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A Study of Genetic Polymorphisms of Milk β-Lactoglobulin, α_{S1}-Casein, β-Casein, and κ-Casein in Five Dairy Breeds

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Gene frequencies of the milk β-lactoglobulin, α_{S1}-casein, β-casein, and κ-casein loci have been estimated from 1663 cows of five dairy breeds. Departure from Hardy–Weinberg equilibrium was found only in the κ-casein system in Jerseys. However, chance alone could have accounted for this single significant finding. Results of pairwise comparisons among the five breeds of allele frequencies at these milk protein loci indicate that of the 40 possible tests, only six comparisons are not significant at the 5% probability level. It would appear that these breeds are characterizable in terms of the gene frequencies of these milk protein loci. Nonindependent assortment of genotypes among these milk protein loci was also studied. The closely linked casein loci were not independent in almost all the breeds where tests could be carried out. The only exception was between the α_{S1}-casein and κ-casein loci in Holsteins. β-Lactoglobulin was independent of the casein loci in all breeds except Brown Swiss, where it was found to be significantly associated with κ-casein. Close linkage is proposed as an important factor for maintaining the observed milk protein polymorphisms.

INTRODUCTION

The four bovine milk proteins, β-lactoglobulin, α_{S1}-casein, β-casein, and κ-casein, are genetically polymorphic in various European and Zebu breeds. Aschaffenburg and Drewry (1957) discovered that the two forms of β-lactoglobulin in milk are controlled by two codominant autosomal alleles, Lg^A and Lg^B, in the Ayrshire, Guernsey, Holstein, and Shorthorn cattle of England. Subsequently, a new allele, Lg^C, was found in Jersey cattle of Australia by Bell (1962), in the African Nguni breed by Osterhoff and Pretorius (1966), and in the Indian and African Zebu breeds by Aschaffenburg *et al.* (1968). Still another allele, Lg^D, was found in German Brown cattle by Meyer

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(1966) and in the French Montebeliarde breed by Grosclaude *et al.* (1966*b*). Kiddy *et. al.* (1965) found two alleles, Lg^A and Lg^B, in Ayrshire, Guernsey, Brown Swiss, Holstein, and Jersey cattle of the United States. From these studies, it was established that β-lactoglobulin A, B, C, and D are controlled by separate codominant autosomal alleles.

Kiddy *et al.* (1964) first demonstrated that α_{S1}-casein A, B, and C are controlled by three codominant autosomal alleles, α_{S1}-Cn^A, α_{S1}-Cn^B, and α_{S1}-Cn^C, while a new allele, α_{S1}-Cn^D, was found in the Flemande breed of France by Grosclaude *et al.* (1966*a*). In order of decreasing electrophoretic mobility at alkaline *p*H, the four variants are α_{S1}-casein A, D, B, and C. Aschaffenburg (1963) first established that β-casein A, B, and C are controlled by codominant autosomal alleles, β-Cn^A, β-Cn^B, and β-Cn^C in Channel Island breeds. Another allele, β-Cn^D, was found later in Indian Zebu cattle by Aschaffenburg (1968). Recently, β-casein A was further separated into β-casein A1, A2, and A3 at acid *p*H by Peterson and Kopfler (1966); these newly found casein variants are also controlled by codominant autosomal alleles, β-Cn^{A1}, β-Cn^{A2}, and β-Cn^{A3} (Kiddy *et al.*, 1966). In increasing electrophoretic mobility at acid *p*H, the five variants are β-casein A3, A2, A1, B, and C. The occurrence of the two forms of κ-casein, A and B, in decreasing electrophoretic mobility at alkaline *p*H was first reported independently by Neelin (1964) in Canada and by Schmidt (1964) in Holland. Later, Woychik (1965) in the United States also confirmed that there are two genetic variants.

Grosclaude *et al.* (1964) in France and King *et al.* (1965) in England both reported nonindependent inheritance of the various casein loci, while Hines *et al.* (1969) in the United States reported close linkage between the α_{S1}-casein and β-casein loci, the α_{S1}-casein and κ-casein loci, and the β-casein and κ-casein loci with recombination frequencies of 0.03, 0.12, and 0.00, respectively. Two excellent review articles are available on these inherited variants of milk, one with emphasis on the distribution of gene frequencies in various breeds of different countries (Aschaffenburg, 1968) and another with emphasis on the chemical and physical properties of these milk protein variants (McKenzie, 1967). Information on the distribution of gene frequencies of these variants in the common dairy breeds of the United States is still very limited, and, as yet, little has been done to compare gene frequencies among breeds or to explore possible nonrandom associations of genotypes among these loci.

The purposes of the present article are (a) to describe the distribution of gene frequencies of β-lactoglobulin, α_{S1}-casein, β-casein, and κ-casein loci in Ayrshire, Brown Swiss, Holstein, Guernsey, and Jersey cattle in Massachusetts and Connecticut; (b) to compare the gene frequencies of these milk protein polymorphic systems among breeds; and (c) to explore nonindependent assortment of genotypes among these milk protein loci in the five dairy breeds.

MATERIALS AND METHODS

A total of 1663 cows of the five dairy breeds Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey were used for genetic typing of the milk β-lactoglobulin, α_{S1}-casein, and κ-casein loci. A skimmed-milk sample of each cow was used for electrophoresis. The vertical polyacrylamide gel electrophoresis cell, model E-C 474 made by E-C Apparatus Company of Philadelphia, and the HEATHKIT model IP-32 power supply unit were used for the various electrophoresis procedures.

Genetic typing of the β-lactoglobulins was carried out by the method of discon-

tinuous polyacrylamide gel electrophoresis[3] in tris–glycine buffer, *p*H 8.3. Genetic typing of α_{S1}-caseins, β-caseins, and κ-caseins was performed by the method of simultaneous typing of these variants with 9% polyacrylamide gel and tris–EDTA–boric acid buffer, *p*H 9.1. β-Casein A was further separated into A1, A2, and A3 with 10% polyacrylamide gel and 7.7% acetic acid. Since all the alleles involved are codominant, gene frequencies of each polymorphic system were calculated by simple counting. Heterogeneity tests were made for each polymorphic system in each breed since the observations on breeds stem from individual herds located at different farms. No significant heterogeneity was found in any of these tests (see Li, 1970). The comparability of allele frequencies of a given locus among breeds was evaluated by $2 \times J$ contingency tables (Mather, 1947), while non-independent segregation of each pair of loci within a given breed was tested by two-way contingency tables (Bancroft, 1968). This latter method of analysis does not, of course, separate the effects of linkage from interloci interaction.

RESULTS AND DISCUSSION

Gene frequencies of the β-lactoglobulin, α_{S1}-casein, β-casein, and κ-casein loci in the five dairy breeds estimated in this study are summarized in Table I. It can be seen from Table I that Lg^B is predominant in most of the dairy breeds, with the exception of Holsteins. The frequency of Lg^B reaches 80% in Ayrshire and 60% in Brown Swiss, Guernsey, and Jersey cattle, but is approximately in equal proportion with Lg^A in the Holstein breed. Only two β-lactoglobulin alleles, Lg^A and Lg^B, were found in the 270 Jersey cows investigated in this study, whereas a rare third allele, Lg^C, has been found in Jerseys of Australia (Bell, 1962).

Of the 1663 cows typed for α_{S1}-casein genotypes, the rare allele α_{S1}-Cn^A was in heterozygous form in three Holstein cows of the same herd (Table I). This rare allele has been found so far only in Holsteins and Red Danish cattle (Kiddy *et al.*, 1964; Larsen and Thymann, 1966). Recently, biochemical analyses of α_{S1}-casein A from Red Danish cattle proved it to be identical to the same protein in the Holstein breed (Farrell *et al.*, 1971). The Ayrshire cows were all found to be homozygous for α_{S1}-Cn^B, and this too is in agreement with results obtained from other studies (Aschaffenburg, 1968; Kiddy *et al.*, 1968). It appears that α_{S1}-Cn^B is also the most common allele in the other four dairy breeds, 94% in Holstein, 98% in Brown Swiss, 79% in Guernsey, and 74% in Jersey. The incidence of α_{S1}-Cn^C was found to be under 5% in Holstein and Brown Swiss but reached 21% and 26% in Jerseys and Guernseys, respectively. It is important to point out that recent biochemical analyses suggest that α_{S1}-Cn^A probably is not a recent mutant (Farrell *et al.*, 1971) even though the frequency of α_{S1}-Cn^A was found to be under 1% not only in this study but in other studies as well (Aschaffenburg, 1968). In addition, α_{S1}-casein A differs in 12 amino acid residues from α_{S1}-casein B and α_{S1}-casein C, whereas the latter two casein variants differ in only one amino acid residue. It has been suggested that α_{S1}-casein A has arisen through deletion of a portion of one of the other two alleles (Aschaffenburg, 1968).

Ayrshire cows were found to be monomorphic for β-Cn^A. But upon further separation of this allele, it was found that β-Cn^{A1} is predominant in Ayrshires. On the other hand, β-Cn^{A2} is the more common allele but decreases in percentage in the Guernsey, Brown Swiss, Jersey, and Holstein breeds. β-Cn^{A3} was found for the first

[3] All electrophoresis methods used in this study were obtained in October 1967 from Dr. C. A. Kiddy, Dairy Cattle Research Branch, USDA, ARS, ASRD, Beltsville, Md. 20705.

Table I. Estimated Gene Frequencies of β-Lactoglobulin, α_{s_1}-Casein, β-Casein, and κ-Casein Systems in the Cows of the Five Dairy Breeds

Breed	β-lactoglobulin system				α_{s_1}-casein system				
	No. of herds	No. of cows	Gene frequency		No. of herds	No. of cows	Gene frequency		
			A	*B*			*A*	*B*	*C*
Ayrshire	5	191	0.17±0.02	0.83±0.02	5	257		1.00	
Brown Swiss	8	259	0.33±0.02	0.67±0.02	8	282		0.98±0.01	0.02±0.01
Guernsey	5	278	0.38±0.02	0.62±0.02	5	296		0.79±0.02	0.21±0.02
Holstein	16	494	0.50±0.02	0.50±0.02	17	541	0.003±0.00	0.94±0.01	0.06±0.01
Jersey	7	270	0.36±0.02	0.64±0.02	7	287		0.74±0.02	0.26±0.02

Breed	β-casein system							κ-casein system			
	No. of herds	No. of cows	Gene frequency					No. of herds	No. of cows	Gene frequency	
			A1	*A2*	*A3*	*B*	*C*			*A*	*B*
Ayrshire	5	202	0.67±0.02	0.32±0.02	0.01±0.00			5	211	0.70±0.02	0.30±0.02
Brown Swiss	7	235	0.15±0.02	0.72±0.02		0.10±0.01	0.03±0.01	8	290	0.41±0.02	0.59±0.02
Guernsey	7	262	0.06±0.01	0.88±0.01		0.01±0.00	0.05±0.01	5	297	0.59±0.02	0.41±0.02
Holstein	18	526	0.49±0.02	0.49±0.02	0.01±0.00	0.01±0.00		17	543	0.75±0.01	0.25±0.01
Jersey	7	298	0.09±0.01	0.54±0.02		0.37±0.02	0.003±0.00	7	297	0.12±0.01	0.88±0.01

Table II. Breed Comparisons for Differences in Gene Frequency of the Four Genetic Systems in the Five Dairy Breeds by $2 \times J$ Contingency Tables

	β-Lg			α_{s1}-Cn			β-Cn			κ-Cn		
reed[a]	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P
A *vs.* B	1	13.3	<0.01	1	5.7	<0.02	4	136.3	<0.01	1	39.3	<0.01
A *vs.* G	1	23.9	<0.01	1	60.5	<0.01	4	202.3	<0.01	1	5.8	<0.02
A *vs.* H	1	60.3	<0.01	2	15.2	<0.01	3	20.3	<0.01	1	2.5[b]	<0.14
A *vs.* J	1	19.5	<0.01	1	78.3	<0.01	4	212.2	<0.01	1	174.6	<0.01
B *vs.* G	1	1.9[b]	<0.16	1	48.6	<0.01	3	37.1	<0.01	1	18.8	<0.01
B *vs.* H	1	20.4	<0.01	2	5.5[b]	<0.06	4	123.8	<0.01	1	93.7	<0.01
B *vs.* J	1	0.7[b]	<0.04	1	67.1	<0.01	3	51.7	<0.01	1	62.5	<0.01
G *vs.* H	1	9.5	<0.01	2	48.1	<0.01	4	171.5	<0.01	1	23.3	<0.01
G *vs.* J	1	0.3[b]	<0.60	1	2.3[b]	<0.14	3	127.7	<0.01	1	141.3	<0.01
H *vs.* J	1	13.2	<0.01	2	74.7	<0.01	4	272.4	<0.01	1	304.0	<0.01

[a] A, Ayrshire; B, Brown Swiss; G, Guernsey; H, Holstein; J, Jersey.
[b] n.s.

time in the Ayrshire breed, reaches only 1% in Holsteins, and was totally absent in Brown Swiss, Guernsey, and Jersey cattle. No β-Cn^{A3} homozygotes were found. β-Cn^{B} was found in four breeds. Its frequency was low in all but the Jersey (36%). β-Cn^{C} was absent in Ayrshires and Holsteins. The latter is in agreement with most reports from other sources since thus far β-Cn^{C} has been reported in only a single Holstein cow (Aschaffenburg, 1968). β-Cn^{C} was found to be under 5% in the other breeds. Two Jersey cows were found to carry β-Cn^{c} (Li, 1970, p. 66); this allele has not previously been reported in this breed (Aschaffenburg, 1968). Information about the β-Cn^{A3} allele is scanty, and one cannot determine whether the situation with respect to this allele is similar to that of α_{S1}-Cn^{A}, a possible ancient mutant (Farrell *et al.*, 1971). Finally, the two κ-casein alleles, κ-Cn^{A} and κ-Cn^{B}, were found in all of the five dairy breeds. κ-Cn^{A} predominates in Holstein cattle, where it reaches 75%, whereas κ-Cn^{B} is the more frequent allele in Jerseys, reaching 88%. In the other breeds, these two alleles are approximately in equal proportions.

The observed genotypic frequencies of each system in all the five breeds follow Hardy–Weinberg expectations with the single exception of the κ-casein system in Jerseys (Li, 1970). The κ-casein locus in Jerseys deviates significantly from expectation at the 5% probability level. Unfortunately, no information exists from other studies to determine whether the absence of the κ-Cn^{A} κ-Cn^{A} genotype in Jerseys is unique to this study or a general phenomenon in this breed. If one considers that there is no obvious excess of κ-Cn^{B} κ-Cn^{B}, that a mere 3% excess of κ-Cn^{A} κ-Cn^{B} heterozygotes exists, and that the frequency of κ-Cn^{A} is relatively low (12%) in Jerseys, there is no convincing evidence to suspect that the κ-Cn^{A} κ-Cn^{A} genotype is at a selective disadvantage in Jerseys. In addition, since a total of 18 independent chi-square tests were made for the various systems for departure from Hardy–Weinberg equilibrium in the five dairy breeds, the number of significant results expected from type 1 errors at the 5% probability level is $(0.05)(18) = 0.90$, which is close to the number actually found. It is concluded that the distribution of gene frequencies of the four milk protein polymorphic systems in these dairy breeds follows Hardy–Weinberg expectations.

Since there are four polymorphic protein systems in each of the five dairy breeds, a total of 40 comparisons for differences in allele frequencies of a given locus between two breeds could be made by $2 \times J$ contingency tables. The results are summarized in Table II. Of these 40 comparisons, most of the results were found to be highly significant, and only six were not significant at the 5% probability level. One of these six comparisons, the difference in allele frequencies at the α_{S1}-casein locus between the Brown Swiss and Guernsey breeds, is actually significant at the 6% probability level. The other five involve the differences in allele frequencies at the β-lactoglobulin locus between the Brown Swiss and Guernsey breeds, the Brown Swiss and Jersey breeds, and the Guernsey and Jersey breeds, at the α_{S1}-casein locus between the Guernsey and Jersey breeds, and at the κ-casein locus between the Ayrshire and Holstein breeds. Comparisons at the β-casein locus are all significant at the 1% probability level among these breeds. These results indicate clearly that gene frequencies at the four milk protein loci are distinctly different among breeds.

For most of the cows used in this study, the four milk protein loci of the same animal were typed. This made it possible to test for nonindependent assortment of genotypes of a given pair of loci within the same breed by ordinary contingency tables. For four loci, there are six possible combinations of pairing two different loci, and thus a total of 30 tests could be made for five different breeds. However, due to the lack of sufficiently large numbers or the absence of alternative genotypes at a

Table III. Detection of Nonindependent Assortment of Genotypes Among the Four Polymorphic Loci in the Five Dairy Breeds by $I \times J$ Contingency Tables

	Ayrshire			Brown Swiss			Guernsey			Holstein			Jersey		
Loci	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P
Lg *vs.* α_{s1}-Cn		n.t.[a]			n.t.		4	6.4	<0.18	2	2.4	<0.30	4	4.8	<0.32
Lg *vs.* β-Cn	2	0.1	<0.97	8	9.1	<0.34	4	6.6	<0.16	4	3.2	<0.53	8	7.6	<0.47
Lg *vs.* κ-Cn	1	0.2	<0.67	4	34.5[b]	<0.01	4	5.9	<0.21	4	4.9	<0.30	2	1.1	<0.60
α_{s1}-Cn *vs.* β-Cn		n.t.			n.t.		4	7.8[c]	<0.10	3	33.7[b]	<0.01	8	48.4[b]	<0.01
α_{s1}-Cn *vs.* κ-Cn		n.t.			n.t.		4	8.7[c]	<0.08	1	0.1	<0.76	2	9.2[b]	<0.01
β-Cn *vs.* κ-Cn	4	7.8	<0.10	6	8.7	<0.19	4	18.8[b]	<0.01	3	6.2[c]	<0.10	3	6.2[c]	<0.10

[a] n.t.—no test.
[b] $P < 0.01$.
[c] $P < 0.10$.

particular locus in a given breed, only 24 tests were made. The results are summarized in Table III. They indicate that nonindependent assortment of genotypes between the α_{S1}-casein and β-casein loci is significant at the 1% probability level in Holsteins and Jerseys and at the 10% probability level in Guernseys and Jerseys.

No tests of independence of the α_{S1}- and β-casein systems were made for Ayrshires and Brown Swiss because of the lack of variation at the α_{S1}-casein locus in these breeds. Nonindependent assortment of genotypes between the α_{S1}-casein and κ-casein loci was also found to be significant in Guernseys and Jerseys but not in Holsteins. Nonindependent assortment of genotypes between the β-casein and κ-casein loci was found to be significant in all the five dairy breeds. In addition, close linkage of the three casein loci was confirmed (Hines *et al.*, 1969). If nonindependent assortment of genotypes among these loci can be attributed to close linkage, one would expect significant results to be detected between any pair of the α_{S1}-casein, β-casein, and κ-casein loci. The results turned out largely as expected. Nonindependent assortment of genotypes between the β-lactoglobulin and the three casein loci was rejected at the higher than 10% probability level with one exception, the β-lactoglobulin and κ-casein loci in Brown Swiss ($0.1 > P$). It is not known why a significant association between α_{S1}-casein and κ-casein loci was not found in Holsteins.

Balanced polymorphisms may best describe the milk protein systems in the five dairy breeds. Differences in selective values of the alleles of any one given locus probably were very small, since no obvious excesses of heterozygotes were found and only one of the 20 chi-square tests indicated significant departure from Hardy–Weinberg equilibrium at the 5% probability level. Other circumstantial evidence comes from the fact that the predominant allele of the β-casein and κ-casein systems was not the same in all the five dairy breeds, as would be predicted for selectively neutral isoalleles by models proposed by Kimura and Crow (1964), Wright (1966), and Kimura (1968).

Balanced polymorphism is usually maintained by overdominance where the rarest allele has a larger than 1% frequency (Fisher, 1930; Ford, 1942). Recently, a surprisingly large number of polymorphic loci (40%) were found in *Drosophila* (Hubby and Lewontin, 1966; Prakash *et al.*, 1969). But if all of these loci are actively under selection and if the selective values at separate loci are independent, an intolerable load arises, as Lewontin and Hubby (1966) have shown. To solve this contradiction, a threshold model with minor modifications has been suggested independently by several authors (Sved *et al.*, 1967; King, 1967; Milkman, 1967). Other mechanisms have also been advanced for maintaining this degree of polymorphism, namely, frequency-dependent selection (Kojima and Yarbrough, 1967), neutral mutation (King and Jukes, 1969; Crow and Kimura, 1970, pp. 322–327; Kimura and Ohta, 1971), soft and hard selection (Wallace, 1970), and linkage disequilibrium under various conditions (Lewontin, 1964*a*,*b*; Sved, 1968; Ohta and Kimura, 1969; Fraser and Burnell, 1970; Franklin and Lewontin, 1970). Despite these efforts, the forces maintaining genetic polymorphisms remain obscure. Nevertheless, one might still wonder whether the effects of truncation selection and assortative mating in these dairy breeds may render the milk protein loci from Hardy–Weinberg equilibrium. It should be noted that the long-term effects of truncation selection and assortative mating would not necessarily be detectable through an examination of deviation from Hardy–Weinberg expectations, for an equilibrium of the latter sort at a single locus could be reached after one generation of random mating and the samples used in this study consist of only one or two generations of cows. In addition, assortative mating and truncation

selection are aimed at metric traits, and often improvement of several such traits is considered in a breeding plan. Therefore, even if these milk protein loci are related to the desirable metric traits under selection, effects of assortative mating and truncation selection on changing allele frequencies of a single locus may be very small and other factors may complicate the situation, for example, linkage disequilibrium. We will return to this subject in later discussion. In any event, the equilibrium observed in these milk protein loci may very well be of unstable type, but without more data and further study this is conjectural.

The gene frequencies of milk protein loci estimated in this study from the five dairy breeds are very close to the results obtained from the same breeds abroad (see Aschaffenburg, 1968) and in other areas of the United States (see Kiddy *et al.*, 1968). The consistency of gene frequencies over a wide range of geographic regions suggests that these milk protein polymorphisms have little interaction with the environment. This is similar to Jamieson's (1966) findings in respect to blood serum transferrin alleles in these dairy breeds. In light of the evidence mentioned previously, the observed significant differences in allele frequencies of these milk protein loci in the five dairy breeds are probably a reflection of the consequence of fluctuation of the population size during the formation of each individual breed.

As mentioned earlier, the three casein loci are in close linkage, while the β-lactoglobulin locus is not linked to the casein loci (Hines *et al.*, 1969). We were interested, therefore, to find out whether these loci have reached gametic equilibrium in these dairy breeds. Significant nonindependent assortment of genotypes was observed only among the closely linked casein loci but not between the β-lactoglobulin locus and the casein loci. In addition, it has been established that the blood serum transferrin locus is not linked to the milk protein loci (Hines *et al.*, 1969), and again no significant linkage disequilibrium exists among the hemoglobulin and serum transferrin loci and the milk protein loci (Li, 1970). The implication of finding no heterozygote advantage at any of the individual milk protein loci but significant linkage disequilibrium when these loci are examined pairwise can be summarized as follows: (a) it may be fruitless to attempt to detect heterozygote advantage through examination of a single locus, and this may be true for most of the other genetic polymorphisms discovered in insects or mammals, for rarely, if ever, is the role of the locus with respect to reproduction or other major functions known; (b) close linkage must have played an important role in maintaining the milk protein polymorphisms in these dairy breeds; and (c) these milk protein loci do not respond to natural selection individually, but rather as an organized group, especially the three closely linked casein loci.

At this juncture, we are obligated to give some possible clues as to why factors such as finite population size, assortative mating, and truncation selection for economic traits in these dairy breeds may have had little effect on reducing the number of milk protein polymorphisms. Indeed, almost all of the major protein components of milk have been found to be genetically polymorphic (McKenzie, 1967). Crow and Kimura (1970, pp. 225–230 and p. 307) have pointed out that the practice of culling off a portion of the population on the basis of certain phenotypic traits in livestock breeding resembles the mode of natural selection assumed by the threshold models proposed by Sved *et al.* (1967), King (1967), and Milkman (1967). Computer simulation studies based on the threshold model proposed by Sved *et al.* (1967) have indicated that finite population size can generate strong linkage disequilibrium, and the latter is important for the stability of a polymorphism at adjacent loci (Sved, 1968). It has also been shown that the strong linkage disequilibrium arising from finite population size

may lead to temporal overdominance of the linked loci even though heterozygote advantage does not exist at any of the relevant loci (Maruyama and Kimura, 1968; Hill and Robertson, 1968; Ohta and Kimura, 1969). As for truncation selection effects, Wills *et al.* (1970) have demonstrated that artificial populations under weak selection as assumed by the threshold model suggested by Milkman (1967) can maintain a high percentage of polymorphisms. Also, Franklin and Lewontin (1970), using the threshold model proposed by King (1967), have obtained similar results to those obtained by Wills *et al.* (1970), although this was not the major interest of their investigation. Finally, positive assortative mating for phenotypic traits without dominance in an artificial population can lead to strong linkage disequilibrium and stable polymorphisms, as shown by Karlin and Scudo (1969). The results of these simulation studies suggest that inbreeding resulting from finite population size, assortative mating, and truncation selection for metric traits may be compensated by strong linkage disequilibrium generated by these same factors. As a result, a large number of milk protein polymorphisms have been maintained in these dairy breeds.

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