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Identification of genetic variation at murine casein loci by RFLP

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Genetic variation in the casein loci of *Mus musculus* (8 different strains) and *Mus spretus* was examined as restriction fragment length variants (RFLVs) using 5 restriction enzymes, *Bam*HI, *Sft*I, *Eco*RI, *Xba*I and *Hind*III. No variation was observed between the inbred strains of *M. musculus*. Between *M. musculus* and *M. spretus* variations in fragment length were observed at different casein loci, revealing restriction fragment length polymorphisms (RFLPs). All the 5 enzymes generated polymorphic fragments in γ-casein locus while α and ε casein loci were polymorphic to four enzymes. The enzymes, *Hind*III and *Xba*I, yielded monomorphic fragments in α and ε-casein loci, respectively. Three enzymes generated polymorphic fragments in κ-casein locus but only two yielded polymorphic fragments at β-casein locus. Among the different casein loci, γ-casein locus was highly polymorphic while β-casein locus exhibited least polymorphism. Thus, RFLVs suggest the occurrence of similar alleles at all the five casein loci viz., α, β, γ, ε and κ of the inbred strains of *M. musculus* and a bi-allelic existence was observed between *M. musculus* and *M. spretus*.

Keywords: Restriction enzymes, murine casein loci, RFLP, *Mus musculus*, *M. spretus*

Introduction

Extensive studies have been carried out on the genetic variation at the casein loci of dairy species of domestic animals. Initially, it was studied at the protein level and later at the DNA level, using restriction fragment length polymorphisms (RFLPs), polymerase chain reaction (PCR), polymerase chain reaction-amplification created restriction sites (PCR-ACRS) and PCR-RFLPs[1]. The value of milk protein polymorphism as a tool in selective breeding has been reviewed in detail by earlier workers[2].

Studies on genetic variation at the murine casein loci are very limited. Analysis at the protein level did not reveal any casein polymorphism in various in-bred strains of mice. However, a protein variant, probably a casein (type not specified) has been observed by urea-gel (*p*H 3.7) electrophoresis in *Mus musculus castaneus*[3]. Linkage data at the murine casein loci is also scanty. Earlier workers mapped the W19H deletion (W-white spotting locus) proximal to the casein cluster and showed that the α and β casein genes are linked in mice[4]. They observed *Taq*I RFLPs in α and β casein loci but were unable to detect a RFLP for γ-casein locus and located the α-and β-casein gene loci in the linkage map of mouse chromosome 5, at E39 and E40, flanked by W-locus and Hmgl7-rs6 locus (current name is Hmgn2-rs6), respectively. In earlier studies, we have generated a physical map of the murine casein locus, which comprised of 5 genes, arranged in a tandem array, in the order as α-β-γ-ε-κ spanning 250 kb of the genomic DNA fragment[5]. The present study reports polymorphic sites with a few selected restriction enzymes at the casein loci of 8 inbred strains of *M. musculus* and between *M. musculus* and *Mus spretus.* The polymorphic information could be used as a tool for the analysis of genetic linkage and to analyze the evolutionary divergence of different species.

Materials and Methods

Genomic DNA Preparation

High molecular weight DNA was prepared from 8 different strains of *M. musculus,* 129, C57/BL6, C3H, DBA, NIH, BALB/c, SJL and CBA and from *M. spretus*[5].

Chemicals and DNA Markers

All the chemicals were procured from Sigma Biochemicals and Reagents, UK. The restriction enzymes and DNA size markers were from New

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England Biolabs, Inc, UK. Positively charged nylon membranes were procured from Boehringer Mannheim, Ltd, UK.

Restriction Enzymes

A panel of 5 restriction enzymes, *Bam*HI, *Sft*I, *Eco*RI, *Xba*I and *Hind*III, were selected for the study. The enzymes, *Sfi*I and *Bam*HI, were selected based on our earlier findings[5] and the other enzymes were selected at random.

Restriction Digestion

For each restriction digestion 10 µg of genomic DNA was used and the reactions were performed as per the manufacturer's instructions by adding the enzyme @ of 4U/µg of DNA. The reaction was stopped by placing the tubes at 65°C for 10 min. Gel loading buffer was added @ 5µLfor every 25 µLof reaction mixture.

Electrophoresis

Digested DNAs along with appropriate DNA size markers were electrophoresed on conventional horizontal gels (0.8% agarose; 1 x TAE buffer: 40 m*M* Tris, 40 m*M* acetate, 1 m*M* EDTA, *p*H 8.0 and ethidium bromide: 0.5 µg/mL) at 1-2 V/cm for 12-16 h except for *Bam*HI and *Sft*I digestions. *Bam*HI and *Sft*I digestions were resolved by pulsed-field conditions.

Southern Blotting

The gels were Southern blotted onto positively charged nylon membranes[5]. The blots were analysed with various casein probes[5], α, γ, ε and κ (cDNA probes) and β (3' genomic fragment, *Sal*I/*Sac*I fragment), labeled with ^{32}P.

Results and Discussion

Restriction fragment length variation analysis of the inbred strains of *M. musculus* showed monomorphic restriction fragments at the casein loci with all selected enzymes (Figs 1-5), which suggests the occurrence of similar alleles in all the 5 casein loci. Since the various strains under study were generated by inbreeding which allows homogenisation of the DNA, the presence of the same allele in all these strains was expected.

Polymorphic differences between *M. musculus* and *M. spretus* were identified for all five casein loci, with the two species apparently fixed for different alleles at each locus. The following combinations of probes and restriction enzymes revealed polymorphic fragments: α-casein-*Bam*HI, *Sfi*I, *Xba*I, *Eco*RI, β-casein-*Bam*HI, *Sfi*I, γ-casein-*Bam*HI, *Sfi*I, *Xba*I, *Hind*III, *Eco*RI, ε-casein-*Bam*HI, *Sfi*I, *Hind*III, *Eco*RI, and κ-casein *Bam*HI, *Xba*I, *Hind*III.

Of the 5 restriction enzymes, all the enzymes except *Hind*III generated polymorphic fragments in the α-casein locus, which showed a two-allele polymorphism (Table 1 & Fig. 1) while the β-casein locus exhibited two-allele polymorphisms only with *Bam*HI and *Sfi*I (Table 1 & Fig. 2).

Two independent *Bam*HI fragments were observed for the α (25 kb) and β (20 kb)-casein loci of *M. spretus* whereas, a common 40 kb fragment was revealed with both α-and β-casein probes in addition

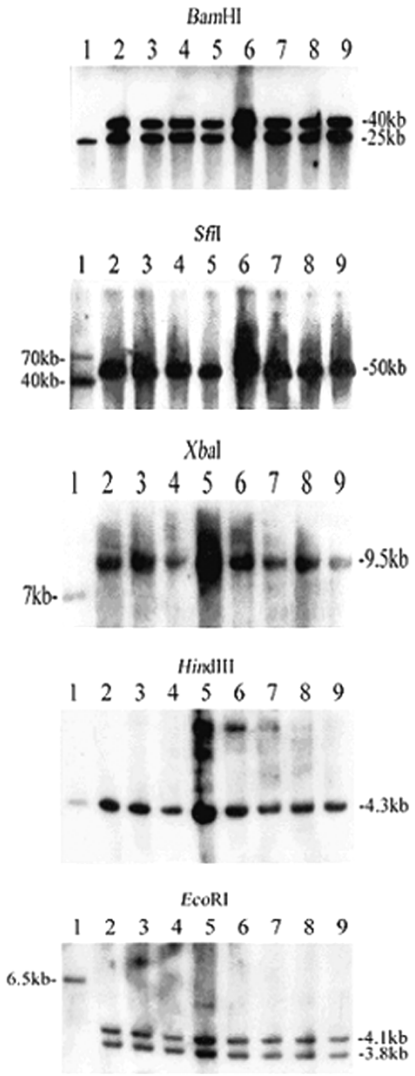

Fig. 1—Murine genomic DNA digested with various restriction enzymes and hybridized with α-casein cDNA probe: Lane -*M. spretus* and lanes 2-9-Different strains of *M. musculus.*

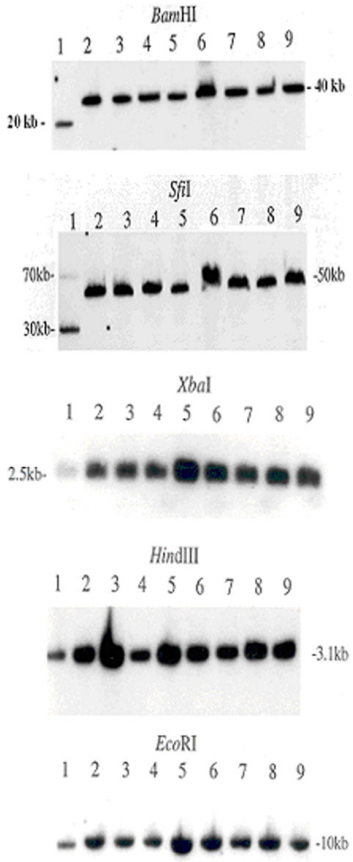

Fig. 2—Murine genomic DNA digested with various restriction enzymes and hybridized with β-casein 3' genomic fragment *(SalI/Sac*I fragment) probe: Lane 1-*M. spretus* and lanes 2-9-Different strains of *M. musculus.*

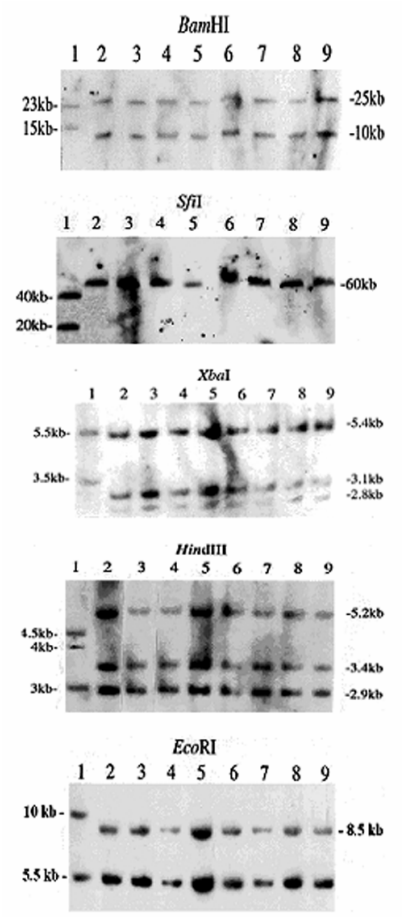

Fig. 3—Murine genomic DNA digested with various restriction enzymes and hybridized with γ-casein cDNA probe: Lane 1-*M. spretus* and lanes 2-9-Different strains of *M. musculus.*

to an independent 25 kb fragment for α-casein locus in *M. musculus*. Thus, the *Bam*HI fragments in *M. spretus* do not indicate any evidence for the linkage between the α-and β-casein genes. The enzyme, *Sfi*I, produced fragments of 70 and 40 kb in the α-casein locus and 70 and 30 kb in the β-casein locus of *M. spretus*, where the smaller fragments together (40 + 30 = 70) constitute the size of the large fragment (70 kb), suggesting that the 70 kb fragment is a partially digested fragment and giving fairly good evidence for the physical linkage of the two genes in *M. spretus.* The low hybridization intensity of the 70 kb fragment compared to the other fragments is also suggestive of partial digestion. This suggests that there is a *Sfi*I site between the α-and β-loci in *M. spretus* with the genes contained in 40 and 30 kb *Sfi*I fragments, respectively against a single 50 kb fragment encompassing both the genes in *M. musculus.* The variation in sizes of *Bam*HI and *Sfi*I fragments between the two species propose the concurrent addition and deletion of theses enzyme sites in *M. spretus.*

As per the physical map of the casein loci generated of *M. musculus*[5], it is evident that the α-and β-casein genes are located very close to each other with an intergenic region of about 8 kb. Comparing the sizes of the *Bam*HI fragments (α + β-loci) the fragments together constitute 45 kb and 65 kb in *M. spretus* and *M. musculus,* respectively. Examining the evolutionary divergence of satellite DNA sequences, a marked reduction in the relative abundance of major satellite DNA was revealed probably due to deletion, in *M. spretus* as compared to *M. musculus*[6,7].

All the 5 enzymes generated polymorphic fragments in the γ-casein locus while at the ε–casein locus polymorphic fragments were observed with 4 enzymes in both the species (Table 1; Figs 3 & 4). Only three enzymes yielded polymorphic fragments

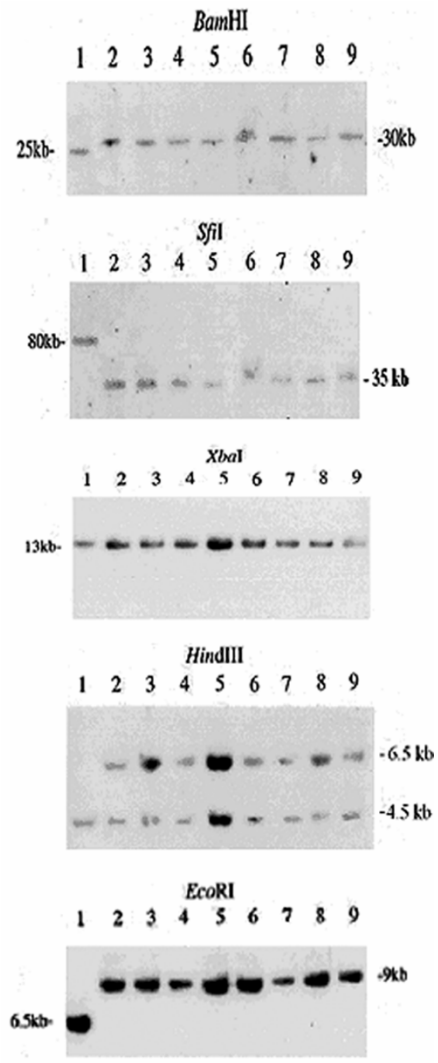

Fig. 4—Murine genomic DNA digested with various restriction enzymes and hybridized with ε-casein cDNA probe: Lane 1-*M. spretus* and lanes 2-9-Different strains of *M. musculus.*

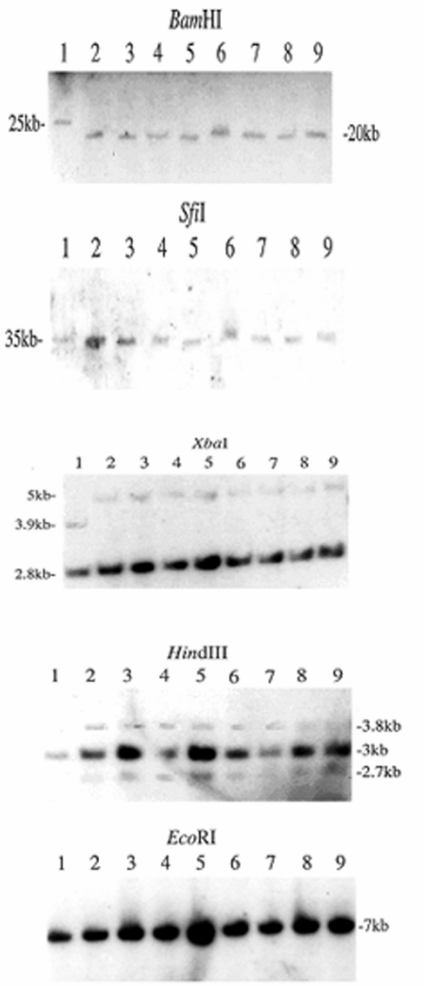

Fig. 5—Murine genomic DNA digested with various restriction enzymes and hybridized with κ-casein cDNA probe: Lane 1-*M. spretus* and lanes 2-9-Different strains of *M. musculus.*

in the κ-casein locus of both the species (Table 1 & Fig. 5). Small variations were also observed in size of *Bam*HI fragments of γ, ε and κ casein loci between *M. spretus* and *M. musculus* while the number of fragments was same in both the species.

Enzyme *Sfi*I generated two fragments (20 & 40 kb) in the γ-casein locus of *M. spretus* as against one fragment (60 kb) in *M. musculus.* This suggests the formation of an additional *Sfi*I site in the γ-locus of *M. spretus* or the loss of the site in *M. musculus.* The *Sfi*I fragment observed in ε-locus was larger in size (~80 kb) in *M. spretus* compared to the fragment in *M. musculus* (35 kb). The variations in sizes might be due to the appearance of new sites as well as deletion of existing sites during evolution. In the γ-casein locus, between the two species, variations in both number and size of fragments were observed with *Xba*I while *Hind*III produced fragments of varying size but of same number in the two species. *Eco*RI produced two fragments, of which one was of similar size in both the species. Polymorphic fragments were detected in ε-casein locus with *Hind*III and *Eco*RI, where one of the fragments produced by the enzyme *Hind*III was of the same size in the two species.

Variations in fragment sizes were also observed at the κ-casein locus with *Xba*I and *Hind*III, where one fragment was of the same size in both the species. Presence of similar sized fragments with an enzyme at the same locus of the two species suggest that, at least two sites for that enzyme are located at similar positions in that particular locus. RFLPs with various

Table 1— DNA fragments generated at the casein loci of *M. musculus* and *M. spretus*

Casein locus	Species of mice	DNA fragments generated (size in kb) Restriction enzymes used				
		*Bam*HI	*Sfi*I	*Xba*I	*Hind*III	*Eco*RI
α	*M. musculus*	25	50	9.5	4.3	3.8
		40				4.1
	M. spretus	25	40	7.0	4.3	6.5
			70			
β	*M. musculus*	40	50	2.5	3.1	10
	M. spretus	20	30	2.5	3.1	10
			70			
γ	*M. musculus*	10	60	2.8	2.9	8.5
		25		3.1	3.4	5.5
				5.4	5.2	
	M. spretus	15	20	3.5	3.0	10
		23	40	5.5	4.0	5.5
					4.5	
ε	*M. musculus*	30	35	13	4.5	9.0
					6.5	
	M. spretus	25	80	13	4.5	6.5
κ	*M. musculus*	20	35	2.8	2.7	7.0
				5.0	3.0	
					3.8	
	M. spretus	25	35	2.8	3.0	7.0
				3.9		

Sizes of monomorphic DNA fragments are underlined.
Approximate sizes of DNA fragments were determined with the help of appropriate DNA size markers electrophoresed along with the restricted DNA.

enzymes have also been identified in the casein loci of other species such as, bovines, ovines, caprines and porcines[8-15].

Crosses between laboratory mice and the highly divergent wild-derived *Mus* species, *M. spretus,* are a powerful tool for the analysis of genetic linkage[16]. The polymorphic information obtained from the restriction analysis can be used to locate the casein gene cluster in the genetic map of mouse chromosome 5. The limited linkage data available for the murine α-and β-casein loci, based on a single/putative recombination event, places them 1 cM (1 cM = ~1.7 Mb in mice) apart[4]. But in the physical map constructed, it has been shown that the α-and β-casein genes are situated only ~8 kb apart[5]. Therefore, the apparent overestimate of the genetic distance between the α and β genes may be explained as a chance observation of a rare event, a genotyping error or a real deviation from the average relationship between genetic and physical distances. An effort has also been made, in collaboration with scientists of Mammalian Genetics Laboratory, NCI-Frederick Cancer Research and Development Center, Maryland USA, to add the casein gene cluster, using κ-casein RFLP, in the mouse gene map developed based on a *M. musculus/M. spretus* cross[17]. As expected, the κ-casein locus maps to chromosome 5 between Pdgfra and Alb1 (current name is Alb) (data not shown). The current mouse genome database provided by Mouse genome informatics, Jackson Laboratory, USA also shows that the casein gene cluster is located between Pdgfra and Alb, spanning a region of 0.3 cM.

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References

1 Martin P, Szymanowsk M, Zwierzchowsk L & Leroux C, The impact of genetic polymorphisms on the protein composition of ruminant milks, *Reprod Nutr Dev,* 42 (2002) 433-459.

2 Ng-Kwai-Hang K F & Grosclaude F, Genetic polymorphism of milk proteins, in *Advanced dairy chemistry-1: Proteins*, edited by P F Fox (Elsevier Science Publishers Ltd, England) 1992, 405- 455.

3 Piletz J E & Ganschow R E, Genetic variation of milk proteins in mice, *Biochem Genet*, 19 (1981) 1023-1030.

4 Geissler E N, Cheng S V, Gusella J F & Housman D E, Genetic analysis of the dominant white-spotting (W) region on mouse chromosome 5: Identification of cloned DNA markers near W, *Proc Natl Acad Sci USA,* 85 (1988) 9635-9639.

5 George S, Clark A J & Archibald A L, Physical mapping of the murine casein locus reveals the gene order as alpha-beta-gamma-epsilon-kappa, *DNA Cell Biol*, 16 (1997) 477- 484.

6 Brown S D M & Dover G A, Conservation of segmental variants of satellite DNA of *Mus musculus* in a related species: *Mus spretus*, *Nature (Lond)*, 285 (1980) 47-49.

7 Matsuda Y & Chapman V M, *In situ* analysis of centromeric satellite DNA segregating in *Mus* species crosses, *Mammal Genome*, 1 (1991) 71-77.

8 Threadgill D W & Womack J E, Genomic analysis of the major bovine milk protein genes, *Nucleic Acids Res*, 18 (1990) 6935-6942.

9 Robitaille G & Petitclerc D, Expression polymorphism of kappa-casein gene in Holstein cows, *J Dairy Res*, 67 (2000) 107-111.

10 Sulimova, G E, Azari M A, Rostamzadeh J, Abadi M R M & Lazebny O E, K-casein gene (CSN3) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker, *Genetika,* 43 (2007) 88-95.

11 Azevedo A L S, Nascimento C S, Steinberg R S, Carvalho M R S, Peixoto M G C D *et al*, Genetic polymorphism of the kappa-casein gene in Brazilian cattle, *Genet Mol Res,* 7 (2008) 623-630.

12 Di Gregorio P, Rando A, Pieragostini E & Masina P, DNA polymorphism at the casein loci in sheep, *Anim Genet,* 22 (1991) 21-30.

13 Leroux C, Martin P, Mahe M F, Leveziel H & Mercier J C, Restriction fragment length polymorphism identification of goat alpha s1-casein alleles: A potential tool in selection of individuals carrying alleles associated with a high level protein synthesis, *Anim Genet,* 21 (1990) 341-351.

14 Yahyaoui H M, Agustina C, Armand S & Josep F M, Genetic polymorphism of the caprine kappa casein gene, *J Dairy Res,* 68 (2001) 209-216.

15 Archibald A L, Couperwhite S, Haley C S, Beattie C W & Alexander L, RFLP and linkage analysis of the porcine casein loci—CASAS1, CASAS2, CASB and CASK, *Anim Genet,* 25 (1994) 349-351.

16 Avner P, Amar L, Dandolo L & Guénet J L, Genetic analysis of the mouse using interspecific crosses, *Trends Genet,* 4 (1988) 18-23.

17 Dietrich W F, Miller J, Steen R *et al*, A comprehensive genetic map of the mouse genome, *Nature (Lond),* 380 (1996) 149-152.