CHARACTERIZATION OF PIEMONTESE BEEF CATTLE USING INDIVIDUAL MULTILOCUS GENOTYPE BASED ON DNA MICROSATELLITES

CARATTERIZZAZIONE DELLA RAZZA PIEMONTESE MEDIANTE IL GENOTIPO MULTILOCUS INDIVIDUALE

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SUMMARY

In the present study a sample of 47 young double-muscled bulls in a performance test, belonging to the Piemontese cattle breed, were divided into subpopulations which were homogeneous for morpho-functional traits, using three different methods. They were, moreover, characterized on a molecular level by a set of 20 microsatellite markers; genetic similarities, genetic distances, Hardy-Weinberg proportions and the coefficients for deficiency of heterozygotes were calculated within and between subpopulations. Phenotypical evaluations, both of morphological nature (somatic measurements), both related to growing traits (weight and daily gain) were available. Also eleven morpho-functional indexes were calculated between and within the aforementioned classes of measurements.

The results indicate an hypothetical relationship between the Individual Multilocus Genotype and the morpho-functional type, supported by greater genetic similarities within than between subpopulations. In particular, genetic homogeneity in the group of subjects considered more distant from the meat-type was greater with respect to the subjects more evolved toward meat aptitude. An interesting genetic distance between this two extreme subpopulations was also recognizable. The coefficients for deficiency of heterozygotes indicate the presence of significantly high homozygosis at six microsatellites, particularly in subjects with greater meat aptitude. Therefore, data show convincing evidence for the existence of precise and distinct phenotypic and genotypic types, in spite of the Piemontese cattle breed's great variability.

Key words: microsatellites, beef cattle, variability, Individual Multilocus Genotype, QTL.

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RIASSUNTO

Nel presente studio, un campione di 47 soggetti appartenenti alla razza bovina da carne Piemontese è stato suddiviso in sottopopolazioni omogenee per caratteri morfo-funzionali. In aggiunta, i soggetti sono stati caratterizzati a livello molecolare mediante un set di 20 marcatori microsatellite; sono state calcolate le similarità genetiche, le distanze genetiche, le proporzioni di Hardy-Weinberg ed i coefficienti per il difetto di eterozigoti entro e tra sottopopolazioni. I risultati indicano una possibile relazione tra il Genotipo Multilocus Individuale, ottenuto mediante i marcatori microsatellite, e la tipologia morfo-funzionale, supportato da similarità genetiche entro-sottopopolazione maggiori rispetto alle similarità genetiche riscontrate tra sottopopolazioni. In particolare, l'omogeneità genetica nel gruppo dei soggetti considerati più distanti dalla tipologia-carne è risultata maggiore rispetto a quella riscontrata all'interno del gruppo dei soggetti meno orientati verso la produzione di carne; le due sottopopolazioni estreme hanno, altresì, rivelato interessanti valori di distanza genetica. I coefficienti per il difetto di eterozigoti indicano la presenza di un significativo livello di omozigosi per sei marcatori microsatellite, particolarmente in soggetti con maggior attitudine carne.

Parole chiave: Piemontese, variabilità, Genotipo Multilocus Individuale, marcatori microsatellite, QTL.

INTRODUCTION

With few exceptions – double muscling (Grobet *et al*., 1998) and meat tenderness (Keele *et al*., 1999) in cattle – most morpho-functional traits in meat animals are quantitative in nature; phenotypes form a continuous distribution since they reflect the action and interaction of many genes, disguised by complex environmental effects. On the whole, the "meat type" in cattle cannot be attributed to a single gene, since it is undoubtedly controlled by the combined action of multiple gene functions, some of which are probably of major effect.

The existence of major genes and their associated genomic markers therefore suggests a promising research goal, aimed at reinforcing the selective progress of quantitative traits using the techniques of Marker Assisted Selection (MAS). Specific methodologies of statistical analysis have been based on the idea that some genes play an important role in determining traits and that it is possible to identify their associated markers which may account for an important part of the total variability (10-20%).

Several Authors are already working toward the goal of realizing a standardized model for the estimation of each "Quantitative Trait",

in which different regions of the genome receive attention and emphasis according to the variance of the QTL (Quantitative Trait Loci) which they explain. The standardized model will permit not only comprehensive evaluation of meat types, but will also afford better examination of combined effects; furthermore it will allow investigation of allele combinations and estimation of non-additive genetic effects such as heterosis and consanguinity (Haley & Wisscher, 1998).

A methodology applied to the analysis of variability and to the estimation of genetic similarity, but which appears to offer good indications for the definition of a complex genotype, is the Individual Multilocus Genotype (IMG), which we have proposed and utilized in previous experiments with encouraging results (Ciampolini *et al*., 1994, 1995, 1996, 2000, 2001, 2003). This method allowed us to characterize and differentiate even genetically similar breeds (Ciampolini *et al*., 2000), but also, by separating similar genotypes into groups, to divide them into subpopulations sharing a similar conformation, thus leading to the hypothesis of a relationship between IMG and morpho-functional type.

This research accompanies a parallel study concerning associations between microsatellites and the aptitude properties of Piemontese beef cattle (Ciampolini *et al*., 2003), and aims to distinguish morphologically homogeneous subpopulations by means of statistical analysis in order to evaluate differences preserved at the genomic level, thus describing the possibility of revealing QTL by means of the Individual Multilocus Genotype.

MATERIALS AND METHODS

The study was conducted on 47 young double-muscled Piemontese bulls in a performance test. Many phenotypical evaluations both of morphological nature (somatic measurements), both related to growing traits (weight and daily gain) were available. Also eleven morpho-functional indexes were calculated between and within the aforementioned classes of measurements.

DNA analysis – DNA was purified from 20 ml samples of peripheral blood, following the method described by Jeanpierre (1987). The PCR reactions and procedures for determining the genotypes of the

microsatellites were carried out according to the methodology described by Vaiman *et al*. (1994).

For this study 20 microsatellites were analyzed; 17 were identified by the INRA laboratories (Vaiman *et al.,* 1994) and the rest by Steffen *et al.* (1993). Information relative to the 20 microsatellites is shown in Table I.

Statistical analysis – Analysis was carried out using the Byosis Program (Swofford & Selander, 1989) for calculating allele frequency, Hardy-Weinberg proportions and excess or defect of heterozygotes, as well as the genetic distance according to Cavalli Sforza & Edwards (1967).

Genetic similarities were estimated according to the method of Ciampolini *et al.* (1995) using the Individual Multilocus Genotype (IMG). Each subject was defined according to its own multilocus genotype (in our case 20 microsatellite loci) consisting of a series of 40 alleles for every animal. To estimate the genetic similarity between

Tab. I. Analysed microsatellites and their polymorphism.

Marker	Locus	Chromosome	Numbers of alleles
INRA 5	D12S4	12	5
INRA 6	D3S9	3	6
INRA 11	D1S6	1	10
INRA 13	D16S10	16	9
INRA 16	D27S20	27	10
INRA 23	D3S10	3	11
INRA 25	D17S6	17	8
INRA 27	D27S16	27	6
INRA 31	D21S12	21	6
INRA 32	D11S9	11	9
INRA 35	D16S11	16	7
INRA 37	D4S26	4	12
INRA 50	D15S5	15	10
INRA 53	D7S6	7	5
INRA 63	D18S5	18	7
INRA 64	D23S15	23	6
INRA 72	D4S11	4	10
ETH 131	D21S4	21	13
ETH 152	D5S1	5	7
ETH 225	D9S1	9	5

two individuals or groups of individuals, the proportion (P) of shared alleles (A) in relation to the 2L possibilities (L = number of loci considered) were calculated. Genetic similarity was measured by P = A/2L and the genetic distance was 1-P. The similarities calculated between each pair of subjects were averaged to obtain similarity values within breeds or subpopulations. To estimate the similarity (or the genetic distance) between breeds or subpopulations the average values of the similarities between every subject of a group and each subject of the comparison group were calculated.

The 47 subjects were then divided according to three different methods into subpopulations which were homogeneous for morpho-functional characteristics; each subpopulation was genetically evaluated for the behaviour of the IMG in the attempt to identify a genetic classification, as determined by any association between genotype and morpho-functional phenotype.

Method 1. With the aid of the morpho-functional measurements carried out during performance tests, a subjective evaluation (referees score) of its greater or lesser meat aptitude was made for each animal. The subjects considered to correspond closely (or less so) to this aptitude were considered "Positive" or "Negative" respectively. Subjects of intermediate type were labelled "Uncertain". Three subpopulations emerged from this: the first, considered to adhere most closely to the criterion of meat characteristics (15 subjects), the second less so, and the third consisting of those subjects with an "uncertain" evaluation.

Method 2. The double-muscled subjects were classified into three distinct classes of quality for each morpho-functional variable. The first class ("Positive") contained those subjects which corresponded more closely to the requirements of the meat type (value of the measurement $\geq$ Mean + SD); those less corresponding to the standard (value of the measurement $\leq$ Mean - SD) were considered to be "Negative". Thus the population was divided into three groups of subjects, the first with mostly positive measurements (11 subjects), the second with prevalently negative measurements (12 subjects) and the third falling within the range Mean $\pm$ SD.

Method 3. The divisions of homogeneous subjects were effected by means of a Cluster Analysis carried out by the "Ward Method" of the "JMP" statistical package (1996) which takes into account all morpho-functional parameters. The population was divisible into

three groups of individuals, each with a low variability; a fourth group, consisting of animals distant from each other and from every other subject in the sample, was excluded from further consideration.

RESULTS

Method 1. Considering the procedure with which subpopulations were established, the differences between somatic measurements of the "Positive" and "Negative" groups were always statistically significant for $P < 0.01$, as seen in the evaluation of the referees (Tab. II).

The differences between the ratios were less significant; the subpopulation of the "Uncertain" group was not always distinct with respect to the other two, from which moreover it differed significantly ($P < 0.05$) regarding many parameters.

Considering this, allele frequencies and the genetic distance estimated according to Cavalli Sforza & Edwards (1967) were calculated only in the two extreme ("Positive" and "Negative") subpopulations. A marked distinction between the two groups was highlighted (arc distance equivalent to 0.256, data not shown).

In addition, the Hardy-Weinberg proportions and the coefficients for deficiency of heterozygotes (data not shown) suggest several microsatellites in disequilibrium; many of these, with a significant deficiency of heterozygotes, are found above all in the "Positive" group.

Variability within the two subpopulations (Tab. III) was lower for the subjects tending less toward the meat aptitude (coefficient of genetic similarity equivalent to 0.371) with respect to those with greater meat aptitude (coefficient 0.333). This confirms results revealed by a previous study carried out on the same breed (Ciampolini *et al.,* 2001), concerning the possible relations between multilocus genotype of DNA microsatellites and the morpho-functional traits as well as the genetic similarity of subjects less evolved toward meat aptitude.

Method 2. Even with the categories created according to these mathematical criteria, obviously subpopulations are characterized by somatic measurements (Table IV) which reflect the choice made and are therefore always significantly ($P < 0.01$) greater in the "Positive"

Tab. II. Averages and Standard Errors of the somatic measurements: 1° Method.

Traits	Positive		Negative		Uncertain	
	Averages	Std Err	Averages	Std Err	Averages	Std Err
Height at withers, cm	119,64 Bb	± 0,422	116,49 A	± 0,453	118,41 Ba	± 0,375
Height at pelvis, cm	127,18 Ba	± 0,402	124,62 Aa	± 0,432	126,08 b	± 0,357
Depth of chest, cm	61,74 Ba	± 0,243	60,19 Aa	± 0,261	61,08 b	± 0,216
Body lenght, cm	143,48 Ba	± 0,678	139,14 Aa	± 0,728	141,60 b	± 0,603
Chest lenght, cm	77,54 Ba	± 0,230	76,07 Aa	± 0,247	76,91 b	± 0,205
Lenght of rump, cm	50,76 Ba	± 0,185	49,57 Aa	± 0,198	50,25 b	± 0,164
Width of brisket, cm	40,84 B	± 0,175	39,42 A	± 0,188	40,38 B	± 0,155
Width of chest, cm	44,17 B	± 0,174	43,11 A	± 0,186	43,87 B	± 0,154
Fore width of rump, cm	43,83 Ba	± 0,225	42,39 Aa	± 0,242	43,21 b	± 0,200
Medium width of rump, cm	46,16 B	± 0,210	44,50 A	± 0,226	45,61 B	± 0,187
Hind width of rump, cm	39,36 B	± 0,184	37,94 A	± 0,198	38,90 B	± 0,163
Lenght of head, cm	44,20 B	± 0,320	42,62 A	± 0,344	43,68 B	± 0,284
Crest girth, cm	183,94 B	± 0,615	179,60 A	± 0,661	182,65 B	± 0,547
Fore cannon girth, cm	19,07 Ba	± 0,081	18,60 Aa	± 0,092	18,87 b	± 0,074
Buttock girth, cm	143,29 b	± 1,453	137,64 a	± 1,639	140,06	± 1,318
Weight at 150 d, kg	162,82	± 1,492	159,12	± 1,603	161,15	± 1,326
Weight at 250 d, kg	328,99 Ba	± 5,159	299,28 Aa	± 5,541	316,24 b	± 4,584
Weight at 350 d, kg	444,10 Ba	± 4,360	416,25 Aa	± 4,683	432,07 b	± 3,874
DG from 150 to 250 d, kg	1,489 B	± 0,037	1,246 A	± 0,039	1,339 A	± 0,033
DG from 150 to 350 d, kg	1,507 B	± 0,026	1,291 A	± 0,028	1,351 A	± 0,023
DG from 250 to 350 d, kg	1,532 b	± 0,050	1,352 a	± 0,054	1,366 a	± 0,044
(9+10)/1 ratio	0,695 b	± 0,002	0,690 a	± 0,002	0,693	± 0,001
4/6 ratio	2,827 Ba	± 0,003	2,806 Aa	± 0,003	2,818 b	± 0,003
4/13 ratio	0,780	± 0,004	0,775	± 0,004	0,775	± 0,003
1/14 ratio	6,278	± 0,015	6,271	± 0,017	6,284	± 0,014
8/3 ratio	0,715	± 0,004	0,717	±0,004	0,718	± 0,003
5/1 ratio	0,648 A	± 0,001	0,653 B	± 0,001	0,650	± 0,001
7/1 ratio	0,341	± 0,001	0,338	± 0,001	0,341	± 0,001
15/18 ratio	0,323	± 0,005	0,330	± 0,005	0,323	± 0,004
15/1 ratio	1,197	± 0,013	1,180	± 0,015	1,181	± 0,012
18/1 ratio	3,711 Bb	± 0,027	3,572 A	± 0,029	3,649 a	± 0,067
18/4 ratio	3,094 Bb	± 0,016	2,990 A	± 0,017	3,051 Ba	± 0,014
Referees score	6,133 Bb	± 0,186	5,326 A	± 0,208	5,556 a	± 0,165

Different letters on the same line mean significant differences.
Capital letters = $P < 0.01$; small letters = $P < 0.05$. DG = Daily Gain.

group. This is equally true for the evaluation of the referees, oriented toward subjects showing greater development and greater daily gain. Instead, the morphological proportions are not clearly definable and

Tab. III. Genetic similarities.

		Average	Range
First Method:			
Within group:	Positive	0,333	0.17-0.47
Within group:	Negative	0,371	0.22-0.52
Within group:	Uncertain	0,359	0.17-0.57
Between groups:	Positive vs Negative	0,381	0.17-0.55
Second Method:			
Within group:	Positive	0,327	0.17-0.52
Within group:	Negative	0,370	0.20-0.52
Within group:	Uncertain	0,362	0.22-0.57
Between groups:	Positive vsNegative	0,380	0.17-0.57
Third Method:			
Within Group 1		0,350	0.22-0.52
Within Group 2		0,355	0.17-0.55
Within Group 3		0,371	0.20-0.57
Within unclassified subjects		0,373	0.27-0.50
Group 1 vs 2		0,368	0.17-0.55
Group 1 vs 3		0,369	0.17-0.57
Group 2 vs 3		0,367	0.17-0.57

only in a few cases do they show significant differences between the groups.

The allele frequencies were calculated for the three groups, as well as the genetic distances according to the method of Cavalli Sforza & Edwards (1967). The results have shown that the "Positive" and "Negative" subpopulations are distant from each other (arc distance 0.276, data not shown).

The Hardy-Weinberg proportions (data not shown) were often respected since only two microsatellites were in disequilibrium, both between the subjects with greater tendency toward meat aptitude as well as in the less "meat-oriented" group; the coefficients for deficiency of heterozygotes indicate a greater tendency to homozygosis (negative values) in subjects with greater or medium meat aptitude, in which five microsatellites were significantly deficient in heterozygotes.

The calculation of genetic similarities (Tab. III), carried out according to the method of Ciampolini *et al.* (1995), indicates greater homogeneity in the group of subjects considered most distant from the meat-type with respect to the "Positive" group (coefficients of simi-

Tab. IV. Averages and Standard Errors of the somatic measurements: 2° Method.

	Positive		Negative		Uncertain	
Traits	Averages	Std Err	Averages	Std Err	Averages	Std Err
Height at withers, cm	120,5 B	± 0,398	116,12 A	± 0,381	118,32 C	± 0,270
Height at pelvis, cm	127,8 B	± 0,415	124,29 A	± 0,397	126,10 C	± 0,281
Depth of chest, cm	62,09 B	± 0,250	60,00 A	± 0,240	61,09 C	± 0,169
Body lenght, cm	144,5 B	± 0,700	138,60 A	± 0,670	141,64 C	± 0,474
Chest lenght, cm	77,87 B	± 0,238	75,88 A	± 0,228	76,92 C	± 0,161
Lenght of rump, cm	51,02 B	± 0,191	49,42 A	± 0,183	50,25 C	± 0,129
Width of brisket, cm	41,35 B	± 0,156	39,36 A	± 0,149	40,21 C	± 0,105
Width of chest, cm	44,61 B	± 0,174	43,09 A	± 0,166	43,70 C	± 0,118
Fore width of rump, cm	44,15 B	± 0,233	42,21 A	± 0,223	43,22 C	± 0,158
Medium width of rump, cm	46,75 B	± 0,193	44,44 A	± 0,185	45,41 C	± 0,131
Hind width of rump, cm	39,88 B	± 0,172	37,92 A	± 0,164	38,71 C	± 0,116
Lenght of head, cm	43,82	± 0,411	43,08	± 0,394	43,67	± 0,278
Crest girth, cm	185,7 B	± 0,594	179,61 A	± 0,569	181,93 C	± 0,402
Fore cannon girth, cm	19,2 B	± 0,086	18,54 A	± 0,091	18,85 C	± 0,057
Buttock girth, cm	142	± 1,735	136,56 a	± 1,829	141,39 b	± 1,144
Weight at 150 d, kg	163,4 b	± 1,690	157,98 a	± 1,618	161,64	± 1,144
Weight at 250 d, kg	335,9 B	± 5,511	296,30 A	± 5,277	315,97 C	± 3,732
Weight at 350 d, kg	450,4 B	± 4,501	412,74 A	± 4,310	432,30 C	± 3,047
DG from 150 to 250 d, kg	0,697 Bb	± 0,048	1,292 A	± 0,046	1,343 a	± 0,032
DG from 150 to 350 d, kg	2.831 B	± 0,036	1,294 Aa	± 0,035	1,391 b	± 0,024
DG from 250 to 350 d, kg	0,778 b	± 0,060	1,296 a	± 0,057	1,456 b	± 0,040
(9+10)/1 ratio	6.287 Bb	± 0,002	0,690 A	± 0,002	0,692 a	± 0,001
4/6 ratio	0,718 B	± 0,003	2,804 A	± 0,003	2,818C	±0,002
4/13 ratio	0,646	± 0,004	0,772	±0,004	0,779	± 0,003
1/14 ratio	0,343	± 0,018	6,275	± 0,019	6,276	± 0,012
8/3 ratio	0,314	± 0,004	0,718	± 0,004	0,716	± 0,003
5/1 ratio	1.117 Aa	± 0,001	0,654 Ba	± 0,001	0,650 b	± 0,001
7/1 ratio	3.737 Bb	± 0,001	0,339 A	± 0,001	0,340 a	± 0,001
15/18 ratio	3.117	± 0,005	0,330	± 0,006	0,327	± 0,004
15/1 ratio	1.475	± 0,015	1,174	± 0,016	1,195	± 0,010
18/1 ratio	1.467 Bb	± 0,029	3,554 A	± 0,028	3,653 Ba	± 0,020
18/4 ratio	1.455 B	± 0,016	2,977 A	± 0,016	3,051 C	± 0,011
Referees score	5.990 b	± 0,224	5,247 a	± 0,215	5,766 b	± 0,155

Different letters on the same line mean significant differences.
Capital letters = $P < 0.01$; small letters = $P < 0.05$. DG = Daily Gain.

larity 0.370 and 0.327 respectively). Even more homogeneous are subjects in the "uncertain" group (coefficient of similarity 0.362).

Method 3. The average values of somatic measurement and referees evaluations for each group of subjects together with the standard deviations are separately reported in Table V. The significant difference between the groups for all measurements (and particularly for the evaluations of the referees) is shown, appearing very high for the more developed subjects, as well as showing the lower variability (*Standard Deviation*, SD) within groups 1, 2 and 3 compared to the variability of the heterogeneous group and of the population as a whole. It must be underlined that these criteria, although taking into account the division into four subpopulations compared to the three used in the previous method, allowed us to obtain particularly homogeneous groups, with a lower standard error for all the measurements, and therefore genetically more distant from each other for submitting to molecular analysis.

In fact, the genetic distance (*arc distance*) estimated according to Cavalli Sforza & Edwards (1967) proved to be equivalent to 0.298 and is greater than that between subjects found in the extreme categories as determined by the previous methods (data not shown). Furthermore, the respect for Hardy-Weinberg proportions is less marked in that different microsatellites proved to be in disequilibrium, especially in the groups with higher values for somatic measurements; also of interest is the presence of homozygotes, significantly high in six microsatellites, especially in the group of subjects with greater somatic development (data not shown).

The genetic similarities estimated according to the method of Ciampolini *et al*. (1995) confirm the high variability within the subpopulation of subjects with greater somatic measurements, and the interesting distance between the two extreme subpopulations (Tab. III).

DISCUSSION

As the results demonstrate, the attempt to set up subpopulations within the sample of subjects analysed, with each group homogeneous for morpho-functional parameters but distinct from the others, was perfectly achieved.

Although the subpopulations set up according to the different

Tab. V. Averages and Standard Errors of the somatic measurements: 3° Method.

	Group 1		Group 2		Group 3	
Traits	Averages	Std Err	Averages	Std Err	Averages	Std Err
Height at withers, cm	116,16 A	± 0,305	117,94 B	± 0,325	120,25 C	± 0,330
Height at pelvis, cm	123,95 A	± 0,209	125,56 B	± 0,172	127,87 C	± 0,209
Depth of chest, cm	59,79 A	± 0,126	60,77 B	± 0,104	62,16 C	± 0,126
Body lenght, cm	138,02 A	± 0,353	140,74 B	± 0,290	144,63 C	± 0,353
Chest lenght, cm	75,69 A	± 0,119	76,61 B	± 0,098	77,93 C	± 0,120
Lenght of rump, cm	49,27 A	± 0,097	50,01 B	± 0,079	51,07 C	± 0,096
Width of brisket, cm	39,15 A	± 0,143	40,14 B	± 0,116	41,09 C	± 0,141
Width of chest, cm	43,05 A	± 0,141	43,74 Ba	± 0,142	44,28 Bb	± 0,144
Fore width of rump, cm	42,02 A	± 0,118	42,92 B	± 0,097	44,21 C	± 0,117
Medium width of rump, cm	44,01 A	± 0,162	45,27 B	± 0,112	46,53 C	± 0,153
Hind width of rump, cm	37,52 A	± 0,148	38,62 B	± 0,108	39,65 C	± 0,140
Lenght of head, cm	43,38	± 0,596	43,19 a	± 0,262	44,31 b	± 0,350
Crest girth, cm	178,28 A	± 0,520	181,78 B	± 0,408	184,80 C	± 0,494
Fore cannon girth, cm	18,44 A	± 0,040	18,75 B	± 0,033	19,19 C	± 0,040
Buttock girth, cm	139,88	± 2,676	139,75	± 0,854	141,88	± 1,734
Weight at 150 d, kg	161,52	± 1,198	161,45	± 1,658	162,80	± 1,371
Weight at 250 d, kg	287,82 A	± 2,354	309,15 B	± 1,796	338,01 C	± 2,277
Weight at 350 d, kg	409,02 A	± 2,267	426,51 B	± 1,864	451,51 C	± 2,265
DG from 150 to 250 d, kg	1,26 a	± 0,054	1,314	± 0,050	1,411 b	± 0,032
DG from 150 to 350 d, kg	1,29	± 0,046	1,385	± 0,035	1,408	± 0,034
DG from 250 to 350 d, kg	1,34	± 0,059	1,481	± 0,061	1,403	± 0,052
(9+10)/1 ratio	0,69 A	± 0,001	0,691 B	± 0,001	0,698 C	± 0,001
4/6 ratio	2,80 A	± 0,002	2,814 B	± 0,001	2,832 C	± 0,002
4/13 ratio	0,77 a	± 0,002	0,774 a	± 0,003	0,783 b	± 0,002
1/14 ratio	6,30 b	± 0,008	6,291	± 0,011	6,266 a	± 0,009
8/3 ratio	0,72 b	± 0,002	0,720 b	± 0,002	0,713 a	± 0,002
5/1 ratio	0,65 b	± 0,001	0,650	± 0,001	0,648 a	± 0,001
7/1 ratio	0,34 Aa	± 0,001	0,340 b	± 0,001	0,342 B	± 0,001
15/18 ratio	0,34 C	± 0,005	0,328 B	± 0,002	0,314 A	± 0,004
15/1 ratio	1,20	± 0,022	1,185	± 0,006	1,180	± 0,015
18/1 ratio	3,52 A	± 0,012	3,616 B	± 0,010	3,755 C	± 0,011
18/4 ratio	2,96 A	± 0,009	3,030 B	± 0,007	3,122 C	± 0,008
Referees score	5,43	± 0,277	5,823	± 0,217	5,592	± 0,172

Different letters on the same line mean significant differences.
Capital letters = $P < 0.01$; small letters = $P < 0.05$. DG = Daily Gain.

methods did not necessarily consist of the same subjects, they always presented highly significant differences between subpopulations for all (or nearly all) somatic measurements while maintaining great

internal homogeneity. In particular, the third method, based on the Cluster Analysis, grouped together individuals which were highly homogeneous for somatic measurements but less so for weight and daily gain.

This result, while representing a simple methodological goal, was extremely important for the following application since it is convincing evidence for the existence of precise and distinct phenotypic and genotypic types, in spite of the Piemontese cattle breed's great variability.

The investigation of relationships between expressed morpho-functional traits and genotype (estimated using the Multilocus Individual Genotype of DNA microsatellites) has provided results of great importance, since the phenotypic subpopulations always proved to be genotypically distant and distinct according to the classic methods of estimating genetic distance (Cavalli-Sforza & Edwards, 1967) as well as for the different behaviour of single markers with respect to Hardy-Weinberg proportions and of the correct presence of heterozygotes.

A surprising aspect, specific for the Piemontese breed and revealed by all the methods used for establishing subpopulations, is that of a greater genetic discordance in subjects phenotypically assigned to the subpopulation tending most toward the meat-type. It was expected that this group would have undergone the greater selective action and therefore would be closer to an "ideal aptitude" implying a genotypic as well as the resulting phenotypic similarity.

The early as well as recent history of this breed (Ciampolini *et al*., 2001), which is noted for its highly-valued meat aptitude, instead fully explains this result. The greater genetic similarity of the base population, already possessing an excellent meat aptitude but still connected to the selective goals of a "double aptitude" (meat and milk), is in opposition to the variability of subjects which at different phenotypic and genotypic levels have only recently been oriented toward a meat specialization.

REFERENCES

CAVALLI SFORZA L.L., EDWARDS A.W.F. (1967). Phylogenetic analysis, models and estimation procedures. Am. J. Hum. Genet., 19: 233-257.

CIAMPOLINI, R. GROHS C., LEOTTA R., LEVEZIEL H., CIANCI, D. (1994). Use of microsatellites to investigate genetic diversity in four Italian beef cattle breeds. XXIV International Conference on Animal Genetics, Prague, 23-28 July.

CIAMPOLINI R., MOAZAMI-GOUDARZI K., VAIMAN D., DILLMANN C., MAZZANTI E., FOULLEY J.L., LEVEZIEL H., CIANCI D. (1995). Individual multilocus genotypes using microsatellite polymorphism permit the analysis of the genetic variability within and between Italian beef cattle breeds. J. Anim. Sci., 73: 3259-3268.

CIAMPOLINI R., MAZZANTI E., LEVEZIEL H., GROHS C., CIANCI D. 1996. Analysis of genetic variability within the Piemontese cattle breed using microsatellite polymorphism and research of association between individual multilocus genotypes and quantitative traits. ISAG Congress, Tours, July.

CIAMPOLINI R., LEVEZIEL H., MAZZANTI E., GROHS C., CIANCI D. (2000). Genomic Identification of the Breed of an Individual or its Tissue. Meat Sci., 54: 35-40.

CIAMPOLINI R., MAZZANTI E., CECCHI F. (2001). La struttura genetica della razza bovina Piemontese. Ann. Fac. Med. Vet., LIV: 113-126.

CIAMPOLINI R. CECCHI F., MAZZANTI E., CIANCI D. (2003). Evaluation of Genetic Variability in Chianina cattle using Genealogical and Molecular Data: Molecular Analysis. Agric. Medit., 133: 89-100.

GROBET L., PONCELET D., ROYO MARTIN L.J., BROUWERS B., PIROTTIN D., MICHAUX C., MENISSIER F., ZANOTTI M., DUNNER S., GEORGES M. (1998). Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. Mamm. Genome, 9: 210-213.

HALEY C.S., WISSCHER P.M. (1998). Strategies to utilize Marker-Quantitative Traits Loci associations. J. Dairy Sci., 81: 85-97.

JEANPIERRE M. (1987). A rapid method for purification of DNA from blood. Nucleic Acids Res., 15: 9611.

J.M.P. User's Guide ver. 3.1.6.2., (1996). S.A.S Institute Inc., Ed. Cary (NC), U.S.A.

KEELE J.W., SHACKELFORD S.D., KAPPES S.M., KOOHMARAIE M., STONE R.T. (1999). A region on bovine chromosome 15 influences beef longissimus tenderness in steers. J.Anim. Sci., 77: 1364-1371.

STEFFEN P., EGGEN A., DIETZ A.B., WOMACK J.E., STRANZINGER G., FRIES R. (1993). Isolation and mapping of polymorphic microsatellites in cattle. Anim. Genet., 24: 121-124.

SWOFFORD D.L., SELANDER R.B. BIOSYS 1. (1989). A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. University of Illinois, Urbana, Illinois.

VAIMAN D., MERCIER D., MOAZAMI-GOURDARZI K., EGGEN A., CIAMPOLINI R., et al. (1994). A set of 99 cattle microsatellites: Characterization, synteny mapping and polymorphism. Mamm. Genome, 5: 288.