Chromosomal Transmission Bias in Laboratory Hybrids Between Wild Strains of the Two European Subspecies of House Mice

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ABSTRACT

Laboratory crosses between wild strains of the two European house mouse subspecies *Mus musculus domesticus* ($2n = 34$) and *M. m. musculus* ($2n = 40$) were performed to analyze the selective processes involved in the non-introgression of centromeric regions of Robertsonian (Rb) fusions in the Danish hybrid zone. The chromosomal analysis of 226 backcross progeny from 22 reciprocal crosses showed that the segregation of the three Rb fusions present did not significantly differ from Mendelian expectations. However, a significant negative correlation was found between Rb transmission rates and the average litter sizes of the F_1 pairs. Among the different models of selection discussed, the most likely one supported the existence of two opposing selective factors resulting in an overall compensation of chromosomal types in the backcross progeny. A two-phase selective process involving embryo competition was postulated with non-Rb carriers being favored during pre-implantation but disadvantaged after implantation. Such balanced selective pressures acting on *musculus* non-Rb centromeres are compatible with the steep slope and off-centered position of the chromosomal cline observed in the Danish hybrid zone. These results suggested that these selective factors may be more related to centromere origin (*musculus* or *domesticus*) than to centromere structure (Rb or non-Rb).

SECONDARY contact zones are often characterized by clinal transitions in the frequencies of genetic markers as well as of morphological traits. Selection against hybrids is the most common postzygotic model of their persistence through time (HUXLEY 1939; BAZYKIN 1969; ENDLER 1977) and has been the focus of extensive theoretical work on cline shape, maintenance and movement (BARTON 1979, 1983; BARTON and BENGTSSON 1986; BARTON and HEWITT 1989; KOHLMAN and SHAW 1991). Using predictions based on the heterozygote disadvantage model, detailed analyses of natural hybrid zones have evaluated and compared the selective effects of genetic differences between hybridizing taxa to infer mechanisms of species formation (BARTON and HEWITT 1985; HEWITT 1988; HARRISON 1990; SZYMURA and BARTON 1991; BARTON and GALE 1993). As the centers of clines for different markers are expected to coincide under this model, observing staggered clines suggests that other processes in addition to underdominance may be involved (HEWITT 1990; HATFIELD *et al.* 1992; SEARLE 1993). However, if such studies detect which genetic differences result in hybrid dysgenesis, how these selective processes operate requires experimental follow-through analyses. In this study, controlled crosses between two house mouse subspecies were performed to characterize the selective forces related to chromosomal differentiation between these two taxa.

Two subspecies of house mice (*Mus musculus domesticus* and *M. m. musculus*) occupy Western and Eastern Europe, respectively, and hybridize along a narrow contact zone extending from Denmark to Bulgaria (HUNT and SELANDER 1973; BOURSOT *et al.* 1993; SAGE *et al.* 1993; FEL-CLAIR *et al.* 1996). Whereas both exhibit the same standard karyotype of 40 acrocentric chromosomes, *M. m. domesticus* displays an astonishing diversity of chromosomal races characterized by various numbers and types of Robertsonian (Rb) fusions whereby two acrocentric chromosomes fuse into a metacentric one. In the Danish portion of the *domesticus/musculus* hybrid zone, *domesticus* populations were found to carry three Rb fusions: Rb(3.8), Rb(2.5) and Rb(6.9) (NANCÉ *et al.* 1990), the centromeric regions of which did not introgress into the *musculus* genome. The chromosomal clines did not coincide with the allozymic center of the hybrid zone and showed increasingly steeper slopes the closer they were to this area (FEL-CLAIR *et al.* 1996). Evidence for underdominance related to Rb heterozygosity has been provided within *domesticus* both by experimental analyses of fertility and the restriction of

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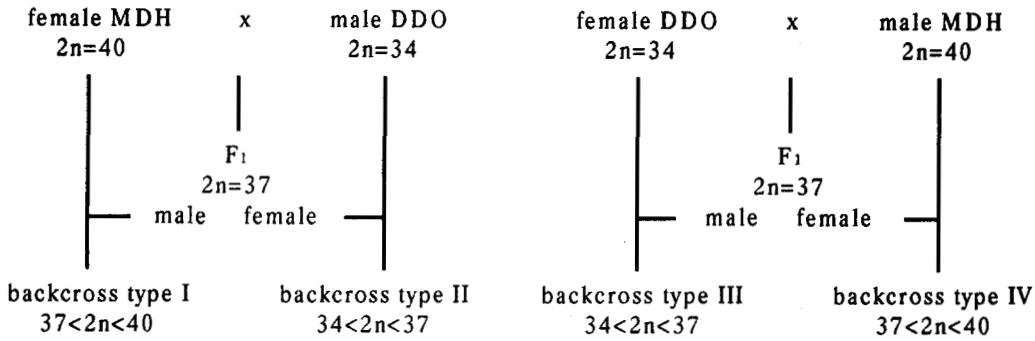

FIGURE 1.—Mating scheme between *musculus* (MDH) and *domesticus* (DDO) mice. The diploid number and the expected range of karyotypes is indicated.

Rb heterozygosity to contact zones with all-acrocentric mice (CATTANACH and MOSELEY 1973; GROPP and WINKING 1981; WINKING 1986; and review in BOURSOT *et al.* 1993; SAGE *et al.* 1993; SEARLE 1993; NACHMAN and SEARLE 1995). Additionally, the level of underdominance is known to increase when Rb fusions are introduced into a laboratory genome (WINKING *et al.* 1988). Based on these data, FEL-CLAIR *et al.* (1996) suggested that the main selective disadvantage compatible with an increase in steepness of the chromosomal clines was genomic underdominance due to the interaction of Rb heterozygosity with the *musculus* foreign background. However, underdominance hypotheses leading to staggered clines are less straightforward. In fact, maintenance of non-coincident clines may involve distortion segregation that theoretical studies have often associated with fixation and diffusion of chromosomal rearrangements (WHITE 1978; HEDRICK 1981; LANDE 1985; MICHALAKIS and OLIVIERI 1993). Although distorted Rb transmission rates have only rarely been detected in wild *domesticus* genomes (HARRIS *et al.* 1986; BRITTON-DAVIDIAN *et al.* 1990; VIROUX and BAUCHAU 1992), they have been more commonly observed when Rb fusions are introduced into laboratory strain genomes and have favored the acrocentric homologues (GROPP and WINKING 1981; ARANHA and MARTIN-DELEON 1994; CORKERY and MARTIN-DELEON 1996). Chromosomal hybrids between wild subspecific strains may thus provide the appropriate experimental tool for detection of meiotic drive (COYNE 1989).

To determine the extent and the nature of the selective effects related to Rb fusions, their segregation was measured in the backcross progeny of F_1 hybrids between Danish strains of Rb *M. m. domesticus* and all-acrocentric *M. m. musculus*. Selective processes compatible with the observed clinal patterns led us to expect differences in chromosomally related embryo mortality between the reciprocal backcrosses due to their distinct genomic backgrounds. Such a situation would result in a correlation between transmission rates and fertility. On the other hand, if meiotic drive were present, a systematic chromosomal transmission bias would be expected as well as the independence between transmission rates and fertility. The relationship between the segregation pattern of the Rb fusions and litter sizes was tested according to these different hypotheses of selection.

MATERIALS AND METHODS

Mice: Two strains of wild house mice from the wild mice genetic repository in Montpellier, France were used. The *M. m. domesticus* strain (DDO) that originated from wild mice captured in Ödis (34 km south of the center of the Danish hybrid zone) is homozygous for three pairs of Robertsonian fusions [$2n = 34$, Rb(3.8), Rb(2.5) and Rb(6.9); NANCÉ *et al.* 1990]. The MDH strain is derived from *M. m. musculus* mice trapped in Hov (40 km north of the center of the Danish hybrid zone) and carries the standard karyotype ($2n = 40$). Both strains have been random-bred in the laboratory for 13 (DDO) and five generations (MDH). Reciprocal crosses between the two strains produced chromosomal F_1 hybrids ($2n = 37$) that were backcrossed either to one of their parents or to another individual from the parental strain to increase the number of backcrosses. The mating scheme and the number of pairs are indicated in Figure 1 and Table 1, respectively. F_1 hybrid mice were paired for an average of 9 months (range: 4–11 months) yielding a total of 299 backcross individuals, 226 of which were karyotyped after weaning. Fertility was measured by litter sizes at weaning for each backcross pair.

Chromosomal analysis: The number of Rb fusions carried by each backcross individual was determined by a karyotypic analysis. Metaphase chromosomes were prepared from yeast-stimulated bone marrow cells (LEE and ELDER 1980) according to the air-drying technique and were observed under a Zeiss Axiophot microscope (1250×) after conventional staining. A minimum of five metaphases was examined for each mouse, one of which was photographed for archiving. F_1 hybrid individuals are all heterozygous for the three Rb fusions and transmit these chromosomes to their backcross progeny either as metacentrics (Rb fusions) or as sets of homologous acrocentrics (dissociated chromosomes). Segregation of these chromosomes yields four classes of backcross individuals (C_0 to C_3) according to the number of metacentrics ($0 \leq M \leq 3$) inherited from the F_1 hybrid parent. These data were determined from the number of Rb fusions scored in the backcross individuals, the karyotype of which differed between reciprocal crosses (see Figure 1). The same procedure provided the level of chromosomal heterozygosity. Individual Rb fusions were not identified. The chromosomal transmission rate of each F_1 hybrid was expressed as the transmission rate of metacentrics (*TRm*) derived from the karyotype data: $TRm = \Sigma M/3n$, n being the number of offspring produced.

Statistical tests: Departure from Mendelian expectations was examined for each type of cross by testing if the number of inherited metacentrics followed a binomial distribution with $P = 0.5$ (*i.e.*, equal to the number of inherited homologous acrocentrics). This test was also performed for each pair.

TABLE 1

Segregation of Rb fusions and litter sizes for the different backcross pairs

Backcross							Litter size										Acrocentric selection model				
Type	No.	C_0	C_1	C_2	C_3	*TRm*	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	*ALS*	*c*	δ	*TRm*	*ALS*	Group
I	1.1	1	10	7	2	0.50	6	4	4	4	5	7	—	—	—	5.0	0.12	0.558	0.48	4.83	3
II	2.1*	0	0	2	0	0.67	2	—	—	—	—	—	—	—	—	2.0	0.59	0.558	0.66	1.81	1
II	2.2*	0	0	4	0	0.67	4	1	—	—	—	—	—	—	—	2.5	0.51	0.558	0.62	2.19	1
II	2.3*	0	1	2	1	0.67	4	—	—	—	—	—	—	—	—	4.0	0.51	0.558	0.62	2.19	1
II	2.4	0	0	2	0	0.67	3	—	—	—	—	—	—	—	—	3.0	0.59	0.558	0.66	1.81	1
II	3.1	2	6	11	0	0.49	6	6	4	5	3	—	—	—	—	4.8	0.14	0.558	0.48	4.70	3
II	3.2	0	2	2	0	0.50	7	—	—	—	—	—	—	—	—	7.0	0.00	0.558	0.44	6.00	4
II	4.1	1	1	2	2	0.61	7	3	2	—	—	—	—	—	—	4.0	0.31	0.558	0.54	3.36	2
II	4.2	6	16	12	2	0.43	3	5	4	7	1	5	7	5	4	4.7	0.08	0.558	0.46	5.27	3
II	4.3	2	8	4	1	0.42	5	6	6	—	—	—	—	—	—	5.7	0.02	0.558	0.45	5.82	4
II	4.4	1	8	4	0	0.41	7	5	4	—	—	—	—	—	—	5.3	0.04	0.558	0.45	5.60	4
II	4.5*	4	4	8	4	0.53	6	6	6	5	7	6	—	—	—	6.0	0.04	0.558	0.45	5.61	4
II	5.1	2	3	2	1	0.42	6	4	—	—	—	—	—	—	—	5.0	0.07	0.558	0.46	5.29	3
II	6.1	0	3	0	0	0.34	5	—	—	—	—	—	—	—	—	5.0	0.05	0.558	0.45	5.54	4
III	7.1	1	2	3	1	0.52	5	2	—	—	—	—	—	—	—	3.5	0.29	0.558	0.53	3.54	2
III	8.1	6	10	4	2	0.36	5	5	8	6	5	—	—	—	—	5.8	0.00	0.558	0.44	6.00	4
IV	9.1	3	4	3	2	0.45	6	2	2	4	—	—	—	—	—	3.5	0.22	0.558	0.50	4.04	2
IV	9.2	1	0	2	1	0.58	5	—	—	—	—	—	—	—	—	5.0	0.22	0.558	0.50	4.06	2
IV	9.3*	1	2	2	0	0.40	7	—	—	—	—	—	—	—	—	7.0	0.00	0.558	0.44	6.00	4
IV	10.1	0	0	4	0	0.67	4	—	—	—	—	—	—	—	—	4.0	0.51	0.558	0.62	2.19	1
IV	11.1	0	4	0	4	0.67	5	4	4	—	—	—	—	—	—	4.3	0.40	0.558	0.57	2.79	1
IV	12.1	0	4	3	1	0.54	7	2	—	—	—	—	—	—	—	4.5	0.22	0.558	0.50	4.05	2
Total		31	88	83	24	0.48															

Type refers to the different crosses described in Figure 1. In the No. column, the first number groups sibling F_1s, the second identifies the F_1 and the asterisk indicates a backcross to one of the parents. C0 to C3 provides the number of individuals that inherit zero to three metacentrics from their F_1 parent. *TRm* is the transmission rate of metacentrics (see text). Litter size indicates the number of progeny per litter that survive to weaning. *ALS* is the average litter size. Estimates for the acrocentric selection model (see text) are provided considering fixed distortion (δ) and variable viability selection (c). Predicted values of *TRm* and *ALS* are calculated using Equations 2 and 3 and $ALS_0 = 6.0$. Group indicates how the pairs were grouped in model F: 1, high selection ($c > 0.35$); 2, intermediate selection ($c < 0.35$); 3, low selection ($c < 0.15$); 4, no selection ($c < 0.05$).

There are three levels of nested factors (mating type, pair and litter) and two variables that can be defined for these three factors (litter size, transmission rate), plus one that can be defined at the pair level (litter rank). The effect of these factors and variables was first analyzed on each variable independently (regression models using the Glim software, BAKER and NELDER 1992). The effect of the mating scheme (effect of backcross types I, II, III and IV, see Figure 1; of backcrossed subspecies, *musculus* I + IV *vs. domesticus* II + III; of sex of the F_1, II + IV *vs.* I + III; and of backcross origin, I + II *vs.* III + IV), of litter sizes and litter ranks on Rb transmission rates were investigated using logistic regression models. The effects of these factors and variables were tested on the proportions of chromosomal heterozygotes in the progeny in a similar manner. The effects of the backcross type and of the litter rank on litter size were fitted assuming a Poisson error on litter size. Second, we analyzed the dependence between the variables (litter size and transmission rate) at the litter and pair levels. At the litter level, a logistic regression can be performed since the litter size of each litter is known exactly and is therefore considered as the independent variable. However, at the pair level, both transmission rates and average litter sizes are estimated, so a Kendall rank correlation corrected for ties was computed.

RESULTS

Pattern of segregation: The chromosomal analysis of the backcross progeny (Table 1) showed an overall transmission ratio slightly in favor of acrocentrics (*TRm* = 0.48; 352 acrocentrics *vs.* 326 metacentrics) but not significantly different from Mendelian expectations ($P = 0.32$). The proportion of each class of individuals (C_0, C_1, C_2, C_3) was consistent with those expected under Mendelian inheritance and independent segregation of each Rb fusion, *i.e.*, 1/8, 3/8, 3/8, 1/8, respectively ($P = 0.80$). No significant departures from Mendelian proportions were observed both within or between backcross types. Additionally, the proportion of Rb heterozygotes was not influenced by the mating scheme (Table 2). Similarly, no noticeable departures from Mendelian proportions were observed within pairs, except in two cases: pair 8.1 showed a higher than expected transmission rate in favor of acrocentrics (*TRm* = 0.36, $P = 0.026$, which was no longer significant after a sequential Bonferroni correction) and pair 11.1 showed a non-independent segregation of each Rb (rejection of binomial inheritance, $P = 0.005$). Transmission rates varied considerably between pairs ranging from 0.33 to 0.67, but no significant differences were found in the distribution of inherited fusions between pairs. Thus, although transmission biases were observed at different levels (total backcross progeny, between

TABLE 2

Tests of the effects of various factors on the segregation of Rb fusions and on litter sizes

Dependent variable or source of variation	d.f.	Rb proportion		Rb heterozygote proportion		Litter size	
		χ^2	P	χ^2	P	χ^2	P
Litter rank	1	0.92	0.34	1	0.32	0.08	0.78
Litter size	1	0.01	0.92	0.16	0.69		
Backcross type	3	3.80	0.28	1.94	0.58	0.67	0.88
Backcrossed subspecies	1	1.92	0.17	0.06	0.81		
F_1 sex	1	1.13	0.29	0.49	0.48		
Backcross origin	1	0.00	1.00	1.06	0.30		
Pairs within backcross type	18	17.50	0.49	17.5	0.49	14.5	0.70
Litter rank within pairs	1	0.07	0.79	0.19	0.66	1.1	0.29
Litter size within pairs	1	3.59	0.058	0.16	0.69		
Per backcross type	3	1.20	0.75	4.26	0.23		

Regression models on litter size, Rb proportion and Rb heterozygote proportion in the backcross progeny. Binomial error was assumed for the number of fusions inherited by the F_1 parent. Poisson error was assumed for litter sizes. d.f. is the number of degrees of freedom involved for each source of variation. χ^2 is the deviance associated with each source of variation. P indicates the level of significance associated with the corresponding likelihood ratio test.

pairs), none yielded significant distortion values. In particular, reciprocal crosses showed no significant differences in chromosomal segregation.

Fertility was measured by the average litter size of pairs and varied from two to seven pups/litter. This litter size was not found to be influenced either by backcross type or litter rank (Table 2). The relationship between the metacentric transmission rate of the different pairs and their average litter size was tested and found to be negatively correlated (Kendall τ, P = 0.0006, Figure 2). Pairs with high average litter sizes showed a transmission bias in favor of acrocentrics, whereas less fertile ones showed a transmission bias in favor of metacentrics. To determine if such a pattern resulted from a litter-size effect or a pair-related trait, the relationship between transmission rates and sizes of litters was tested (see Table 2). While no individual litter-size effect was detected (P = 0.78), a nearly significant positive correlation was detected within pairs (P = 0.058). This suggested that transmission rates and litter sizes were also related within pairs, but the trend was opposite to that observed for mean litter sizes, *i.e.*, mice from the smaller litters of a pair tended to inherit more acrocentrics.

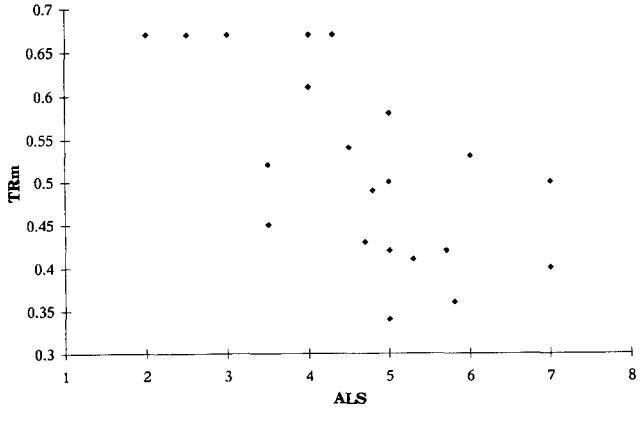

FIGURE 2.—Correlation between the metacentric transmission rate (*TRm*) and the average litter size (*ALS*) of each pair.

Nature of the chromosomal transmission bias: The observed correlation between transmission rates and average litter size indicated that chromosomally related embryo loss was occurring in these crosses. Two types of selection models can account for this loss: an underdominant selection model or selection against one of the chromosomal types. However, these models predict patterns of segregation bias that were not observed in our data, since reciprocal crosses did not differ in their transmission rates nor was there a systematic distortion over all crosses. This suggested that more than one selective factor was involved and that the behavior of these forces was antagonistic resulting in an overall compensation between chromosomal types. Such a situation could arise if a meiotic drive process (or any soft-selection process that mimics meiotic drive) was involved. Indeed, if both processes, *i.e.*, distortion and viability selection, were present and favored opposite chromosomal types, their effects could compensate each other and yield an apparent Mendelian transmission rate. Additionally, the existence of a correlation between transmission rates and average litter sizes required that one, at least, of these processes vary in intensity between pairs leading to variable compensation levels among pairs. Different possibilities were compatible with the observed segregation pattern, depending on which chromosome type (metacentrics or acrocentrics) was favored by distortion and thus disadvantaged by viability selection and which factor varied between pairs. To identify the variable selective factor and the chromo-

somal type under selection, a model was constructed to test the effects of the different assumptions on the relationship between transmission rate and average litter size.

Distortion and viability-selection models: Consider the probability d of observing in an implanted embryo a given metacentric inherited from its F_1 parent, the probability I that an implanted embryo carries k (k varies from 0 to 3) metacentrics is

$$I(M = k) = \binom{3}{k} d^k (1 - d)^{(3-k)}.$$

$d - 1/2$ is thus the advantage of metacentrics conferred by distortion. Consider now the probability J that an embryo survives to weaning and carries k metacentrics:

$$J(M = k) = (1 - c)^{ak + (1-a)(3-k)},$$

where c reflects the intensity of the viability selection and a the type of chromosome that is counter-selected (if $a = 1$, metacentrics are counter-selected; if $a = 0$, acrocentrics are, and if heterozygotes are selected against, $a = 1$ for backcrosses on *musculus* and $a = 0$ for backcrosses on *domesticus*). Considering that all the observed offspring have survived the selective process and that selection is the same and multiplicative for the three chromosomes, the probability K of observing a backcross individual at weaning that carries k metacentrics can be written as

$$K(M = k) = \frac{I(M = k)J(M = k)}{\sum_k I(M = k)J(M = k)}. \quad (1)$$

Let us note now for convenience $\delta = ad + (1 - a)(1 - d)$, the distortion of the chromosomal type that is counter-selected. The expected transmission rate (TR) of this chromosomal type is thus

$$E(TR) = \frac{1}{3} \cdot \sum_k kK(M = k) = \frac{\delta(1 - c)}{1 - c\delta}. \quad (2)$$

The expected average litter size (ALS) is the fraction of surviving embryos among those initially present (ALS_0):

$$ALS = ALS_0 \sum_k K(M = k) = ALS_0(1 - \delta c)^3. \quad (3)$$

There are two ways of expressing ALS as a function of $E(TR)$: solving (2) and (3) either by considering c variable and δ constant or by considering c constant and δ variable that leads to, respectively,

$$ALS = ALS_0 \left(\frac{\delta - 1}{TR - 1}\right)^3$$

that is an increasing function of TR (case 1)

$$ALS = ALS_0 \left(\frac{1 - c}{1 - c + cTR}\right)^3$$

that is a decreasing function of TR. (case 2)

Only two hypotheses are compatible with a negative correlation between the metacentric transmission rate (TRm) and average litter size: in case 1, acrocentrics need to be considered as the chromosomal type under selection ($a = 0$), whereas metacentrics are in case 2 ($a = 1$). Selection against heterozygotes cannot theoretically produce the observed correlation unless a majority of backcrosses are on *domesticus*, which is the case for our data. These three models were therefore explicitly compared by evaluating their likelihood. Litter size can be approximated by a Poisson distribution with a mean $ALS_0(1 - \delta c)^3$. So, the likelihood of observing a litter of n individuals and their respective karyotype is

$$P(ALS = n) \cdot \prod_{i=1}^{n} K(M = k_i). \quad (4)$$

The likelihood for the whole progeny of a pair is thus the product of the likelihood of each litter. Maximum likelihood estimates of c and δ were computed using the Metropolis algorithm adapted from N. H. BARTON (SZYMURA and BARTON 1986). ALS_0 was evaluated from the average litter size of *musculus* × *domesticus* crosses from the same experiment ($ALS_0 = 6$, data in FEL-CLAIR 1995) and the Poisson distribution of these litter sizes was also tested (FISHER 1950) and not rejected ($P = 0.78$). The full model includes two parameters per backcross pair, and its likelihood was taken as the reference (model A). The total interpair deviance was estimated by fitting both mean c and mean δ (model B). In case 1 (acrocentric selection, model C, $a = 0$), variation of both TR and ALS is explained by variation of c, whereas in case 2 (metacentric selection, model D, $a = 1$), this variation is explained by that of δ. The heterozygote selection model is derived from the same equations with $a = 1$ for backcrosses on *musculus* and $a = 0$ for backcrosses on *domesticus* (model E). In each case, the variable parameter was estimated for the 22 pairs. The relative plausibility of these models was evaluated using the Akaike information criterion (AIC = deviance + 2 × number of degrees of freedom). Models and results are detailed in Table 3. The model providing the best fit (model C, AIC = 58.2) indicates that the chromosomal type under selection is the acrocentric state. The maximum likelihood estimates for c and δ in this model are indicated in Table 1. The fitted global bias due to distortion is 6% ($\delta = 0.56$) and is significantly different from zero ($P = 0.0034$). The model that considers metacentrics as the selected chromosomal state is 16 times less likely than the acrocentric one. The pair factor in both models is not significant compared to the minimum model because many pairs share common estimates. Grouping pairs sharing similar estimates into four classes (model F) does not result in a significant increase of residual deviance [likelihood ratio test, χ^2 (18 d.f.) = 1.13, $P = 1$] and leads to a significance level of the same order as the Kendall rank correlation previously computed [group factor, likelihood ratio

TABLE 3

Description, comparison and test of the different models of selection

Description				Comparison of models				G-test			
Model	Type under selection	Parameters δ	Parameters c	Deviance	d.f.	AIC	% TD	*vs.*	ΔDev	Δd.f.	*P*
A	—	22	22	0.00	44	88	100				
B	—	1	1	34.06	2	38.06	0				
C	Acrocentrics	1	22	12.25	23	58.25	64	B	21.8	21	0.41
D	Metacentrics	22	1	15.64	23	61.64	54	B	18.4	21	0.62
E	Heterozygotes	1	22	22.09	23	68.09	35	B	12.0	21	0.94
F	Acrocentrics	1	4	13.38	5	23.38	61	B	20.7	3	0.0001
G	Metacentrics	4	1	16.17	5	26.17	53	B	17.9	3	0.0005
H	Acrocentrics	—	22	20.85	22	64.85	39	C	8.6	1	0.0034
I	Acrocentrics	1	4	30.76	5	40.8	10	B	3.3	3	0.35

Comparison of different hypotheses of selection involving viability selection (c) and distortion (δ). The chromosome type under selection is either the metacentrics ($a = 1$) or the acrocentrics ($a = 0$) or the heterozygotes (see text). Parameters indicate the kind (c or δ) and the number of fitted parameters for each model. The deviance is twice the difference between the log-likelihood of the model (for the 22 pairs calculated using Equation 4) and the log-likelihood of the full model (model A). AIC (Akaike information criterion) is deviance + 2 d.f. %TD refers to the percent of total deviance explained by the model. Likelihood ratio tests are performed *vs.* the model indicated in column *vs.* The difference in deviance follows a χ^2 with Δd.f. degrees of freedom. In models F and G, four estimates of the variable parameters were performed since many pairs shared common estimates in models C and D, respectively. Model I is identical to model F, but pairs were grouped by backcross type. Model H was fitted to test if δ was significantly different from 0.5 in model C.

test, χ^2 (3 d.f.) = 20.68, $P = 0.00012$]. These four classes do not correspond to groups sharing the same backcross type; when pairs are grouped according to their backcross type (see model I in Table 3), the model does not account for a significant part of the deviance.

DISCUSSION

The chromosomal analysis did not support a Rb heterozygote-related effect as expected, since differences in embryo mortality were not observed between the reciprocal backcrosses due to their distinct genomic backgrounds. Instead, results suggested that a minimum of two opposing selective factors were present and that the target of selection involved the acrocentric chromosomes and not the metacentric ones. Moreover, the relation between transmission rates and litter sizes followed opposite trends when considered between or within pairs. Between pairs, *TRm* and *ALS* were negatively correlated, whereas a positive trend was observed within pairs. Two processes may thus be distinguished: a pair-effect and a within-pair litter-effect. We will discuss them in turn and provide hypotheses for the underlying biological mechanisms.

Distortion mechanism: Among the different models tested explaining interpair deviance of both *TRm* and *ALS*, the most likely one involved two selective factors: distortion in favor of *musculus* acrocentric chromosomes ($\delta = 0.56$) and a pair-specific selection against them. Most crosses performed in this study involved female F_1s. However, a similar pattern between transmission rates and fertility was found in a previous series of similar backcrosses comprising a majority of male F_1 hybrids (13 males out of a total of 16 F_1; NANCE *et al.* 1990). These crosses involved the progeny of wild mice from two localities within the Danish hybrid zone where only Rb(3.8) was segregating. In these backcrosses, the same model (selection on acrocentrics) was found to be 10^3 times more likely than selection on metacentrics and significantly better than the minimal model [χ^2 (17 d.f.) = 37.54, $P = 0.001$, calculated from data in NANCÉ *et al.* 1990 without grouping pairs]. These results suggested that segregation distortion with similar effects would be occurring in both female and male F_1s. Such a feature is not compatible with meiotic drive since this process is expected to differ between sexes (for review see LYTTLE 1993; RUVINSKY 1995; HAIG and BERGSTROM 1995). This indicated that the biased transmission rates probably did not result from a distortion mechanism involving gamete formation in F_1s, but rather from a type of soft selection occurring at some stage during embryo development. In particular, competition between chromosomally differentiated embryos during the preimplantation stage would lead to the observed pattern. We therefore postulated a two-phase selective process, one occurring during preimplantation with no embryo mortality, and the other after implantation resulting in embryo loss. The early selection process relied solely on the chromosomal type of the embryo and thus led to a constant excess of acrocentric-bearing embryos regardless of the cross. The late selection process occurring after implantation varied in intensity among pairs. This could be due to

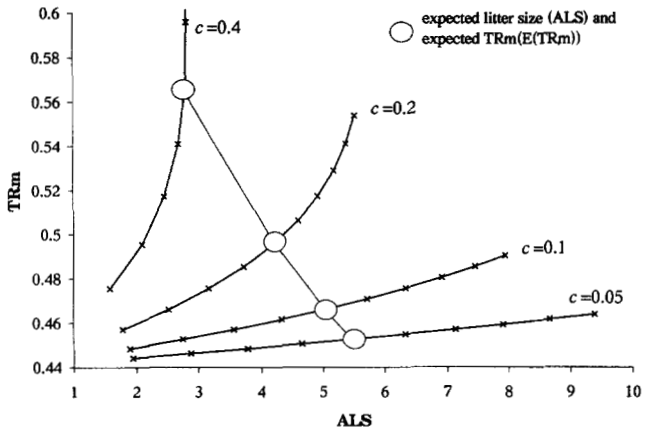

FIGURE 3.—Relationship between metacentric transmission rate (*TRm*) and litter size assuming competition for implantation favoring acrocentrics and viability selection against them enhanced by competition between implanted embryos (see text). Variation in the viability selection level creates a negative correlation between expected litter size and expected *TRm*, whereas random fluctuations in the number of competing embryos for a same level of selection create a positive trend.

incompatibilities between acrocentrics and genomic factors leading to variable embryo lethality.

Viability-selection mechanism: Such an acrocentric selection model would account for the correlation between transmission rates and average litter size between pairs. It would not, however, predict the within-pair litter effect observed. The existence of a positive trend between *TRm* and *LS* within pairs indicated that the higher the litter size, the more acrocentrics were counter-selected. This may be interpreted as a density-dependent selection with competition between implanted embryos increasing the disadvantage of acrocentrics. This can be expressed as follows.

We define ALS_0 as the average litter size before selection. Let us denote by n the number of competing embryos within a litter with $E(n) = ALS_0$. We suppose that the disadvantage of acrocentrics increases with increasing density, say that it equals cn/ALS_0. *TRm* and *LS* (the number of embryos that survive) thus become

$$TRm = \frac{\delta(cn - ALS_0)}{c\delta n - ALS_0} \quad \text{(see Eq. 2)}$$

$$LS = n\left(\frac{ALS_0 - c\delta n}{ALS_0}\right)^3 \quad \text{(see Eq. 3)}$$

In a reasonable range of variation of n around $E(n)$, random fluctuations will in most cases generate a positive correlation within pairs between *TRm* and *LS*. It should be noted that at the pair level, the acrocentric counter-selection depends not only on the acrocentric disadvantage but also on parameters that could vary from one female to another (average number of ova shed, uterine capacity). Figure 3 represents *LS* as a function of *TRm* for varying values of c, and for a range of n that corresponds to 99% of a Poisson distribution with ALS^0 as the mean, for $ALS_0 = 6$ and for $\delta = 0.56$. Thus, competition between implanted embryos that disadvantages acrocentric-rich bearers is compatible with both within- and between-pair effects. The litter-effect may be only due to random variation in the number of competing embryos, and the pair-effect may be caused by differences in the intensity of embryo competition between females.

Pre- and postimplantation selection: The segregation pattern of Rb fusions in backcrosses between the two house mouse subspecies thus suggested that competition between embryos may be occurring at two stages of embryogenesis, pre- and postimplantation, and that antagonistic selective processes were associated with each stage. Embryo competition during preimplantation may be related to differences in rates of development between chromosomal types allowing acrocentric-rich embryos to rapidly saturate more implantation sites. Differences in developmental rates of preimplantation mouse embryos have been described in experiments comparing normal and delayed mating (ISHIKAWA *et al.* 1992). On the other hand, during postimplantation, resource competition enhanced by a limited uterine capacity varying between females would disadvantage acrocentric-rich embryos. Biased recovery of the homogeneously staining region (HSR) on chromosome *1* in *M. m. domesticus* was attributed to differences in survival rates of postimplantation embryos (WEICHENHAN *et al.* 1996). Further experimental work is necessary to unravel the mechanisms underlying these selective processes.

Consequence for the hybrid zone: In the Danish hybrid zone, the limited introgression of *domesticus* Rb fusions into the *musculus* genome was shown to be restricted to the centromeric regions of the chromosomes. Underdominant selective processes related to interactions between the centromeres of the Rb chromosomes and the *musculus* genomic background were postulated (FEL-CLAIR *et al.* 1996). However, the present chromosomal analysis indicated that underdominance was not the relevant model of selection and suggested the existence of opposing selective pressures acting on centromeres in hybrids. The clinal pattern of Rb(3.8) in the Danish hybrid zone between *M. m. domesticus* and *M. m. musculus* is very steep and is restricted to the *domesticus* part of the zone (FEL-CLAIR *et al.* 1996). For this cline to be maintained, *musculus* centromeres have to be favored in the *musculus* part of the zone and vice versa for *domesticus*. This selective gradient is predicted to change across the hybrid zone depending on the introgression level but the switch from a *musculus* selective advantage to a *domesticus* one may not necessarily occur at the 50% level of introgression (depending on the relation of dominance, penetration and epistasis of the genes involved). In fact, the centers of these clines will be determined by the position where both selection

forces exactly compensate each other along the introgression gradient (see HALDANE 1948). The interpretation of the Rb clines involving antagonistic selective processes requires thus that the level of compensation between them vary with the subspecific background. Our experimental data, however, showed no background effect, which may be due to two reasons. First, the variability between pairs may have outweighed the expected differences due to the background. Second, the level of introgression in the F_1s and backcrosses may be too close to the level at which the opposing selection forces counterbalance each other for its effect to be detectable. An experimental design to detect a background effect would require following transmission ratio and fertility in successive generations of backcrosses.

In conclusion, the levels of selection must vary with the genetic background for the cline to be maintained and do not compensate in the center of the zone but in the *domesticus*-type hybrids, which results in the staggering of the Rb cline *vs.* allozymes. The centromeric selective effects observed in the experimental *domesticus/musculus* hybrids are then not related to the underdominant selective process commonly associated with Rb fusions in the *domesticus* genome. Even if underdominance due to Rb heterozygosity cannot be discarded in these crosses, this study shows that it is not the predominant selective factor operating in these hybrid genomes. The description of this chromosomal hybrid zone may therefore provide insight for the understanding of staggered clines in tension zones.

Subspecific centromeric differentiation: In addition to minor changes in heterochromatin as well as nucleolar organizer regions (see BOURSOT *et al.* 1993) and Rb fusions in Denmark, chromosomal differentiation between *M. m. domesticus* and *M. m. musculus* involves differences in the molecular structure of the centromeric satellite DNAs (REDI *et al.* 1990). Using Rb fusions as centromeric markers does not allow us to differentiate incompatibilities due to a particular centromeric state (metacentric or acrocentric) from those related to centromere origin (*domesticus* or *musculus*). However, the fact that the selective effects shown in this study are very different from those commonly associated with fusions suggests that the chromosomal incompatibilities observed in the hybrid zone would not be related to the state of the centromere but to its origin. Such an observation illustrates that centromeres may be subjected to specific selective pressures unrelated to chromosomal rearrangements. The impact on hybridization would be important since many centromeres that are inevitably well spread over the genome may be involved (BARTON and BENGTSSON 1986).

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