Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1629–1633, March 1987
Genetics

Detailed ordering of markers localizing to the Xq26-Xqter region of the human X chromosome by the use of an interspecific *Mus spretus* mouse cross

(restriction fragment length polymorphisms/cross-reacting human probes/human–mouse homologies/pedigree analysis/recombination analysis)

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Communicated by Mary Lyon, November 17, 1986 (received for review October 7, 1986)

ABSTRACT **Five probes localizing to the Xq26-Xqter region of the human X chromosome have been genetically mapped on the mouse X chromosome using an interspecific cross involving *Mus spretus* to a contiguous region lying proximally to the Tabby (*Ta*) locus. Pedigree and recombinational analysis establish the marker order as being *Hprt-FIX*-c11-*G6PD*-St14-1. The size of this contiguous region is such that the X-linked muscular dystrophy (*mdx*) mouse mutation probably maps within this segment. This in turn suggests that it is highly improbable that the mouse *mdx* locus represents a model for Duchenne muscular dystrophy (*DMD*). It is, however, compatible with the idea that this mutation may correspond in man to Emery Dreifuss muscular dystrophy. The high frequency of restriction fragment length polymorphisms found in this interspecific system for all the human cross-reacting probes examined up until now, using only a limited number of restriction enzymes, suggests that the *Mus spretus* mapping system may be of great potential value for establishing the linkage relationships existing in man when conserved chromosomal regions are concerned and human/mouse cross-reacting probes are available or can be obtained.**

In view of the large number of hereditary diseases showing X chromosomal-linked inheritance, considerable effort has been expanded both in generating flanking molecular markers for use in prenatal diagnosis and in establishing an accurate map of the human X chromosome (1). More than 200 such X chromosome loci have been regionally localized using a combination of *in situ* hybridization, deletion mapping analysis, and, to a lesser extent, family studies (1).

Progress has been made in the establishment of an accurate detailed genetic map of the whole human X chromosome by the identification of molecular probes that are capable of either detecting polymorphisms in a series of overlapping family panels, thus allowing the analysis of a series of nested three-point crosses (2), or being used for multipoint analysis (3). However, such efforts are hindered by the relative paucity of probes identifying restriction fragment length polymorphisms (RFLPs) occurring with high frequencies in human populations that are capable of being used in such extensive family studies. One approach to improving this situation involves the isolation of probes recognizing hypervariable mini-satellite sequences (4) so that RFLPs can be identified that are present at a high frequency in human families.

A second possible approach involves the use of other experimental systems such as the mouse in which genetic analysis can be facilitated. For such an approach to be valid and to possess advantages over mapping in man itself, the following four basic conditions must be met: (*i*) Cross-reacting molecular probes and/or conserved phenotypic traits must exist. (*ii*) Linkage relationships must be maintained between the two species for the chromosome in question, at least over small genetic distances. (*iii*) At least some basic information as to the chromosomal breakpoints and inversions having occurred during the evolution of the two species must be available. (*iv*) RFLPs must be easier to generate and/or recombinational analysis facilitated compared to man. The human and mouse X chromosomes appear to fulfill at least three of these four basic conditions. The overall high degree of conservation of sequences on the human and murine X chromosomes has been well documented (5–7) (P.A., L.A., D.A. unpublished results). Moreover, three breakpoints involving two inversions have been postulated to be sufficient to explain the structural rearrangement having occurred between the X chromosomes of the two species (5). While this may represent a minimal estimate, the number of breakpoints and inversions can hardly be very much larger than this (P.A., L.A., D.A., unpublished results).

The use of interspecies *Mus spretus* crosses has allowed the fourth condition to be met since the high levels of allelism innate to such crosses for both X-linked (7, 8) and autosomal sequences (9, 10) not only allows analysis of loci showing little variation in intra-species crosses but also confers the advantage of allowing simultaneous recombinational and pedigree analysis of the results (7).

Here we report on the use of this system to order five loci localizing to the human Xq26-Xqter interval on the mouse X chromosome. The probes either cross-reacted sufficiently with the mouse to be analyzed directly or to allow homologous mouse probes to be generated that could in turn be analyzed. The finding that the locus order found in the mouse may be compatible with that thought to exist in man over a distance corresponding to approximately one-fifth of the total mouse X chromosome suggests that this approach may be useful in obtaining genetic information relevant to other regions of the human X chromosome and in defining further the regions of the mouse X chromosome containing loci homologous to important human disease loci.

MATERIALS AND METHODS

Mouse Strains. The mouse strains used have been described (8).

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Abbreviation: RFLP, restriction fragment length polymorphism.
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Cross Construction and Progeny Analysis. To localize X chromosome-specific molecular sequences, the backcross represented in Fig. 1 was established. In addition to the loci subsequently defined by polymorphic X chromosome probes, this cross segregated for the coat marker *Ta*, the neurological mutation jimpy *jp*, and allelic forms of the *Hprt* gene derived from either the *Mus musculus domesticus* or *Mus spretus* species. Observations of backcross progeny for segregation of the *Ta* and *jp* markers was carried out 15–30 days after birth. Progeny were then dissected, and organs such as the brain, liver, lungs, and kidney were flash frozen and stored in liquid nitrogen. DNA preparation from these organs, restriction enzyme digests, and electrophoresis were carried out as described (8). Gel transfers to Hybond-N membrane (Amersham, England) were made according to the manufacturers instructions, and the DNAs cross-linked to the membrane.

Probes. The probes used, their human gene mapping nomenclature, their origins, and the provisional locus symbols for the mouse are described in Table 1. The G28-B probe is a 2.7-kilobase (kb) repetitive free sequence subcloned from a 14.4-kb sequence, G28 (6). This was isolated by screening a mouse genomic DNA library cloned in λEMBL3 by P. Baldacci and M. Cochet, with the human probe pGD3 mapping on the 3′ side of the human *G6PD* gene and within the associated *GdX* locus (6).

Probe Preparation and Blot Hybridization. Probes were either nick-translated (pHPT5, G28B, or c11) or random-primed (FIX and St14-1) using [α-^{32}P]dCTP and [α-^{32}P]dTTP (800 Ci/mmol; 1 Ci = 37 GBq) using standard procedures. Hybridization and washing conditions were as in Table 2.

RESULTS

Meiotic recombinant progeny from the interspecies cross detailed in Fig. 1 segregated not only for the X-linked coat color marker *Ta* and *Hprt* but also for RFLPs corresponding to a further 20 typed loci. The position on the X chromosome of four of the key markers is shown in Fig. 1*B*. Probes 100, 52, and 45 have been described (8).

Direct localization of human probes on a mouse DNA test panel requires that stringency conditions be defined that allow observation of cross-hybridization to mouse DNA. Of

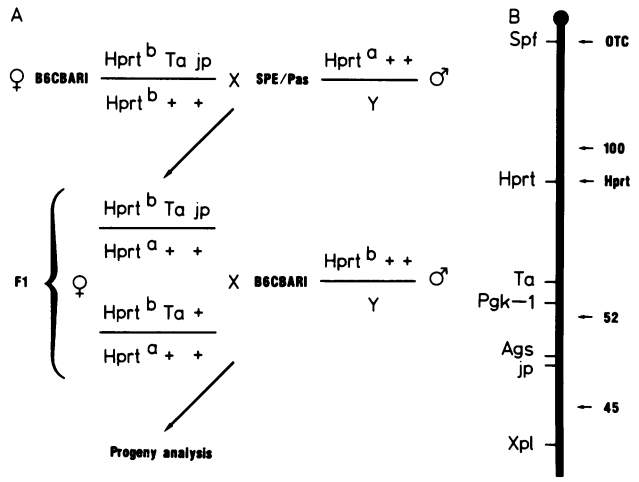

FIG. 1. (*A*) Configuration of the interspecies backcross used for probe localization. F_1 females *Ta jp*/++ and F_1 females *Ta*+/++ were used, their genotypes being characterized subsequently by progeny testing. (*B*) Position of four of the key markers used in this study on the mouse X chromosome. These were *OTC* (synonymous with the Sparse-fur loci *Spf*), 100, 52, and 45 (corresponding to loci *DXPas5*, *DXPas2*, and *DXPas1*).

the three human probes used directly, c11 and St14-1 were found to give fairly strong hybridization signals under the reduced stringency conditions defined here.

The FIX probe on the other hand gave consistently very weak signals, suggesting a low degree of homology between the human and mouse sequences. While the c11 and St14-1 probes detect RFLPs on *Taq* I-digested DNAs between the B6CBARI and *Mus spretus* mouse strains, out of the seven enzymes tested only *Msp* I produced RFLPs that were detected between the two strains with the FIX probe. Typical examples of Southern blot profiles of backcross progeny for the c11, St14-1, and FIX probes, are shown in Fig. 2.

The RFLPs indicated have been verified as being X linked through the following procedures and observations: (*i*) a panel of somatic cell hybrids either having or lacking the murine X chromosome was examined; (*ii*) the observation that male progeny never had more than one of the restriction fragments whereas 50% of female progeny had a doublet indicating the simultaneous presence of the two allelic forms; (*iii*) comparison of the hybridization patterns of *Mus spretus*-derived RFLPs with that found in DNAs from female B6CBARI mice and male (B6CBARI × SPE/Pas)F_1 mice. Any bands present in female SPE/Pas DNA but absent from the male F1 DNA must be X chromosome linked (Fig. 2*D*).

The hybridization pattern detected by probes c11, G28B, and pHPT5 on the segregating backcross progeny are relatively simple, and in the case of c11 all the bands detected by this probe can be unambiguously assigned to the X chromosome (data not shown). St14-1 on the other hand gives very complicated hybridization patterns (Fig. 2*C*) under the reduced stringency conditions used here, and autosomally encoded sequences are revealed in addition to the X-linked sequences (Fig. 2*D*).

While primarily pedigree analysis has been used to determine the location of the *FIX* gene, all the other sequences have been localized using both pedigree and recombination frequency analysis. An example of the use of pedigree analysis allowing correlation of the cross-over events that have occurred on the X chromosome of each animal with the haplotype detected by a particular probe is shown in Fig. 3 for *Hprt*, c11, and *G6PD* (*GdX*). If backcross animals 58 and 22 show that all three sequences must be distal to the centromere-*OTC*-100 marker segment, animals 21 and 29 equally show that these sequences must be proximal to *Ta*. Within this defined segment animal 31 places c11 distal to *Hprt* whereas animal 27 places it proximal to *G6PD*. Similar analysis carried out using FIX and St14-1 results in the establishment of the following gene order: centromere-*OTC*-100 (*Hprt*-FIX-c11-*G6PD*-St14-1)-*Ta*. Twenty of the entire panel of 37 informative progeny showing recombination between loci 100 and *Ta* that were used in the pedigree analysis to map the five loci described in this paper are shown in Fig. 4. A further 123 backcross progeny failed to show recombination within this region and were noninformative. The recombination frequency data obtained are presented in Table 3. They are in agreement with those obtained from the pedigree analysis and indicates equally that the linked loci flanked by the *Hprt* and St14-1 sequences must lie distal to marker 100 (*DXPas 5*) and proximal to the *Ta* locus.

DISCUSSION

The framework for using interspecific mice crosses between inbred strains of the *Mus musculus domesticus* and *Mus spretus* species for assigning genes to particular linkage groups (9) and for the high resolution mapping of particular murine chromosomes (6–8, 10) has been established. Our studies on the X chromosome have, moreover, established at least for this chromosome, that not only is the gene order obtained the same, but equally the recombination frequencies

Table 1. List of X-chromosomal probes used

Human probe	Human gene workshop symbol	RFLP (if any) in man	Holder	Ref.	Regional assignment	Name of homologous mouse probe isolated (if any)	Provisional mouse locus symbol
pHPT5	*HPRT*	*Bam*HI *Taq* I	Caskey	11, 12	Xq26-q27.3	—	*Hprt*
FIX	*FIX*	*Msp* I *Xmn* I *Dde* I	Mandel	13	Xq26-q27.3	—	*Cf-9*
c69, c11	*DXS 144*	*Taq* I	Mandel	14	Xq26-q28	—	*DXPas 6*
St14-1	*DXS 52*	*Taq* I *Msp* I	Mandel	14	Xq28		*DXPas 8*
pGD3	*G6PD (GdX)*		Luzzatto	15, 16	Xq28	G28A G28B G28C G28D	*Gdx-(G6PD)*

The anonymous human probes c11 and St14-1 have been listed in the text under their probe nomenclature rather than under their human workshop symbols. The mouse locus designations have also been avoided in the interests of clarity but are presented here for references.

observed in such interspecific crosses are similar to those observed in intraspecific crosses (7) (P.A., L.A, unpublished data).

This validation of the *Mus spretus* mapping system suggested that we could take advantage of the intrinsically great genetic diversity existing between these two parental mouse strains to generate RFLPs for loci, including coding sequences already regionally localized in man, to map such loci in the mouse by recombinational analysis. Such an analysis obviously depends on the isolation of probe sequences having a high enough degree of species cross-reactivity either to be used directly or to be used for the isolation of homologous murine probes by preliminary screening of cloned murine libraries. Both approaches have been used in the present work. While studies with species cross-reacting probes have and will provide further information as to the chromosomal rearrangements that have occurred between man and mouse (P.A., L.A., unpublished data), we hoped that the murine system could also be used to define the order of genes within maintained chromosomal regions that are common to both the mouse and human X chromosome. For this to occur X chromosome linkage relationships must be maintained in mice and humans, at least over small genetic distances.

The human Xq26-Xqter region was an obvious candidate model system for such a comparative human: *Mus spretus* mapping analysis for the following reasons: (*i*) the *Hprt* gene localizing in man to Xq26 had already been localized in the mouse (12); (*ii*) the region in man contains many important genes including *Hprt, G6PD, FVIII (HEMA), FIX (HEMB)* as well as disease loci for adrenoleukodystrophy, Emery Dreifuss muscular dystrophy, and the fragile-X site linked to a mental retardation syndrome (*FRAXA*) (1); and (*iii*) at least five species cross-reacting sequences were known to exist for the region.

Table 2. Probes

Probe	Temp.	Hybridization and wash conditions
FIX	50°C	5% (wt/vol) dextran sulfate; wash: 2× SSC/0.05% $NaDodSO_4$ at 50°C
St14-1	60°C	5% (wt/vol) dextran sulfate; wash: 2× SSC/0.05% $NaDodSO_4$ at 50°C
c11	42°C	40% (vol/vol) formamide; wash: 1× SSC/0.05% $NaDodSO_4$ at 60°C
pHPT5	42°C	40% (vol/vol) formamide/5% (wt/vol) dextran sulfate; wash: 0.2× SSC/0.05% $NaDodSO_4$ at 60°C
G28B	42°C	50% (vol/vol) formamide; wash: 0.4× SSC/0.05% $NaDodSO_4$ at 60°C

Both prehybridization and hybridizations (30–50 hr) were in 5× SSC/5× Denhardt's solution/deproteinized salmon sperm DNA at 20 μg/ml. (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0.) (1× Denhardt's = 0.02% polyvinylpyrrolidine, 0.02% bovine serum albumin, 0.02% Ficoll.) The FIX, pHPT5, and G28B probes were routinely used as inserts; the c11 and St14-1 probes were used in the form of plasmids.

Our results suggest that the human Xq26-Xqter region is conserved as a contiguous region in the mouse and has allowed the gene order *Hprt-FIX*-c11-*G6PD*-St14 to be established. This order differs from the *Hprt*-c11-*FIX*-St14 order suggested by Mandel and coworkers (3, 13) for the Xq26-Xqter region in man by the relative orientations of the c11 and *FIX* loci. While this discrepancy could be due to the presence of a small internal inversion within the contiguous region of the mouse X chromosome corresponding to Xq26-Xq28 in man, other alternative explanations cannot be ruled out. It has for instance been established in man that recombination functions for the *FIX-FRAXA* linkage localizing to Xq26-Xq27.3 show apparent heterogeneity in different families; no statistically significant heterogeneity being found for the adjacent *FRAXA*-St14 (Xq27.3-Xq28) interval (3). Such observations of inherited differences in recombination frequencies for the human *FIX-FRAXA* interval could well imply the occurrence in the human population of variant chromosomal arrangements, such as inversions within this region, and this could account for the discrepancies between the murine gene order reported here and the human data reported by Mandel and coworkers (13). It is interesting to note in this respect that S. Adolphe, M. Djalali, P. Steinbach, and H. Hameister (personal communication) have observed the presence of fragile X sites in the mouse mapping cytologically to the XC-XD region of the X chromosome.

The recombination data we have obtained in the mouse differ from that observed in man in two respects. First, closely linked markers such as *Hprt* and *FIX* have been reported to show little or no linkage to loci in the *G6PD*/St14 cluster even in normal families (2, 3, 14). In the mouse on the other hand, the Hprt and G28B probes show clear linkage to each other. Second, in man, no intervening locus at an intermediate distance between the two Xq26-Xqter and Xq28-Xqter clusters has been described, while in the mouse the c11-defined locus clearly lies midway between the two clusters. Within each cluster itself on the other hand the recombination frequencies observed parallel those observed in man (3, 13).

One mouse mutation of particular interest that has been localized several centimorgans distal to *Hprt*, within the *Hprt–Ta* interval, is X-linked muscular dystrophy (*mdx*) (17–19). Mice carrying this mutation have symptoms that have been variously interpreted as suggesting that this

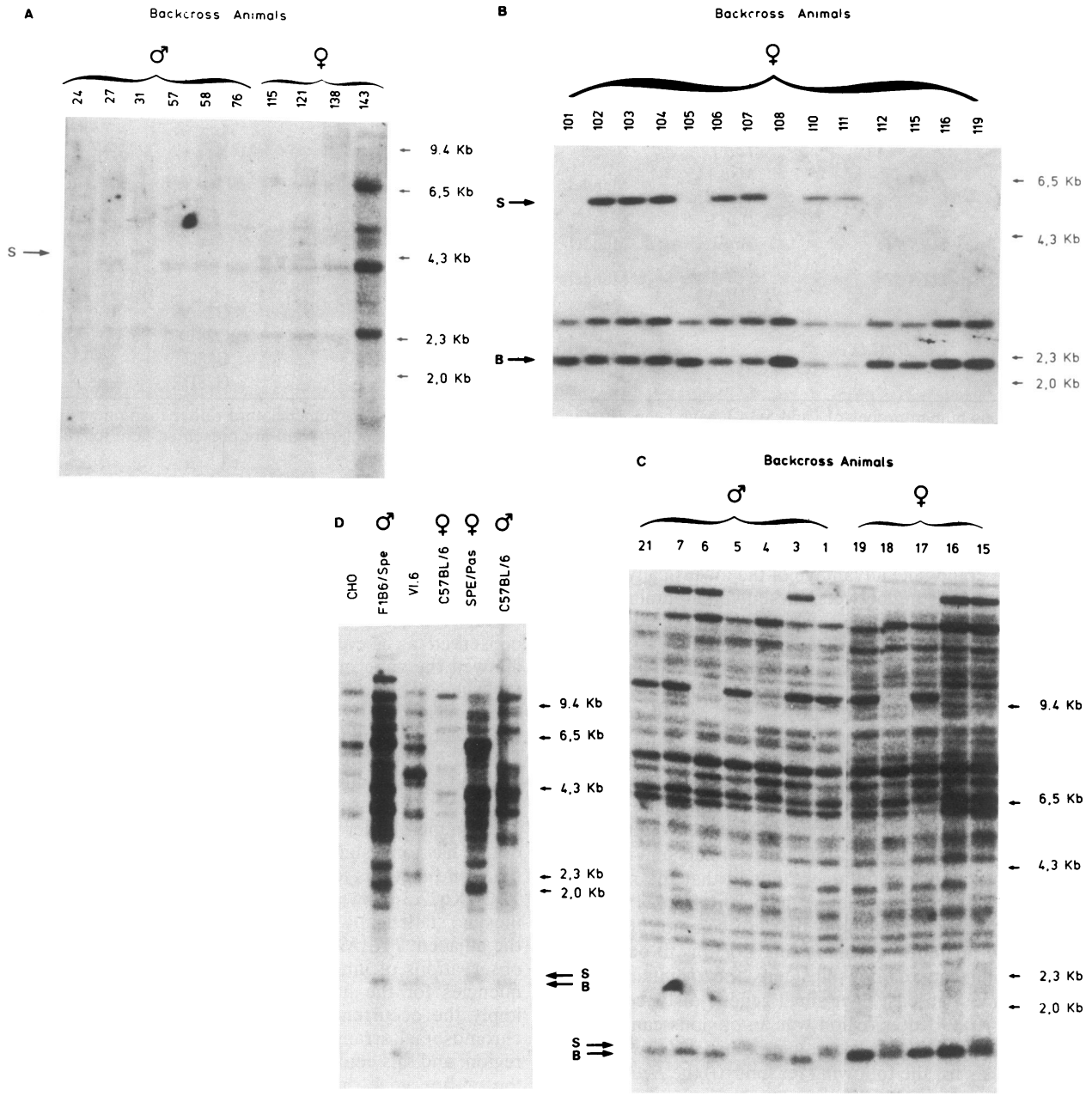

FIG. 2. Hybridization patterns on Southern blots of segregating progeny from the cross described in Fig. 1 with the FIX probe (*A*), the c11 probe(*B*), the St14-1 probe (*C*), and the St14-1 probe (*D*) on a blot designed to allow identification of X-linked sequences. In *A*, *B*, and *C*, the numbers of the backcross animals refer to the identification codes of individual backcross animals. The symbol "S" refers to X-linked *Mus spretus*-derived RFLPs, and "B" refers to X-linked B6CBARI derived RFLPs. The S- and B-derived X-linked polymorphism used for establishing the mapping data are indicated by an arrow together with the corrresponding allelic notation. In *D*, CHO refers to the control parent DNA from a Chinese hamster ovary cell line CH3g and VI-6 to a Chinese hamster–mouse somatic cell hybrid containing mouse chromosomes X and 16. The lower band arrowed, absent from the CHO track but present in the F_1 B6/Spe male, VI-6, and C57BL/6 female and male tracks but absent from the SPE/Pas female track is the X-linked B-derived allelic form. The band running slightly above in the SPE/Pas lane, absent from all the other lanes, corresponds to the X-linked S-derived allelic form.

mutation might be analogous to the Duchenne muscular dystrophy mutation occurring in man (17).

Our finding that the entire region between *Hprt* and the ST14 defined locus in the mouse extends over some 18 centimorgans may well imply that this mutation lies within this span. Since the recombination values observed here in our *Mus spretus* cross for *Hprt* and *Ta* are slightly high when compared to published data, direct comparison with the published *Hprt–mdx* data obtained in a different cross system could, however, lead to incorrect inferences being drawn.

Comparison of the ratio % recombination *Hprt–mdx*/% recombination *Hprt–Ta* obtained from published data (18, 19) with that obtained in our experiments for the ratio % recombination *Hprt*–St14/% recombination *Hprt–Ta* (both the recombination frequencies measured in our experiments being calculated on an identical panel of animals) should however suffer less from this shortcoming. Using data extrapolated from Chapman *et al.* (18) on the linkage relationships of *mdx* with *Hprt* and *Pgk*, we obtain values of (0.31 vs. 0.61) for these calculations indicating that *mdx* may lie within the chromosomal span delimited by *Hprt* and St14. Use of data obtained for *mdx* in relation to *Hq* and *Mo* by Bulfield and Isaacson (19) leads to values of (0.67 vs. 0.61). These data support the contention that *mdx* probably lies within the chromosomal span corresponding to the human Xq26-Xq28/Xqter region and, therefore, suggests that *mdx*

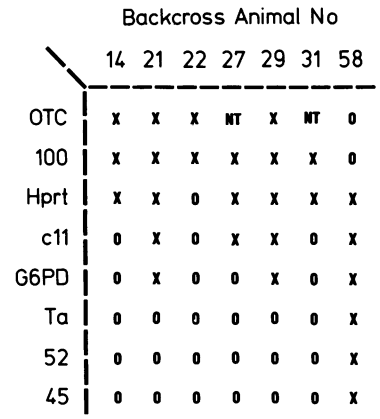

FIG. 3. Use of pedigree analysis of individual progeny from the *Mus spretus* backcross to localize the *Hprt*-c11-*GdX (G6PD)* loci between locus 100 *(DXPas 5)* and the *Ta* coat color gene. The haplotypes illustrated for female progeny correspond to that of the X chromosome of maternal origin. X, B6CBARI-derived allelic form of the locus; O, SPE/Pas-derived allelic form of the locus. The numbers identify the different backcross progeny animals.

cannot correspond to the human *DMD* locus unless an interstitial translocation has occurred. This must for the moment appear unlikely, and we would suggest, therefore, that the *mdx* mutation corresponds to Emery Dreifuss dystrophy, which has been reported to be linked to color blindness (20) and would therefore be expected in the mouse to localize within the *Hprt*–St14 span close to, or at the position of *mdx*. As we have not had the *mdx* mutation segregating within the cross described here, completely definitive conclusions as to the nature of the *mdx* locus must await such crosses as well as studies bearing on the localization of species cross-reacting sequences isolated from the *DMD* region in man.

While major strides have been made in establishing a genetic map of the human X chromosome using a combination of physical mapping methods and multifactor and pairwise two-factor crosses allied to the use of sophisticated computational programs (2), it appears likely from our analysis that the *Mus spretus* mapping system may have a useful complementary role to play in establishing high resolution linkage maps of particular regions of this chromosome. While all extrapolations from the murine to the human system must be carefully controlled, the high degree of polymorphism generated using few restriction enzymes (8) and the

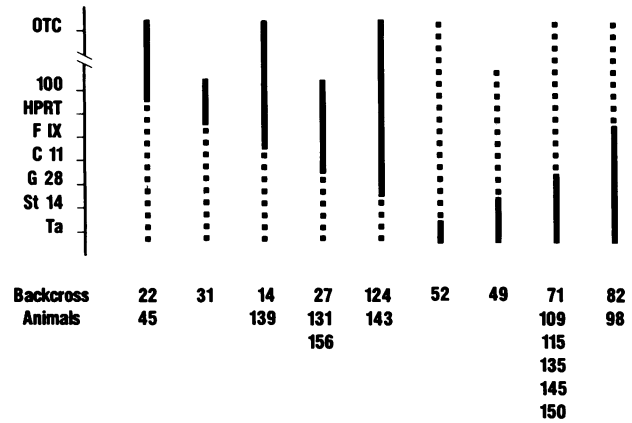

FIG. 4. Summary representation of chromosomes derived from 20 animals showing meiotic recombination in the chromosomal segment extending from the proximal marker 100 to the distal *Ta* marker. The solid lines correspond to the loci derived from B6CBARI and the broken lines to *Mus spretus*-derived loci.

Table 3. Two point linkage data

Locus	*Hprt*	*FIX*	*c11*	*G28(G6PD)*
Hprt		(3/98) 3%		
c11	(7/107) 6.5%	(3/95) 3%		
G28(G6PD)	(21/186) 11.2%	—	(9/105) 8.5%	
St14	(19/105) 18.0%	—	(9/68) 13.2%	(3/99) 3%
Ta	(37/143) 25.8%	—	—	—

Recombination frequencies for the various recombination intervals defined by the *Hprt, FIX, c11, G28(G6PD), St14*, and *Ta* loci. %, % recombinants per total number of animals analyzed for the interval in question. The number in parentheses is the number of recombinant animals per total number of animals analyzed for the interval.

capacity to carry out pedigree analysis using over 20 defined and genetically mapped markers on panels of over 200 informative recombinant backcross animals for each and every locus showing RFLPs confers specific advantages on the *Mus spretus* mapping system, which should prove of great use in establishing the structure of the mouse and human X chromosomes.

1. Goodfellow, P. N., Davies, K. E. & Ropers, H. H. (1985) *Cytogenet. Cell Genet.* **40,** 296–352.
2. Drayna, D. & White, R. (1985) *Science* **230,** 753–758.
3. Camerino, G., Oberle, I., Wrogemann, K., Arveiler, B., Hanauer, A., Raimondi, E. & Mandel, J. L. (1986) *Nucleic Acids Res.*, in press.
4. Wong, Z., Wilson, V., Jeffreys, A. J. & Thein, S. L. (1986) *Nucleic Acids. Res.* **14,** 4605–4616.
5. Buckle, V. J., Edwards, J. H., Evans, E. P., Jonasson, J. A., Lyon, M. F., Peters, J., Searle, A. G. & Wedd, N. S. (1985) *Cytogenet. Cell Genet.* **40,** 594.
6. Amar, L. C., Mattei, M. G., Arnaud, D., Cambrou, J. & Avner, P. (1986) *Somatic Mol. Cell Genet.*, in press.
7. Dautigny, A., Mattei, M. G., Morello, D., Alliel, P. M., Pham Dinh, D., Amar, L., Arnaud, D., Simon, D., Mattei, J. F., Guénet, J. L., Jollès, P. & Avner, P. (1986) *Nature (London)* **321,** 867–869.
8. Amar, L. C., Arnaud, D., Cambrou, J., Guénet, J. L. & Avner, P. R. (1985) *EMBO J.* **4,** 3695–3700.
9. Robert, B., Barton, P., Minty, A., Daubas, P., Weyder, A., Bonhomme, F., Catalan, J., Chazottes, D., Guénet, J. L. & Buckingham, M. (1985) *Nature (London)* **314,** 181–183.
10. Bucan, M., Yang-Feng, T., Colberg-Poley, A., Wolgemuth, D. J., Guénet, J. L., Francke, U. & Lehrach, H. (1986) *EMBO J.* **5,** 2899–2905.
11. Brennand, J., Chinault, C. A., Konecki, D. S., Melton, D. W. & Caskey, T. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1950–1954.
12. Konecki, D. S., Brennand, J., Fuscoe, J. C., Caskey, T. C. & Chinault, A. C. (1982) *Nucleic Acids Res.* **10,** 6763–6775.
13. Camerino, G., Mattei, M. G., Mattei, J. F., Jaye, M. & Mandel, J. L. (1983) *Nature (London)* **306,** 701–704.
14. Mandel, J. L., Arveiler, B., Camerino, G., Hanauer, A., Heilig, R., Koenig, M. & Oberlé, I. (1986) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
15. Toniolo, D., d'Urso, M., Martini, G., Persico, M., Tufano, V., Battistuzzi, G. & Luzzato, L. (1984) *EMBO J.* **3,** 1987–1995.
16. Martini, G., Toniolo, D., Vulliamy, T., Luzzato, L., Dono, R., Viglietto, G., Paonessa, G., d'Urso, M. & Persico, M. G. (1986) *EMBO J.* **5,** 1849–1855.
17. Bulfield, G., Siller, W. G., Wight, P. A. L. & Moore, K. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1189–1192.
18. Chapman, V. M., Murawski, M., Miller, D. & Swiatek, D. (1985) *Mouse News Lett.* **72,** 120.
19. Bulfield, G. & Isaacson, J. H. (1985) *Mouse News Lett.* **72,** 97.
20. Boswankel, E., Walker, A., Hodgson, S., Benham, F., Bobrow, M., Davies, K., Dubowitz, V. & Grenata, C. (1985) *Cytogenet. Cell Genet.* **40,** 586.