

Genetic structure of milk proteins in Argentinian Holstein and Argentinian Creole cattle

M. A. POLI¹ and A. G. ANTONINI²

¹ *Inst. Genética, CICA-INTA, C.C.25, 1712-Castelar, Argentina*

² *CIGEBA, FCV, UNLP, 58 y 118, 1900-La Plata, Argentina*

POLI, M. A. and ANTONINI, A. G. 1991. Genetic structure of milk proteins in Argentinian Holstein and Argentinian Creole cattle. — *Hereditas* 115: 177–182. Lund, Sweden. ISSN 0018-0661. Received June 17, 1991. Accepted October 17, 1991

The genetic structure of milk proteins was studied in two completely different breeds: Argentinian Holstein (dairy breed; $n = 377$) and Argentinian Creole cattle (native breed; $n = 161$). Fairly large similarities were observed among Argentinian Holstein allelic frequencies reported in the present study and the values described in the literature for other Holstein populations. The high frequency of α_{s1} -Cn gene was the most remarkable gene frequency difference between Holstein and Creole breeds. Gametic phase disequilibrium was found among three casein loci but not between casein and β -lactoglobulin loci. Both breeds showed the same gametic disequilibrium in β -Cn A/k-Cn A and β -Cn A/k-Cn B haplotypes; however, the α_{s1} -Cn B/ β -Cn A haplotype was only in disequilibrium in Holstein breed. In general, the gametic phase disequilibrium found in both breeds was similar to those described for other breeds. The possible causes of gametic disequilibrium are discussed, and the results suggest that natural selection could be the most likely explanation.

Mario A. Poli, Inst. Genética, CICA-INTA, C.C. 25, 1712-Castelar, Argentina

Bovine milk has about 30.5 g/l proteins, 85 % of which consist of casein, 13 % of lactoglobulin, lactoalbumin, and serum albumin, and the rest is a small amount of immunoglobulins and blood serum proteins (EIGEL et al. 1984).

Since ASCHAFFENBURG and DREWRY (1955) described the presence of different β -lactoglobulin types in cow's milk, many studies have been carried out on the genetic polymorphisms of proteins in milk (ASCHAFFENBURG and THYMANN 1965; KIDDY et al. 1972; MACKINLAY and WAKE 1964; LI and GAUNT 1972; SEIBERT et al. 1987).

The first evidence of casein variation was also reported by ASCHAFFENBURG and DREWRY (1955), who noted that β -caseins exist in three forms (A, B, C). THOMPSON et al. (1962) observed that also α_{s1} -casein has three alternative forms (A, B, C). Later, NEELIN (1964) described genetic variation in k-caseins.

In milk proteins, as in some other multiple gene models, an allelic association can occur within gametes, and different factors can affect the gametic frequencies and gametic phase equilibrium. Different authors described linkage disequilibrium between loci in different breeds, and they estimated the recombination frequencies values to about 5–10 % (GROSCLAUDE et al. 1964,

1965; LARSEN and THYMANN 1966; HINES et al. 1969; MERLIN and DI STASIO 1982; GRAML et al. 1986; TEJEDOR et al. 1987; GELDERMANN et al. 1985). These authors assumed the possibility of linked loci explaining differences between observed and expected haplotype frequencies.

The Argentinian Holstein (AH) is the most important dairy breed used in this country. The first cows were introduced in Argentina from The Netherlands in 1883 (INCHAUSTI and TAGLE 1967), and since then the breeders have been importing animals. During the last three decades, breeders also have imported semen and embryos, principally from Canada and USA. In Argentina, as in the rest of the world, AH has been intensively selected for milk yield.

On the other hand, Argentinian Creole (ACr) is a "native" *Bos taurus* breed different from Cuban Creole, described by HERNANDEZ et al. 1983, which is a crossbreed between *Bos taurus* and *Bos indicus*. ACr's ancestries were the former cows brought by Columbus during the American conquest period. Herds sampled in this study had never been crossed with any other artificially selected breed. This breed has been supported by natural selection for nearly five centuries, and it is well adapted to hard environmental conditions.

For all these reasons ACr is an interesting and valuable genetic resource. The phenotype, genetic and performance characteristics of ACr are described in GANADO BOVINO CRIOLLO (1986).

The present study was undertaken to investigate the genetic polymorphisms of milk proteins and the status of the genetic relationship of these loci in two completely different breeds: Argentinian Holstein and Argentinian Creole cattle.

Material and methods

Milk samples were collected from 377 AH (three herds) and 161 ACr (four herds) cattle.

Methods described by PETERSON (1963) and WOYCHIK (1964) and by KIDDY et al. (1972) were applied for the genetic typing of both casein and lactoglobulins, respectively. These methods do not allow the distinction among β -caseins A_1 , A_2 and A_3 .

Direct counting was used to estimate allelic frequencies, and to test Hardy-Weinberg equilibrium, a chi-square analysis was performed.

Differences between genotypic frequency distributions were tested by the G test of independence (SOKAL and ROHLF 1979).

The gametic phase disequilibrium was estimated by the equation: $D_{ij} = f_{ij} - p_i q_j$, in which f_{ij} represents the gametic frequencies for $A_i B_j$, and $p_i q_j$ are allelic frequencies of A_i and B_j in the two loci. If all gametes are identifiable it is possible to estimate D_{ij} , but for the casein loci, coupling and repulsion of the double heterozygote cannot be distinguished. In order to overcome this impediment, the gametic frequencies were calculated by iterative method (CEPELLINI et al. 1955) and the statistical significance of the disequilibrium was tested by χ^2 (WEIR and COCKERHAM 1978).

Results

Table 1 shows the allelic and genotypic frequencies and Hardy-Weinberg equilibria for the α_{s1} , β , and k-casein loci and the β -lactoglobulin locus. It can be seen that only the k-casein locus was in disequilibrium in AH ($P < 0.001$) and only the β -lactoglobulin locus regarding ACr ($P < 0.01$). Allelic frequencies presented show narrow differences for the β -Cn, k-Cn and β -Lg loci, and a large difference for α_{s1} -Cn.

Table 1. Genotypic and allelic frequencies and Hardy-Weinberg equilibrium

Locus	Breed	Genotypes			Alleles		χ^2
		BB	BC	CC	B	C	
α_{s1} -Cn	AH	0.934	0.063	0.002	0.966	0.034	0.71 ns
	ACr	0.596	0.360	0.043	0.776	0.223	0.23 ns
β -Cn	AH	0.989	0.010	0.000	0.995	0.005	0.00 ns
	ACr	0.857	0.143	0.000	0.928	0.071	0.95 ns
k-Cn	AH	0.727	0.102	0.170	0.778	0.222	139.4***
	ACr	0.559	0.385	0.056	0.751	0.248	0.02 ns
β -Lg	AH	0.194	0.476	0.330	0.460	0.539	0.17 ns
	ACr	0.297	0.392	0.310	0.494	0.506	7.32**

AH: Argentinian Holstein

ACr: Argentinian Creole

** : $P < 0.01$

*** : $P < 0.001$

ns: non-significant

The β -Cn BB genotype was absent in both breeds, and a deficiency of heterozygotes AB can be observed in both k-Cn (AH) and β -Lg (ACr) (Table 1).

Genotypic frequencies and G values are drawn in Fig. 1. It is possible to see that AH and ACr had significantly different genotypic distributions regarding α_{s1} , β , k-Cn, and β -Lg.

The heterozygosity mean (\bar{H}) value also was different between breeds: AH = 0.17 ± 0.1 and ACr = 0.32 ± 0.05 and, as could be predicted for a native breed, \bar{H} was higher for ACr than for AH.

The allelic frequencies in different Holstein populations, taken from the literature and the present study, are summarized in Table 2. Fairly large similarities can be noticed among AH allelic frequencies reported in the present study and the values found by MCLEAN et al. (1984), NG KWAI HANG et al. (1984), and TEJEDOR et al. (1987) for other Holstein populations, especially regarding the casein loci. However, small differences were found between our values and those reported in other studies regarding the β -lactoglobulin locus, as shown in Table 2.

The gametic phase disequilibrium (D) between casein and β -lactoglobulin loci are given in Table 3, and the values are expressed as squares of the gametic correlation (r^2). The sign of D and the statistical significance are added.

Non-significant gametic disequilibrium was found between the casein loci and the β -lactoglobulin locus. AH and ACr showed the same gametic disequilibrium in β -Cn A/k-Cn A and β -Cn A/k-Cn B, but the α_{s1} -Cn B/ β -Cn A disequilibrium was only significant in AH.

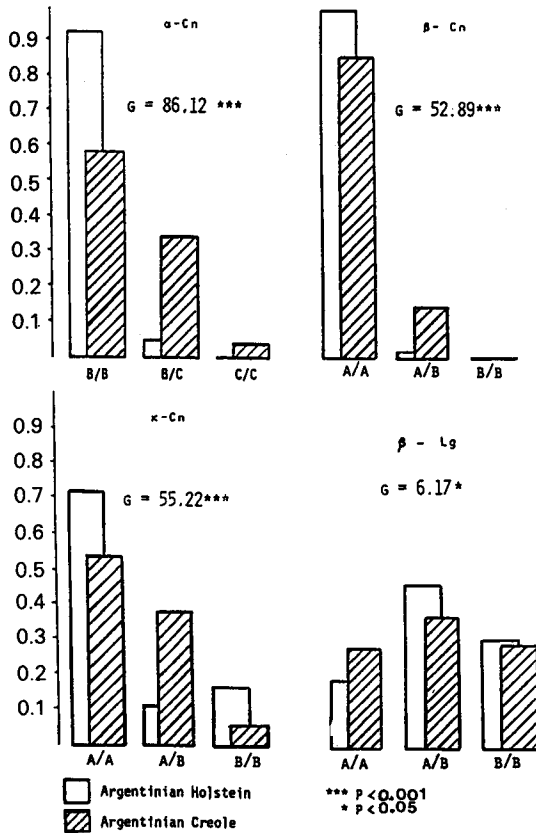


Fig. 1. Genotypic distribution of the milk proteins.

Discussion

A breed of farm animals is not usually a Mendelian population where several conditions are given (large number of individuals, random mating, etc.). Therefore, there are some limitations to

applying the Hardy-Weinberg law. It can be assumed that the breeds used in this study have similar Hardy-Weinberg limitations. Thus, the allelic frequency, genotype and mean heterozygosity of milk proteins can be used as the first tools to describe these populations.

The high frequency of the α_{s1} -casein gene, which is the most remarkable gene frequency difference between AH and ACr (Table 1) could be the result of a long-term artificial selection for high milk yield, as was pointed out by LIN et al. (1986).

Genotypic frequencies and G values (Table 1; Fig. 1) should be taken as merely indicative of differences between breeds because of the small number of genetic markers analyzed.

Many parameters, such as similar allelic frequencies, gametic disequilibria, quantity of semen and embryos imported from Canada and USA, the same selective criteria applied by breeders and phenotypic characteristics, lead us to suggest that AH could be considered as any other Holstein population around the world. Therefore, Argentina was not out of the world "Holsteinization" process mentioned by CUNNINGHAM (1981).

Theoretically, neutral alleles in natural populations at different loci could be associated at random, and thus haplotype frequencies could be in Hardy-Weinberg equilibrium. Nevertheless, non-random gametic association can be observed more frequently than expected from the Hardy-Weinberg law. This gametic disequilibrium could arise from mating structure, finite size population, migration, or selection.

In farm populations, when no historical information is available, it is very difficult to find a cause for the gametic disequilibrium. In the past, different factors such as strong inbreeding could account for gametic disequilibrium, and these factors may

Table 2. Allelic frequencies in different Holstein populations

Locus	Allele	1	2	3	Mean \pm SD	Present study
α_{s1} -Cn	B	0.970	0.963	0.983	0.972 \pm 0.01	0.965
	C	0.027	0.037	0.034	0.026 \pm 0.01	0.034
β -Cn	A	0.993	0.964	0.983	0.980 \pm 0.01	0.994
	B	0.007	0.035	0.014	0.018 \pm 0.01	0.005
κ -Cn	A	0.744	0.678	0.767	0.729 \pm 0.04	0.718
	B	0.256	0.322	0.233	0.270 \pm 0.04	0.221
β -Lg	A	0.387	0.386	0.363	0.378 \pm 0.01	0.460
	B	0.613	0.614	0.637	0.621 \pm 0.01	0.539

1: NG KWAI HANG et al. 1984
 2: MCLEAN et al. 1984
 3: TEJEDOR et al. 1987

Cn: Casein
 Lg: Lactoglobulin

Table 3. r^2 values of the milk protein gametes

Gamete	AH				ACr			
		N	r^2 ($\times 10^4$)		N	r^2 ($\times 10^4$)		
α_{s1} -Cn/ β -Cn	BA	526	915.78***	+	213	33.26 ns	-	
	BB	0	-		63	33.26 ns	+	
	CA	24	420.83 ns	-	19	33.05 ns	+	
	CB	0	-		3	30.96 ns	-	
α_{s1} -Cn/ k -Cn	BA	296	41.89 ns	+	176	8.09 ns	-	
	BB	77	20.78 ns	-	55	0.93 ns	+	
	CA	10	0.10 ns	+	51	0.93 ns	+	
	CB	1	0.30 ns	-	15	0.93 ns	-	
α_{s1} -Cn/ β -Lg	BA	236	13.98 ns	-	120	11.39 ns	-	
	BB	288	14.93 ns	+	108	8.93 ns	+	
	CA	11	18.18 ns	+	33	3.72 ns	-	
	CB	9	322.20 ns	-	31	3.72 ns	+	
β -Cn/ β -Lg	AA	243	0.71 ns	+	140	19.60 ns	-	
	AB	298	71.78 ns	-	130	11.20 ns	+	
	BA	4	4.75 ns	+	13	19.94 ns	+	
	BB	3	71.77 ns	+	9	10.95 ns	-	
β -Cn/ k -Cn	AA	229	949.66***	+	223	1289.93***	+	
	AB	77	685.71*	-	53	1277.00**	-	
	BA	0	-		5	1283.46 ns	-	
	BB	3	0.24 ns	+	7	1283.46 ns	+	
k -Cn/ β -Lg	AA	121	4.96 ns	-	112	6.68 ns	-	
	AB	171	4.68 ns	+	106	0.09 ns	-	
	BA	31	216.13 ns	+	48	382.58 ns	+	
	BB	39	5.13 ns	-	26	115.02 ns	-	

AH: Argentinian Holstein ***: $p < 0.001$
ACr: Argentinian Creole **: $p < 0.01$
N: Number of gametes *: $p < 0.05$
-; +: Sign of D ns: non-significant

remain today. As proposed by HEDRICK (1983), the cause of gametic disequilibrium must be attributed, in a strict sense, over the time and space. It is necessary to know the population's history in order to calculate size migration, non-random mating, and linkage distance between the loci involved. Perhaps the most rational way to do it is to consider each possible cause of gametic disequilibrium and to eliminate those less important causes in the population under study until only one cause remains (HEDRICK 1983).

WEIR and HILL (1980) stated that mating structure has no significant effect on the gametic disequilibrium when the effective population size (N_e) is large. We estimated the effective population size in ACr ($N_e = 109$) and in AH ($N_e = 237$) large enough values to avoid mating structure as a principal cause of disequilibrium in these breeds. Besides, as mentioned by DEGOS and BODMER (1972) very high levels of inbreeding are necessary to cause significant gametic phase disequilibrium.

Gametic phase disequilibria can also be created by the mixing of two populations which are genet-

ically different. In other words, the allelic frequencies of both loci in the two populations must be different, and these differences must be large enough in order to create gametic disequilibrium. Moreover, the migration rate during the mixture influences gametic disequilibrium. Migration rate in AH has been so high, especially during the last three decades, because AH was absorbed by imported Holstein and the mixture effect today would be low. Allelic frequencies presented in Table 2 indicate that AH and other Holstein populations are fairly similar. It is evident that possible differences that could have existed in the past have disappeared, and that AH could be considered similar to Canadian or American Holstein.

The ACr samples were taken from four herds with founders derived from the North-West Argentina region in the earlier '40s. According to the available information about gene frequencies, genotypic distribution, phenotypic characteristics, and historical data, the ACr cattle studied may be considered as one population (POLI 1989). Thus, the population mixture effect could be considered a minimum.

GRAML et al. (1986) summarized the D values among casein loci in various cattle breeds. Our correlation gametic values (r^2), shown in Table 3, agree with those of GRAML et al. (1986) and TEJEDOR et al. (1987). The haplotypes β -Cn A/k-Cn A and β -Cn A/k-Cn B (Table 3) were similar in AH and ACr like in other sixteen breeds. Moreover, in this study D values for AH and ACr had the same sign. The r^2 value of the α_{s1} -Cn B/ β -Cn A haplotype (Table 3) was only significant in AH, and it is shared by eighteen other breeds (GRAML et al. 1986), but with opposite sign. Another exception, reported in the literature, was described by MERLIN and DI STASIO (1982) in Aosta Red Pied breed.

GELDERMANN et al. (1985) found that the α_{s1} -Cn B/ β -Cn A haplotype was in disequilibrium, and they related this result to a higher milk yield. Holstein is a breed intensively selected for high milk yield, but it is difficult to accept that the artificial selection could be the cause of that gametic disequilibrium. The artificial selection explanation is also contradicted by the results on Jersey by LARSEN and THYMANN (1966) and KING et al. (1965), a breed selected for high milk fat content, where this haplotype was negatively correlated with high milk yield.

THOMSON (1977) noted that the selection operating on one locus or on closely linked neutral loci can also generate gametic disequilibrium. If we consider fitness as the ability of an animal to produce progeny, milk yield is an important and crucial trait in mammals. The cattles have developed under the guide of natural forces in the early times and supplemented by artificial selection when they were domesticated. Therefore, in the past they must have developed the capacity to produce the quality and quantity of milk necessary to ensure a good start for the calf's survival and adequate growth.

In this study, we found three haplotypes in disequilibrium, which were previously reported in different breeds (dairy, beef, dual purpose, native breeds), widespread around the world. It is possible to speculate that those haplotypes in disequilibrium shared by many different breeds, would be connected with a minimum quality and quantity of milk and that they were naturally selected in the past. Probably, they could be connected with milk components rather than high milk yield and, as suggested by GONYON et al. (1987) and HAENLEIN et al. (1987), they could be useful markers for milk component traits.

As was discussed above, the structural mating, finite size population, or migration could be the less appropriate mechanism to explain the gametic disequilibrium found among milk protein gene loci in AH and ACr. We consider that natural selection could be the most important cause.

Acknowledgements. — We wish to thank Ing. E. Favret for valuable discussions, Dra. L. Semorile and Ing. R. Wolff for their critical reading of the manuscript, and M. C. Argerich for the technical assistance.

References

- ASCHAFFENBURG, R. and DREWRY, J. 1955. Occurrence of different beta-lactoglobulins in cow's milk. — *Nature* 176: 218–219
- ASCHAFFENBURG, R. and THYMANN, M. 1965. Simultaneous phenotyping procedure for the principal proteins of cow's milk. — *J. Dairy Sci.* 48: 1524–1526
- CEPELLINI, R., SINISCALCO, M. and SMITH, C. A. B. 1955. The estimation of gene frequencies in a random-mating population. — *Hum. Genet.* 20: 97–115
- CUNNINGHAM, E. P. 1981. European Friesian — the Canadian and American invasion. — In: *Methods and Experiences with "In Situ" Preservation of Farm Animals*. 1990 (ed. I. BODO), FAO Animal Production and Health, paper 80, p. 85–102
- DEGOS, L. and BODMER, W. F. 1972. The effects of inbreeding on a two locus system. — In: *Histocompatibility Testing* (eds J. DAUSSET and J. COLOMBANI), Munksgaard, Copenhagen, p. 545–547
- EIGEL, W. N., BUTLER, J. E., ERNSTROM, C. A., FARRELL, H. M., HARWALKAR, W. R., JENNESS, R. and WHITNEY, R. 1984. Nomenclature of Proteins of Cow's Milk: Fifth revision. — *J. Dairy Sci.* 67: 1599–1631
- GANADO BOVINO CRIOLLO 1986. — *Ed. Subcomité Asesor del Arido Subtropical Argentino*, 224 pp
- GELDERMANN, H., PIPER, U. and ROTH, B. 1985. Effects of marked chromosome section on milk performance in cattle. — *Theor. Appl. Genet.* 70: 138–146
- GONYON, D. S., MATHER, R. E., HINES, H. C., HAENLEIN, G. F. W., ARAVE, C. W. and GAUNT, S. N. 1987. Associations of bovine blood and milk polymorphisms with lactation traits: Holstein. — *J. Dairy Sci.* 70: 2585–2598
- GRAML, R., BUCHBERGER, J. and PIRCHNER, F. 1986. Genetic disequilibrium between the α_{s1} , β , k-Casein and the β -Lactoglobulin loci of the Bavarian Brown and Bavarian Simmental cattle. — *Génét. Sélect. Evol.* 18: 1–10
- GROSCLAUDE, F., GARNIER, J., RIBADEAU-DUMAS, B. and JEUNET, R. 1964. Etreite dépendance des loci controlant le polymorphisme des caseines α_{s1} et β . — *C. R. Acad. Sci. (Paris)* 259: 1569–1571
- GROSCLAUDE, F., PUJOLLE, J., GARNIER, J. and RIBADEAU-DUMAS, B. 1965. Déterminisme génétique des caseins K du lait de vache; étroite liaison du locus k-Cn avec les loci α_{s1} et β -Cn. — *C. R. Acad. Sci. (Paris)* 261: 5229–5232
- HAENLEIN, G. F. W., GONYON, D. S., MATHER, R. E. and HINES, H. C. 1987. Associations of bovine blood and milk polymorphisms with lactation traits: Guernseys. — *J. Dairy Sci.* 70: 2599–2609
- HEDRICK, P. W. 1983. Genetics of populations. — *Ed. Science Books International*, 386 pp.
- HERNANDEZ, M. H., GRANADO, A. and PEREZ-BEATO, O. 1983. Polimorfismo de seis sistemas sanguíneos y cinco lácteos en vacas de la raza Criollo de Cuba. — *Rev. Cub. Cienc. Vet.* 14: 253–260
- HINES, H. C., KIDDY, C. A., BRUN, E. W. and ARAVE, C. W. 1969. Linkage among cattle blood and milk polymorphisms. — *Genetics* 62: 401–412

- INCHAUSTI, D. and TAGLE, E. C. 1967. Bovinotecnia. — *Ed. El Ateneo*, p. 481–492
- KIDDY, C. A., ROLLINS, R. E. and ZIKAKIS, J. P. 1972. Discontinuous polyacrylamide electrophoresis for β -lactoglobulins typing of cow's milk. — *J. Dairy Sci.* 55: 1506–1507
- KING, J. W. B., ASCHAFFENBURG, R., KIDDY, C. A. and THOMPSON, M. P. 1965. Non independent occurrence of α_1 and β -casein variants of cow's milk. — *Nature* 206: 324–325
- LARSEN, R. and THYMAN, M. 1966. Studies on milk protein polymorphism in Danish cattle and the interaction of the controlling genes. — *Acta Vet. Scand.* 7: 189–205
- LI, F. H. and GAUNT, S. N. 1972. A study of genetic polymorphisms of milk β -lactoglobulin, α_1 -Casein, β -Casein and K-Casein in five dairy breeds. — *Biochem. Genet.* 6: 9–20
- LIN, C. Y., MCALLISTER, A. J., NG KWAI HANG, K. F. and HAYES, J. F. 1986. Effects of milk protein loci on first lactation production in dairy cattle. — *J. Dairy Sci.* 69: 704–712
- MACKINLAY, A. G. and WAKE, R. G. 1964. The heterogeneity of K-Casein. — *Biochem. Biophys. Acta* 93: 378–386
- MCLEAN, D. M., GRAHAM, E. R. B., PONZONI, R. W. and MCKENZIE, H. A. 1984. Effects of milk protein genetic variants on milk yield and composition. — *J. Dairy Res.* 51: 531
- MERLIN, P. and DI STASIO, L. 1982. Study on milk proteins loci in some decreasing Italian cattle breeds. — *Ann. Génét. Sél. Anim.* 14: 17–28
- NEELIN, J. M. 1964. Variant of K-casein revealed by improved starch gel electrophoresis. — *J. Dairy Sci.* 47: 506–510
- NG KWAI HANG, K. F., HAYES, J. F., MOXLEY, J. E. and MONARDES, H. G. 1984. Association of genetic variants of casein and milk serum proteins with milk, fat and protein production by dairy cattle. — *J. Dairy Sci.* 67: 835–840
- PETERSON, R. F. 1963. High resolution of milk protein obtained by gel electrophoresis. — *J. Dairy Sci.* 46: 1136–1139
- POLI, M. A. 1989. Polimorfismo proteico en bovinos Criollo y Nelore. — *Abstr. XX Congreso Argentino de Genética, Bahía Blanca, 17-20/9, 1989*, p. 37
- SEIBERT, B., ERHARDT, G. and SENFT, B. 1987. Detection of a new K-Casein variant in cow's milk. — *Anim. Genet.* 18: 269–272
- SOKAL, R. R. and ROHLF, F. J. 1979. Biometria. 1 Ed. — *Es-pañola. Ed. Blume*, 655 pp.
- TEJEDOR, T., ALTARRIBA, J. and ZARAZAGA, I. 1967. Estructura alotípica de la población Holstein-Friesian en relación con los loci de proteínas lácteas. — *Genética Ibérica* 39: 85–103
- THOMPSON, M. P., KIDDY, C. A., PEPPER, L. and ZITTLE, C. A. 1962. Variations in the α_1 -Casein of individual cow's milk. — *Nature* 195: 1001–1002
- THOMSON, G. 1977. The effects of a selected locus on linked neutral loci. — *Genetics* 85: 753–788
- WEIR, B. C. and COCKERHAM, C. C. 1978. Testing hypothesis about linkage disequilibrium with multiple alleles. — *Genetics* 88: 633–642
- WEIR, B. S. and HILL, W. G. 1980. Effect of mating structure on variation in linkage disequilibrium. — *Genetics* 95: 477–488
- WOYCHIK, J. H. 1964. Polymorphism in K-Cn of cow's milk. — *Biochem. Biophys. Commun.* 16: 267–271