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Genetic disequilibria between the α_{S1}-, β-, κ-casein and the β-lactoglobulin loci of the *Bavarian Brown* and *Bavarian Simmental* cattle [1]

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Summary

Genetic disequilibria between 3 casein loci and between them and the β-lactoglobulin locus were estimated for a *Simmental* and a *Braunvieh* sample of about 2 000 cows each. Between the casein loci, disequilibria were statistically significant but between them and the independent lactoglobulin locus, disequilibria were smaller and statistically insignificant. In general, sign and magnitude of the casein loci disequilibria were similar between the 2 breeds.

Key words : Linkage disequilibrium, gamete frequency, casein, β-lactoglobulin, cattle.

Résumé

Déséquilibres génétiques entre les locus α_{s1}-, β-, κ-caséine et β-lactoglobuline chez les bovins des races Brune *et* Simmental de Bavière

On a estimé les déséquilibres génétiques entre 3 locus des caséines et entre ceux-ci et le locus de la β-lactoglobuline dans des échantillons *Simmental* et *Braunvieh* d'environ 2 000 vaches chacun. Les déséquilibres entre les locus des caséines sont statistiquement significatifs mais, entre ceux-ci et le locus indépendant de la lactoglobuline, les déséquilibres sont plus faibles et statistiquement non significatifs. En général, le signe et l'importance des déséquilibres entre les locus des caséines sont similaires dans les 2 races.

Mots clés : Déséquilibre de « linkage », fréquence gamétique, caséine, β-lactoglobuline, bovins.

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I. Introduction

Neutral alleles at different loci should be in Hardy-Weinberg and in linkage equilibrium in large panmictic populations.

Linkage disequilibria can occur because selection may cause an association between gene B at locus Lg saj and gene A at locus α_{S1}-Cn. Alternatively, disequilibrium may be a consequence of random drift (HILL & ROBERTSON, 1968) or it may result from mixing of 2 previously isolated and genetically different populations. Also gene frequency changes due to selection at a locus may generate linkage disequilibrium between 2 adjacent neutral loci (THOMSON, 1977). CROW & KIMURA (1970) show that weak linkage and weak epistasis may sustain a stable disequilibrium.

Linkage between casein loci (GROSCLAUDE *et al.*, 1964, 1965, 1978 ; LARSEN & THYMANN, 1966 ; HINES *et al.*, 1969) is one of the few linkages hitherto known in cattle. The recombination frequency between casein loci is 5 p. 100 or less (GROSCLAUDE *et al.*, 1964 ; HINES *et al.*, 1969 ; LARSEN, 1970), that between casein loci and the lactoglobulin locus 1/2. It appears to be of interest to investigate the status of linkage equilibria among casein loci and between these and the β-lactoglobulin locus in German cattle breeds not investigated before.

II. Material and methods

Casein and lactoglobulin genotypes were determined in 2 rather large samples of *Bavarian Simmentals* (FV) (N = 2 262) and *Bavarian Brown* cattle (BV) (N = 2 139) and gene frequencies were estimated therefrom (GRAML *et al.*, 1984 a, b). The *Simmental* can be considered as a closed dual purpose breed while the *Bavarian Brown* sample embraces about 70 p. 100 of *Bavarian Brown* × *Brown Swiss* crosses of various degrees. The *Brown Swiss* share of the genotypes is in most cows less than 50 p. 100. The principal aim of the investigation was to estimate the effects of milk protein genes on milk constituents but also the heritabilities of these were to be estimated. Therefore

TABLE 1

Number and age of the animals.

		Daughters	Dams	Granddams	Total (1)
Number	FV	1 076	918	95	2 262
	rBV	140	121	21	312
	tBV	485	367	39	2 139
Parity	FV	1.5	5.1	7.8	3.6
(Lactations)	rBV	2.4	6.2	8.5	4.5
	tBV	1.6	5.4	8.1	3.8

(1) Inclusive residual animals (FV : 173, rBV : 30, tBV : 1 248) of different ages.
FV *Bavarian Simmental.*
rBV Purebred *Bavarian Brown* cattle (residual population).
tBV Total *Bavarian Brown* cattle (crossbreds of different degrees and purebreds).

for the FV sample, data collection was organized in such a way that at least 2 daughter-dam pairs were located at any one farm. In some cases a cow was both daughter and dam. However, some single animals were also included. In the BV sample, all cows in a herd were studied. Therefore, the daughter-dam pairs comprised about half of the animals. Numbers of animals in the 2 breeds and in the different categories are given in table 1.

The determination of the protein types has been described elsewhere (GRAML *et al.*, 1984 a). Suffice here to state that samples were not tested in acid gels, which precluded differentiation of β-Cn A^1, A^2, A^3. For 3 casein loci and the β-lactoglobulin locus, 84 different genotype combinations were detectable in the *Simmental* material and 91 in the *Bavarian Brown* sample.

Several linkage disequilibria are possible if multiple alleles exist at the loci : $D_{ij} = f_{ij} - p_i q_j$, where f_{ij} represents the gametic frequency for $A_i B_j$, p_i and q_j, the allelic frequencies of the genes A_i and B_j at the 2 loci. When m alleles are at the first locus, and n alleles at the second, mn linkage disequilibria are possible. There are (m − 1) (n − 1) independent coefficients (WEIR, 1979). All disequilibria D_{ij} are estimable only if all gametes, including those of double heterozygotes, are identifiable. For the casein loci, coupling and repulsion double heterozygotes cannot be distinguished. Therefore, gamete frequencies were estimated by allocation (CEPPELLINI *et al.*, 1955).

The statistical significance of the disequilibria was tested by a χ^2 with one degree of freedom as has been suggested by WEIR & COCKERHAM (1978) :

$$\chi^2_{ij} = N r^2_{ij}$$

$$r^2_{ij} = \frac{D^2_{ij}}{[p_i (1 - p_i) q_j (1 - q_j)]}.$$

Here D_{ij} represents the disequilibrium between loci i and j and p_i, q_j the gene frequencies at the 2 loci. r_{ij} denotes the gametic correlation and N equals the number of gametes in the sample.

In our samples the rare alleles D of β-lactoglobulin and C of β-casein have, as a consequence, low gamete frequencies which possibly could fake disequilibria. Therefore, in a second analysis these alleles were pooled with alleles A and B of the respective loci. The significance was tested by

$$\chi^2_{IJ} = N r^2_{IJ}$$

$$r^2_{IJ} = \frac{D^2_{IJ}}{[p_I (1 - p_I) q_J (1 - q_J)]}.$$

similar to the quantities given above but under conditions

$$p_I = \sum_{i \in I} p_i \qquad q_J = \sum_{j \in J} q_j$$

$$p_{IJ} = \sum_{i \in I} \sum_{j \in J} p_{ij} \qquad D_{IJ} = \sum_{i \in I} \sum_{j \in J} D_{ij}$$

TABLE 2

Frequencies of the casein gametes ($\times 10^3$).

Gamete		Daughters			Dams			Granddams			Total (1)		
		FV	rBV	tBV	FV	rBV	tBV	FV	rBV	tBV	FV	rBV	tBV
α_{s1}-Cn/β-Cn	BA	769	518	738	783	651	695	747	545	618	778	586	699
	BB	89	359	197	69	245	227	111	296	229	80	278	207
	BC	47	57	21	39	30	26	37	91	86	43	56	33
	CA	93	66	41	109	62	48	105	68	67	98	73	54
	CB	2	0	3	0	12	4	0	0	0	1	6	6
	CC	0	0	0	0	0	0	0	0	0	0	1	1
α_{s1}-Cn/κ-Cn	BA	635	425	406	604	442	439	558	432	466	620	405	400
	BB	271	510	551	288	483	507	337	500	465	281	515	539
	CA	70	56	18	69	22	18	53	0	0	68	37	23
	CB	24	9	25	39	53	36	52	68	69	31	43	38
β-Cn/κ-Cn	AA	626	292	340	611	316	336	531	268	295	618	314	328
	AB	238	292	443	278	396	408	321	368	377	258	345	425
	BA	76	189	79	59	143	118	80	141	136	67	121	88
	BB	14	170	116	12	115	112	31	132	105	14	163	125
	CA	3	0	5	3	5	3	0	23	35	3	7	7
	CB	43	57	17	37	25	23	37	68	52	40	50	27

(1) Inclusive residual animals.
FV : *Bavarian Simmental.*
rBV : Purebred *Bavarian Brown* cattle (residual population).
tBV : Total *Bavarian Brown* cattle (crossbreds of different degrees and purebreds).

TABLE 3

r^2-values of the casein gametes.

Gamete		Sample	N	r^2 ($\times 10^4$) (1)
α_{s1}-Cn/β-Cn(2)	BA	FV	3 520	132*** –
		rBV	366	250** –
		tBV	2 990	61*** –
	BC	FV	195	49 +
		rBV	35	32 +
		tBV	141	6 +
	CB	FV	5	74 –
		rBV	4	188 –
		tBV	26	50 –
α_{s1}-Cn/κ-Cn(3)	BA	FV	2 805	1 +
		rBV	253	2 –
		tBV	1 711	6 +
β-Cn/κ-Cn(4)	AA	FV	2 796	96*** +
		rBV	196	5 –
		tBV	1 403	19 +
	BA	FV	303	76 +
		rBV	76	20 +
		tBV	376	1 +
	CA	FV	14	795 –
		rBV	4	79 –
		tBV	30	67 –
α_{s1}-Cn/β-Cn(5)	BA	FV	3 520	130*** –
		rBV	366	243** –
		tBV	2 990	61*** –
β-Cn/κ-Cn(5)	AA	FV	2 796	94*** +
		rBV	196	5 –
		tBV	1 403	20 +

(1) Daughters, dams, granddams and residual animals pooled.
(2) BB = CB, CA = BA, CC = BC, sign of D reverse.
(3) CA = BA, sign of D equal, BB, CB = BA, sign of D reverse.
(4) AB = AA, BB = BA, CB = CA, sign of D reverse.
(5) Estimation with collapsed data (pooled alleles β-Cn B, C).
N Number of the gametes.
FV *Bavarian Simmental.*
rBV Purebred *Bavarian Brown* cattle (residual population).
tBV Total *Bavarian Brown* cattle (crossbreds of different degrees and purebreds).
+, – Sign of D.
*, **, *** 5 p. 100, 1 p. 100, 0.1 p. 100 significance.

III. Results

Frequencies of casein gametes are given in table 2. They are fairly similar between breeds and between age groups albeit the gametes α_{s1}-Cn^B β-Cn^B and α_{s1}-Cn^B κ-Cn^B have a somewhat higher frequency in BV than in FV while the reverse is true for the BA gametes.

No significant linkage disequilibrium was found between the casein loci and the β-lactoglobulin locus. The estimated disequilibria between casein loci are given in table 3 as squares of the gametic correlations together with the sign of D. In table 3 rare gametes are included, i.e. r_{ij}^2 values are shown. Several disequilibria are statistically significant and that is true for combinations between all 3 casein loci. Also the signs of the disequilibria tend to be the same in both breeds. Also in table 3 disequilibria computed from pooled frequencies are given and some of the disequilibria are statistically significant.

IV. Discussion

The similarity of the casein loci disequilibria in all samples and the fact that some are significant statistically indicate that the disequilibria are real. As mentioned before, disequilibria may be caused by random drift in small populations, recent hybridization and selection. WEIR & HILL (1980) showed that in populations of limited effective size (N_e) loci with recombination frequency c should have a linkage disequilibrium between them which can be approximated by

$$\sigma_{D_{ij}}^2 \approx \frac{(1 - c_{ij})^2 + c_{ij}^2}{2\, N_e\, c_{ij}\, (2 - c_{ij})} + \frac{1}{n_{ij}},$$

$$\sigma_{D_{ij}}^2 = E\,(D_{ij}^2)/E\,[p_i\,(1 - p_i)\,q_j)\,(1 - q_j)] \approx r_{ij}^2.$$

The approximation is good in particular if loci are independent. The effective population size of FV appears to be around 140 (PIRCHNER, 1983). Since the daughter sample is 1 076 (n_{ij}) one may expect $r^2 = .0033$ between independent loci and $r^2 = .0341$ between linked casein loci with are somewhat larger than the values observed. However, the effective size of the *Braunvieh* population must be larger since much of it consists of *Braunvieh* × *Brown Swiss* crosses.

The tBV sample consists largely of rBV × BS crosses and disequilibria are expected if the gamete frequencies in the respective parent populations are sufficiently different. GRAML *et al.* (1984 b) report little difference between the gene frequencies of *Bavarian Braunvieh* (rBV) and *Brown Swiss* and as evident from table 2 gamete frequencies appear to be rather similar between rBV and tBV. Also r^2 values of tBV tended to be smaller than in rBV. Therefore it appears that the hybridization between rBV and BS is not the major cause of the linkage disequilibria observed.

However, the similarity in sign and extent of linkage disequilibria between *Fleckvieh* and *Braunvieh* appears to us to be an indicator for selection as a cause of the disequilibria. It must be pointed out that the 2 breeds even though close in terms of their ultimate genealogy (KIDD & PIRCHNER, 1971) have been separated for a long time.

TABLE 4

Disequilibria between casein loci in various cattle populations.

Population	Country	n	α_{s1}-Cn/β-Cn			α_{s1}-Cn/κ-Cn	β-Cn/κ-Cn			Reference of haplotype or genotype frequencies
			BA	BC	CB	BA	AA	BA	CA	
Aosta Black Pied	Italy	268	−***		−	+*	+***			MERLIN and DI STASIO (1982)
Aosta Red Pied	Italy	242	+	−	+	+	−	+	−	MERLIN and DI STASIO (1982)
Bavarian Brown	Germany	312	−**	+	−	−	−	+	−	Own results
Bav. Brown involv. BS crosses	Germany	2 139	−***	+	−	+	+	−	−	Own results
Bavarian Simmental	Germany	2 262	−***	+	−	+	+***	+	−	Own results
Bav. Simmental × Red Holstein	Germany	73	−	+	−	+	−			BUCHBERGER (1985) (1)
Black and White	Denmark	109	−			−	+***			LARSEN and THYMANN (1966)
Black a. White × Holstein Fries.	Germany	155	−			+	−			BUCHBERGER (1985) (1)
Blonde of Aquitaine	France	161	−*			−	−			GROSCLAUDE (1974)
Brown Swiss	U.S.A.	250	−	+	−					KING *et al.* (1965)
Chianina	Italy	120	−**	+	−	−**	+	+	−	MERLIN and DI STASIO (1982)
Flamande	France	298	−			−***	−			GROSCLAUDE (1974)
Grey Alpine (South Tyrol)	Italy	172	−**	+	−	+**	−*	+*	−	MERLIN and DI STASIO (1982)
Grey Alpine (North Tyrol)	Austria	91	−*	+	−					GROSCLAUDE (1973) (2)
Guernsey	Britain	170	−	+	−					KING *et al.* (1965)
Holstein	France	281	−			−*		+		GROSCLAUDE (1974)
Holstein Friesian	U.S.A.	6 531	−***		−	−***	+***			HINES *et al.* (1977)
Jersey	Denmark	160	−**			+**	+***			LARSEN and THYMANN (1966)
Jersey	Britain	353	−***							KING *et al.* (1965)
Montbéliarde	France	350	−***	+	−	−	−**	+*	−	GROSCLAUDE (1974)
Murbodner	Austria	82	−	+	−	+	+	+	−	GROSCLAUDE (1973) (2)
Murnau-Werdenfelser	Germany	161	−			+**	−			BUCHBERGER (1985) (1)
Normande	France	318	−***	+	−	+	+***	−	−	GROSCLAUDE (1974)
Piedmontese	Italy	572	−***	+	−	+***	+**	−	−	VOGLINO and CARIGNANO (1975)
Piedmontese	Italy	214	−***	+	−	+	+	+	−	MERLIN and DI STASIO (1982)
Pinzgauer	Germany	249	−*	+	−	+	+***	−	−	BUCHBERGER (1985) (1)
Pinzgauer	Austria	76	−	+	−	+	+***	−	−	GROSCLAUDE (1973) (2)
Pinzgauer (South Tyrol)	Italy	211	−*	+	−	−	+***	+	−	MERLIN and DI STASIO (1982)
Red Danes	Denmark	252	−	+	−	+	+***	−	−	LARSEN and THYMANN (1966)
Tarentaise	France	286	−***	+	−	+***	−***	+	+*	GROSCLAUDE (1974)
Valdostana	Italy	461	−			−	−*			VOGLINO and CARIGNANO (1975)
Yellow Franconia	Germany	960	−	+	+	−*	+***	−	−	BUCHBERGER (1985) (1)
Positive sign			1	20	2	17	17	10	1	
Negative sign			31	1	21	11	11	7	16	
Mean of r^2 ($\times 10^4$)			286	89	236	338	607	507	530	
Standard deviation of r^2 ($\times 10^4$)			385	96	408	653	918	870	730	

*, **, *** 5 p. 100, 1 p. 100, 0.1 p. 100 significance. (1) Unpublished results. (2) Personal communication.

Their respective areas of distribution were sharply delimited and any hybridization was frowned upon and it certainly did not occur between the principal breeding centers in the *Simmental* on one hand and *Eastern Switzerland* and *Western Austria* on the other.

In table 4 we have listed linkage disequilibria between casein loci in various cattle breeds which have been reported in the literature or which could be computed from gamete frequencies given in the respective publications. It is evident that linkage disequilibria between α_{s1}-Cn and β-Cn are identical in sign in nearly all breeds investigated. The one exception is a sample of *Red Pied Aosta.* In contrast linkage disequilibria between the α_{s1}-Cn and the κ-Cn locus vary between breeds. In our *Bavarian Braunvieh* sample (tabl. 3) the linkage disequilibrium was negative between the respective BA alleles at the 2 loci and it changed to rather strong positive disequilibrium in the rBV granddams, but when all age groups were combined no linkage disequilibrium of any size seemed to exist. The linkage disequilibria between the β-Cn and the κ-Cn loci where BA resp. CA gametes are involved vary between breeds. The disequilibrium between the A alleles at both loci differs between breeds but this may be caused by the lack of differentiation between the A^1, A^2 and A^3 alleles at the β-Cn locus which however did not prevent the recognition of the disequilibria between the β-Cn and the α_{s1}-Cn loci. In our tBV sample the disequilibrium between β-Cn B and κ-Cn A was contrary in sign to the majority of the disequilibria in the other breeds. The significant negative disequilibrium is caused by the high frequency of respective gametes in the rBV sample thus possibly be the exception explainable by the recent crossbreeding.

The similarity of the disequilibria between the α_{s1}-Cn and β-Cn loci not only between our 2 breeds but also in many other breeds, and the evidence of epistatic contributions to the genetic variance (GRAML, 1982) lead us to suggest that selection is a cause, possibly an important one, of disequilibria between milk protein gene loci.

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