

Study on milk proteins loci in some decreasing Italian cattle breeds ⁽¹⁾

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Summary

The genetic polymorphism of the structural loci of five of the six main lactoproteins, α -lactalbumin, β -lactoglobulin, α_{s1} -, β - and x -casein, was examined in *Grey Alpine*, *Norica-Pinzgau*, *Aosta Black Pied*, *Aosta Red Pied*, *Piedmont* and *Chiana* cattle breeds.

The analysis is concerned with the gene frequencies at the five loci, the frequencies of the allelic combinations controlled by the genetic unit formed by the three casein loci, the linkage disequilibrium in this cluster of loci, the genetic distance between the populations and the heterozygosity.

I. - Introduction

In recent years the disappearance of local livestock breeds has been the argument of international meetings and conferences, where the causes of the reduction of the genetic variability and the reasons and methods for its conservation have been amply discussed. Emphasis has been laid upon the importance of investigating the genetic structure of decreasing populations (e.g., the Round Table on « The conservation of animal genetic resources » at the Ist World Congress on Genetics Applied to Livestock Production, Madrid, 1974).

This work is part of a wide program of investigations on biochemical and immunological polymorphisms in Italian cattle. It intends to describe the milk proteins loci and variability in six decreasing breeds : *Grey Alpine* (GA), *Norica-Pinzgau* (NP), *Aosta Black Pied* (ABP), *Aosta Red Pied* (ARP), *Piedmont* (P) and *Chiana* (CH). The sizes of the breeds in the last twenty years are reported in table 1.

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TABLE 1

Sizes of the six breeds.
Effectif des six races considérées.

	End of '50s (1)	1973-1974 (1)	1977-1978 (2)
<i>Grey Alpine</i>	300 000	90 000	50 000
<i>Norica Pinzgau</i>	40 000	20 000	15 000
<i>Aosta Black Pied</i>	17 000	10 000	18 000
<i>Aosta Red Pied</i>	118 000	50 000 (?)	108 000
<i>Piedmont</i>	700 000	640 000	537 000
<i>Chiana</i>	527 000	210 000	162 000

(1) LAUVERGNE (1975).

(2) Different sources.

II. - Material and methods

Milk samples were collected from 172 *Grey Alpine*, 211 *Pinzgau*, 268 *Aosta Black Pied*, 242 *Aosta Red Pied*, 214 *Piedmont* and 120 *Chiana* cows.

Electrophoresis was performed in basic buffer (ASCHAFFENBURG & MICHALAK, 1968) for the detection of α -lactalbumin, β -lactoglobulin, α_{s1} , β - and κ -casein variants. Casein samples were also run with the buffer of PETERSON & KOPFLER (1966) to subtype β -casein A into A¹, A² and A³.

The frequencies of the allelic combinations controlled by the genetic unit formed by α_{s1} -Cn, β -Cn and κ -Cn loci were calculated by the iterative method (CEPPELINI *et al.*, 1955) and the linkage disequilibrium in this cluster of loci was examined for loci pairs (WEIR & COCKERHAM, 1978).

The genetic relationships between the six breeds were evaluated by two methods suggested by BOUQUET & GROSCLAUDE (1968) and by NEI & ROYCHOUDHURY (1974).

The genetic variability was evaluated for the single locus, for the whole of the loci considered and for the casein cluster (NEI, 1978).

III. - Results

All investigated loci reveal polymorphism (table 2) and generally the results agree with those previously obtained for *Pinzgau* (MÁCHA & MÜLLEROVA, 1970 ; HORVÁTH & MÉSZÁROS, 1972) for *Aosta Red Pied* (BETTINI & MASINA, 1972 ; VOGLINO & CARIGNANO, 1975), for *Piedmont* (BETTINI & MASINA, 1972 ; MARIANI & RUSSO,

1973; VOGLINO & CARIGNANO, 1975) and for *Chiana* (BETTINI & MASINA, 1972; RUSSO & MARIANI, 1974).

Important observations are concerned with the rare variants which may suggest interesting relationships between breeds, even if it is necessary to keep in mind that electrophoretically identical variants may derive from different alleles and so conclusions drawn from the presence of rare variants should be supported by biochemical investigations.

TABLE 2

Allele frequencies.
Fréquences alléliques.

Locus	Allele	B r e e d					
		GA	NP	ABP	ARP	P	CH
α -La	A	.0	.0	.0	.0	.033	.013
	B	1.0	1.0	1.0	1.0	.967	.987
β -Lg	A	.564	.284	.427	.430	.276	.304
	B	.436	.713	.573	.566	.724	.696
	D	.0	.003	.0	.004	.0	.0
α_{s1} -Cn	D	.0	.0	.002	.0	.002	.0
	B	.741	.735	.882	.901	.809	.742
	C	.259	.265	.116	.099	.189	.258
β -Cn	A ¹	.160	.284	.110	.128	.304	.458
	A ²	.613	.611	.466	.812	.514	.404
	A ³	.003	.0	.0	.0	.002	.0
	B	.175	.031	.424	.054	.147	.046
	C	.049	.074	.0	.006	.033	.092
κ -Cn	C	.012	.0	.0	.0	.0	.0
	A	.517	.794	.317	.337	.526	.358
	B	.471	.206	.683	.663	.474	.642

The α -La A variant occurs in *Piedmont* and *Chiana* breeds, both considered of Podolic origin. Since the *Chiana* is supposed the oldest Italian cattle breed and was a sacrificial beast in ancient Rome (MASON, 1966), it is worth considering also the hypothesis that the presence of the α -La A variant is the result of introgression from *Bos indicus*, possibly brought to Rome as tribute (BAKER & MANWELL, 1980). The presence of this variant in *Piedmont* breed is in agreement with the hypothesis, supported by several studies on genetic polymorphisms and by the presence of bifid processes to the last thoracic vertebra, that the breed derives from cross-breeding of Aurochs type with zebu from Pakistan (MALETTO, 1973).

The finding in *Pinzgau* and *Aosta Red Pied* breeds of β -Lg D variant, first described in *Montbéliarde* (GROSCLAUDE *et al.*, 1966 a), is in agreement with the classification recently proposed by BAKER & MANWELL (1980), who consider these breeds derived from the same group, namely Upland *brachyceros*. ASCHAFFENBURG (1968) had already observed that the β -Lg^D allele was a characteristic of cattle of the *Simmental* type, although its occurrence in *Danish Jersey* suggested that the D variant may be more widely distributed. Subsequent studies on other cattle breeds showed that this variant is prevalently present in those which are considered of the same origin; so the β -Lg^D allele may be a very useful marker in differentiating breeds.

The rare α_{s1} -Cn D, discovered in *Flamande* (GROSCLAUDE *et al.*, 1966 a) and then observed in five additional French breeds (GROSCLAUDE, 1979), was till now described in four Italian breeds: *Brown Alpine*, *Reggio*, *Rendena* and *Chiana* (RUSSO & MARIANI, 1978); its occurrence in *Aosta Black Pied* and *Piedmont* shows that in Italian breeds the D variant is rather spread although with low frequencies.

The very rare β -Cn A³ variant was only observed in *Grey Alpine* and *Piedmont* breeds.

TABLE 3

Frequencies of α_{s1} -Cn — β -Cn — x-Cn combinations.
Fréquences des combinaisons α_{s1} -Cn — β -Cn — x-Cn.

	B r e e d					
	GA	NP	ABP	ARP	P	CH
BA ¹ A0480	.2632	.0249	.0300	.1422	.0501
BA ¹ B1062	.0173	.0852	.0887	.1344	.4082
BA ² A2307	.2765	.2142	.2730	.2373	.0829
BA ² B1346	.0807	.1433	.4620	.1150	.0630
BA ² C0112	—	—	—	—	—
BA ³ A0029	—	—	—	.0	—
BBA1486	.0205	.0642	.0190	.0841	.0458
BBB0097	.0039	.3506	.0260	.0627	.0
BCA0	.0018	—	.0	.0	.0
BCB0492	.0707	—	.0021	.0327	.0917
BCC0002	—	—	—	—	—
CA ¹ A0	.0038	.0	.0033	.0243	.0
CA ¹ B0057	.0001	.0	.0061	.0006	.0
CA ² A0852	.2208	.0112	.0077	.0376	.1795
CA ² B1515	.0334	.0958	.0693	.1241	.0788
CA ² C0002	—	—	—	—	—
CA ³ A	—	—	—	—	.0023	—
CBA0020	.0063	.0008	.0031	.0002	.0
CBB0141	.0001	.0079	.0056	.0002	.0
CCA0	.0009	—	.0007	.0	.0
DA ¹ B	—	—	.0	—	.0023	—
DA ² A	—	—	.0019	—	.0	—

— : No combination due to absence of an allele in the breed.

BA²A : α_{s1} -CnB — β -CnA¹ — x-CnA, etc.

We indicated as $x\text{-Cn}^C$ the postulated allele which determines the new variant observed in *Grey Alpine* (Di STASIO & MERLIN, 1979); however, the hypothesis on the existence of this allele was not till now confirmed since we lack segregation data, while biochemical analysis is not yet completed.

Apart from *Piedmont* breed in which $\alpha_{s1}\text{-Cn}$ locus reveals a statistically significant lack of homozygote genotypes CC, the other breeds are in genetic equilibrium for all loci examined.

Table 3 shows the results of the analysis of $\alpha_{s1}\text{-Cn} - \beta\text{-Cn} - x\text{-Cn}$ cluster. The $\alpha_{s1}\text{-Cn}^B - \beta\text{-Cn}^{A^2} - x\text{-Cn}^A$ haplotype, predominant in almost all the breeds of *Bos taurus* origin, is the most frequent in *Grey Alpine*, *Pinzgau* and *Piedmont* breeds; the $\alpha_{s1}\text{-Cn}^B - \beta\text{-Cn}^{A^1} - x\text{-Cn}^B$ and $\alpha_{s1}\text{-Cn}^B - \beta\text{-Cn}^{A^2} - x\text{-Cn}^B$ combinations are the predominant ones in *Chiana* and *Aosta Red Pied* respectively; $\alpha_{s1}\text{-Cn}^B - \beta\text{-Cn}^B - x\text{-Cn}^B$ haplotype is predominant in *Aosta Black Pied* and shows a frequency similar to that observed in *Normande* (GROSCLAUDE *et al.*, 1966 b).

With regard to $\beta\text{-Cn}^{A^3}$, it was pointed out by GROSCLAUDE (1979) that in European breeds this allele appears associated with $\alpha_{s1}\text{-Cn}^C$, while our investigations show it associated both with $\alpha_{s1}\text{-Cn}^C$ (in *Piedmont*) and with $\alpha_{s1}\text{-Cn}^B$ (in *Grey Alpine*). On the basis of the phylogenetic tree proposed by GROSCLAUDE we may suppose that the $\beta\text{-Cn}^{A^3}$ variant observed in *Grey Alpine* is not the same as the one observed in other European breeds; obviously, this hypothesis should be confirmed by biochemical investigations.

It is worth noting the presence at $\alpha_{s1}\text{-Cn} - \beta\text{-Cn}$ cluster of some types considered as recombinants: $\alpha_{s1}\text{-Cn}^C - \beta\text{-Cn}^{A^1}$ and $\alpha_{s1}\text{-Cn}^C - \beta\text{-Cn}^B$. The first was observed in *Grey Alpine*, *Pinzgau*, *Aosta Red Pied* and *Piedmont*; the second was observed in *Grey Alpine*, *Aosta Black Pied*, *Aosta Red Pied* and *Piedmont*. In table 3 we indicated also the $\alpha_{s1}\text{-Cn}^C - \beta\text{-Cn}^C$ type for which we have no direct evidence.

The existence of these recombinants do not alter the linkage disequilibrium which is significant between all the three loci, with the exception of *Pinzgau*, where it is significant only between $\alpha_{s1}\text{-Cn}$ and $\beta\text{-Cn}$ and between $\beta\text{-Cn}$ and $x\text{-Cn}$; in *Aosta Red Pied* we did not observe linkage disequilibrium between the three loci (table 4).

TABLE 4

Linkage disequilibrium.
Déséquilibre de linkage.

Breed	D		
	$\alpha_{s1}\text{-Cn} - \beta\text{-Cn}$	$\alpha_{s1}\text{-Cn} - x\text{-Cn}$	$\beta\text{-Cn} - x\text{-Cn}$
<i>Grey Alpine</i>	0.078**	0.049*	0.058*
<i>Norica Pinzgau</i>	0.092***	0.021	0.056***
<i>Aosta Black Pied</i>	0.054**	0.025*	0.079***
<i>Aosta Red Pied</i>	0.004	0.019	0.010
<i>Piedmont</i>	0.064**	0.039*	0.017*
<i>Chiana</i>	0.154***	0.087***	0.118***

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

The existence of polymorphism of the α -lactalbumin system in *Piedmont* and *Chiana* breeds allowed us to study the relationships between the α -La and β -Lg loci in breeds of *Bos taurus* origin. On the basis of the genotypic distributions we found no association between the two loci, in accordance with the data reported on *Malagasy zebu* (GROSCLAUDE *et al.*, 1974) and on *Somali zebu* (Di STASIO *et al.*, 1979). However, considering the low frequency of α -La^A allele, it is necessary to extend the study on a larger number of samples.

The genetic connections between the six breeds examined were evaluated by two methods, trying to make the most of the limited number of loci studied. The method described by BOUQUET & GROSCLAUDE (1968) expresses the genetic resemblance (R) as an index ranging between 0 and 1; we calculated R using genotypic frequencies at β -Lg, α_{s1} -Cn, β -Cn and x -Cn loci instead of the allelic frequencies at the single loci. The measure of genetic distance proposed by NEI & ROYCHOUDHURY (1974) intends to estimate the number of net codon differences per locus between populations; of the three different suggested estimates we calculated the minimum distance, D_m .

In the present work the two methods gave similar results (tables 5 and 6).

TABLE 5

Index of genetical resemblance.
Indice de ressemblance génétique.

Compared breeds	R
<i>Piedmont - Grey Alpine</i>	0.616
<i>Piedmont - Chiana</i>	0.505
<i>Piedmont - Pinzgau</i>	0.491
<i>Piedmont - Aosta Black Pied</i>	0.470
<i>Grey Alpine - Aosta Black Pied</i>	0.467
<i>Grey Alpine - Pinzgau</i>	0.447
<i>Aosta Red Pied - Aosta Black Pied</i>	0.344
<i>Aosta Red Pied - Grey Alpine</i>	0.326
<i>Aosta Red Pied - Piedmont</i>	0.290
<i>Chiana - Grey Alpine</i>	0.266
<i>Aosta Red Pied - Pinzgau</i>	0.248
<i>Chiana - Pinzgau</i>	0.230
<i>Chiana - Aosta Red Pied</i>	0.168
<i>Pinzgau - Aosta Black Pied</i>	0.163
<i>Chiana - Aosta Black Pied</i>	0.143

TABLE 6

Genetic distance.
Distances génétiques.

	GA	NP	ABP	ARP	P	CH
GA	—	β -Lg = .078* α_{s1} -Cn = .0 β -Cn = .018* x-Cn = .073* D _m = .042	β -Lg = .019* α_{s1} -Cn = .020* β -Cn = .044* x-Cn = .043* D _m = .032	β -Lg = .018* α_{s1} -Cn = .025* β -Cn = .028* x-Cn = .035* D _m = .027	β -Lg = .083* α_{s1} -Cn = .005 β -Cn = .016 x-Cn = .0 D _m = .026	β -Lg = .068* α_{s1} -Cn = .0 β -Cn = .076* x-Cn = .027* D _m = .043
NP		—	β -Lg = .020* α_{s1} -Cn = .022* β -Cn = .106* x-Cn = .227* D _m = .094	β -Lg = .021* α_{s1} -Cn = .028* β -Cn = .035* x-Cn = .209* D _m = .073	β -Lg = .0 α_{s1} -Cn = .006 β -Cn = .013* x-Cn = .072* D _m = .023	β -Lg = .0 α_{s1} -Cn = .0 β -Cn = .037* x-Cn = .190* D _m = .057
ABP		—	—	β -Lg = .0 α_{s1} -Cn = .0 β -Cn = .128* x-Cn = .0 D _m = .032	β -Lg = .023* α_{s1} -Cn = .006 β -Cn = .059* x-Cn = .044 D _m = .033	β -Lg = .015* α_{s1} -Cn = .020* β -Cn = .180* x-Cn = .002 D _m = .054
ARP			—	—	β -Lg = .024* α_{s1} -Cn = .008* β -Cn = .065* x-Cn = .036* D _m = .033	β -Lg = .016 α_{s1} -Cn = .025* x-Cn = .001 β -Cn = .141* D _m = .046
P					—	β -Lg = .001 α_{s1} -Cn = .005 β -Cn = .025* x-Cn = .028* D _m = .015
CH						—

We found the highest similarity between *Piedmont* and *Grey Alpine*, and between *Piedmont* and *Chiana*, according to the hypothesis of their common Podolic origin.

The investigated loci were utilized as marker to get a relative estimate of the genetic variability of the populations (table 7).

Grey Alpine and *Piedmont* breeds showed high values both at the whole of the loci and at the casein cluster, whereas the other breeds, especially *Aosta Red Pied*, showed a lower variability perhaps depending on their small sizes, limited breeding areas and adopted mating systems.

TABLE 7

Genetic variability.
Variabilité génétique.

	GA	NP	ABP	ARP	P	CH
α -La0	.0	.0	.0	.063	.025
β -Lg493	.413	.491	.497	.400	.425
α_{s1} -Cn385	.391	.208	.179	.311	.385
β -Cn567	.540	.592	.323	.622	.619
x -Cn512	.328	.434	.449	.500	.462
Average391	.335	.345	.290	.379	.383
Casein cluster862	.794	.791	.700	.865	.774

IV. - Discussion and conclusions

In small populations, and also in large ones divided by their reproductive structure in many sub-units, we observe an accentuated phenomenon of reduction of the effective population size. In fact, the genetic variability in a population depends upon the rate of the allelomorphous genes in the genotype, on the frequencies of which act the evolutionary process (natural selection, migration, genetic drift, etc.) and the deviations from the random mating determined by adopted mating systems. The investigations on marker loci allow us to evaluate the evolution of the populations by means of gene frequencies, whose variations may be indicative of changes occurring in the whole genotype.

We could estimate the modifications of the genetic variability in three of the breeds examined: *Chiana*, for which the previous study on milk proteins was completed with the analysis of the acid variants of the β -casein (BETTINI & MASINA, 1972), *Piedmont* and *Aosta Red Pied*, for which were calculated also the casein haplotype frequencies (VOGLINO & CARIGNANO, 1975).

In the *Chiana* breed we observed that the heterozygosity values are increased for all loci, with the exception of the β -Cn locus almost unvaried. However, from our results the *Chiana* compared with the other breeds shows a relatively high variability for the single loci and for the whole of the loci considered, but a low variability for the casein cluster. This situation, together with the absence of recombinant types, which are related with the genetic effective population size, shows a certain uniformity of the breed.

From available information on the pedigree of analyzed cows it was possible to identify the genotype of two bulls employed in A.I. which revealed the same genotype : α_{s1} -Cn^C — β -Cn^{A2} — x -Cn^A/ α_{s1} -Cn^B — β -Cn^{A1} — x -Cn^B. The use in A.I. of these bulls may explain the relatively high frequencies of α_{s1} -Cn^C — β -Cn^{A2} — x -Cn^A and α_{s1} -Cn^B — β -Cn^{A1} — x -Cn^B types in the breed. Our purpose is to identify the genotype of the other bulls in A.I. in order to have also for the males the informations on the five loci examined.

With respect to *Piedmont* breed we calculated that the heterozygosity values both at the single casein loci and at the casein cluster are increased. This may be a result due to the preferential and large utilization in A.I. of bulls possessing alleles with low frequencies which in a short time spread in the population. This result is in agreement with the data reported by Di STASIO *et al.* (1977) based on the study of some blood biochemical polymorphisms.

On the contrary, in *Aosta Red Pied* breed we observed a reduction of the variability in all cases. We may suppose that the limited breeding areas, the particular geographic environment and inbreeding are the main causes of this situation, although a low genetic variability may be an original characteristic of the breed.

Up to the present the problem on the critical value of the variability, beyond which the negative effects on productive and reproductive characteristics appear, is distant from a satisfactory answer, involving many and complex phenomena whose discussion is beyond the aim of the present paper. However, the presence in these breeds of recombinant types at the casein cluster suggests that their population size is up to now not much reduced.

Indeed the rather limited number of samples examined may affect the conclusions presented here, but our results will be completed with those obtained by laboratories which studied other markers (about twenty loci) in the same breeds. It is hoped that our genetic investigations will contribute to a rather comprehensive picture of these cattle populations, some of them being to-day in hard need of an immediate action to be saved.

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Résumé

Analyse du polymorphisme des lactoprotéines dans quelques races bovines italiennes d'effectifs décroissants

Le polymorphisme génétique de cinq des six principales lactoprotéines (α -lactalbumine, β -lactoglobuline, α_{s1} , β - et x -caséine) a été étudié dans six races bovines autochtones italiennes d'effectifs décroissants : *Grise Alpine*, *Norica-Pinzgau*, *Valldôtaine pie noire*, *Valldôtaine pie rouge*, *Piémontaise* et *Chianine*.

Les cinq loci se sont révélés polymorphes, avec les allèles déjà décrits dans les races bovines européennes. La mise en évidence des allèles rares (α -La^A, β -Lg^D, α_{s1} -Cn^D, β -Cn^{A3}) peut contribuer à révéler d'intéressantes relations entre races.

L'examen des combinaisons alléliques contrôlées par l'unité génétique formée des trois loci des caséines a montré que les combinaisons les plus fréquentes sont α_{s1} -Cn^B — β -Cn^{A2} — x -Cn^A dans les races *Grise Alpine*, *Pinzgau* et *Piémontaise*, α_{s1} -Cn^B — β -Cn^{A1} — x -Cn^B dans la *Chianine*, α_{s1} -Cn^B — β -Cn^{A2} — x -Cn^B dans la *Valldôtaine pie rouge* et α_{s1} -Cn^B — β -Cn^B — x -Cn^B dans la *Valldôtaine pie noire*.

En ce qui concerne le variant β -Cn^{A3} on souligne qu'il a été trouvé associé non seulement avec α_{s1} -Cn^C, comme rapporté par GROSCLAUDE (1979) pour les races européennes, mais aussi avec α_{s1} -Cn^B (dans la race *Grise Alpine*). Cela pourrait signifier qu'il s'agit de variants dérivant de mutations différentes, hypothèse qui doit être confirmée par des études biochimiques.

Des combinaisons considérées comme recombinantes ont été trouvées dans toutes les races sauf la *Chianine*. Néanmoins le déséquilibre de linkage entre les loci des caséines est accentué dans toutes les races, à l'exception de la *Valldôtaine pie rouge*.

L'existence de polymorphisme de l' α -lactalbumine dans la *Piémontaise* et la *Chianine* a permis d'étudier les relations entre les loci α -La et β -Lg. La répartition des génotypes aux deux loci concorde avec celle qui est attendue dans l'hypothèse d'indépendance génétique.

Les relations génétiques entre les six races ont été étudiées avec deux méthodes (BOUQUET & GROSCLAUDE, 1968 ; NEI & ROYCHOUDHURY, 1974) qui ont donné à peu près les mêmes résultats, en montrant la plus haute ressemblance entre la *Piémontaise* et la *Grise Alpine*, et entre la *Piémontaise* et la *Chianine*.

La variabilité génétique des six races a été estimée, et pour trois d'entre elles, comparée avec les données des travaux précédents. Dans les races *Chianine* et *Piémontaise* il y aurait une augmentation et dans la *Valldôtaine pie rouge* une diminution de la variabilité génétique.

Riassunto

Studio sui polimorfismi proteici del latte in alcune razze bovine italiane in fase di contrazione numerica

Il lavoro concerne lo studio del polimorfismo genetico di cinque delle sei principali lattoproteine (α -lattoalbumina, β -lattoglobulina, α_{s1} , β - e x -caseina) in sei razze bovine autoctone italiane in fase di contrazione numerica : *Grigio Alpina*, *Norica-Pinzgau*, *Valldostana pezzata nera*, *Valldostana pezzata rossa*, *Piemontese* e *Chianina*.

Tutti i loci esaminati sono risultati polimorfi, rivelando gli alleli già descritti nelle razze bovine europee. In particolare, sono stati evidenziati alleli rari (α -La^A, β -Lg^D, α_{s1} -Cn^D, β -Cn^{A3}) la cui presenza può contribuire a rivelare interessanti relazioni fra le razze.

L'esame delle combinazioni alleliche controllate dall'unità genetica formata dai tre loci delle caseine ha rivelato che le combinazioni più frequenti sono α_{s1} -Cn^B — β -Cn^{A2} — x -Cn^A nelle razze *Grigio Alpina*, *Pinzgau* e *Piemontese*, α_{s1} -Cn^B — β -Cn^{A1} — x -Cn^B nella *Chianina*, α_{s1} -Cn^B — β -Cn^{A2} — x -Cn^B nella *Valldostana pezzata rossa* e α_{s1} -Cn^B — β -Cn^B — x -Cn^B nella *Valldostana pezzata nera*.

Riguardo alla variante β -Cn A³ si sottolinea che è stata trovata associata non soltanto con α_{s1} -Cn C, come riportato da GROSCLAUDE (1979) per le razze europee, ma anche con α_{s1} -Cn B (nella razza *Grigio Alpina*). Ciò potrebbe significare che si tratta di varianti che derivano da mutazioni differenti; naturalmente tale ipotesi deve essere confermata da studi biochimici.

In tutte le razze, ad eccezione della *Chianina*, sono state trovate combinazioni considerate ricombinanti. Tuttavia il disequilibrio da linkage fra i tre loci delle caseine è accentuato in tutte le razze, tranne che nella *Valdostana pezzata rossa*.

Lo studio delle relazioni fra i loci α -La e β -Lg, reso possibile dall'esistenza di polimorfismo dell' α -lattoalbumina e della β -lattoglobulina nelle razze *Piemontese* e *Chianina*, ha mostrato che la ripartizione dei genotipi ai due loci concorda con quella attesa nell'ipotesi di indipendenza genetica.

Le relazioni genetiche fra le sei razze sono state studiate con due metodi (BOUQUET e GROSCLAUDE, 1968; NEI e ROYCHOUDHURY, 1974) che hanno dato risultati simili; la più alta somiglianza è stata osservata fra la *Piemontese* e la *Grigio Alpina*, e fra la *Piemontese* e la *Chianina*.

Infine, si è valutata la variabilità genetica delle sei razze e, per tre di esse, la si è confrontata con i dati desunti da lavori precedenti. Per le razze *Piemontese* e *Chianina* è stato messo in evidenza un aumento, mentre per la *Valdostana pezzata rossa* una diminuzione della variabilità genetica.

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