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Genetic analysis of testis weight and fertility in an interspecies hybrid congenic strain for Chromosome X

Rosemary W. Elliott, Darla R. Miller,* R. Scott Pearsall, Colleen Hohman, Yuke Zhang, Diane Poslinski, Debra A. Tabaczynski, Verne M. Chapman**

Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, New York State Department of Health, 666 Elm Street, Buffalo, New York 14263, USA

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Abstract. A hybrid congenic strain, C57BL/6J.SPRET-*Hprt*^{a}, carrying 17 map units of Chromosome (Chr) X from *Mus spretus* on a background of C57BL/6J, has the novel phenotype of low fertility associated with small testis weight. In histological cross-section, many of the tubules in the testes of these congenic mice are empty except for Sertoli cells, while the other tubules appear to be normal. The gene, interspecific hybrid testis weight 1 (*Ihtw1*) causing this phenotype, has been fine mapped by using the strategy of generating subcongenic strains from recombinants within the congenic region. Genetic and phenotypic analysis of the subcongenic strains has defined a critical region of 1.8 map units for *Ihtw1*. This region of the genetic map is orthologous to the region on human Chr X containing the gene for the Borjeson-Forssman-Lehman syndrome, an inherited disease in which males show microorchidism.

Introduction

Matings between mice from *M. spretus* and inbred strains, which are derived largely from *M. musculus domesticus*, produce viable fertile females and viable infertile males. This hybrid male sterility is consistent with Haldane's rule (Haldane 1922), which states: "When in the F1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous [heterogametic or XY] sex." As an example, interspecific hybrid males from C57BL/6J and *M. spretus* parents are infertile (Guénet et al. 1990). This infertility has been associated with a lack of X//Y pairing in the pseudoautosomal region (PAR) (Matsuda et al. 1991). In humans as well as in mice, failure of the X-Y chromosome pairing is often associated with meiotic arrest and subsequent degeneration of primary spermatocytes before metaphase II (Speed and Chandley 1990; Matsuda et al. 1991, 1992; Hale et al. 1993; Ashley et al. 1994).

In addition to loci on the sex chromosomes, autosomal genes on Chr 17 have been implicated in hybrid sterility. Hybrid sterility 1 (*Hst1*) causes sterility in male F_1s between the inbred strain C57BL/10, carrying *Hst1*^{s}, and PWK, an inbred strain of *M. m. musculus*. Similar F_1 hybrids between another inbred strain, C3H, carrying *Hst1*^{f}, and PWK are fertile (Forejt and Ivanyi 1974). There is a fine structure map of the region (Gregorova et al. 1996) and a YAC contig spanning the gene (Trachtulec et al. 1994). The *Hst1* gene is encoded in a 580-kb YAC, and four candidate genes in the region have been identified. Four other hybrid sterility loci on proximal Chr 17, *Hst4* (Pilder et al. 1991), *Hst5*, and *Hst6* (Pilder et al. 1993) and *Hst7* (Pilder, 1997), were found by analyzing crosses involving selected laboratory strains, *M. spretus*, and mice carrying *t*-mutations. Recent molecular studies have identified the gene involved in *Hst6* as an axonemal dynein (Samant et al. 1999; Fosella et al. 2000).

A genetic resource showing decreased fertility in hybrid males was developed by Verne Chapman. It is a congenic strain, C57BL/6J.SPRET-*Hprt*^{a}, in which a portion of Chr X from *M. spretus* was transferred to a C57BL/6J background by using a standard congenic backcross protocol, selecting for the *Hprt*^{a} allele. The chromosomal region from *M. spretus* is 17 cM long, and extends from *DXMit48* to *DXMit60*. This congenic strain is associated with two phenotypes, one involving placental weight in females and one involving testis weights in males. Hybrid placental dysplasia has already been described (Zechner et al. 1996). Placentas from C57BL/6J.SPRET-*Hprt*^{a} weigh about 0.2 g, compared with placentas in C57BL/6J, which weigh about 0.1 g. The larger placentas have an excess of spongiotrophoblast cells and glycogen cells (Hemberger et al. 1999).

In this study, we examine the phenotype found in males from C57BL/6J.SPRET-*Hprt*^{a}. The congenic males breed, but fertility is low. These hybrid congenic males have small testes in which some of the tubules, as seen in cross-section, contain developing sperm, while the other tubules contain only Sertoli cells. The locus responsible for this phenotype has been named *Ihtw1* (interspecific hybrid testis weight 1). Using the approach of making subcongenic mice, we have refined the position of *Ihtw1* to a 1.8 cM region on Chr X.

Materials and Methods

Animals and DNA. Mice were bred in the colony maintained at Roswell Park Cancer Institute (RPCI). DNA was obtained from 2 mm of tail added to 0.15 ml of 50 mM NaOH, heated to 95°C for 10 min, mixed thoroughly, and neutralized with 0.21 ml of 60 mM Tris, pH 8, 1.7 mM EDTA.

Congenic strain. Congenic strain C57BL/6J.SPRET-*Hprt*^{a} is also called AT24 (allelic transfer 24). It was constructed by mating (C57BL/6J × *M. spretus*) F_1 with C57BL/6J and selecting females that were heterozygous for *Hprt*. These females were then mated with C57BL/6J males, and their female progeny, heterozygous for *Hprt*, were used for the next generation. This process was repeated until generation N12, at which point the strain was homozygosed by mating females heterozygous for *Hprt*^{a} with sibling males hemizygous for *Hprt*^{a}. The line has been maintained by brother-sister mating and is now at generation N12F35. The ends of the congenic region were determined by using PCR of microsatellite sequences.

Subcongenic strains. These were obtained by mating the congenic strain AT24 with C57BL/6J to obtain F_1 animals. Recombination in the F_1 ani-

* *Present address:* Life Sciences Division, Oak Ridge National Laboratory, PO Box 2009, Oak Ridge, TN 37831, USA.

** *Present address:* Department of Cell Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA.

Correspondence to: R.W. Elliott; E-mail: rosemary.elliott@roswellpark.org

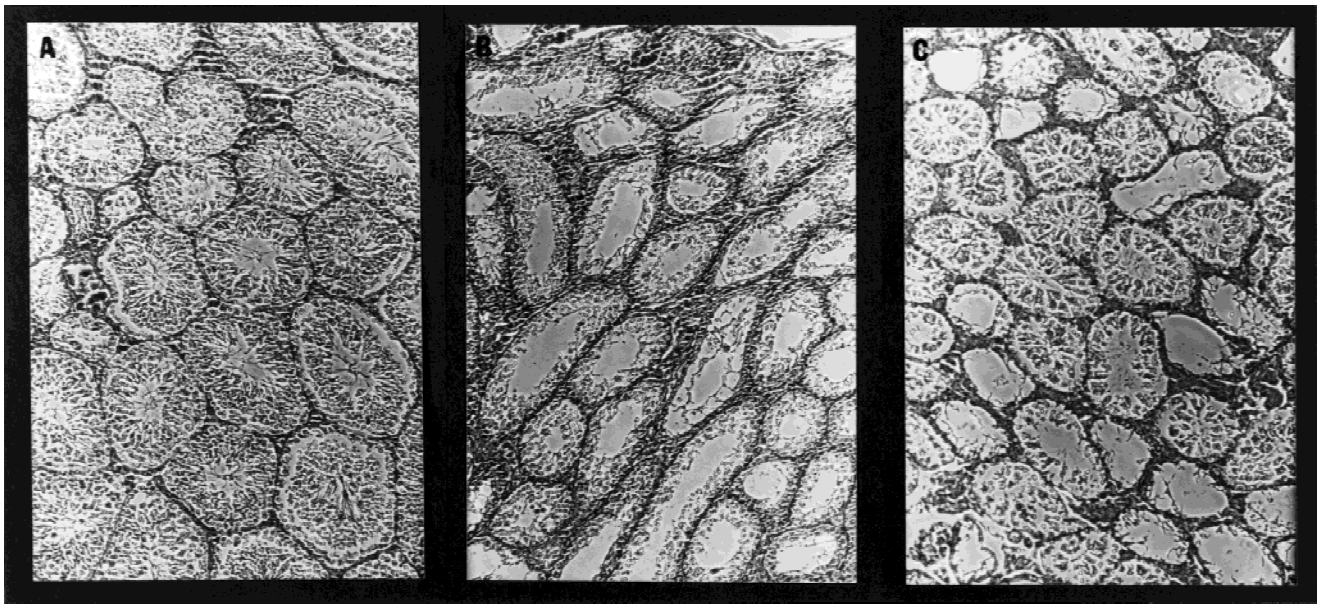

Fig. 1. Histological sections of testes. Testis sections were prepared as described in Materials and methods and stained with hematoxylin and eosin. **A,** testis from C57BL/6J mouse. **B,** testis from an infertile (C57BL/6J × *M. spretus*) F_1 hybrid mouse. **C,** testis from C57BL/6J.SPRET-*Hprt*^{a} mouse.

mals was identified by PCR typing of progeny from a backcross to C57BL/6J. Animals with crossovers in the interval were mated with C57BL/6J. To obtain homozygous females for each subcongenic strain, recombinant male progeny were mated with heterozygous females carrying the same recombination. Each congenic strain was maintained by brother-sister mating. Crosses between subcongenic strains, 101 × 212 and 394 × 374, were performed in both directions. Progeny were crossed to C57BL/6J, and recombinants were identified by PCR typing. The lines were maintained by brother-sister mating.

Testis weight. At 10 weeks of age or slightly older, male mice were sacrificed and weighed. Pairs of testes were dissected and weighed.

Histology. Testes were pierced several times with a needle, fixed in 10% formalin overnight, washed in 70% ethanol, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin and observed under standard conditions.

PCR. PCR was carried out in multiwell Falcon microtest plates in the PTC-100-96 thermal cycler (MJ Research Inc.) in a 10-μl volume containing 8–10 ng of genomic DNA, 1–2.5 pmole of each primer, and 0.2 unit of Taq polymerase. The reaction mixture also contained 0.05% bovine serum albumin, 1.5 mM $MgCl_2$, 10 mM Tris HCl, 50 mM KCl pH 8.3, and 1 μl 2% cresol red dye in 60% sucrose. The cycling parameters used were: 94°C for 3 min, 94°C for 45 s, 55°C for 40 s, 72°C for 40 s, for 36 cycles, with the last extension time 5 min. Analysis of the reaction product was performed by electrophoresis on neutral acrylamide gels in 0.5× Tris/borate buffer, pH 8.3 for 1–2 h at 300 volts. Gels were stained with ethidium bromide and photographed.

Results

Congenic strain. The congenic strain AT24 contains alleles derived from C57BL/6J, except for a region on Chr X surrounding *Hprt* in which alleles are derived from *M. spretus.* Animals from AT24 have testes weighing 0.110 ± .072 g, whereas in animals from C57BL/6J and *M. spretus*, testis weights are 0.230 ± 0.013 g and 0.190 ± 0.020 g, respectively. A comparison of breeding history from 58 mating pairs of AT24 and of C57BL/6J shows that the average number of litters per mating pair was 2.2 ± 1.4 for AT24, and 5.8 ± 1.7 for C57BL/6J.

Histology of the testis from AT24 animals illustrates a further element in the phenotype. Examination of the cross-section of a formalin-fixed testis shows that only about half of the tubules contain developing sperm. This is shown in Fig. 1, which compares cross sections of testes from C57BL/6J, (C57BL/6J × *M. spretus*) F1, and C57BL/6J.SPRET-*Hprt*^{a}. The testis from C57BL/6J (Fig. 1A) shows normal spermatogenesis, with all tubules containing developing sperm. The tubules in the testis from an infertile (C57BL/6J × *M. spretus*) F_1 hybrid (Fig. 1B) contain Sertoli cells, spermatogonia, and primary spermatocytes. However, the spermatocytes stop development between meiosis I and II because of lack of pairing in the PAR. In contrast, many of the tubules from the congenic strain (Fig. 1C) contain normally developing spermatozoa, while some of the tubules contain only Sertoli cells.

Typing the congenic strain with microsatellite markers showed that the proximal boundary lies between *DXMit50* and *DXMit48.* These are at positions 14.1 and 14.2 on the consensus map. The distal boundary lies between *DXMit60* and *DXMit61,* which are at positions 30.75 and 30.9 on the consensus map. The congenic insert is thus about 17 map units in length, or about 22% of the length of the consensus recombinational map of Chr X. The gene causing the phenotype has been called *Ihtw1,* and it maps within the congenic interval.

Subcongenic strains. To refine the map position for *Ihtw1,* subcongenic strains were made from the congenic strain C57BL/6J.SPRET-*Hprt*^{a} as described in Methods. Eleven recombinant progeny were identified and bred to make homozygous subcongenic strains (Fig. 2). Four of the recombinants were males and seven were females. A further male recombinant did not breed. Four more subcongenic strains were obtained after secondary recombination events occurred in three of the developing subcongenic strains. The limits of the genetic interval from *M. spretus* were determined by microsatellite typing and are shown in Fig. 2 for the 15 subcongenic strains.

When the subcongenic strains were homozygous, testis weights were obtained and are shown in Table 1 and at the bottom of Fig. 2. In nine of the strains, testis weights were indistinguishable from testis weights from C57BL/6J. In three of the strains, 24-212, 24-374, and 24-394, testis weights were significantly lower than testis weights for C57BL/6J. For three other strains, 24-86, 24-137, and 24-729, testis weights were intermediate be-

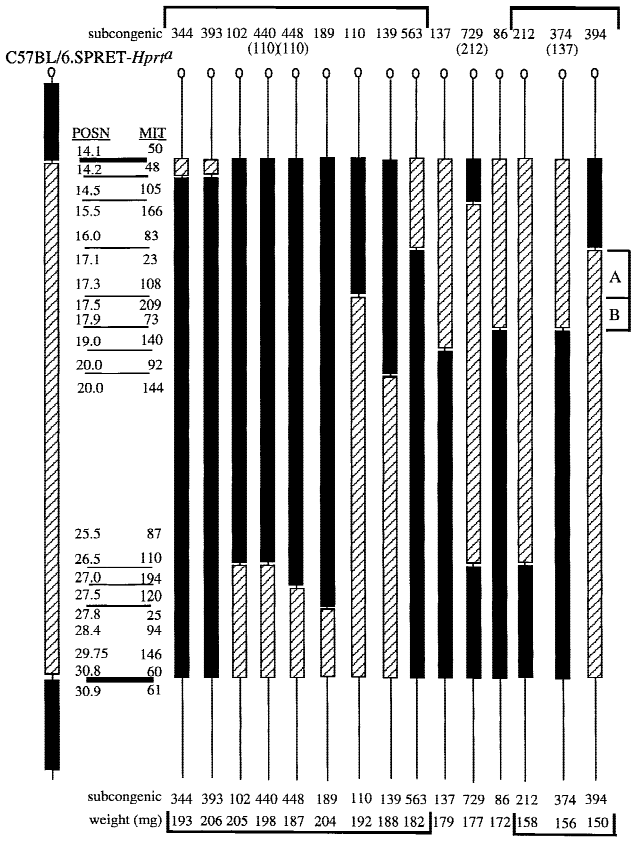

Fig. 2. Schematic maps of subcongenic strains. A diagrammatic map of the AT24 hybrid congenic strain is shown on the left. Filled black regions indicate inheritance from C57BL/6J, and hatched regions indicate inheritance from *M. spretus.* The positions (POSN) of several MIT markers are indicated, as obtained from the 2000 consensus map of Chr X (http://www.informatics.jax.org/ccr/searches/contents.cgi?&year=2000&chr=X). Similar diagrams of each of 15 subcongenic strains are presented on the right. Each breakpoint is marked with a horizontal line on the list of markers at the left. The name of each subcongenic strain is at the top and bottom of each map, and the average testis weight is shown at the bottom. Recombination in strain 24-110 yielded strains 24-440 and 24-448, while recombination in strains 24-137 and 24-212 yielded strains 24-374 and 24-729, respectively. The group of strains with testis weights indistinguishable from C57BL/6J are bracketed at the left. The three strains with testis weights significantly smaller are bracketed at the right. Strains 24-86, 24-137, and 24-729, whose weights are intermediate, are placed between these two groups. The critical region is shown by a heavily outlined box to the right, divided into regions A and B.

tween these levels. The testis weights for the subcongenic mice were compared with mice carrying alleles from C57BL/6J. Control mice were either siblings carrying alleles from C57BL/6J, derived in the breeding of the subcongenic strain (Table 1), or previously bred C57BL/6J males. Highly significant differences between the subcongenic strain and control C57BL/6J were observed when the testis weight of the subcongenic strain was below 0.16 g.

Histological analysis of sections of the testes from some of the subcongenic strains has been performed to determine whether they contain empty tubules. Sections from the strains with smaller testes, such as 24-394 and 24-374, possessed some tubules that were empty except for Sertoli cells. Sections from strains with larger testes, such as 24-102 and 24-189, were like the C57BL/6J control and did not have these empty tubules. Thus, among the subcongenic strains, the presence of empty tubules appears to correlate with testis weight.

Comparison of the intervals from *M. spretus* within these subcongenic strains allows us to narrow the genetic region likely to

Table 1. Testis weights in subcongenic strains. Testes weights were determined from males between 10 weeks and 6 months of age. For some strains, control males were obtained from progeny of the cross used to generate the respective subcongenic strain.

Subcong.	Weight (g) ± SD (No.)	Control wt (g) ± SD (No.)	*p*
110	0.192 ± 0.014 (33)	0.211 ± 0.011 (5)	
102	0.205 ± 0.015 (26)	0.187 ± 0.024 (35)	
139	0.188 ± 0.011 (15)	0.198 ± 0.021 (12)	
189	0.204 ± 0.025 (22)	0.204 ± 0.020 (25)	
344	0.193 ± 0.016 (15)	0.195 ± 0.014 (10)	
393	0.205 ± 0.009 (13)		
440	0.201 ± 0.015 (28)		
448	0.195 ± 0.195 (13)		
563	0.189 ± 0.017 (20)		
729	0.177 ± 0.014 (8)		
86	0.172 ± 0.017 (47)	0.196 ± 0.020 (11)	0.0035
137	0.179 ± 0.019 (34)	0.197 ± 0.023 (9)	0.053
212	0.158 ± 0.019 (30)	0.211 ± 0.011 (9)	0.0000005
374	0.156 ± 0.016 (16)	0.214 ± 0.020 (6)	0.00045
394	0.150 ± 0.013 (11)	0.196 ± 0.014 (6)	0.00008
AT24	0.112 ± 0.012 (57)		
Cross 110 × 212			
S1	0.125 ± 0.014 (22)		
S2	0.206 ± 0.008 (11)		
P1	0.170 ± 0.009 (7)		
P2	0.182 ± 0.021 (9)		
P3	0.171 ± 0.010 (9)		
D1	0.192 ± 0.011 (13)		
D2	0.186 ± 0.009 (6)		

p values were obtained using a two-tailed *t* test, without assuming equal variances.

contain *Ihtw1* (Fig. 2). The ends of the three subcongenic strains with the smallest testis weights suggest the length of the critical region. The loci included are from *DXMit23* at position 17.1 to *DXMit75* at position 18.9, a distance of 1.8 map units. This has been divided into regions A and B based on the breakpoint in subcongenic strain 24-110. This strain contains DNA from C57BL/6J for region A and from *M. spretus* for region B. However, it has a testis weight similar to C57BL/6J, suggesting that region A contains *Ihtw1*. The results suggest the presence of a major locus in the critical region, and a possible second locus required to give the testis weight found in AT-24. Comparison of strains 24-212 and 24-729 (derived from 24-212) suggests that replacing the interval containing *DXMit48* and *DXMit105* with DNA from C57BL/6J causes a suppression of the small testis weight phenotype observed in 24-212. Strains 24-86 and 24-374 appear to have crossed over at the same position, but have somewhat different weights. This suggests that there may be a difference in the site of recombination, but that the marker resolution is not adequate to demonstrate this.

Genetic map. To obtain a more defined genetic map of the congenic region with higher resolution, and to determine the map positions of some of the candidate genes for the variation in testis weight, we have examined three interspecies crosses between *M. spretus* and C57BL/6J. These crosses have been typed for many of the same MIT markers, but different genes have been typed on each cross. To obtain a reliable map order, it is helpful to analyze markers typed in the same cross. Cross BSBCE uses 230 male progeny that have been typed for 142 loci on Chr X in our laboratory, including both genes and MIT markers (Chapman et al. 1994; Disteche et al. 1998). The use of male progeny allows us to follow the segregation of dominant markers, such as *DXMit75*, which does not amplify DNA from *M. spretus.* This cross was typed for all the MIT markers in the proximal half of the AT24 interval that were variant between the two parents. DNA from the BSBJ and BSSJ crosses from The Jackson Laboratory (TJL) was also typed in the AT24 interval for as many markers as possible, but as their progeny included both males and females, typing of dominant markers was not performed.

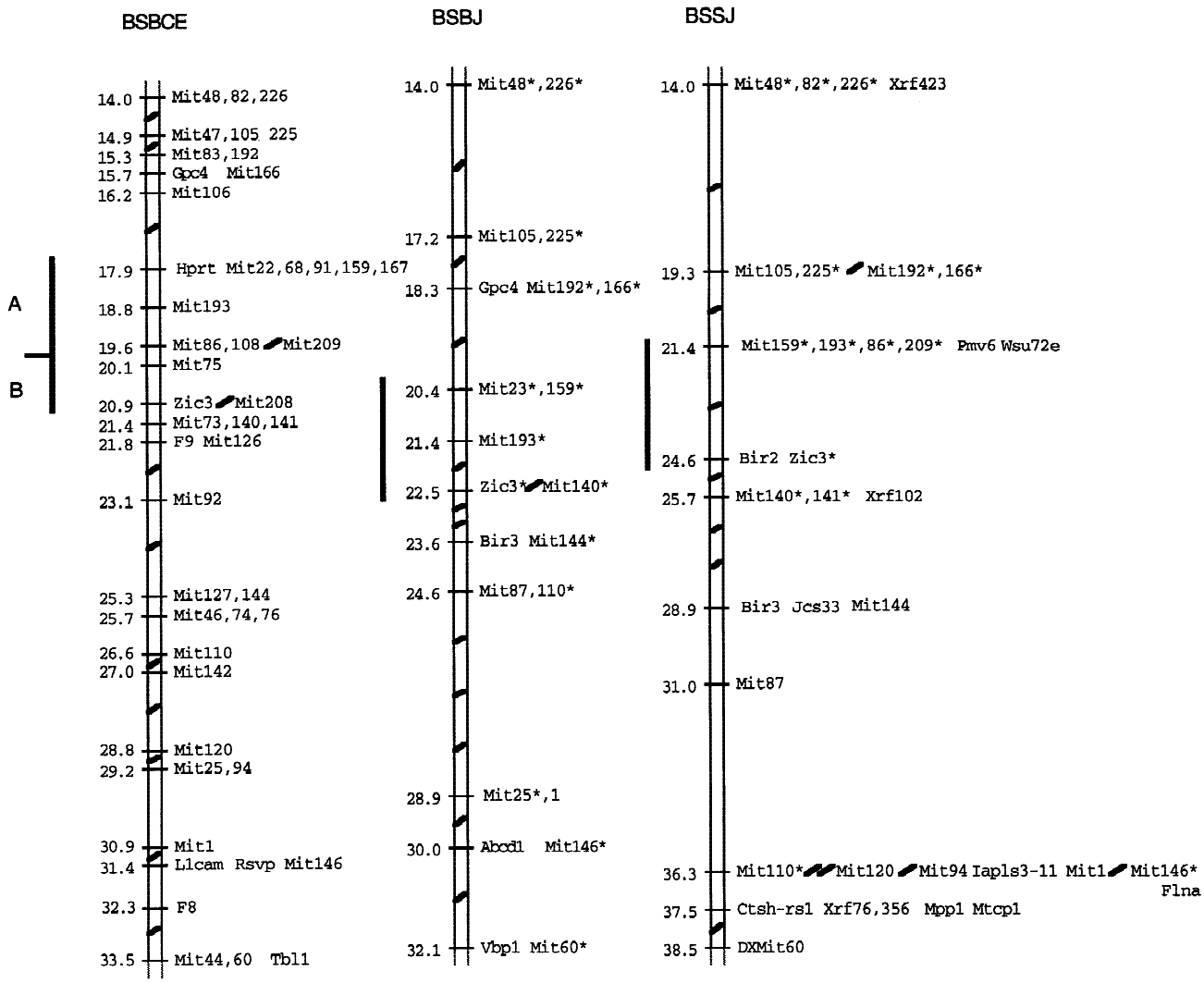

Fig. 3. Genetic maps of three interspecies backcrosses. Interspecific backcrosses between C57BL/6J and *M. spretus* were used to obtain three maps of the AT24 congenic region. Data were entered into the Map manager computer program, which generated the maps. Cross BSBCE, based on 230 male progeny, was type for 48 loci in the congenic interval. This cross was initiated by Verne Chapman, and typing was performed in his laboratory and in that of Rosemary Elliott. BSBJ and BSSJ are the public domain backcrosses from TJL and are each based on 94 progeny. They have been typed within the congenic region for 22 and 36 loci respectively. Markers typed for this project in these two crosses are identified with an asterisk. Maps are oriented with the centromeric end at the top. Within a bin, markers are listed in map order, if this is known, or in alphabetical order. The position of *DXMit48*, the most proximal marker in the congenic region, was defined for the maps as 14, the position given in the chromosome committee report. The positions of other markers was obtained from the data. The critical region is identified by using a heavy vertical line to the left of each map. The positions of the 12 recombinational events in the subcongenic strains are indicated by heavy diagonal bars placed in the chromosome stem at the appropriate interval. Where there is no crossover in the map, the position of a crossover in the subcongenics is identified by a diagonal heavy line in the correct map position within a bin. Data for the public crosses have been submitted to TJL and will be found at http://www.jax.org/resources/documents/cmdata/

Maps from these three crosses are shown in Fig. 3. The critical region is indicated to the left of each map by a heavy vertical line. Where the same markers are typed, the marker order in the three crosses is the same. This order also agrees with the order generated by the crossovers in the subcongenic strains. The positions of these crossovers are shown by short diagonal lines on the map stem and within some bins in Fig. 3. The three crosses give different length estimates of the congenic region, ranging from 18.1 map units for BSBJ to 24.5 map units for BSSJ. Comparison with the consensus map indicates that several markers show small differences in marker order between the maps in Fig. 3 and the consensus map. These have not been specifically identified. Loci where the positions are significantly different from the consensus map include genes *F9* (clotting factor 9) and *Zic3* (zinc finger protein of the cerebellum 3), both typed using PCR, and *DXMit108*, *208*, *225*, and *226*.

To further check genes whose primers did not show genetic variation, several genes were typed on the T31 panel of RH markers. These loci included *Agtr2*, *Ant2*, *Api3*, *F9*, *Vbp1*. *Agtr2* and *Ant2* are placed proximal to the congenic region, consistent with their known map positions. *Api3*, previously not regionally mapped, lies between *DXMit50* and *DXMit48*, at the proximal boundary of the congenic region. We do not know whether it is inside the region. The position of *F9* is consistent with the position we found in the BSBCE backcross, rather than that given in the consensus map. *Vbp1* is proximal to *DXMit60*, suggesting it is within the congenic region. This has been confirmed by Southern blotting experiments (Hemberger, unpublished). RH mapping data

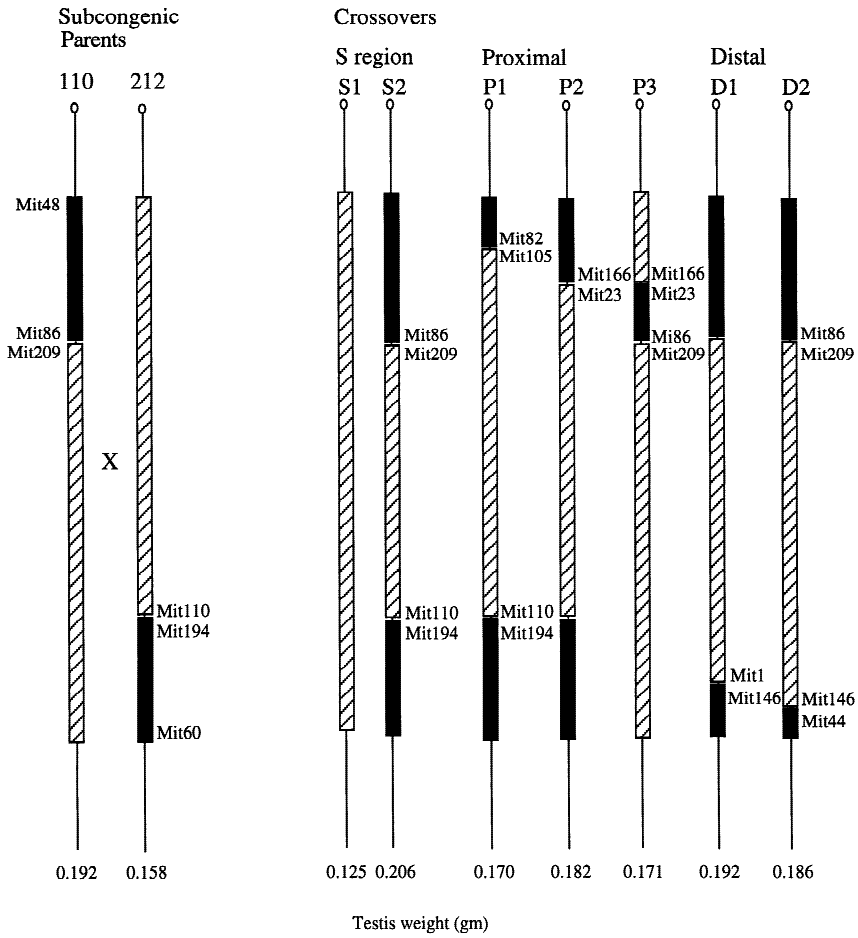

Fig. 4. Schematic maps of progeny of 24-110 × 24-212 cross. The parents of the cross are diagrammed at the left, with the same labeling conventions as in Fig. 2. The classes of recombinant progeny are shown at the right. The two progeny classes obtained by recombination within the common region from *M. spretus* are labeled "S region." Crossovers in the proximal region where DNA from 24-110 is from C57BL/6J and DNA from 24-212 is from *M. spretus* are labeled "Proximal" and the recombinant classes numbered P1, P2, or P3. Crossover positions are indicated by placing the number of the MIT markers proximal and distal to the breakpoint. Note that the reciprocal recombinants P2 and P3 had crossovers in the same genetic interval, between *DXMit106* and *DXMit23.* Crossovers in the distal interspecies region are labeled "Distal", and the classes numbered D1 and D2. Average testis weights from Table 1 are given below each diagrammed recombinant.

have been submitted to http://www.jax.org/resources/documents/cmdata/.

Crosses between subcongenic strains. None of the subcongenic strains had testis weights as low as their AT24 parent (0.11 gm). Two hypotheses were developed to explain this finding. (i) AT24 contains an autosomal region from *M. spretus* that is required for the full phenotype, and this was replaced by a region from C57BL/6J during the breeding for the subcongenic strains. (ii) Besides *Ihtw1,* there is second locus, *Ihtw2,* within the 17-cM subcongenic region that is required for the full phenotype.

To test hypothesis 1, we have done two experiments. First, we performed a genomic scan of AT24, by using 350 genetic markers distributed over all the autosomes. The markers were chosen so that if the hypothetical autosomal region were of significant length, such as 10 map units, it would have been identified. All 350 autosomal loci tested were homozygous for alleles from C57BL/6J; none was homozygous for alleles from *M. spretus* or heterozygous like the (BXS) F_1. Thus, there is no evidence for a cryptic autosomal congenic region of significant length, although we cannot eliminate the possibility of a congenic region shorter than 10 map units.

As a second experiment to determine whether an autosomal locus is involved, we have performed a cross between two subcongenic strains to reconstitute the original congenic strain. Subcongenic strains, 24-110 and 24-212 (testis weights 0.192 g and 0.158 g) were crossed, and hybrid progeny were crossed to C57BL/6J. The 186 backcross progeny were typed for relevant markers. The desired recombinant animals were identified, as well as several other recombinants whose genotypes are shown in Fig. 4. Testis weights from strains developed from these recombinants are shown in Table 1 and Fig. 4. Reciprocal crossovers (S1 and S2) within the *M. spretus* region were identified. S1 contains 17 map units of continuous genetic region from *M. spretus.* Its testis weight of 0.125 ± 0.0135 g (N = 22) is not significantly different from the 0.112 ± 0.012 g found in the original AT24 congenic strain. Reconstitution of the original phenotype when the genotype was reconstituted suggests that the absence of testis weight of 0.11 g among the subcongenic strains was not associated with the loss of an autosomal genetic region from *M. spretus.* It is more likely that hypothesis 2 is correct and a second locus within the 17-cM congenic region is required to obtain the full phenotype.

Five other recombinants among the progeny were made homozygous (see Fig. 4). They were generated by recombination between C57BL/6J and *M. spretus* DNA within the congenic region. The most interesting of these (Proximal 3, P3) is a double recombinant that has an interval of 1.0 cM from C57BL/6J within the 17-cM congenic region from *M. spretus.* This interval is almost identical to that associated with part A of the critical interval (Fig. 2). Testis weights from these males were 0.17 ± 0.011 g (N = 7). This provides confirmation that this portion of the critical region contains genes involved in the small testis phenotype, as the testis weight is significantly different (t test) from the testis weight for AT24 ($p < 0.0000002$) and from C57BL/6J ($p = 0.00013$). The position of the proximal crossover event in P3 cannot be distinguished from that in 24-394, 24-563, or recombinant P2. It is possible that this interval contains a stretch of DNA with a high probability of crossing over between C57BL/6J and *M. spretus.* Recombinants S2, D1, and D2 carry the proximal crossover from subcongenic strain 24-110, but contain varying amounts of

C57BL/6J DNA from 24-212 at the distal end of the AT24 congenic region. They all have testis weights indistinguishable from 24-110. Recombinant P1 appears to have the same genetic content and testis weight as subcongenic 24-729. Both these strains and recombinant P2 carry the critical region from *M. spretus*, but have both proximal and distal regions from C57BL/6J. Possibly one of these regions contains a suppressor of the testis weight phenotype.

Discussion

In the studies presented here, we have described the generation of a hybrid congenic strain carrying 17 map units from *M. spretus* on a C57BL/6J background. The phenotype associated with this strain includes low fertility in the male, associated with low testis weights. The major locus contributing to this phenotype has been named *Ihtw1* and maps in the 17 map unit hybrid interval on Chr X. The low testis weight is correlated with the presence of unfilled tubules in the testis. The empty tubules lack spermatogonia and developing sperm, but retain Sertoli cells. Sertoli cells are required to support the maintenance of spermatogonia and their development into mature sperm. A simple model to account for the small testis size is that the product of a gene in the congenic interval is important in the development of sperm, possibly by determining the number of spermatogonia, or the life span of spermatogonial stem cells. This protein interacts with the product of a second locus, elsewhere in the genome. When these two genes come from the same parent, normal numbers of spermatogonia are produced and sperm development is normal. When one gene comes from *M. spretus* and the other from C57BL/6J, the protein interaction is less efficient, and sperm development is limited. This could be owing to a decrease in the numbers of spermatogonia or possibly to difficulties in the interaction between Sertoli cells and spermatogonia in the developing testis.

We have established 15 subcongenic strains whose genotypes and phenotypes have allowed us to refine the mapping of *Ihtw1* to a chromosomal interval of 1.8 map units. Three previously typed mapping panels have been used to search for genes in the critical interval, and this analysis has been extended by typing genes in the RH panel. Only two of the genes mapped in the crosses in Fig. 3 are within the critical region. These are *Hprt*, in part A of the critical region, mapped in the BSBCE cross, and *Zic3*, at the distal end of part B of the critical region, mapped in all three backcrosses. One EST, *DXWsu72e*, was mapped in the critical region in the BSSJ backcross (Ko et al. 1998). Two other genes, *Fgf13* and *Tnfsf5* have been placed in the critical region in the consensus map. These were mapped in the Frederick cross (Smallwood et al. 1996) and are at the proximal end of a fairly long interval. Their position is not yet well defined relative to markers in the crosses in Fig. 3. The gene *Gpc4* has been mapped in the BSBCE and BSBJ backcrosses proximal to the critical region. The critical interval appears to be a gene-poor region, in contrast to the distal part of the congenic region, which appears to be gene-dense. This observation is supported by sequence data for human Chr X from (http://www.ncbi.nlm.nih.gov/genome/seq/HsHome.html). Sequenced contigs in the region orthologous to the interval contain very few genes and ESTs.

There is evidence of variation in positions of recombination events between the three crosses. A comparison of the maps in Fig. 3 suggests that, in the proximal region of the congenic interval, relatively more crossovers have occurred in the two crosses from TJL than in the BSBCE cross. The interval between *DXMit48* and the *Hprt* cluster is 3.9 map units for the BSBCE cross and 6.4 and 7.4 map units in the two crosses from TJL. The critical region is 1.8 map units on the consensus map. It is about 3 map units in the BSBCE map, while it is less than 2 map units in the BSBJ and a little over 3 map units in the BSSJ maps. The distal end of the map of the BSSJ cross appears to be compressed relative to the two BSB crosses. The distance between *DXMit110* and *DXMit60* is 2.2 map units in BSSJ, while in BSBCE and BSBJ it is 6.9 and 7.5 map units respectively. Just proximal to this, however, in BSSJ, there is a 5.3 map unit interval between *DXMit87* and *DXMit110*, while these markers are in the same bin in the BSBJ cross. The chromosomal region near *DXMit187* has the highest transmission distortion ratio in cross BSSJ, with only 13 heterozygotes and 81 homozygotes. It is interesting to speculate that recombination frequency in this region may be affected by one or more of the Dcsx (distortion controlling) loci mapped in this vicinity (Montagutelli et al. 1996).

Recombination that occurred in the generation of the subcongenic strains involved crossing mice with the *M. spretus*-derived interval to C57BL/6J, and then backcrossing to C57BL/6J. These crosses were more like the BSB crosses, and the distribution of crossovers, as illustrated by the diagonal bars in Fig. 3, is more similar to the distribution of crossovers in these maps. Most of these crossovers occurred only once in the generation of the various subcongenics. However, crossovers occurred twice in the most proximal interval. In the third interval, crossovers occurred three times, while in the eighth interval, between *DXMit101* and *DXMit194*, independent crossovers occurred four times. Considering all the data from the three crosses and the subcongenic construction, it appears highly likely that crossover events between C57BL/6J and *M. spretus* are not randomly distributed, but occur in preferred regions.

The 17 map unit interval within the congenic region is orthologous with a continuous region of human Chr X containing Xq26, Xq27, and Xq28. The proximal end of the congenic region is in the middle of this orthologous region between mouse and human. The distal end of the congenic region coincides with the homology break point between Xq28 and Xp22.3. The interval orthologous to Xp22.3 contains the single locus *Tbl1* (Disteche et al. 1998). Within the human map orthologous to the congenic interval there are two loci known to affect testis weight in man, FMR1 (fragile X mental retardation 1) and BFLS (Borjeson-Forssman-Lehman syndrome). The FMR1 locus is associated with macroorchidism in man, while in BFLS affected males show mental retardation, often have epilepsy, and have genitalia of reduced size (Borjeson et al. 1962). Several studies have placed the locus for BFLS syndrome in the region between HPRT and F8 (Mathews et al. 1989; Turner et al. 1989). The mouse orthologs of these genes are both in the 17 map unit congenic interval. A favored candidate for mutation leading to BFLS is SOX3. This is a transcription factor that maps on human Xq27.1 (Mumm et al. 1997) and is at position 24.5 in the mouse (Collignon et al. 1996), within the congenic interval. It is transcribed mainly in the brain, but is also transcribed in the testis. Its map position in the mouse is distal to the critical region we have defined, but it is possible that SOX3 interacts with the protein product of *Ihtw1*. However, a recent study has shown that, in one family with BFLS, FGF13 is involved in a duplication (Gecz et al. 1999). This makes FGF13 an excellent candidate for BFLS. It is possible that *Ihtw1* is a mouse model for part of the BFLS phenotype. Supporting this is the genetic finding that BFLS maps in an interval orthologous to the interval containing *Ihtw1*. Further, both BFLS patients and AT24 mice have small gonads. Also, according to the consensus map, *Fgf13* may be within the critical region.

Part of the critical region that we have identified has been deleted in ES cells by irradiation followed by selection against *Hprt* (Kushi et al. 1998; Thomas et al. 1998). Some of the ES cells carrying small deletions were used to generate chimeric mice, and germline chimeras were obtained from three deletions. Some males hemizygous for the deletion were runted or died at birth (Kushi et al. 1998), suggesting the presence of a gene important for development in this deleted region.

The finding of low fertility in interspecific congenic hybrids is of great interest because it suggests that the speciation process may involve difficulties in protein-protein interactions in the develop-

ment of male germ cells. A receptor-ligand pair or a pair of interacting transcription factors would be good candidates for this interaction. The identification of the gene function of *Ihtw1* and the genes with which it interacts will be important in unraveling the molecular events in sperm development.

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References

Ashley T, Ried T, Ward DC (1994) Detection of nondisjunction and recombination in meiotic and postmeiotic cells from XY^{Sxr} [XY, Tp(Y)1Ct] mice using multicolor fluorescence *in situ* hybridization. Proc Natl Acad Sci USA 91, 524–528

Borjeson M, Forssman H, Lehman O (1962) An X-linked, recessively inherited syndrome characterized by grave mental deficiency, epilepsy and endocrine disorder. Acta Med Scand 171, 13–21

Chapman VM, Keitz BT, Bishop DF (1994) Genetic linkage of the erythroid-specific delta-aminolevulinate synthase gene (Alas2) to the distal region of the mouse X chromosome. Mann Genome 5, 741

Collignon J, Sockanathan S, Hacker A, Cohen-Tannoudji M, Norris D et al. (1996) A comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2. Development 122, 509–520

Disteche CM, Dinulos M, Bassi MT, Elliott RW, Rugarli EI (1998) Mapping of the murine *Tbl1* gene reveals a new rearrangement between mouse and human X chromosomes. Mamm Genome 9, 1062–1064

Forejt J, Ivanyi P (1974) Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.). Genet Res 24, 189–206

Fosella J, Samant SA, Silver LM, King SM, Vaughan KT et al. (2000) An axonemal dynein at the hybrid sterility 6 locus: implications for t haplotype-specific male sterility and the evolution of species barriers. Mamm Genome 11, 8–15

Gecz J, Baker E, Donnelly A, Ming JE, McDonnald-McGinn DM et al. (1999) Fibroblast growth factor homologous factor 2 (FHF2): gene structure, expression and mapping to the Borjeson-Forssman-Lehmann syndrome region in Xq26 delineated by a duplication breakpoint in a BFLS-like patient. Hum Genet 104:56–63

Gregorova S, Mnukovafajdelova M, Trachtulec Z, Capkova J, Loudova M et al. (1996) Sub-milliMorgan map of the proximal part of mouse Chromosome 17 including the hybrid sterility 1 gene. Mamm Genome 7, 107–113

Guénet JL, Nagamine C, Simon-Chazottes D, Montagutelli X, Bonhomme F (1990) Hst-3: an X-linked hybrid sterility gene. Genet Res 56, 163–165

Haldane JBS (1922) Sex ratio and unisexual sterility in hybrid animals. J Genet 12, 101–109

Hale DW, Washburn LL, Eicher EM (1993) Meiotic abnormalities in hybrid mice of the C57BL/6J × *Mus spretus* cross suggest a cytogenetic basis for Haldane's rule of hybrid sterility. Cytogenet Cell Genet 63, 221–234

Hemberger MC, Pearsall RS, Zechner U, Orth A, Otto S et al. (1999) Genetic dissection of X-linked interspecific hybrid placental dysplasia in congenic mouse strains. Genetics 153, 383–390

Ko MSH, Threat TA, Wang X, Horton JH, Cui Y et al. (1998) Genome-wide mapping of unselected transcripts from extraembryonic tissue of 7.5-day mouse embryos reveals enrichment in the t-complex and under-representation on the X chromosome. Hum Mol Genet 7, 1967–1978

Kushi K, Edamura K, Noguchi M, Akiyama K, Nisi Y, Sasai H (1998) Generation of mutant mice with large chromosomal deletion by use of irradiated ES cells—analysis of large deletion around Hprt locus of ES cells. Mamm Genome 9, 269–273

Mathews KD, Ardinger HH, Nishimura DY, Buetow KH, Murray JC, Bartley JA (1989) Linkage localization of Borjeson-Forssman-Lehmann syndrome. Am J Med Genet 34, 470–474

Matsuda Y, Hirobe T, Chapman VM (1991) Genetic basis of X-Y chromosome dissociation and male sterility in interspecific hybrids. Proc Natl Acad Sci USA 88, 4850–4854

Matsuda Y, Moens PB, Chapman VM (1992) Deficiency of X and Y chromosomal pairing at meiotic prophase in spermatocytes of sterile interspecific hybrids between laboratory mice (*Mus domesticus*) and *Mus spretus.* Chromosoma 101, 483–492

Montagutelli X, Turner R, Nadeau JH (1996) Epistatic control of non-Mendelian inheritance in mouse interspecific crosses. Genetics 143, 1739–1752

Mumm S, Zucchi I, Pilia G (1997) SOX3 gene maps near DXS984 in Xq27.1, within candidate regions for several X-linked disorders. Am J Med Genet 72, 376–378

Pilder SH (1997) Identification and linkage mapping of Hst7, a new *M. spretus/M. m. domesticus* chromosome 17 hybrid sterility locus. Mamm Genome 8, 290–291

Pilder SH, Hammer MF, Silver LM (1991) A novel mouse chromosome 17 hybrid sterility locus: implications for the origins of t haplotypes. Genetics 129, 237–246

Pilder SH, Olds-Clarke P, Phillips DM, Silver LM (1993) Hybrid sterility-6: a mouse t complex locus controlling sperm flagellar assembly and movement. Dev Biol 159, 631–642

Samant SA, Fossella J, Silver LE, Pilder S (1999). Mapping and cloning recombinant breakpoints demarcating the hybrid sterility 6-specific sperm tail assembly defect. Mamm Genome 10, 88–94

Smallwood PM, Munoz-Sanjuan I, Tong P, Macke JP, Hendry SH et al. (1996) Fibroblast growth factor (FGF) homologous factors: new members of the FGF family implicated in nervous system development. Proc Natl Acad Sci USA 84, 9850–9857

Speed RM, Chandley AC (1990) Prophase of meiosis in human spermatocytes analyzed by EM microspreading in infertile men and their controls and comparisons with human oocytes. Hum Genet 8, 547–554

Thomas JW, LaMantia C, Magnuson T (1998) X-ray-induced mutations in mouse embryonic stem cells. Proc Natl Acad Sci USA 93, 1114–1119

Trachtulec Z, Vincek V, Hamvas RM, Forejt J, Lehrach H et al. (1994) Physical map of mouse chromosome 17 in the region relevant for positional cloning of the hybrid sterility 1 gene. Genomics 23, 132–137

Turner G, Gedeon A, Mulley J, Sutherland G, Rae J et al. (1989) Borjeson-Forssman-Lehmann syndrome: clinical manifestations and gene localization to Xq26-27. Am J Med Genet 34, 463–469

Zechner U, Reule M, Orth A, Bonhomme F, Strack B et al. (1996) An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrid. Nat Genet 12, 398–403