Biological Journal of the Linnean Society, 2005, **84**, 379–393. With 2 figures

The genus Mus as a model for evolutionary studies
Edited by J. Britton-Davidian and J. B. Searle

Postzygotic isolation between the two European subspecies of the house mouse: estimates from fertility patterns in wild and laboratory-bred hybrids

JANICE BRITTON-DAVIDIAN[1]*, FABIENNE FEL-CLAIR[1], JOËLLE LOPEZ[1], PAUL ALIBERT[2] and PIERRE BOURSOT[3]

[1]*Laboratoire de Génétique et Environnement, Institut des Sciences de l'Evolution (UMR 5554) CC065, Université Montpellier 2, 34095 Montpellier cedex 05, France*
[2]*Biogeosciences – Dijon UMR-CNRS 5561 Equipe «Différenciation et Espèces», Bâtiment Gabriel, 6 Bd Gabriel, Université de Bourgogne, 21000 Dijon, France*
[3]*Laboratoire Génome, Populations, Interactions, Adaptations (UMR 5171), CC063, Université Montpellier 2, 34095 Montpellier cedex 05, France*

Received 31 October 2003; accepted for publication 7 October 2004

We assessed the fertility (reproductive success, litter size, testis weight, spermatocyte-to-spermatid ratio) of F_1s and backcrosses between different wild-derived outbred and inbred strains of two mouse subspecies, *Mus musculus domesticus* and *M. m. musculus*. A significant proportion of the F_1 females between the outbred crosses did not reproduce, suggesting that female infertility was present. As the spermatocyte-to-spermatid ratio was correlated with testis weight, the latter was used to attribute a sterile vs. fertile phenotype to all males. Segregation proportions in the backcrosses of F_1 females yielded 11 (inbred) to 17% (outbred) sterile males, suggesting the contribution of two to three major genetic factors to hybrid male sterility. Only one direction of cross between the inbred strains produced sterile F_1 males, indicating that one factor was borne by the *musculus* X-chromosome. No such differences were observed between reciprocal crosses in the outbred strains. The involvement of the X chromosome in male sterility thus could not be assessed, but its contribution appears likely given the limited introgression of X-linked markers through the hybrid zone between the subspecies. However, we observed no sterile phenotypes in wild males from the hybrid zone, although testis weight tended to decrease in the centre of the transect.

ADDITIONAL KEYWORDS: Haldane's rule – hybrid sterility – hybrid zone – incompatibility – reproductive isolation – spermatocyte-to-spermatid ratio – testis weight – X chromosome.

INTRODUCTION

The genetic basis and evolution of reproductive isolation between taxa is a key component for understanding speciation, and has been the focus of renewed interest in the past decade (Coyne & Orr, 1998; Jiggins *et al*., 2001; Turelli, Barton & Coyne, 2001; Wu, 2001; Saetre *et al*., 2003). Although the emphasis has recently shifted to patterns of prezygotic isolation and chromosomal effects (Noor *et al*., 2001; Rieseberg, 2001; Via, 2001; Delneri *et al*., 2003; Hey, 2003; Navarro & Barton, 2003), the genetic architecture of postzygotic isolation has been thoroughly studied in only a few biological models, such as the elegant introgression constructs performed between *Drosophila* (Coyne & Orr, 1998; Wu & Hollocher, 1998; Tao, Hartl & Laurie, 2001; Tao *et al*., 2003), *Helianthus* taxa (Rieseberg, Whitton & Gardner, 1999; Rieseberg,

*Corresponding author. E-mail: britton@isem.univ-montp2.fr

2001), and very recently between house mouse strains derived from *Mus musculus domesticus* and *M. m. molossinus* (Oka *et al*., 2004). Postzygotic isolation is characterized by two components, hybrid inviability and sterility, both of which follow Haldane's rule (Haldane, 1922): if hybrids of only one sex are sterile or inviable in a species cross, that sex is nearly always the heterogametic one (Coyne & Orr, 1998). The most agreed upon genetic basis for the evolution of reproductive isolation follows the Dobzhansky–Muller model, which involves epistatic interactions between two or more incompatible genes (Orr, 1995; Laurie, 1997; Butlin, 1998). Such a model is supported by studies on hybrid sterility in *Drosophila*, which suggest the incompatibilities involve many genes distributed throughout the whole genome in tightly linked clusters (Tao *et al*., 2001). However, most of these analyses have investigated patterns of reproductive isolation between species, most likely leading to an overestimate of the number of genes required to initiate reproductive isolation, due to the cumulative rate of increase of epistatic incompatibilities with divergence (Orr, 1995; Coyne & Orr, 1998; Wu & Hollocher, 1998; Turelli *et al*., 2001). Alternatively, hybrid zones between genetically divergent taxa appear as appropriate models for detecting the emergence of hybrid sterility, and monitoring its effect on genic introgression. Although hybrid sterility patterns have been studied in several hybrid zones, in only a few cases has its genetic architecture been addressed (Virdee & Hewitt, 1994; Jiggins *et al*., 2001; references in Laurie, 1997).

The house mouse is an excellent model for the study of postzygotic isolation for two reasons. First, it is in this taxon that the first sterility gene in mammals was described. In pioneering studies involving crosses between wild and laboratory mice, Forejt and collaborators (Forejt & Ivanyi, 1975; Forejt, 1981; Gregorova *et al*., 1996; Trachtulec *et al*., 1997, 2005) uncovered the sterility gene *Hst-1* present on chromosome 17; incompatibilities between different sets of alleles in two subspecies of the European house mouse, *Mus musculus domesticus* and *M. m. musculus,* yielded progeny in which males were sterile and females fertile, thus in agreement with Haldane's rule. In addition, epistatic interactions between *Hst-1* and at least two other loci were suspected from backcross data (Forejt, 1981; Forejt, 1996). Second, the hybrid zone between these two subspecies, which extends from Denmark to Bulgaria, has been the focus of extensive research (for review see Boursot *et al*., 1993; Sage, Atchley & Capanna, 1993). In analyses across several transects of the hybrid zone, the most striking pattern observed has been the extremely limited introgression of sex chromosome markers as compared with randomly chosen autosomal markers (Tucker *et al*., 1992; Dod *et al*., 1993; Prager, Boursot & Sage, 1997). Indications of hybrid unfitness due to disruption of coadapted gene systems were provided by the higher intestinal worm loads of hybrid populations compared with parental forms (Sage *et al*., 1986; Moulia *et al*., 1991, 1993), whereas, another trait, developmental stability of tooth characters, showed a better performance in hybrids indicating heterosis (Alibert *et al*., 1994; Auffray *et al*., 1996). However, very few studies have attempted to perform direct measures of hybrid unfitness such as inviability or infertility, and their contribution to the structure of the hybrid zone.

The aim of this study was to evaluate several fertility parameters in laboratory-bred progeny both between outbred and between inbred strains derived from populations of the two European house mouse subspecies, *M. m. domesticus* and *M. m. musculus*. In addition, wild males from the hybrid zone between the two subspecies in Denmark were analysed. Components of the genetic architecture of hybrid sterility were inferred from fertility phenotypes, and their contribution to genic introgression between subspecies is discussed.

MATERIAL AND METHODS

Crosses

Two series of crosses were performed between wild-derived strains of the two subspecies of the European house mouse. The first consisted of outbred strains maintained since 1998 at the 'Conservatoire Génétique de la Souris Sauvage' in Montpellier, originating from two localities at both edges of the hybrid zone between these subspecies in Denmark: MDH (Hov; *Mus musculus musculus*) and DDO (Ödis; *M. m. domesticus*; Fel-Clair *et al*., 1996). Allozyme analyses of ten diagnostic loci of the original sampled populations showed that the mice were slightly introgressed, those from Hov carrying 1.5% of *domesticus* alleles and those from Ödis 11% of *musculus* alleles (Alibert *et al*., 1997). In addition, the DDO strain had a diploid number of $2n = 34$ due to the presence of three pairs of Robertsonian fusions [Rb(3.8), Rb(2.5) and Rb(6.9)], whereas MDH carried the standard karyotype for the house mouse ($2n = 40$ acrocentric chromosomes; Lenormand *et al*., 1997). The second series of crosses involved two inbred strains, maintained at the Institut Pasteur in Paris, France, both carrying the standard karyotype, one originating from Lhotka in the Czech Republic (PWK; Gregorova & Forejt, 2000) and the other from Toulouse in France (WLA).

Mice were housed in the same standard conditions of light/dark (12 h/12 h) and temperature (20 °C). All pairs were maintained for a minimum of 4 months, with reproducing individuals kept paired until they

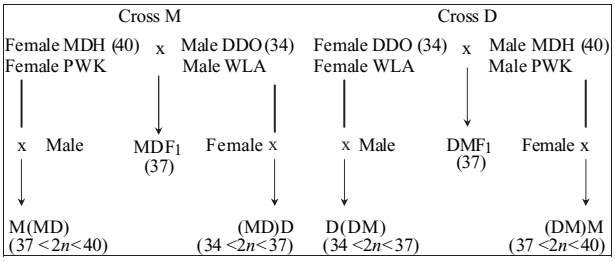

Figure 1. Mating scheme between the *musculus* and *domesticus* Danish (MDH × DDO) and inbred (PWK × WLA) strains. Diploid numbers are indicated in parentheses for the Danish strains at the different levels of crosses. The nomenclature for the different crosses is as follows: letters M (*musculus*) and D (*domesticus*) refer to the subspecific origin of the parents, the female always preceding the male partner. In the backcrosses, the F_1 is indicated in parentheses.

produced enough progeny for all analyses. Cages were checked every 2 days for birth of litters. The mating protocol involved intrasubspecific control crosses, reciprocal intersubspecific crosses as well as reciprocal backcrosses (Fig. 1). Within the latter, two classes were distinguished: true backcrosses (T), and strain backcrosses (S) in which the father or the mother was substituted by an individual of the corresponding parental strain. The nomenclature for the different crosses is as follows: letters M (*musculus*) and D (*domesticus*) specify the subspecific origin of the parents, the female always preceding the male partner. In the backcrosses, the F_1 individual is indicated in parentheses. All inbred backcrosses involved only female F_1s. Details of the number of pairs per type of cross are provided in Tables 1 and 2.

Wild mice

In total, 91 males from 31 localities along a transect through the hybrid zone in Denmark were studied. Previous allozyme analyses of ten diagnostic loci between the two subspecies allowed us to assign a hybrid index (Hi) to the populations from which these mice originated (see Fel-Clair *et al*., 1996, for details). Individuals were then grouped into five classes of Hi values varying from 0.0 to 1.0, the latter values corresponding to non-introgressed *M. m. musculus* and *M. m. domesticus*, respectively.

Fertility estimates

Two parameters of fertility were estimated from the crosses: reproductive success, i.e. the number of pairs that produced progeny, and litter size. The latter was measured between birth and weaning (21 days). The sex ratio was determined at weaning. The mice were killed when adult (> 12 g), and the body weight was recorded as well as the fresh weight of each testis for males.

The data collected for the wild males from the hybrid zone involved only body and testis weight. The right testis was weighed after fixation in Bouin's solution, and these values were converted to fresh weight by extrapolation from a subset of testes measured before and after fixation in the laboratory-bred individuals (Fel-Clair, 1995).

Histology

Thirty-nine adult males from the Danish crosses were used for a detailed analysis of spermatogenesis: two DDO, two MDH, 14 MDF_1, ten DMF_1, six (MD)D and five (DM)M (see Fig. 1 for explanation of crosses). After weighing the testes, the right testis was fixed in Bouin for at least 1 month before being processed. After progressive dehydration, the testis was embedded in plastic paraffin (Histomed), after which routinely prepared 7-μm-thick serial sections were obtained and placed on slides. After removing the paraffin, slides were stained with the periodic acid-Schiff reaction and counterstained with Groat haematoxylin and eosin. Fifteen truly transverse cross-sections of the seminiferous tubules per male were selected for scoring by light microscopy, each separated by at least 50 μm. The 12 stages of the seminiferous epithelium cycle were identified according to the criteria of Oakberg (1956) and Leblond & Clermont (1952) on the basis of acrosome formation and its progressive maturation. All stages were examined and the numbers of primary spermatocytes and round spermatids were scored, according to the method of Garagna *et al*. (1989). Cell counts were corrected using the Abercrombie (1946) correction and used to calculate the mean ratio of primary spermatocytes (b) to round spermatids (a), based on the relative occurrence of these two cell types at stages I–VIII of the seminiferous cycle. This spermatocyte-to-spermatid ratio (SSR) provides an indication of the overall cell death occurring between the primary spermatocyte and round spermatid stages in the testis, the expected ratio being 1 : 4 if spermatogenesis proceeds unimpaired. The percentage of germ cell death for each individual was calculated as: $100 \times [1 - (a/4b)]$. Following Garagna *et al*. (1989), the proportion of defective tubules defined as the proportion of tubules with an SSR lower than 1 : 3 was calculated for each individual. Additionally, in each cross-section, the number of Sertoli cells was counted and expressed per 100 μm of perimeter of the seminiferous tubule. All observations were made with a Zeiss Axiophot microscope, and cell counts were performed under a ×100 oil-immersion objective ×10 ocular ×1.25 optovar. Diameters of the cross-sectioned

Table 1. Reproductive and fertility parameters in the MDH × DDO series of crosses

MDH/DDO cross	R	NR	Litters		Sex of progeny		RTW ($\times 10^{-3}$)	
			N	Size ± SE	Male	Female	*N*	Mean ± SE
Intrasubspecies								
MDH × MDH	7	1	44	5.4 ± 2.1	110	109	94	7.7 ± 0.7
DDO × DDO	6	2	31	7.2 ± 2.2	93	103	71	8.9 ± 0.7
Mean	13	3	75	6.1 ± 2.3				8.4 ± 0.9
Intersubspecies								
MDF_1	8	0	41	5.4 ± 1.8	119	94	62	2.6 ± 0.4
DMF_1	11	0	33	6.4 ± 1.9	112	86	79	2.3 ± 0.3
Mean	19	0	74	5.9 ± 1.9				2.4 ± 0.4
True backcrosses								
$(MD)D^T$	6	9	11	4.7 ± 1.8	28	20	22	7.1 ± 2.0
$(DM)M^T$	2	11	4	4.25 ± 2.3	3	4	3	6.0 ± 0.4
$D^T(DM)$	0	2	–					
Strain backcrosses								
$(MD)D^S$	10	28	29	4.9 ± 1.7	67	63	51	6.4 ± 2.0
$(DM)M^S$	5	24	11	4.3 ± 1.7	26	19	21	6.7 ± 2.2
$M^S(MD)$	1	12	6	5.2 ± 1.1	8	22	3	9.4 ± 1.5
$D^S(DM)$	2	7	7	5.6 ± 2.1	19	17	15	6.0 ± 2.0
Total backcrosses								
(MD)D	16	37	40	4.9 ± 1.7	95	83	73	6.6 ± 2.0
(DM)M	1	12	6	5.2 ± 1.1	8	22	3	9.4 ± 1.5
M(MD)	7	35	15	4.3 ± 1.8	29	23	24	6.7 ± 2.0
D(DM)	2	9	7	5.6 ± 2.1	19	17	15	6.0 ± 2.0
Mean	26	93	68	4.8 ± 1.9			115	6.6 ± 2.1

R, number of reproductive pairs; NR, number of pairs that produced no progeny; sex distribution of progeny at weaning; RTW, relative testis weight. The nomenclature for the different crosses is as follows: letters M (*musculus*) and D (*domesticus*) refer to the subspecific origin of the parents, the female always preceding the male partner. In the backcrosses, the F_1 is indicated in parentheses. The two types of backcrosses are distinguished, the superscript ^{T} referring to the true backcrosses and ^{S} to those in which the father or the mother was substituted by an individual of the corresponding parental strain.

tubules, and of spermatocytes and round spermatids, were measured with a micrometer ocular.

Statistical analyses

Crosses were compared using χ^2 and Fisher tests for data on reproductive success and Wilcoxon–Mann–Whitney tests as well as analyses of variance for data on litter size. In the latter, cross, pair and litter rank were taken into account. The distribution of testis weight was compared within and between crosses using the non-parametric Wilcoxon–Mann–Whitney test for unpaired data. The contributions of genotype and chromosomal state to relative testis weight were tested by analyses of variance. Variation between the histological parameters was tested using Kendall Tau correlations, and comparisons between crosses were performed with the Mann–Whitney *U*-test. Corrections for multiple tests were made using the sequential Bonferroni procedure where applicable (Dunn–Sidak method, see Sokal & Rohlf, 1995), and corrected levels are provided after the probability values as follows: NS, non-significant; *< 0.05; ** < 0.01; *** < 0.001.

RESULTS

Reproductive success and litter size

Data on reproductive success, i.e. the proportion of pairs that yielded progeny, the number of litters as well as mean litter size are reported in Table 1 for the Danish strains and Table 2 for the inbred strains. Comparative tests showed no significant differences in reproductive success in either of the two sets of crosses between parental pairs (all $P > 0.21$), between reciprocal interracial crosses (all $P > 0.71$) or between these two categories (all $P > 0.17$), even though the latter tended to perform better than the former (Tables 1, 2). By contrast, differences between the two strains were present for crosses involving an F_1 hybrid. In the Dan-

Table 2. Reproductive and fertility parameters in the PWK × WLA series of crosses

			Litters		Sex of progeny		RTW($\times 10^{-3}$)	
PWK/WLA cross	R	NR	*N*	Size ± SE	Male	Female	*N*	Mean ± SE
Intrasubspecies								
PWK × PWK	4	4	8	5.0 ± 1.3	24	16	23	7.9 ± 1.6
WLA × WLA	3	0	18	4.7 ± 1.4	33	42	29	9.2 ± 2.1
Mean	7	4	26	4.8 ± 1.4	57	58	52	8.6 ± 2.0
Intersubspecies								
MDF_1	3	1	10	5.0 ± 1.8	34	24	24	3.3 ± 0.4
DMF_1	2	1	11	5.2 ± 1.5	30	27	30	5.9 ± 1.1
Mean	5	2	21	5.1 ± 1.6	64	51	54	4.8 ± 1.6
True backcrosses								
$(MD)D^T$	3	0	19	6.8 ± 1.0	56	73	52	8.2 ± 2.2
$(DM)M^T$	3	0	16	4.4 ± 1.7	39	31	33	7.3 ± 2.6
Mean	6	0	35	5.7 ± 1.8	95	104	85	7.9 ± 2.4
Strain backcrosses								
$(MD)D^S$	7	0	33	6.4 ± 1.2	100	115	74	7.6 ± 2.4
$(DM)M^S$	8	0	25	5.4 ± 2.3	58	53	48	7.9 ± 1.9
Mean	15	0	58	5.9 ± 1.8	158	168	122	7.7 ± 2.2
Total backcrosses								
(MD)D	10	0	52	6.6 ± 1.3	156	188	126	7.9 ± 2.3
(DM)M	11	0	41	5.0 ± 2.1	97	84	81	7.6 ± 2.2
Mean	21	0	93	5.9 ± 1.8	253	272	207	7.8 ± 2.3

R, number of pairs that reproduced; NR, number of pairs that produced no progeny; sex distribution of progeny at weaning; RTW, relative testis weight. The nomenclature for the different crosses is as follows: letters M (*musculus*) and D (*domesticus*) refer to the subspecific origin of the parents, the female always preceding the male partner. In the backcrosses, the F_1 is indicated in parentheses. The two types of backcrosses are distinguished, the superscript ^{T} referring to the true backcrosses and ^{S} to those in which the father or the mother was substituted by an individual of the corresponding parental strain.

ish strains, a significant reduction in the reproductive performance of F_1 females was observed in backcrosses (23% reproductive success) compared with the intra- (81%) and intersubspecific (100%) ones (all comparisons: $P < 0.001$, ***), whereas no differences were observed between these categories in the inbred strains (all $P > 0.09$). The subspecific origin of the parents in the Danish strain had no effect on the reproductive success of the F_1s within backcrosses (all $P > 0.31$), nor did the type of backcross (true vs. strain backcrosses: all $P > 0.32$). In addition, the Danish crosses involving F_1s did not differ significantly between sexes ([(MD)D + (DM)M] vs. [M(MD) + D(DM)]: $P > 0.19$), although F_1 males (13%) tended towards a lower reproductive success than females (23%).

Comparisons of mean litter size showed a significant reduction in the Danish strains as a whole for crosses involving an F_1 compared with parental ones (backcrosses vs. intra- and interstrain crosses: all $P < 0.0002$, ***). When parental strains were compared, mean litter size in DDO was found to be significantly higher than that of MDH ($P = 0.002$, **) and of all other crosses (all $P < 0.001$, ***) except DMF_1 ($P = 0.11$). As the latter also involved a DDO female, these results suggest that part of the differences in mean litter size observed between crosses may have a maternal origin. Further tests using an analysis of variance confirmed these results, but highlighted the presence of variability between pairs leading to a significant pair effect in almost all comparisons ($P = 0.0001$, ***). Additionally, although a litter rank effect was detected in some cases, this factor alone did not influence litter size ($P > 0.08$). In the inbred crosses, comparisons involving parental and F_1 mice showed again no significant differences in mean litter size (all $P > 0.42$), although values were slightly higher in the latter. The only significant difference revealed by the Wilcoxon–Mann–Whitney tests was related to a higher mean litter size in backcrosses involving MDF_1s compared with the others (all $P < 0.003$, **). These results were confirmed by the analysis of variance, which showed in addition a significant pair effect in four of the ten comparisons (all $P < 0.02$, *). No litter rank effect on mean litter size was observed (all $P > 0.17$).

Sex ratio of progeny (Tables 1, 2) was scored at weaning in all crosses and showed no significant departures from a 1 : 1 ratio in either set of strains (χ^2 tests, all $P > 0.01$, NS).

Testis weight and histological analysis

An additional parameter of fertility in males was measured by testis weight in both series of crosses and in wild hybrids. As testis weight was found to be positively correlated with body weight in some of the crosses and in wild mice (crosses: *** < P < NS; wild mice: *< P < NS; Table 3), the data were expressed as relative testis weight values (RTW) in all individuals (Tables 1, 2). The distribution of RTW within crosses showed similar results in the two sets of strains. Values of RTW were highest in the parental individuals, *domesticus* mice having significantly larger RTW than *musculus* mice in the Danish strains ($P < 0.0001$, **), but not in the inbred strains ($P = 0.015$, NS). F_1 hybrids displayed the lowest RTW values regardless of the cross, although significant differences were apparent according to the subspecific origin of the parents: RTW was higher in the MDF_1 than in the DMF_1s in the Danish strains ($P < 10^{-6}$, ***), whereas the opposite was observed for the inbred strains ($P < 0.0001$, ***). Backcross individuals exhibited a level of RTW that was intermediate between the parental and the F_1 mice and showed similar values between reciprocal crosses (outbred crosses: $P > 0.80$; inbred crosses: $P = 0.44$). All other comparisons with backcrosses were significant (outbred crosses: all $P < 0.005$, *; inbred crosses: all $P < 0.002$, **), except those involving parental *musculus* (MDH vs. [M^S(MD) + D^S(DM)]: $P = 0.04$, NS; PWK vs. [(MD)D^{T+S} and (DM)M^{T+S}]: all $P > 0.69$).

The mean relative testis weight in wild hybrid males varied per hybrid index class (Hi) from 0.0055 to 0.0067 (Table 3). The distribution of RTW according to genotype showed a decrease in RTW in the intermediate Hi class (Hi3) compared with the least introgressed populations (Hi1 and Hi5). However, an analysis of variance of these data indicated that Hi was not a significant component of the variance in relative testis weight in these mice ($P = 0.09$).

The histological analysis involved a subset of 39 individuals from the Danish crosses. Five parameters were scored: SSR, tubule diameter (Diameter), the percentage of defective tubules (DT%), the number of Sertoli cells per 100 μm of tubule diameter (S/100μ) and the percentage of germ cell death (GCD%) which was estimated from the SSR (Table 4). All of these parameters showed correlated variation among individuals. The SSR varied continuously from 3 to 0, zero corresponding to sterile individuals in which complete spermatogenic arrest was observed occurring before the spermatid stage (Fig. 2). The decrease in SSR was correlated with a reduction in diameter of the tubules ($P = 2 \times 10^{-11}$, ***), suggesting an overall decrease in size of the testis. The relationship between testis size and SSR was tested using the relative weight of the testes, and was found to be significant ($P = 10^{-12}$, ***). Thus, the decrease in SSR was associated with a reduction in relative testis weight. These results are in agreement with previous studies indicating a correlation between total sperm production and testis size. Conversely, the number of Sertoli cells per 100 μm of diameter increased significantly as testis weight ($P = 10^{-7}$, ***) and SSR decreased ($P = 4 \times 10^{-5}$, ***). The DT% parameter provides an indication of the variation in SSR between tubules within the testis. With a threshold SSR value of 1 : 3, the observed DT% showed that variation between tubules was generally low, never falling below 50% in the parental mice, and reaching 100% in a large majority of the F_1s and backcrosses. Comparisons between crosses indicated that SSR was highest in the parental individuals, lowest in the F_1s (MDF_1 and DMF_1 were not significantly different, $P = 0.81$) and intermediate in the backcrosses. Mann–Whitney *U*-tests showed that these differences were significant except for F_1 vs. backcrosses (all $P < 0.0002$, **). The distribution of RTW among levels of crosses followed a similar trend, but the differences were significant only between parental and F_1 individuals ($P = 0.002$, **).

Table 3. Distribution among the five hybrid index classes (Hi with corresponding ranges) of the mean relative testis weight (RTW) and correlation values between testis and body weight for the sample of wild males from the Danish hybrid zone

Hybrid index class	*N*	Correlation	*P*	RTW ($\times 10^{-3}$) ± SE
0.81 < Hi5 < 1.00	22	0.596	0.003*	6.7 ± 0.2
0.61 < Hi4 < 0.80	22	0.447	0.037	6.1 ± 0.3
0.41 < Hi3 < 0.60	19	0.536	0.018	5.5 ± 0.3
0.21 < Hi2 < 0.40	14	0.301	0.295	6.4 ± 0.4
0.00 < Hi1 < 0.20	14	0.557	0.039*	6.0 ± 0.4

SE, standard error; *P*, probability value of the correlation; *$P < 0.05$ after Bonferroni correction.

Table 4. Body measurements and histological parameters for the 39 individuals from the MDH × DDO series of crosses

Type	Ind	SSR	RTW ($\times 10^{-3}$)	TW	BW	S/100µ ± SE	Sertoli	Diameter ± SE	GCD%	DT%
MDH	46	3.00 ± 0.33	8.1	137.4	17.0	1.87 ± 0.29	11.3	192.5 ± 7.4	25.0	56.3
	37	2.96 ± 0.52	9.0	139.1	15.5	2.00 ± 0.33	11.5	183.5 ± 8.9	26.1	58.8
DDO	33	2.70 ± 0.41	8.8	172.7	19.5	2.38 ± 0.56	12.9	172.7 ± 11.3	32.5	73.7
	49	2.67 ± 0.47	10.2	153.6	15.0	2.44 ± 0.66	13.8	180.3 ± 9.0	33.2	80.0
MDF_1	278	2.85 ± 0.72	8.3	187.5	22.5	1.79 ± 0.22	10.3	183.0 ± 15.1	28.7	68.8
	85	2.41 ± 0.57	7.4	181.5	24.5	1.76 ± 0.26	10.0	180.7 ± 9.8	39.7	93.8
	88	2.00 ± 0.81	6.8	149.0	22.0	1.85 ± 0.54	9.9	170.6 ± 9.1	50.0	86.7
	1193	1.50 ± 0.46	5.5	135.2	24.0	1.87 ± 0.45	10.3	175.7 ± 9.9	62.5	100.0
	294	1.31 ± 0.27	5.4	129.0	24.0	2.19 ± 0.51	10.9	157.6 ± 7.4	67.2	100.0
	154	1.30 ± 0.32	5.3	116.0	22.0	2.64 ± 0.45	12.4	150.0 ± 9.9	67.5	100.0
	151	1.10 ± 0.15	5.1	107.4	21.0	2.14 ± 0.45	10.6	158.0 ± 6.8	72.5	100.0
	635	1.12 ± 0.53	4.8	135.3	28.0	2.34 ± 0.54	11.5	156.7 ± 13.6	72.0	100.0
	169	0	2.1	62.0	29.5	4.00 ± 0.83	15.2	121.0 ± 9.1	100.0	100.0
	504	0	3.1	81.0	26.0	3.26 ± 0.74	11.3	110.4 ± 8.1	100.0	100.0
	326	0	2.9	81.0	28.0	2.86 ± 1.24	10.6	110.6 ± 7.1	100.0	100.0
	716	0	2.8	78.0	28.0	3.44 ± 0.94	12.0	111.1 ± 8.0	100.0	100.0
	171	0	2.5	68.0	27.5	4.29 ± 1.51	15.1	111.8 ± 8.8	100.0	100.0
	170	0	2.4	57.0	23.5	3.61 ± 1.08	12.9	113.9 ± 8.9	100.0	100.0
DMF_1	790	1.90 ± 0.23	7.0	164.6	23.5	1.88 ± 0.33	10.3	174.5 ± 12.5	52.5	100.0
	442	1.70 ± 0.47	7.8	250.9	32.0	1.88 ± 0.30	10.4	176.5 ± 13.2	57.5	94.4
	652	1.70 ± 0.31	6.4	149.8	23.5	2.03 ± 0.47	11.9	186.5 ± 11.7	57.5	100.0
	73	1.09 ± 0.28	4.6	100.8	22.0	2.33 ± 0.43	11.6	158.4 ± 12.9	72.7	100.0
	75	0.82 ± 0.23	4.5	97.5	21.5	2.55 ± 0.47	11.8	147.0 ± 7.7	79.5	100.0
	77	0.80 ± 0.37	4.3	102.5	24.0	2.29 ± 0.31	10.2	142.1 ± 10.0	80.0	100.0
	76	0.47 ± 0.21	3.8	86.5	22.5	3.03 ± 0.64	13.8	144.9 ± 11.6	88.2	100.0
	102	0	2.6	47.5	18.0	4.41 ± 1.51	16.5	119.1 ± 10.0	100.0	100.0
	106	0	2.8	55.4	19.5	4.49 ± 0.81	15.4	109.2 ± 8.5	100.0	100.0
	103	0	3.2	47.5	15.0	4.39 ± 0.52	15.3	108.7 ± 7.5	100.0	100.0
(MD)D	880	2.6 ± 0.34	8.7	191.8	22.0	2.04 ± 0.42	11.9	184.9 ± 11.8	35.0	80.0
	1207	2.3 ± 0.27	10.4	206.7	20.0	1.66 ± 0.25	10.1	193.3 ± 11.0	42.5	100.0
	891	2.13 ± 0.32	10.2	174.2	17.0	2.15 ± 0.23	13.1	193.9 ± 14.6	46.7	100.0
	677	1.84 ± 0.27	7.7	145.8	19.0	2.22 ± 0.43	12.8	183.6 ± 14.5	54.0	100.0
	1213	1.27 ± 0.38	4.2	105.0	25.0	2.83 ± 0.63	15.3	170.4 ± 10.7	68.4	100.0
	1225	0	4.5	74.2	16.5	2.73 ± 0.51	11.8	137.3 ± 9.6	100.0	100.0
(DM)M	971	2.21 ± 0.39	9.1	194.9	21.5	1.94 ± 0.240	11.8	193.1 ± 12.1	44.8	100.0
	888	1.29 ± 0.27	4.4	114.3	26.0	1.86 ± 0.423	12.1	172.8 ± 18.3	67.7	100.0
	676	1.2 ± 0.31	4.8	101.8	21.0	2.35 ± 0.346	11.4	154.0 ± 9.7	70.0	100.0
	816	0	1.8	58.1	31.5	3.15 ± 0.825	13.8	141.3 ± 15.8	100.0	100.0
	890	0	2.5	49.4	20.0	3.79 ± 0.540	15.6	130.8 ± 8.6	100.0	100.0

SSR, spermatocyte-to-spermatid ratio; RTW ($\times 10^{-3}$), relative testis weight; TW, testis weight (mg); BW, body weight (g); S/100µ, number of Sertoli cells/100 µm of tubule diameter; Sertoli, number of Sertoli cells per cross-section; Diameter, tubule diameter; GCD%, percentage of germ cell death; DT%, percentage of defective tubules. See Fig. 1 for the nomenclature of crosses.

HYBRID MALE STERILITY

Among the F_1 males examined in the histology analysis, seven were involved in backcross pairs; of these, three produced offspring ($1.5 \pm 0.5 < SSR < 1.9 \pm 0.2$; $RTW > 0.0055$) and four produced none ($0 < SSR < 1.12 \pm 0.53$; $0.002 < RTW < 0.0048$). As SSR and RTW were highly correlated, RTW values for these individuals were used to establish a fertility phenotype threshold: mice having an $RTW \geq 0.0055$ were considered as fertile (F), and those with an $RTW < 0.0045$ were classed as sterile (S). Thus, the continuous variables (SSR and RTW) were transformed into two discrete characters (S and F). Such an approach is supported by fertility data showing that sterility in house mice occurs below a value of about

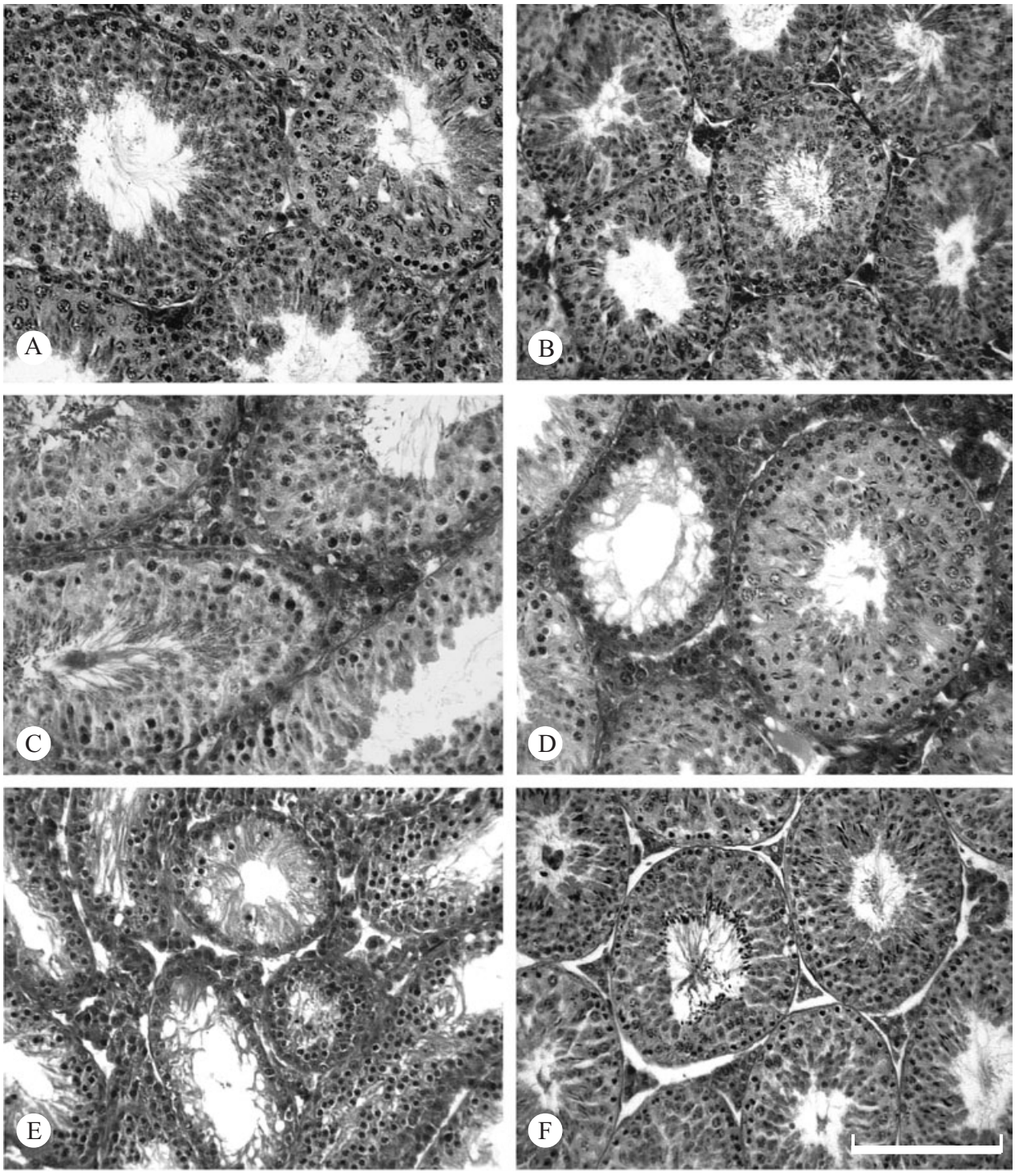

Figure 2. Histological sections of testes. Fertile parental males: A, DDO; B, MDH. C, fertile F_1 male which produced progeny. D, potentially fertile F_1 male; note juxtaposition of defective and functional seminiferous tubule cross-sections. E, sterile F_1 male; note absence of spermatozoa in tubule lumen. F, fertile backcross male with functional tubules. Scale bar = 100 μm.

10% of the normal sperm concentration (Searle & Beechey, 1974), suggesting that the threshold level of spermatogenic potential (SSR) could be a limiting factor in fertility. The RTW-based fertility score allowed us then to examine the segregation of this phenotype in the progeny of the outbred and inbred crosses. In the crosses involving the Danish strains, 178 F_1 males could thus be scored, 169 of which were sterile, yielding a mean sterility rate of 95% ($MDF_1 = 94\%$; $DMF_1 = 95\%$). Among the 81 mice analysed in the backcrosses, the proportion of sterile individuals decreased considerably to a value of only 17% [(MD)D = 17%; (DM)M = 18%]. A similar pattern was observed in the inbred strains for the backcross progeny, which showed a mean proportion of 11% sterile individuals [12% in the (MD)D and 9% in the (DM)M for a total of 193 males analysed; the latter were not significantly different: $P = 0.64$]. However, the intersubspecific reciprocal inbred crosses yielded highly contrasting results in which 100% of the MDF_1s were sterile, whereas none of the DMF_1s were. Several conclusions can be drawn from the data in the inbred crosses. First, as all F_1s have the same autosomal hybrid genome, but differ in the origin of the sex chromosomes, these results indicate that the latter contribute to sterility in these crosses. Second, the proportion of sterile males in these backcrosses is close to a 1 : 8 ratio (Fisher exact test, $P = 0.64$), but lower than a 1 : 4 ratio ($P = 0.0003$), thus indicating that at least two sterility factors in addition to the sex chromosomes are segregating in these crosses. In the Danish strains, as sterile F_1 males were present in both reciprocal crosses, the contribution of the sex chromosomes could not be assessed. In addition, the proportion of sterile males in the backcrosses was slightly higher although not significantly different from that in the inbred backcrosses (Fisher exact test, $P = 0.64$), nor was it significantly different either from a 1 : 4 ($P = 0.25$) or 1 : 8 ($P = 0.51$) segregation ratio. As three Rb fusions are present in the *domesticus* Danish mice, the contribution of chromosomal heterozygosity to hybrid sterility in these crosses was investigated, because it is known to affect spermatogenesis, particularly when the rearrangements occur on a foreign background (Redi & Capanna, 1988; Winking, Dulić, & Bulfield, 1988; Scriven, 1992; Hauffe & Searle, 1998; Castiglia & Capanna, 2000). As all F_1 individuals were heterozygous for the three Rb fusions, an analysis of variance was performed on backcrosses to check the contribution of chromosomal rearrangements to reduction in testis weight (for chromosomal data, see Lenormand *et al*., 1997). Results of the tests indicated that neither the total number of Rb fusions nor the total number in a heterozygous state contributed significantly to RTW (Rb: $P = 0.49$; Het: $P = 0.18$). Among the different effects tested, only an interaction between the subspecific origin of the backcross (DDO, MDH) and backcross type (true, strain or total) was significant ($P = 0.04$).

DISCUSSION

Male and female hybrid infertility

This study provides an extensive investigation of sterility patterns in hybrids between *M. m. domesticus* and *M. m. musculus*, as it combines the assessment of fertility using various parameters in laboratory F_1 and backcrosses, as well as in wild males from a hybrid zone between the two subspecies.

Crosses between the two subspecies irrespective of their origin led to the production of sterile F_1 males; these results are in accordance with previous studies (Forejt & Ivanyi, 1975). However, F_1 female infertility is also apparent in the Danish crosses from the reproductive performance data, as a significant proportion did not reproduce, and those that did had smaller litter sizes than intra- or intersubspecific crosses. Although the physiological origin of this infertility was not determined, it is of note that none of these females presented uterine scars after 6–9 months in the presence of a male. A primary rather than a behaviourally based origin of this sterility is supported by the high mating performance of the intersubspecific pairs. In addition, the barren F_1 females and sterile males originated from distinct pairs, indicating that the genetic factors involved differed between sexes. This pattern is consistent with data on *Drosophila* suggesting that loci causing hybrid sterility are sex-specific (Hollocher & Wu, 1996; Laurie, 1997; Orr, 1997). No similar reduction in female F_1 fertility was observed in the inbred strain crosses nor in previous reports (Forejt & Ivanyi, 1975; Gregorova & Forejt, 2000). These results indicate that variability for this trait is present within the subspecies. Regardless, hybrid dysfunction was more pronounced in male than female F_1s, in accordance with the dominance and faster male theories for Haldane's rule (Wu & Davis, 1993; Laurie, 1997; Hollocher, 1998; Presgraves & Orr, 1998).

Results of the histological analysis indicated that several components of testicular function were altered, the most obvious one being the spermatogenic efficiency as measured by the SSR score. In all sterile mice with an SSR = 0, a precocious meiotic arrest with no germ cells differentiating into spermatocytes or spermatids was observed. These results are in agreement with previous studies in hybrids between the two subspecies (Forejt, 1981; Yoshiki *et al*., 1993). The absence of differentiating germ cells is associated with a decrease in tubule diameter and relative testis weight. As RTW increases, spermatids appear in the sections until sufficient sperm are formed to ensure

fertility. Although testis weight is known to be under polygenic control (Chubb, 1992; Le Roy *et al*., 2001), results indicate that efficiency of spermatogenesis is the major determinant of RTW in these individuals. Sertoli cells, by contrast, show an almost two-fold increase in number in the sterile mice compared with the parental mice. This contrasts with observations showing that small-sized testes are generally associated with a decrease in the Sertoli cell population (Chubb, 1992). As in other non-seasonally breeding species, the number of Sertoli cells is fixed early in development in house mice (Kluin, Kramer & de Rooij, 1984; Russell & Peterson, 1984), suggesting that the increase in number observed in the sterile mice may be related to developmental perturbations. However, as testes sizes greatly differ in sterile vs. parental individuals, recording the number of Sertoli cells per 100 μm of tubule diameter may artificially inflate the scores in smaller testes. When absolute numbers of Sertoli cells per cross-section are compared, the inverse relationship between RTW and number of Sertoli cells remains significant ($P = 0.038$), with an 11% increase per cross-section of testis in the sterile individuals. This modification in Sertoli cell number may be related to a dysfunctional expression of the Y-linked sex-determining gene (*Sry*), because it is known to regulate the differentiation of Sertoli cells (Cooke & Saunders, 2002) and their postnatal mitotic activity (Chubb, 1992). Furthermore, several studies have indicated that Sertoli cell function and structure were not affected in similar subspecific hybrids (Forejt & Ivanyi, 1975; Yoshiki *et al*., 1993; but see Chubb & Nolan, 1987). These studies suggest that sterility factors in *domesticus*/*musculus* hybrids most likely involve defective cell-to-cell contact between Sertoli and germ cells (De Kretser, 1990).

Genetic architecture of hybrid male sterility

The correlation between the SSR and RTW led us to determine threshold values of fertility, which were used to attribute a sterile vs. fertile phenotype to all males. On the basis of the proportion of sterile males and asymmetric distribution of this phenotype in the inbred reciprocal crosses, a tentative scheme for the genetic determination of sterility can be established (Table 5). In inbred crosses, the asymmetric sterility patterns of the F_1s indicates that a sterility factor is linked to a sex chromosome of one but not the other strain. Segregation of the X and Y chromosomes from both subspecies in relation to the sterility phenotype proportions indicates that a Y chromosome is not involved, but rather that the sex-chromosome sterility factor is borne by the *musculus* X (Table 5). Under this hypothesis, sterile backcross males carry the *musculus* X sterility factor and two autosomal factors. The low proportion of sterile males in the backcrosses further suggests that sterility is caused by epistatic interactions involving heterozygosity at these autosomal loci, although the nature of the incompatibilities requires proper assessment. Interpretation of the genetic basis of sterility in the Danish crosses is less straightforward, as sterile F_1s are present in both directions of crosses, yielding no information on the

Table 5. Segregation of alleles at three epistatically interacting loci compatible with the observed distribution of sterile males

Cross M			Genotype	Cross D			Genotype
Parents	*domesticus* *musculus*	Male Female	$AABBX^dY^d$ $aabbX^mX^m$	Parents	*musculus* *domesticus*	Male Female	$aabbX^mY^m$ $AABBX^dX^d$
MDF_1		Male Female × *domesticus*	$AaBbX^mY^d$ $AaBbX^mX^d$ $AABBX^dY^d$	DMF_1		Male Female × *musculus*	$AaBbX^dY^m$ $AaBbX^dX^m$ $aabbX^mY^m$
(MD)D		Male	$AABBX^dY^d$ $AABbX^dY^d$ $AaBBX^dY^d$ $AaBbX^dY^d$ $AABBX^mY^d$ $AABbX^mY^d$ $AaBBX^mY^d$ $AaBbX^mY^d$	(DM)D		Male	$AABBX^dY^m$ $AABbX^dY^m$ $AaBBX^dY^m$ $AaBbX^dY^m$ $AABBX^mY^m$ $AABbX^mY^m$ $AaBBX^mY^m$ $AaBbX^mY^m$

Two autosomal loci are involved with alleles A and B in *domesticus*, and a and b in *musculus*; X^dY^d and X^mY^m refer to the sex chromosomes in *domesticus* and *musculus*, respectively. The genotype of sterile males is highlighted in F_1 hybrids and backcrosses; the latter refer only to crosses involving a female F_1: dark shading corresponds to results in the PWK × WLA cross, and light shading to additional sterile genotypes in the DDO × MDH cross.

role of the sex chromosomes. In addition, the low number of backcross progeny makes the phenotypic proportions uncertain, because the latter do not significantly differ from either 1 : 4 and 1 : 8 proportions, nor from those in the inbred strains. As such, they would not be incompatible with the involvement of X-linked sterility factors, provided these were carried by both the *domesticus* and the *musculus* X chromosomes, in which case the expected proportions of sterile males would be 1 : 4. Furthermore, in the Danish cross, the variability in the percentage of sterile males between pairs (50–100%) suggests that both incompatible and compatible alleles are segregating in the strains, in accordance with their outbred origin and previous studies (Forejt & Ivanyi, 1975).

This is the first report highlighting the contribution of an X-linked gene to hybrid male dysfunction in *domesticus*/*musculus* crosses. Data on the genetic basis of incompatibilities leading to hybrid breakdown in house mice have very recently been produced using a new consomic construct in which the X chromosome of an *M. m. molossinus* strain was introduced into the genome of a predominantly *M. m. domesticus* mouse (Oka *et al*., 2004). Males of this consomic strain are sterile and show reduced testis weight and abnormal sperm head morphology. The analysis revealed that the incompatibilities were due to interactions between X-linked genes and autosomal and/or Y-linked genes. However, as the impaired fertility of these hybrids is related to defective sperm function rather than to deficiency in sperm number as is the case in the *domesticus*/*musculus* hybrids, the incompatibilities most likely involve different genes and/or interactions. Hybrid sterility between *M. spretus* and the laboratory mouse is also known to involve the X chromosome, and it is thought to be associated with impairment of the pseudo-autosomal region (Matsuda, Hirobe & Chapman, 1991). More recently, an *M. spretus* X-factor affecting testis weight in a laboratory mouse background was reported (Elliott *et al*., 2001). Interestingly, the fertility dysfunction in this case involves a gene responsible for ligand transcription factors required for the normal development of male germ cells. Other known sterility factors in the mouse are carried by the t-haplotype, a variant of chromosome 17 embedded in four chromosomal inversions, with the properties of a transmission distorter, and bearing homozygous male sterility factors. This sterility phenotype is also expressed when these t-haplotype loci are made heterozygous with *M. spretus* alleles, which is the reason why they are often referred to as hybrid sterility genes. However, they apparently coincide with the transmission distortion loci of the t-haplotypes, and act by causing sperm flagella development impairment (Schimenti, 2000, and references therein), a completely different sterility phenotype from the one we observed in our crosses. The *Hst-1* locus, identified in *domesticus*/*musculus* hybrids (Forejt & Ivanyi, 1975; Forejt, 1981), was also mapped onto chromosome 17 (Forejt & Ivanyi, 1975; Forejt, 1981; Gregorova *et al*., 1996; Trachtulec *et al*., 1997), and causes histological hybrid male sterility phenotypes very similar to those we observed here (Forejt, 1981; Yoshiki *et al*., 1993; Gregorova & Forejt, 2000). In addition, our results on backcross fertility segregation showing full arrest to complete fertility in hybrid males support the arguments presented in several studies according to which *Hst-1* and three or more independently segregating hybrid sterility loci are involved, and that epistatic interaction of all or most of these loci are required for complete sterility (Forejt, 1981; Yoshiki *et al*., 1993; Forejt, 1996). However, whether *Hst-1* is involved in our crosses remains to be assessed by further molecular analyses.

A large body of studies exploring the mechanisms underlying Haldane's rule have documented the widespread role of the X chromosome in hybrid heterogametic male sterility (see Wang, 2003, and references therein; Laurie, 1997; Tao *et al*., 2001, 2003). The results presented here show that hybrid dysfunction in *domesticus* × *musculus* hybrids also follows these two rules of speciation, namely that hybrid sterility factors involve X-chromosome incompatibilities and evolve faster in males than in females (Coyne & Orr, 1989).

Evolution of hybrid sterility and effect on genic introgression

The genetic architecture of hybrid sterility in *domesticus* × *musculus* progeny is in accordance with the evolution of postzygotic isolation involving epistatic interactions between incompatible alleles in the two subspecies, which are not deleterious on their own genetic background (Gavrilets, 1997). The existence of such incompatibilities also agrees with the expected evolution of isolating mechanisms as by-products of genetic divergence in allopatry (Mayr, 1963). These two subspecies originated from an Indo-Pakistani cradle, have been separated for 500 000 years (Boursot *et al*., 1996; Din *et al*., 1996) and have only recently come into contact (5000–1000 years ago; Auffray, Vanlerberghe & Britton-Davidian, 1990). Thus, divergence at these loci is expected to have occurred in allopatry, and to be widespread throughout the two subspecies. This is confirmed by the diversity of laboratory strains having a predominantly *M. m. domesticus* genome that were tested for incompatibility with wild *musculus* males, although all the latter were sampled in only three regions near or within the hybrid zone (Denmark, Prague and Bulgaria; Forejt & Ivanyi, 1975; Vanlerberghe *et al*., 1986;

but see Vyskočilová *et al.*, 2005, this issue). Additional sampling from other areas will establish the extent of the geographical distribution and variability of hybrid sterility genes in the two subspecies. The inbred and outbred crosses produced contrasting results: lack vs. presence of sterile females, as well as asymmetry vs. symmetry in F_1 male sterility between reciprocal crosses. This disparity suggests that incompatible alleles are currently accumulating within the subspecies, leading to different levels of hybrid dysfunction depending on the geographical origin of the mice tested (Orr, 1995; Coyne & Orr, 1998; Turelli *et al*., 2001).

Given the epistatic nature of male hybrid dysfunction involving several genes, only a low frequency of sterile individuals is expected to occur within the hybrid zone, because recombination will reduce the probability of occurrence of the incompatible allele combination (Virdee & Hewitt, 1994). That this may be the case is shown by the RTW values of the wild hybrid males examined, which were above the sterility threshold described, suggesting that none carried the hybrid sterility phenotype. However, RTW decreased in the centre of the hybrid zone where admixture of the two subspecific gene pools is expected to be the highest. These results, albeit not significant, suggest that sterile male hybrids may occur at a low frequency in this area of the hybrid zone. Theoretical studies have demonstrated that epistatic interacting incompatibilities can build up very strong barriers to neutral gene flow (Gavrilets, 1997), and that among these, reciprocal X-autosome incompatibilities are the strongest isolating mechanisms (Wang, 2003). That the X chromosome participates in hybrid breakdown in the *domesticus–musculus* hybrid zone is supported by reports of the very limited introgression of X-linked markers in several transects (Tucker *et al*., 1992; Dod *et al*., 1993; see also Božíková *et al*., 2005; Dod *et al*., 2005; Payseur & Nachman, 2005, all this issue). An intriguing point that remains to be elucidated is the absence of a role of the Y chromosome. In three different transects across the hybrid zone, the Y chromosome also showed extremely limited introgression between the subspecies (Tucker *et al*., 1992; Dod *et al*., 1993; Prager *et al*., 1997; see also Dod *et al*., 2005, this issue), and a world-wide study of variation in the house mouse failed to reveal any region of admixture of Y chromosomal types, contrary to what prevails for all other markers tested (Boissinot & Boursot, 1997; Karn *et al*., 2002). Thus, it is likely that the Y chromosome is involved in hybrid incompatibilities. The fact that all the F_1 males in the inbred crosses carrying a *musculus* Y are fertile indicates that at least the *musculus* Y of PWK is not involved in hybrid sterility. However, this does not allow us to exclude a contribution, albeit complex, of the Y in the Danish crosses to hybrid male sterility. Additional studies are required to determine the nature of the X-linked sterility factors, as well as that of the incompatibilities involving the Y chromosomes.

NOTE ADDED IN PROOF

During the processing of our manusript, Storchová and colleagues published a study demonstrating X-linked hybrid male sterility using consomic constructs in which the X chromosome of *M. m. musculus* (PWD/Ph strain) was introgressed into the genetic background of the C57BL/6J inbred strain (predominantly of *M. m. domesticus* origin). Storchová *et al.* 2004. Genetic analysis of X-linked hybrid sterility in the house mouse. *Mammalian Genome* **15:** 515–524.

ACKNOWLEDGEMENTS

Special thanks are extended to F. Bonhomme for providing the Danish mouse strains and breeding facilities, and to J.-L. Guénet from the Institut Pasteur in Paris, France, for gift of mice from the PWK and WLA inbred strains. The technical assistance of K. Manolakou, A. Orth, J. Catalan, J. Grobert and L. Meslin was greatly appreciated, as was the management of the animals by A. Ziegel. The statistical assistance of G. Ganem is gratefully acknowledged, as well as that of J.-C. Auffray. The project was supported by grants from the GREG (Groupement de Recherches et d'Etudes sur le Génome; décision No.83/94), and ACC-SV7 (Actions Concertées Coordonnées en Sciences du Vivant, No.9507044), as well as by funds from the ISEM-UMR5554. The research was part of a collaborative project partially supported by a grant from the Biotechnology Programme of the Commission of European Communities (No.BIO2CT-920476). This is publication ISEM No.2004-013.

REFERENCES

Abercrombie M. 1946. Estimation of nuclear population from microtome sections. *Anatomical Record* **94:** 239–247.

Alibert P, Fel-Clair F, Manolakou K, Britton- Davidian J, Auffray J-C. 1997. Developmental stability fitness, and trait size in laboratory hybrids between European subspecies of the house mouse. *Evolution* **51:** 1284–1295.

Alibert P, Renaud S, Dod B, Bonhomme F, Auffray J-C. 1994. Fluctuating asymmetry in the *Mus musculus* hybrid zone: a heterotic effect in disrupted co-adapted genomes. *Proceedings of the Royal Society of London B* **258:** 53–59.

Auffray J-C, Alibert P, Latieule C, Dod B. 1996. Relative warp analysis of skull shape across the hybrid zone of the house mouse (*Mus musculus*) in Denmark. *Journal of Zoology, London* **240:** 441–455.

Auffray J-C, Vanlerberghe F, Britton-Davidian J. 1990. The house mouse progression in Eurasia: a palaeontological

and archaeozoological approach. *Biological Journal of the Linnean Society* **41:** 13–25.

Boissinot S, Boursot P. 1997. Discordant phylogeographic patterns between the Y chromosome and mitochondrial DNA in the house mouse: selection on the Y chromosome? *Genetics* **146:** 1019–1034.

Boursot P, Auffray J-C, Britton-Davidian J, Bonhomme F. 1993. The evolution of house mice. *Annual Review of Ecology and Systematics* **24:** 119–152.

Boursot P, Din W, Anand R, Darviche D, Dod B, Von Deimling F, Talwar GP, Bonhomme F. 1996. Origin and radiation of the house mouse: mitochondrial DNA phylogeny. *Journal of Evolutionary Biology* **9:** 391–415.

Božíková E, Munclinger P, Teeter KC, Tucker PK, Macholán M, Piálek J. 2005. Mitochondrial DNA in the hybrid zone between *Mus musculus musculus* and *Mus musculus domesticus*: a comparison of two transects. *Biological Journal of the Linnean Society* **84:** 363–378.

Butlin R. 1998. What do hybrid zones in general, and the *Chorthippus parallelus* zone in particular, tell us about speciation. In: Howard DJ, Berlocher SH, eds. *Endless forms. Species and speciation*. Oxford: Oxford University Press, 367–378.

Castiglia R, Capanna E. 2000. Contact zone between chromosomal races of *Mus musculus domesticus*. 2. Fertility and segregation in laboratory-reared and wild mice heterozygous for multiple Robertsonian rearrangements. *Heredity* **85:** 147–156.

Chubb C. 1992. Genes regulating testis size. *Biology of Reproduction* **47:** 29–36.

Chubb C, Nolan C. 1987. Mouse hybrid sterility and testicular function. *Biology of Reproduction* **36:** 1343–1348.

Cooke HJ, Saunders PT. 2002. Mouse models of male infertility. *Nature Review Genetics* **3:** 790–801.

Coyne JA, Orr HA. 1989. Two rules of speciation. In: Otte J, Endler J, eds. *Speciation and its consequences*. Sunderland, MA: Sinauer Associates, Inc, 180–207.

Coyne JA, Orr HA. 1998. The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society of London B* **353:** 287–305.

De Kretser DM. 1990. Germ cell–Sertoli cell interactions. *Reproduction, Fertility and Development* **2:** 225–235.

Delneri D, Colson I, Grammenoudi S, Roberts IN, Louis EJ, Oliver SG. 2003. Engineering evolution to study speciation in yeasts. *Nature* **422:** 68–72.

Din W, Anand R, Boursot P, Darviche D, Dod B, Jouvin-Marche E, Orth A, Talwar GP, Cazenave P-A, Bonhomme F. 1996. Origin and radiation of the house mouse: clues from nuclear genes. *Journal of Evolutionary Biology* **9:** 519–539.

Dod B, Jermiin LS, Boursot P, Chapman VH, Nielsen JT, Bonhomme F. 1993. Counterselection on sex chromosomes in the *Mus musculus* European hybrid zone. *Journal of Evolutionary Biology* **6:** 529–546.

Dod B, Smadja C, Karn RC, Boursot P. 2005. Testing for selection on the androgen-binding protein in the Danish mouse hybrid zone. *Biological Journal of the Linnean Society* **84:** 447–459.

Elliott RW, Miller DR, Pearsall RS, Hohman C, Zhang Y, Poslinski D, Tabaczynski DA, Chapman VM. 2001. Genetic analysis of testis weight and fertility in an interspecies hybrid congenic strain for Chromosome X. *Mammalian Genome* **12:** 45–51.

Fel-Clair F. 1995. Etude de la zone d'hybridation entre *Mus musculus domesticus* et *Mus musculus musculus* au Danemark: rôle de la différenciation chromosomique (fusions centriques, organisateurs nucléolaires) et estimation de la fertilité. DPhil Thesis, Université Montpellier II.

Fel-Clair F, Lenormand T, Catalan J, Grobert J, Orth A, Boursot P, Viroux M-C, Britton-Davidian J. 1996. Genomic incompatibilities in the hybrid zone between house mice in Denmark: evidence from steep and non-coincident chromosomal clines for Robertsonian fusions. *Genetical Research* **67:** 123–134.

Forejt J. 1981. Hybrid sterility gene located in the T/t-H-2 supergene on chromosome 17. In: Reisfeld RA, Ferrone S, eds. *Current trends in histocompatibility. Immunogenetic and molecular profiles*. London: Plenum Press, 103–131.

Forejt J. 1996. Hybrid sterility in the mouse. *Trends in Genetics* **12:** 412–417.

Forejt J, Ivanyi P. 1975. Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.). *Genetical Research* **24:** 189–206.

Garagna S, Zuccotti M, Searle JB, Redi CA, Wilkinson PJ. 1989. Spermatogenesis in heterozygotes for Robertsonian chromosomal rearrangements from natural populations of the common shrew, *Sorex araneus*. *Journal of Reproduction and Fertility* **87:** 431–438.

Gavrilets S. 1997. Hybrid zones with Dobzhansky-type epistatic selection. *Evolution* **51:** 1027–1035.

Gregorova S, Forejt J. 2000. PWD/Ph and PWK/Ph inbred mouse strains of *Mus m. musculus* subspecies – a valuable resource of phenotypic variations and genomic polymorphisms. *Folia Biologica* **46:** 31–41.

Gregorova S, Mnukova-Fajdelova M, Trachtulec Z, Capkova J, Loudova M, Hoglund M, Hamvas R, Lehrach H, Vincek V, Klein J, Forejt J. 1996. Sub-milliMorgan map of the proximal part of mouse Chromosome 17 including the hybrid sterility 1 gene. *Mammalian Genome* **7:** 107–113.

Haldane JBS. 1922. Sex-ratio and unisexual sterility in hybrid animals. *Journal of Genetics* **12:** 101–109.

Hauffe HC, Searle JB. 1998. Chromosomal heterozygosity and fertility in house mice (*Mus musculus domesticus*) from Northern Italy. *Genetics* **150:** 1143–1154.

Hey J. 2003. Speciation and inversions: chimps and humans. *Bioessays* **25:** 825–828.

Hollocher H. 1998. Reproductive isolation in *Drosophila*: how close are we to untangling the genetics of speciation? *Current Opinion in Genetics and Development* **8:** 709–714.

Hollocher H, Wu CI. 1996. The genetics of reproductive isolation in the *Drosophila simulans* clade: X versus autosomal effects and male versus female effects. *Genetics* **143:** 1243–1255.

Jiggins CD, Linares M, Naisbit RE, Salazar C, Yang ZH, Mallet J. 2001. Sex-linked hybrid sterility in a butterfly. *Evolution* **55:** 1631–1638.

Karn RC, Orth A, Bonhomme F, Boursot P. 2002. The complex history of a gene proposed to participate in a sexual isolation mechanism in house mice. *Molecular Biology and Evolution* **19:** 462–471.

Kluin PM, Kramer MF, de Rooij DG. 1984. Proliferation of spermatogonia and Sertoli cells in maturing mice. *Anatomy and Embryology* **169:** 73–78.

Laurie CC. 1997. The weaker sex is heterogametic: 75 years of Haldane's rule. *Genetics* **147:** 937–951.

Le Roy I, Tordjman S, Samour-Migliore D, Degrelle H, Roubertoux PL. 2001. Genetic architecture of testis and seminal vesicle weights in mice. *Genetics* **158:** 333–340.

Leblond CP, Clermont Y. 1952. Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the 'periodic acid-fuchsin sulfurous acid' technique. *American Journal of Anatomy* **90:** 167–215.

Lenormand T, Fel-Clair F, Manolakou K, Alibert P, Britton-Davidian J. 1997. Chromosomal transmission bias in laboratory hybrids between wild strains of the two European subspecies of house mice. *Genetics* **147:** 1279–1287.

Matsuda Y, Hirobe T, Chapman VM. 1991. Genetic basis of X–Y chromosome dissociation and male sterility in interspecific hybrids. *Proceedings of the National Academy of Sciences, USA* **88:** 4850–4854.

Mayr E. 1963. *Populations, species and evolution.* Cambridge, MA: Belknap Press.

Moulia C, Aussel JP, Bonhomme F, Boursot P, Nielsen JT, Renaud F. 1991. Wormy mice in a hybrid zone: a genetic control of susceptibility to parasite infection. *Journal of Evolutionary Biology* **4:** 679–687.

Moulia C, Le Brun N, Dallas J, Orth A, Renaud F. 1993. Experimental evidence of genetic determinism in high susceptibility to intestinal pinworm infection in mice: a hybrid zone model. *Parasitology* **106:** 387–393.

Navarro A, Barton NH. 2003. Accumulating postzygotic isolation genes in parapatry: a new twist on chromosomal speciation. *Evolution* **57:** 447–459.

Noor MAF, Grams KL, Bertucci LA, Reiland J. 2001. Chromosomal inversions and the reproductive isolation of species. *Proceedings of the National Academy of Sciences, USA* **98:** 12084–12088.

Oakberg EF. 1956. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. *American Journal of Anatomy* **99:** 391–413.

Oka A, Mita A, Sakurai-Yamatani N, Yamamoto H, Takagi N, Takano-Shimizu T, Toshimori K, Moriwaki K, Shiroishi T. 2004. Hybrid breakdown caused by substitution of the X chromosome between two mouse subspecies. *Genetics* **166:** 913–924.

Orr HA. 1995. The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics* **139:** 1805–1813.

Orr HA. 1997. Haldane's rule. *Annual Review of Ecology and Systematics* **28:** 195–218.

Payseur BA, Nachman MW. 2005. The genomics of speciation: investigating the molecular correlates of X chromosome introgression across the hybrid zone between *Mus domesticus* and *Mus musculus*. *Biological Journal of the Linnean Society* **84:** 523–534.

Prager EM, Boursot P, Sage RD. 1997. New assays for Y chromosome and p53 pseudogene clines among East Holstein house mice. *Mammalian Genome* **8:** 279–281.

Presgraves DC, Orr HA. 1998. Haldane's rule in taxa lacking a hemizygous X. *Science* **282:** 952–954.

Redi CA, Capanna E. 1988. Robertsonian heterozygotes in the house mouse and the fate of their germ cells. In: Daniel A, ed. *The cytogenetics of mammalian autosomal rearrangements*. New York: Alan R. Liss, Inc, 315–359.

Rieseberg LH. 2001. Chromosomal rearrangements and speciation. *Trends in Ecology and Evolution* **16:** 351–358.

Rieseberg LH, Whitton J, Gardner K. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* **152:** 713–727.

Russell LD, Peterson RN. 1984. Determination of the elongate spermatid–Sertoli ratio in various mammals. *Journal of Reproduction and Fertility* **70:** 635–641.

Saetre GP, Borge T, Lindroos K, Haavie J, Sheldon BC, Primmer C, Syvanen AC. 2003. Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proceedings of the Royal Society of London B* **270:** 53–59.

Sage RD, Atchley WR, Capanna E. 1993. House mice as models in systematic biology. *Systematic Biology* **42:** 523–561.

Sage RD, Heyneman D, Lim K-C, Wilson AC. 1986. Wormy mice in a hybrid zone. *Nature* **324:** 60–63.

Schimenti J. 2000. Segregation distortion of mouse t haplotypes – the molecular basis emerges. *Trends in Genetics* **16:** 240–243.

Scriven PN. 1992. Robertsonian translocations introduced into an island population of house mice. *Journal of Zoology, London* **227:** 493–502.

Searle AG, Beechey CV. 1974. Sperm-count, egg-fertilization and dominant lethality after x-irradiation of mice. *Mutation Research* **22:** 63–72.

Sokal RR, Rohlf FJ. 1995. *Biometry*. New York: Freeman.

Tao Y, Hartl DL, Laurie CC. 2001. Sex-ratio segregation distortion associated with reproductive isolation in *Drosophila*. *Proceedings of the National Academy of Sciences, USA* **98:** 13183–13188.

Tao Y, Zeng ZB, Li J, Hartl DL, Laurie CC. 2003. Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. II. Mapping hybrid male sterility loci on the third chromosome. *Genetics* **164:** 1399–1418.

Trachtulec Z, Mnukova-Fajdelova M, Hamvas RM, Gregorova S, Mayer WE, Lehrach HR, Vincek V, Forejt J, Klein J. 1997. Isolation of candidate hybrid sterility 1 genes by cDNA selection in a 1.1 megabase pair region on mouse chromosome 17. *Mammalian Genome* **8:** 312–316.

Trachtulec Z, Mihola O, Vlcek C, Himmelbauer H, Pačes V, Forejt J. 2005. Positional cloning of the Hybrid sterility 1 gene: fine genetic mapping and evaluation of two candidate genes. *Biological Journal of the Linnean Society* **84:** 637–641.

Tucker PK, Sage RD, Warner J, Wilson AC, Eicher EM. 1992. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. *Evolution* **46:** 1146–1163.

Turelli M, Barton NH, Coyne JA. 2001. Theory and speciation. *Trends in Ecology and Evolution* **16:** 330–342.

Vanlerberghe F, Dod B, Boursot P, Bellis M, Bonhomme F. 1986. Absence of Y-chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. *Genetical Research* **48:** 191–197.

Via S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology and Evolution* **16:** 381–390.

Virdee SR, Hewitt GM. 1994. Clines for hybrid dysfunction in a grasshopper hybrid zone. *Evolution* **48:** 392–407.

Vyskočilová M, Trachtulec Z, Forejt J, Piálek J. 2005. Does geography matter in hybrid sterility in house mice? *Biological Journal of the Linnean Society* **84:** 663–674.

Wang RX. 2003. Differential strength of sex-biased hybrid inferiority in impeding gene flow may be a cause of Haldane's rule. *Journal of Evolutionary Biology* **16:** 353–361.

Winking H, Dulić B, Bulfield G. 1988. Robertsonian karyotype variation in the European house mouse, *Mus musculus*; survey of present knowledge and new observations. *Zeitschrift für Säugetierkunde* **53:** 148–161.

Wu CI. 2001. The genic view of the process of speciation. *Journal of Evolutionary Biology* **14:** 851–865.

Wu CI, Davis AW. 1993. Evolution of postmating reproductive isolation – the composite nature of Haldane's Rule and its genetic bases. *American Naturalist* **142:** 187–212.

Wu CI, Hollocher H. 1998. Subtle is nature. The genetics of species differentiation and speciation. In: Howard DJ, Berlocher SH, eds. *Endless forms. Species and speciation*. Oxford: Oxford University Press, 339–351.

Yoshiki A, Moriwaki K, Sakakura T, Kusakabe M. 1993. Histological studies on male sterility of hybrids between laboratory and wild mouse strains. *Development, Growth and Differentiation.* **35:** 271–281.