used for genotyping.

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Received March 23, 2003
Accepted November 25, 2003

Corresponding Editor: James Womack