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Genomic incompatibilities in the hybrid zone between house mice in Denmark: evidence from steep and non-coincident chromosomal clines for Robertsonian fusions

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Summary

The pattern of chromosomal variation is investigated in house mice from the Danish hybrid zone between the translocation-prone *Mus musculus domesticus* and the chromosomally conservative *M. m. musculus*. The cytogenetic analysis confirmed the non-introgression of three pairs of Robertsonian (Rb) fusions from *M. m. domesticus* into the *M. m. musculus* genome. The geographic distribution of two of these Rb fusions was shown to follow staggered chromosomal clines which increased in steepness the closer they were to the centre of the hybrid zone as defined by allozymes. Analysis of alternate hypotheses suggests that chromosomal differentiation of the Danish *domesticus* occurred after contact was established with *musculus*. The staggering of the clines would reflect the order of arrival of the Rb fusions into the hybrid zone. Several models with different processes of underdominance of the chromosomal heterozygotes are discussed to account for the difference in width between clines. A selective model with increasing levels of genomic underdominance due to interaction with a progressively enriched *musculus* genome provides the best fit for the observed pattern. Selection against Rb fusions with little effect on the recombination of linked allozyme markers supports the view that no reduction in gene flow due to chromosomal heterozygosity is yet apparent through the hybrid zone and that only the centromeric segments of the Rb fusions are incompatible with the *musculus* genome.

1. Introduction

Subspecies of the house mouse *Mus musculus* have identical G-banded 2n = 40 acrocentric karyotypes and present only minor differences in the quantity and localization of heterochromatin and nucleolar organizer regions (review in Boursot *et al.* 1993). Robertsonian (Rb) translocations which are formed by the fusion of two acrocentric chromosomes occur almost exclusively in *Mus musculus domesticus* in geographically separated populations found in Europe and North Africa (see Bauchau, 1990 for a review). The factors involved in the formation, the selective impact, as well as the evolutionary consequences of these chromosomal rearrangements are still under debate although numerous theoretical analyses and genetic surveys of wild populations have been performed (see references in Boursot *et al.* 1993; Sage *et al.* 1993; Searle, 1993; Nachman & Searle, 1995).

The existence of Rb fusions in *M. m. domesticus* from Denmark has allowed us to analyse the interaction of these rearrangements with a chromosomally conservative genome, *M. m. musculus*. These two subspecies interact along a narrow hybrid zone crossing Europe from Denmark to Bulgaria. The introgression of genic, molecular and morphological characters has been extensively studied by different authors in Denmark (Hunt & Selander, 1973; Ferris *et al.* 1983; Boursot *et al.* 1984; Vanlerberghe *et al.* 1986; Gyllensten & Wilson, 1987; Vanlerberghe *et al.* 1988*a*; Nancé *et al.* 1990; Dod *et al.* 1993; Alibert *et al.* 1994), Bulgaria (Vanlerberghe *et al.* 1986; Vanlerberghe *et al.* 1988*b*), southern Germany (Sage *et al.* 1986*a*; Sage *et al.* 1986*b*; Tucker *et al.* 1992) and more recently northern Germany (Prager *et al.* 1993). The most salient features of this hybrid zone involve

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the introgression of most allozymic, mitochondrial and morphological markers, whereas that of sex chromosomes (Vanlerberghe *et al.* 1986; Dod *et al.* 1993; Tucker *et al.* 1992) is more limited (Ferris *et al.* 1983; Boursot *et al.* 1984; Gyllensten & Wilson, 1987; Vanlerberghe *et al.* 1988*a*). In a previous cytogenetic study in Denmark, Nancé *et al.* (1990) showed that the three pairs of fusions present in *M. m. domesticus* did not introgress into the *M. m. musculus* genome and that one pair of these fusions presented a very steep cline. These data suggested that fusions were counterselected when in a *musculus* background. The extensive introgression observed for allozymic markers located on the arms of these chromosomes led Nancé *et al.* to postulate that selection was operating preferentially on the centromeres of the Rb fusions.

In the present study, the previous chromosomal analysis of populations from the hybrid zone in Denmark was extended by further sampling to specify the interaction between the subspecific and the chromosomal differentiation. The aim of this study was to analyse the chromosomal clines of the different Rb fusions to determine their dynamics and contribution to post-zygotic isolation in the hybrid zone between the two subspecies.

2. Material and methods

(i) *Mice*

408 house mice were live-trapped in farm buildings and stores during three field trips in October 1990, 1991 and 1992. Trapping was organized along two transects, an Eastern transect where previous samples had been collected in 1984, 1985 and 1987 and a second one, 25 km west of the first one, where the hybrid zone was described in Hunt & Selander (1973) as being wider (Fig. 1). The data presented here include chromosomal and allozymic data from localities sampled in 1984, 1985, 1987 published in Nancé *et al.* (1990) and Dod *et al.* (1993).

(ii) *Cytogenetic analysis*

Chromosome preparations were made from yeast-stimulated bone marrow cells (Lee & Elder, 1980) according to the air-drying technique and were observed under a Zeiss Axiophot microscope. Chromosomes were identified after G-banding according to the method of Seabright (1971) and the nomenclature of Cowell (1984) from photographs of three to five metaphase plates for each individual.

(iii) *Electrophoretic analysis*

The samples were assigned to one or the other subspecies by the electrophoretic analysis of seven

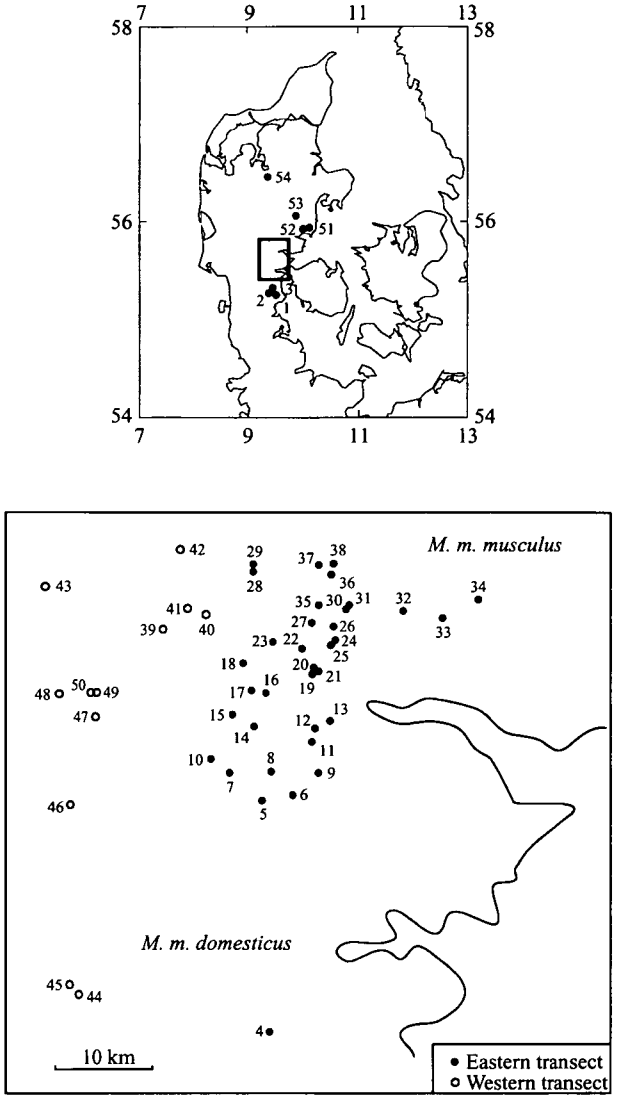

Fig. 1. Map of Denmark showing sampled localities. Insert details distribution of populations along the transects through the hybrid zone: 1, Kastvraa; 2, Harken; 3, Simmersted; 4, Odis; 5, Egtved; 6, Ammitsbolvej; 7, Torskind; 8, Odsted; 9, Mejling Skovvej; 10, Bindeballe; 11, Jerlev Ville; 12, Jerlev; 13, Villstrup; 14, Ravning; 15, Store-Lihme; 16, Balle; 17, Sodover; 18, Gammelby; 19, Rugballe 1; 20, Rugballe 2; 21, Rugballe 3; 22, Jelling; 23, Molvang; 24, Horup 1; 25, Horup 2; 26, Brandbjerg; 27, Hygum; 28, Givskud 1; 29, Givskud 2; 30, Vindelev Molle; 31, Vindelev Stormgaard; 32, Lindved; 33, Baastrup; 34, Losning; 35, Sandvad; 36, Adal 1; 37, Adal 2; 38, Fousing; 39, Lindeballe; 40, Smidstrup; 41, Norskov; 42, Give; 43, Filskov; 44, Maltbaek Mark; 45, Maltbaek; 46, Vorbasse; 47, Skoldbjerg; 48, Trollund; 49, Plougslund 1; 50, Plougslund 2; 51, Hov; 52, Gossmer; 53, Klank; 54, Foulum.

diagnostic loci which show alternate alleles diagnostic for each subspecies (*Es1, Es2, Pgm1, Mpi1, Gpd1, Np, Sod1*) (Hunt & Selander, 1973; Bonhomme *et al.* 1984). Tissue samples and starch gel buffers were prepared according to Pasteur *et al.* (1987). Genotypic data for all the localities studied (1984, 1985, 1987 and 1990–1992) will be published elsewhere.

Table 1. *Chromosomal analysis of Danish mice. Sample numbers are detailed in Fig. 1. The locality name is followed by the year of sampling. Data for 1984, 1985 and 1987 are taken from Nancé* et al. *1990. Homozygosity (2), heterozygosity (1) and absence (0) of Rb fusions are indicated. K = number of mice karyotyped; G = number of G-banded karyotypes; km = position to the centre of the zone*

						Rb fusions					
km	No.	Locality	K	2n	G	2·5	3·8	6·9	9·13	4·17	10·11
−45·7	1	Kastvraa 90	1	39	1	1	0	0	0	0	0
			3	38	1	1	0	1	0	0	0
					2	1	1	0	0	0	0
			1	37	1	2	1	0	0	0	0
			1	36	0						
			3	35	1	1	2	1	0	1	0
					2	2	2	1	0	0	0
			2	34	2	2	2	2	0	0	0
−45·7	2	Harken 90	1	35	1	1	2	2	0	0	0
			1	35	1	1	2	1	0	1	0
			11	34	11	2	2	2	0	0	0
−45·7	3	Simmersted 90	1	39	1	0	1	0	0	0	0
			2	38	1	1	1	0	0	0	0
					1	1	0	1	0	0	0
			2	37	2	1	2	0	0	0	0
			1	36	1	1	2	1	0	0	0
			2	35	2	1	2	2	0	0	0
			1	34	1	2	2	2	0	0	0
−33·7	4	Odis 84	1	35	1	2	2	1	0	0	0
			5	34	5	2	2	2	0	0	0
−12·3	5	Egtved 85	6	38	6	0	2	0	0	0	0
			9	37	5	1	2	0	0	0	0
				37	3	0	2	1	0	0	0
			8	36	7	1	2	1	0	0	0
					1	2	2	0	0	0	0
			3	35	3	2	2	1	0	0	0
−12·3	5	Egtved 90	1	37	1	1	2	0	0	0	0
−11·4	6	Ammitsbolvej 90	1	38	1	0	2	0	0	0	0
			3	37	3	1	2	0	0	0	0
		Ammitsbolvej 92	1	37	1	1	2	0	0	0	0
−10·4	7	Torskind 84	4	38	4	0	2	0	0	0	0
			2	37	2	1	2	0	0	0	0
		Torskind 85	7	37	6	1	2	0	0	0	0
					1	0	2	1	0	0	0
			7	36	5	2	2	0	0	0	0
					1	1	2	1	0	0	0
					1	1	2	0	0	0	1
			1	35	1	2	2	1	0	0	0
		Torskind 91	3	38	2	0	2	0	0	0	0
			5	37	5	1	2	0	0	0	0
			2	36	2	2	2	0	0	0	0
−9·7	8	Odsted 90	1	37	1	1	2	0	0	0	0
			1	36	1	2	2	0	0	0	0
		Odsted 91	1	37	1	1	2	0	0	0	0
		Odsted 92	9	38	9	0	2	0	0	0	0
			3	37	3	1	2	0	0	0	0
−9·2	9	Mesling Skouvej 90	1	38	1	0	2	0	0	0	0
−9·3	10	Bindeballe 92	1	40							
			2	37	1	1	2	0	0	0	0
			1	36	1	2	2	0	0	0	0
			1	35	1	2	2	1	0	0	0
−6·6	11	Jerlev Ville 92	2	38	2	1	1	0	0	0	0
			1	36	1	2	2	0	0	0	0
−5·2	12	Jerlev 91	12	38	11	0	2	0	0	0	0
			4	37	4	1	2	0	0	0	0
		Jerlev 92	18	38	16	0	2	0	0	0	0
					1	1	1	0	0	0	0
−4·3	13	Vilstrup 92	2	40	0						
			1	38	1	0	2	0	0	0	0
−6·0	14	Ravning 92	5	39	5	0	1	0	0	0	0

Table 1. (*cont.*)

km	No.	Locality	K	2n	G	Rb fusions 2·5	3·8	6·9	9·13	4·17	10·11
			3	38	3	0	2	0	0	0	0
−5·5	15	Store-Limhe 87	2	40	0						
			5	39	5	0	1	0	0	0	0
			1	38	1	0	2	0	0	0	0
−3·1	16	Balle 92	8	40	0						
			1	39	1	0	1	0	0	0	0
			2	38	2	0	2	0	0	0	0
−2·9	17	Sodover 85	4	40	0						
			1	39	1	0	1	0	0	0	0
			1	38	1	0	2	0	0	0	0
		Sodover 91	1	38	1	0	2	0	0	0	0
		Sodover 92	1	40	0						
−0·9	18	Gammelby 87	2	40	0						
−0·7	19	Rugballe 1, 87	8	38	1	0	2	0	0	0	0
−0·7	20	Rugballe 2, 91	3	40	0						
−0·7	21	Rugballe 3, 92	2	40	0						
1·5	22	Jelling 87	3	40	0						
		Jelling 90	3	40	0						
		Jelling 91	1	40	0						
1·5	23	Molvang 90	2	40	0						
		Molvang 92	1	40	0						
2·1	24	Horup 1, 90	8	40	0						
2·4	25	Horup 2, 91	20	40	0						
2·4	26	Brandbjerg 91	19	40	0						
		Brandbjerg 92	1	40	0						
3·7	27	Hygum 90	1	40	0						
6·5	28	Givskud 1, 87	6	40	0						
		Givskud 1, 91	14	40	0						
6·5	29	Givskud 2, 91	5	40	0						
6·0	30	Vindelev Molle 85	5	40	0						
		Vindelev Molle 91	2	40	0						
6·0	31	Vindelev Stormgaard 91	15	40	0						
		Vindelev Stormgaard 92	10	40	0						
6·1	32	Lindved 90	4	40	0						
6·0	33	Baastrup 87	22	40	0						
8·1	34	Losning 84	6	40	0						
		Losning 85	9	40	0						
5·4	35	Sandvad 90	8	40	0						
8·1	36	Adal 1, 90	3	40	0						
8·1	37	Adal 2, 91	1	40	0						
8·0	38	Fousing 90	1	40	0						
		Fousing 91	4	40	0						
		Fousing 92	1	40	0						
0·8	39	Lindeballe 92	4	40	0						
2·9	40	Smidstrup 92	4	40	0						
			1	39	1	0	0	1	0	0	0
1·7	41	Norskov 92	4	40	0						
7·6	42	Give 92	6	40	0						
1·8	43	Filskov 92	2	40	0						
−31·9	44	Maltbaek mark 92	1	36	1	1	2	0	1	0	0
			3	35	1	1	2	1	1	0	0
					1	2	2	0	1	0	0
			1	34	1	2	2	1	1	0	0
−31·4	45	Maltbaek 92	1	36	1	2	2	0	0	0	0
			4	35	3	2	2	0	1	0	0
					1	0	2	1	1	1	0
			3	34	3	2	2	0	2	0	0
−15·8	46	Vorbasse 92	1	38	1	0	2	0	0	0	0
			4	37	3	1	2	0	0	0	0
					1	0	2	0	1	0	0
			7	36	4	2	2	0	0	0	0
					2	1	2	0	1	0	0
			2	35	1	2	2	0	1	0	0

Table 1. (*cont.*)

km	No.	Locality	K	2n	G	Rb fusions 2·5	3·8	6·9	9·13	4·17	10·11
−7·7	47	Skoldbjerg 92	2	37	2	1	2	0	0	0	0
			4	36	4	2	2	0	0	0	0
			1	35	1	2	2	0	1	0	0
−6·3	48	Trollund 92	6	37	5	1	2	0	0	0	0
					1	2	1	0	0	0	0
			5	36	4	2	2	0	0	0	0
5·7	49	Plougslund 1, 92	1	38	1	0	2	0	0	0	0
			6	37	5	1	2	0	0	0	0
					1	2	1	0	0	0	0
			4	36	3	2	2	0	0	0	0
			1	35	1	2	2	0	1	0	0
−5·7	50	Plougslund 2, 92	1	38	0						
			7	37	6	1	2	0	0	0	0
			3	36	2	2	2	0	0	0	0
24·3	51	Hov 84	6	40	0						
		Hov 85	2	40	0						
		Hov 87	4	40	0						
		Hov 91	1	40	0						
		Hov 92	2	40	0						
24·3	52	Gossmer 91	27	40	0						
		Gossmer 92	14	40	0						
41·5	53	Klank 85	8	40	0						
62·9	54	Foulum 90	15	40	0						

(iv) *Statistical analysis*

The chromosomal data are treated as a locus with two alleles, a metacentric form and a two-acrocentric one. Hardy–Weinberg expectations and linkage disequilibrium were tested using the program GENEPOP 1.2 developed by Raymond & Rousset (1995). Populations sampled during different years were pooled for the study of geographic variation in the distribution of the Rb fusions and allozymes. The orientation and the centre of the hybrid zone as determined by the allozyme data of Hunt & Selander (1973) were followed. Geographic distances between localities along the eastern transect were measured after orthogonal projection onto this orientation.

The model used to fit both chromosomal and allozymic clines corresponds to a solution of the diffusion equation in one dimension associated with an underdominant locus:

$$p = \frac{e^{w(x-c)}}{1+e^{w(x-c)}}$$ Bazykin (1969),

x is the geographic distance along the transect and p represents the frequency of the marker. In the case of strict underdominance (heterozygote fitness is $1-s$ and homozygotes fitness is 1), $w = \sqrt{(2s/\sigma^2)}$ with σ the standard deviation in distance between parents and offspring and s the selection coefficient. Although more elaborate models may yield better cline fits for the allozyme data (Syzmura & Barton, 1991), the heterozygote disadvantage model was used here for comparative purposes.

The width of a cline is described as the inverse of the maximum slope (Endler, 1977) which in this case is given by:

$$\left[\frac{\partial p}{\partial x}\right]^{-1}_{x=c} = \left|\frac{4}{w}\right|.$$

The centre of a cline, c, is the position of the maximum change in frequency

$$\left(\left[\frac{\partial^2 p}{\partial x^2}\right]_{x=c} = 0\right).$$

The centre and width of the clines were estimated by maximum likelihood using the Metropolis algorithm adapted from N. H. Barton (Szymura & Barton, 1986). Input data were genotypes and an F factor (departure from Hardy–Weinberg proportions) was estimated for each locality. The centres and widths of the different clines were compared independently using a likelihood ratio test (see Szymura & Barton, 1986). Cline characteristics for Rb fusions were tested against those of the seven individual allozymic clines.

Cline parameters were estimated for Rb(3·8) and Rb(2·5) using samples along the eastern transect (localities 4–23). The sampling scheme yielded insufficient data to fit a reliable cline for Rb(6·9). Genotypic data from localities 1–38 and 51–54 were included in the allozymic clinal analysis.

Table 2. *Characteristics of clines. Values are given in km. Confidence intervals (95%) are estimated from log likelihood distributions (such as* $2LL < 2LL_{max} - \chi^2$ *with 1* D.F.)

Clines	Centre	Confidence interval		Width	Confidence interval	
Rb(2·5)	−12·0	−12·9	−11·1	11·5	9·1	15·0
Rb(3·8)	−4·2	−4·8	−3·7	5·0	3·7	6·9
Allozyme consensus	−0·4	−0·9	0·1	28·6	27·0	30·4

Table 3. *Tests of concordance and coincidence for the different clines*

	Concordance				Coincidence			
	LL1	LL0	ΔLL	D.F.	LL1	LL0	ΔLL	D.F.
Allozymes	−2622·3	—	—	—	−2678·6	—	—	—
Rb(3·8)	−60·3	—	—	—	−60·3	—	—	—
Rb(2·5)	−108·6	—	—	—	−108·6	—	—	—
Allozymes + Rb(3·8)	−2718·1	−2682·7	35·4	1 ***	−2762·7	−2738·9	23·8	1 ***
Allozymes + Rb(2·5)	−2739·8	−2730·9	8·9	1 ***	−2850·1	−2787·2	62·9	1 ***
Rb(3·8) + Rb(2·5)	−175·6	−168·9	6·7	1 ***	−230·8	−168·9	62·0	1 ***

LL1: loglikelihood value with constraint on slope or centre.
LL0: loglikelihood value without constraint.
ΔLL: LL0–LL1. 2ΔLL follows a χ^2 with D.F. degrees of freedom.
*** $P < 0{\cdot}001$.

3. Results

(i) *Patterns of chromosomal variation*

Chromosomal number varied from 2n = 40 to a minimum of 2n = 34 (Table 1). Rb fusions were present in 27 localities, all except two of which were polymorphic. Six different Rb fusions were identified by G-banding: Rb(2·5), Rb(3·8), Rb(6·9), Rb(9·13), Rb(10·11) and Rb(4·17). Four of these Rb fusions were previously described in Nancé *et al.* (1990), while Rb fusions Rb(4·17) and Rb(9·13) are newly identified for this region. None of the Rb fusions observed are unique to Denmark although this combination of metacentrics is not found elsewhere (see Bauchau, 1990). Rb(4·17) is present in Italy, Rb(2·5) is present in Germany and Greece, Rb(3·8) and Rb(10·11) in Germany and Italy, Rb(6·9) in Greece and Rb(9·13) in former Yugoslavia.

As shown in the previous analysis, all-acrocentric karyotypes are distributed in the northern half of the area studied and inhabited by *M. m. musculus*, while chromosomal variation is restricted to the southern half where *M. m. domesticus* prevails. These results confirm the lack of introgression of Rb fusions into a predominantly *musculus* genome. In the *domesticus* populations, a complex pattern prevails with a North–South gradient in the mean number of Rb fusions and an East–West differentiation in the composition of fusions. The most widespread fusion is Rb(3·8) which is present in both transects and fixed in almost all southern localities. Rb(2·5) is almost never found fixed in a locality, but decreases in frequency from South to North. It always occurs in populations carrying Rb(3·8). Rb(6·9) is found in association with the two preceding Rb fusions in the south and Rb(9·13) is restricted to the western transect. Rb(10·11) and Rb(4·17) are rare fusions and limited to a few individuals only. If the northern limits of chromosomal variability are well described by the sampling scheme, the southern boundary is not. The slight decrease in frequency of Rb(2·5) and Rb(6·9) in the southernmost localities may imply additional changes in chromosomal variability further south (contact with all-acrocentric mice, another chromosomal race?). The presence of alternative Rb fusions in the eastern and western transects suggests that the mice belong to chromosomally differentiated populations. Contact between these two types of populations is observed in two localities: Maltbaek (44, 45) where both Rb(6·9) and Rb(9·13) are present.

Chromosomal rearrangements were identified in 235 individuals. 48% were homozygous while 52% were heterozygous consisting mostly of single heterozygotes (104 mice), i.e. carrying only one Rb fusion in a heterozygous state. Multiple heterozygotes could be subdivided into double (23 mice) and triple heterozygotes (4), three of which were complex heterozygotes, since they carried the two monobrachially homologous Rb fusions (Rb(6·9) and Rb(9·13)). Significant departures from Hardy–Weinberg proportions were tested in samples of six or more individuals

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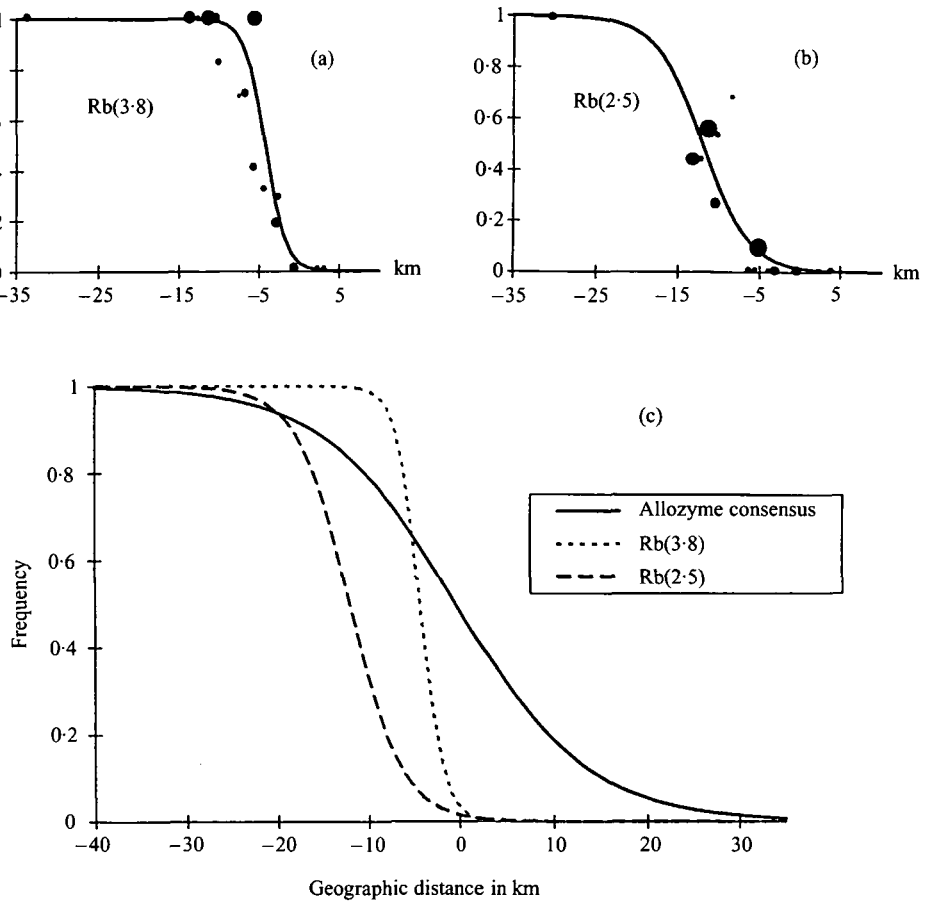

Fig. 2. Geographical representation of the clines for the different markers. Clines were fitted to a logistic model by maximum likelihood. (a) and (b) chromosomal clines with weighted samples. (c) Rb and allozymic clines. The latter is represented as a consensus cline for comparative purposes.

and were observed in only one case (Rb(3·8) at Jerlev 91). The combination of tests for all variable Rb fusions did not allow us to reject Hardy–Weinberg proportions in any locality. Linkage disequilibrium among Rb fusions was tested and only one significant result (1/21) was observed which would be expected by chance (Rice, 1989). No linkage disequilibrium was detected between Rb(3·8) and the *Es-1* and *Es-2* loci (located respectively 33 and 42 centimorgans from the centromere of chromosome 8). In localities sampled over several successive years, only one case (Rb(2·5) in Torskind 84 and 85) showed significant variation from one year to the next.

(ii) *Clinal analysis*

Estimates of cline widths and centres are provided for both Rb(3·8) and Rb(2·5) in Table 2. The clinal parameters (centres and widths) of the different markers were compared as follows: Rb(3·8) *v.* Rb(2·5), Rb(3·8) *v.* allozymes, Rb(2·5) *v.* allozymes. Likelihood ratio tests showed that all comparisons were significant (Table 3). These results indicate that the chromosomal clines are staggered and that the clines for at least two of the fusions and that of the allozymes differ in width. These clinal patterns are illustrated in Figure 2.

4. Discussion

(i) *Chromosomal variation*

The Danish Rb populations analysed in this study show a more complex situation than that previously described by Nancé *et al.* (1990). Two additional Rb fusions have been identified, Rb(4·17) and Rb(9·13). Rb(4·17) is present in only a few populations whereas Rb(9·13) characterizes the western transect suggesting that two groups of populations exist in this region of Denmark differentiated by monobrachially homologous fusions: Rb(9·13) and Rb(6·9). Contact between the two groups of populations is observed in two localities where both fusions are present. Hybrids carrying these two fusions are expected to show a relatively severe impairment of fertility related to the formation of a quadrivalent at meiosis (Gropp & Winking, 1981). Furthermore, the three Rb fusions (3·8), (6·9) and (2·5) were previously considered as fixed in the South of the zone studied, whereas the present analysis suggests that additional chromosomal differences may be occurring further south.

(ii) *Non-coincidence of clines*

The distribution of Rb fusions in Denmark shows that the different clines are staggered within *M. m.*

Table 4. *Relative selective coefficients maintaining clines at equilibrium*

Clines compared	
Rb(3·8)/Rb(2·5)	5·3
Rb(3·8)/Allozymes	32·7
Rb(2·5)/Allozymes	6·2

domesticus and decrease in width as they are closer to the centre of the *M. m. domesticus*/*M. m. musculus* hybrid zone.

Coincidence of clines for multiple markers is a predicted feature of tension zones which are by definition maintained by a balance between selection and dispersal. When individual clines produced by a heterozygote disadvantage overlap, they are predicted to move together due to linkage disequilibrium between the selected loci and attraction by density troughs (Barton, 1980; Hewitt & Barton, 1980; Barton & Hewitt, 1983, 1985; Hewitt, 1989; Barton & Gale, 1993). Non-coincidence of clines has been observed in only a few hybrid zones and is considered to result from the recent spread of a new mutant and/or from selective processes maintaining the clines staggered (Hewitt, 1989; Searle *et al.* 1993). Studies of contact zones between Rb populations and those carrying the standard karyotype within *M. m. domesticus* show various patterns. In some cases, the clines for different Rb fusions coincide (Corti *et al.* 1990), while in others, the presence of intermediate homozygote populations carrying some of the Rb fusions results in a staggered clinal pattern. Such clines have best been described in Rb populations from Scotland (Searle, 1991; Searle *et al.* 1993), but have also been observed in Belgium and Italy (Searle, 1993). The staggering of the chromosomal clines in Denmark can be explained by either of the two processes mentioned above.

(iii) *Non-concordance of the clines*

The difference in steepness between both Rb clines and that of allozymes can best be explained by selective processes associated with underdominance of Rb heterozygotes.

Experimental studies on the deleterious effect of heterozygosity for Rb fusions are available for *M. m. domesticus*. During meiosis, pairing of Rb fusions with homologous acrocentrics produces trivalents which are subject to non-disjunction, thereby increasing the percentage of aneuploid fertile gametes. Chromosomal heterozygotes will thus show a decrease in litter size due to post-zygotic losses of aneuploid embryos (Searle, 1993). The rates of aneuploidy in wild heterozygotes are very low (< 5%) when only one trivalent is present, but increase in triple heterozygotes (13%; Scriven, 1992) and reach considerable values when heterozygosity involves 7–9 Rb fusions (Capanna *et al.* 1977; Gropp & Winking, 1981; Winking *et al.* 1988). Moreover, these rates were shown to increase when Rb fusions were transferred to a foreign genomic background. Winking *et al.* (1988) observed a 30-fold increase of resorbed implants when Rb(8·17) was transferred from a wild mouse to a laboratory genome.

Thus, two origins of underdominance can be distinguished. The first one which we will call 'epistatic underdominance' relates to the increase in the disadvantage of multiple Rb heterozygotes over single Rb heterozygotes. The second type of underdominance, 'genomic underdominance', considers that the disadvantage of a Rb heterzygote is greater on a hybrid background than on a *domesticus* genome. The latter is supported by the fact that where single Rb clines within *M. m. domesticus* have been studied (21–27 km in Italy, Montgelard, 1985; 30 km in Belgium, Bauchau *et al.* 1990; 20–30 km in Scotland, Searle *et al.* 1993), they are all wider than those observed in Denmark.

Using the underdominance model, the relative values of the differences in mean fitness can be calculated by the square of the ratio in cline widths (Table 4). The results show that the reduction in mean fitness is much higher for Rb(3·8) than that for Rb(2·5). The same observation holds when the ratios of selective pressures are estimated between chromosomal and allozymic markers. These results suggest that Rb fusions in the hybrid zone are not only under different intensities of selective pressure but that the level of underdominance is higher for Rb fusions than for allozymes.

(iv) *Interpretation of the chromosomal cline patterns*

Since house mice have a standard karyotype of 40 acrocentric chromosomes, Rb fusions are considered as derived characters which may or may not have been present in *domesticus* mice when the Danish hybrid zone was established (Auffray *et al.* 1992; Auffray, 1993). Therefore, two hypotheses that rely on the chromosomal structure of the subspecies at the time of contact are discussed to account for the non-coincidence and non-concordance of the Rb and allozymic clines.

Pre-contact chromosomal differentiation

This hypothesis considers that the contact occurred between chromosomally differentiated subspecies, with *domesticus* populations homozygous for the three Rb fusions. At the time of the contact, the chromosomal clines would have coincided with the allozymic and molecular ones and resulted in the production of triple Rb heterozygotes. The transition from a congruence of clines to their present staggering implies not only that selective factors be involved in their movement, but that they be strong enough to

counter the attraction forces of the tension zone. Displacement of clines in secondary contacts have received very little theoretical attention. Mathematical analysis and simulation of the contact between chromosomally differentiated races of *Sorex araneus* predicted that the more severe selection of complex heterozygotes *v.* simple heterozygotes will move the clines apart (Hatfield *et al.* 1992). Similarly, movement of clines for genes involved in hybrid dysfunction was postulated to account for the lack of hybrid disadvantage in the hybrid zone between *Chorthippus parallelus parallelus* and *C. p. erythropus* in the Pyrenees (Virdee & Hewitt, 1994). In the Danish case, two processes need to be explained: (i) the non-coincidence of the Rb and allozymic clines and (ii) the staggering of the Rb clines. The latter can be achieved through epistatic underdominance which will tend to reduce chromosomal unfitness by favouring single over multiple Rb heterozygotes (Barton & Bengtsson, 1986). However, this process as well as the displacement of the initially coincident Rb and allozymic clines require the presence of acrocentric chromosomes homologous to the Rb fusions. These may have two origins: *musculus* by introgression or *domesticus* by fission. In the first case, a very strong relative advantage of the originally *musculus* acrocentrics must be assumed to push the Rb clines more than 11 km from the centre of zone and from each other. The alternative hypothesis involves the existence of *domesticus* acrocentrics produced by fission which have not yet been documented in house mice and would be unlikely to occur according to recent telomeric sequence data (Nanda *et al.* 1995). Thus, the hypothesis of a pre-contact chromosomal differentiation requires levels of selection and mutation processes that have no support from experimental data.

Post-contact chromosomal differentiation

The contact is considered as having occurred between chromosomally undifferentiated (2n = 40, standard karyotype) subspecies. Non-coincidence of the Rb clines would result from the independent and successive spread of the three Rb fusions which would have originated within *domesticus* populations further south and dispersed up to the hybrid populations. Two alternative models can account for the observed differences in cline widths.

Density trough. The first one makes no assumption of underdominance for Rb heterozygotes, but relies on tension zone effects. As the Rb fusions move into the hybrid zone, they become trapped in a density trough. The difference in steepness between the Rb(3·8) cline and that of Rb(2·5) is predicted by their distance to the centre of the hybrid zone. If the width of the Rb(3·8) cline provides a rough measure of the distance over which the density trough is reducing gene flow, then it is reasonable to assume that the Rb(2·5) cline which is 11·6 km away from the centre of the hybrid zone, is located outside of the density trough (Barton & Hewitt, 1985). In this case, the model would not predict that this cline have a smaller width than that of single Rb clines observed elsewhere within *M. m. domesticus* (Montgelard, 1985; Bauchau *et al.* 1990; Searle *et al.* 1993).

Underdominance. The alternative model integrates both epistatic and genomic underdominance of chromosomal heterozygotes to explain the observed clinal pattern. The spread of each Rb fusion occurs within a pool of *domesticus* acrocentrics which become progressively enriched in *musculus* genes. This results in a gradient in the intensity of genomic underdominance which would account for the difference in width of the Rb clines depending on their position within the hybrid zone. A difference in time of spread of the Rb fusions would in itself be sufficient to explain the non-coincidence of the Rb clines. However, a mild epistatic underdominance would contribute to slacken the spread of Rb(2·5) or maintain it separate from the Rb(3·8) cline (Barton & Bengtsson, 1986). As the Rb fusions approach the centre of the zone, the *domesticus* chromosomes (Rb and acrocentrics) interact with *musculus* acrocentrics which may further increase genomic underdominance due to centromeric incompatibilities. If the latter are predominant and if the fitness reduction of Rb/*musculus* hybrids is higher than that of the two other chromosomal types, *domesticus* acrocentrics would be expected to persist in the hybrid zone forming a buffer between *musculus* acrocentrics and *domesticus* Rb chromosomes. Two arguments suggest, however, that the present day clinal pattern of Rb(3·8) does not involve a *domesticus* acrocentric buffer. First, interaction between Rb and *domesticus* acrocentric chromosomes will not produce a cline with a slope as steep as that of Rb(3·8). Secondly, the proximity of the Rb(3·8) cline to the centre of the hybrid zone would lead us to expect the extinction by drift of the buffering *domesticus* acrocentrics. Thus, centromeric incompatibilities alone are unlikely to account for the Rb(3·8) clinal pattern. The most likely hypothesis for the non-coincidence and non-concordance of the Rb and allozymic clines involves genomic underdominance due to interaction with the *musculus* background as the main selective disadvantage. Epistatic underdominance if present would contribute to the staggering of the chromosomal clines. Similarly, both sources of genomic underdominance, i.e. genetic and centromeric incompatibilities, may explain the more than five times higher selection level against Rb(3·8) than Rb(2·5). Finally, a balance between these two types of genomic incompatibilities and attraction of the tension zone may account for the position of the Rb(3·8) cline within *domesticus* and the absence of introgression of the Rb fusions into the *musculus* genome.

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Gene flow. A post-contact chromosomal differentiation suggests that the flow of allozyme markers through the hybrid zone would have occurred prior to the arrival of the Rb fusions. The introgression of allozyme markers would then have been established before the barrier to Rb fusions became effective. Some Rb fusions are known to inhibit recombination when heterozygous in the *domesticus* genome (Davisson & Akeson, 1993) which would result in subsequently lowering the flow of allozymes carried by the chromosomal arms of the Rb fusions. However, there is no indication of linkage disequilibrium between Rb(3·8) and the *Es-1* and *Es-2* loci in the hybrid populations which suggests that this may not be the case. Several reasons may account for these observations: (i) Rb fusions have only recently moved into this area, (ii) there is no inhibition of recombination in wild genomes or only over a small segment, or (iii) most likely, linkage disequilibrium is too weak to be detected, particularly since clines are staggered (Szymura & Barton, 1986).

(v) *Evolution of the hybrid zone*

The study of Rb fusions in the Danish hybrid zone has allowed us to investigate the effect of the evolution of a post-contact genetic differentiation on gene exchange between the two subspecies. This situation differs from most studies of contact zones in which markers differentiated in allopatry. The *musculus* genome acts as a strong barrier impeding the flow of Rb fusions to an extent similar to that found for molecular markers on the X and Y chromosomes (Vanlerberghe *et al.* 1986; Dod *et al.* 1993). However, whereas hybrid unfitness due to differentiation of sex chromosomes has been extensively investigated, that related to newly acquired chromosomal rearrangements is less well documented. Hybrid unfitness due to chromosomal heterozygosity would be expected to contribute to a reduction in gene flow through the hybrid zone. In the case of the house mice, there is no indication that chromosome differentiation has yet modified the flow of genes through the hybrid zone. Selection against Rb fusions with little or no effect on the recombination of chromosomal arm markers (allozymes) agrees with the view that only the centromeric segments of Rb fusions are blocked by the barrier. The effect of centromeric incompatibilities on hybrid fertility is unclear and may involve either mechanical incompatibilities such as pairing deficiencies leading to non-disjunction and aneuploidy, or non-mechanical ones such as relocation of recombination events resulting in hybrid breakdown (Coates & Shaw, 1982, 1984). The consequences of chromosomal heterozygosity on hybrid unfitness in the Danish mice will need to be determined by assessing aneuploidy and recombination rates for chromosomally heterozygote hybrids.

Redi *et al.* (1990) showed that the organization of the centromeric satellite DNA sequences differed between the two subspecies and suggested that this differentiation may be involved in the occurrence of centric fusions in *M. m. domesticus* and their near absence in *M. m. musculus* (Zima *et al.* 1990). Such structural differences support the idea that centromeric incompatibilities may exist between the two subspecies. However, this leads us to question whether the absence of introgression is restricted to the centromeres of Rb fusions due to structural modifications, or represents a general feature common to the centromeres of the entire chromosomal complement. The analysis of diagnostic centromeric markers of acrocentric chromosomes will allow us to determine the extent of their introgression and the role of centromeric differentiation in the two subspecies of house mice.

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