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Minisatellite linkage maps in the mouse by cross-hybridization with human probes containing tandem repeats

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ABSTRACT **Tests of 29 human variable number of tandem repeat probes in inbred mouse lines showed that 80% (23/29) cross-hybridize, and 48% (14/29) produce multiple band, minisatellite polymorphisms (fingerprint patterns). Minisatellite-type polymorphisms detected by 11 probes were characterized in eight different strains; on average, 240 polymorphic differences were detected between pairs of strains. Reproducible fingerprint patterns permit the study of the segregation of the minisatellite polymorphisms in experimentally designed crosses. As an example, we constructed primary minisatellite genetic linkage maps containing 346 polymorphic bands, distributed in 101 groups of closely linked systems, from genotypes on a recombinant inbred panel (C57BL/6J × DBA/2J); 38 of the groups were assigned by linkage to 15 autosomal chromosomes. The minisatellite genetic maps of C57BL/6J and DBA/2J can be applied in other linkage studies involving these strains.**

Studies of genetic disease have been greatly aided by recent efforts to characterize and map DNA polymorphism (reviewed in ref. 1). In principle, chromosome assignments can now be obtained for the genes responsible for Mendelian diseases in humans, if sufficient families are available for linkage studies. Identification of the genes implicated in multifactorial traits, or those responsible for rare Mendelian disease for which multiple-case families are difficult to obtain, may be greatly aided by linkage investigations in animal models. Experimental crosses from inbred lines in the mouse often provide suitable models to identify candidate loci or chromosome regions for investigation in humans.

Although the density of mapped loci in the mouse approaches that in humans, linkage studies in inbred lines are often hampered by a paucity of informative markers. Many restriction fragment length polymorphisms have been defined within inbred mouse strains, but these are often diallelic systems that are not polymorphic between all strains (2, 3). Linkage studies with these systems are often laborious, as they require the use of many different restriction enzymes. Jeffreys *et al.* (4, 5) and others (6) have shown that minisatellite polymorphisms, based on differences in the number of tandem repeats of short DNA sequences, overcome some of these limitations. Genomic libraries of human DNA have been screened with oligomeric sequences derived from some of these tandemly repeated elements, or minisatellite fragments isolated from gels have been cloned, to identify probes that detect unique, highly polymorphic, or variable number of tandem repeats (VNTR) loci in humans (7–9). Single locus human VNTRs have been shown to cross-hybridize and to reveal complex minisatellite polymorphisms (genetic fingerprints) in many other species (5, 6).

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Minisatellite polymorphisms have been applied for linkage studies in domestic animals, for which large pedigrees of simple structure can be collected (6, 10). This method allows rapid screening of a significant portion of the genome. Recently, Georges *et al.* (10) have obtained linkage between a minisatellite band detected by a human VNTR probe and a gene responsible for muscular hypertrophy in cattle. Jeffreys *et al.* (5) studied minisatellite polymorphisms defined by human probes 33.6 and 33.5 in several different inbred mouse strains and examined the segregation of bands in a C57BL/6J × DBA/2J recombinant inbred (RI) panel. These two probes detected at least 13 distinct loci, of which 8 could be assigned to specific chromosomes by linkage in the RI panel. These results suggest that minisatellite polymorphisms are widely dispersed in the mouse genome and that they may be a source of large numbers of polymorphic markers for linkage studies with inbred mouse strains.

In this paper, we make a systematic investigation of polymorphisms detected by 11 different minisatellite sequences, including 10 probes corresponding to human VNTR loci, in eight different inbred mouse strains. We report mapping studies in the C57BL/6J × DBA/2J RI panel.

MATERIALS AND METHODS

DNA on the inbred mouse lines DBA/2J, C3H/He, 129/Sv, C57BL/6J, BALB/c, SJL/J, NOD/Lt, and SPE/Pas (*Mus spretus*) was obtained from animals raised in the Pasteur Institute. Initial tests for cross-hybridization were undertaken with C57BL/6J and SPE/Pas. DNA from the C57BL/6J (male) × DBA/2J (female) RI panel (B × D) consisting of 26 lines was purchased from The Jackson Laboratory. A public data base of 142 independent markers was used for linkage studies in the B × D panel (11).

Twenty-nine VNTR probes were studied, including the following, which detect fingerprint patterns in the mouse: pYNZ2 (D1S57) (12), pYNH24 (D2S44) (13), pEFD134.7 (D3S45) (14), pAW101 (D14S1) (15), pEWRB2.3 (unpublished), pYNH37.3 (D17S28) (16), pYNZ22 (D17S5) (17), and pRMU3 (D17S24) (18). The retinoblastoma probe p68RS2.0 (RB1) (19) was a gift from T. Dryja, and the insulin probe pINS310 (20) was a gift from G. Bell. Probe pSP.2.5.EI, containing a 2.5-kilobase fragment of mouse DNA homologous to part of the *Drosophila per* gene (6, 21), was obtained from M. Young. Five probes (pYNZ2, pYNZ22, pYNH24, p68RS2.0, and pINS310) contain a repetition that includes the consensus sequence GNNNNTGGG (where N = unspecified nucleotide) (7, 9, 19, 20), and a sixth (pYNH37.3) is also likely to possess this consensus repeat (7). The remaining probes contain other core sequences, or unknown sequences.

Preparation of probe DNA, digestion, and techniques for Southern blots followed standard methods (22), except di-

Abbreviations: VNTR, variable number of tandem repeats; RI, recombinant inbred; lod score, logarithm of odds score.

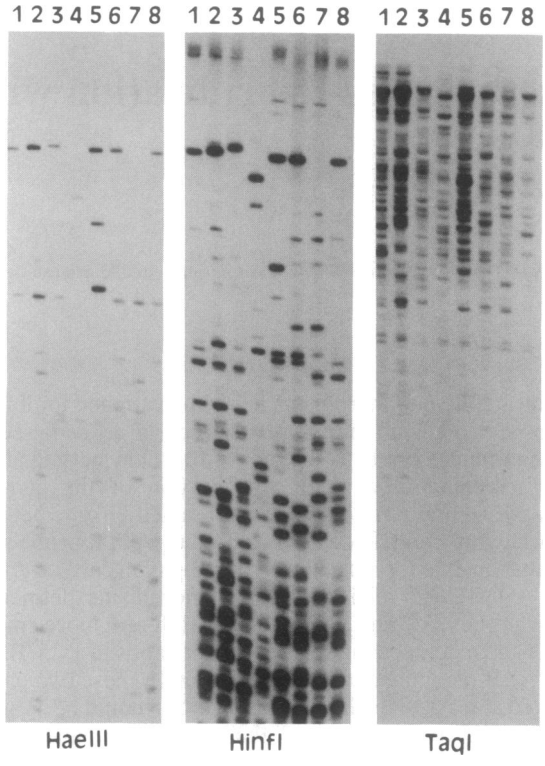

FIG. 1. Hybridization of a human VNTR probe (pYNZ2) to DNA from eight strains of mice digested by *Hae* III, *Hin*fI, and *Taq* I. The number above each lane indicates the strain: 1, DBA/2J; 2, BALB/c; 3, 129/Sv; 4, SPE/Pas; 5, SJL/J; 6, NOD/Lt; 7, C57BL/6J; 8, C3H/He.

gested DNAs were transferred onto charged nylon membrane (Gelman or Pall) under alkaline conditions (23). For detection of hybridization of human probes to mouse DNA, hybridization was in 6× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), 2× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 2 mM sodium phosphate, 0–100 μg of salmon sperm DNA per ml, 1% SDS, and 40% formamide at 37°C or 42°C, with a final wash in 2× SSC/0.1% SDS at 60°C. Hybridization conditions for fingerprint conditions were as described by Georges *et al.* (6), with a final wash in 0.5–2× SSC/0.1% SDS at 55–65°C. Four restriction enzymes (*Taq* I, *Msp* I, *Pst* I, and *Eco*RI) were used for identification of cross-hybridizing probes, and three were used to investigate minisatellite polymorphisms (*Hae* III, *Hin*fI, and *Taq* I).

Autoradiograms were interpreted independently by two observers, and polymorphic bands were labeled by a sequential number corresponding to their positions of migration. Bands are identified by a label of the form *x-y-z-p*, where *x* is the identification number of the probe (1 = pSP.2.5.EI, 2 = pYNZ2, 3 = pEFD134.7, 4 = pYNH24, 5 = pEWRB2.3, 6 = pAW101, 7 = p68RS2.0, 8 = pINS310, 9 = pYNH37.3, 10 = pYNZ22, 11 = pRMU3), *y* is the identification number of the enzyme (1 = *Taq* I, 2 = *Hae* III, 3 = *Hin*fI), *z* is the sequential number of the band for a particular probe/enzyme combination, and *p* is the parental origin of the band—i.e., *p* identifies that parental line in which the band was present. In the B × D panel, P1 = C57BL/6J and P2 = DBA/2J.

Polymorphic bands and other genetic markers from a public data base were examined for compatibility with Mendelian segregation in the B × D panel by a χ^2 test for 50% transmission. Pairwise linkage relationships were evaluated by direct counts of recombinant and nonrecombinant lines, and logarithm of odds scores (lod scores) and recombination estimates were calculated by standard methods (24, 25). A critical level corresponding to $P < 0.01$ according to table 1 of Silver and Buckler (26) was used to judge the significance of linkage. All recombinants were verified for bands within the same group by reexamination of the original autoradiograms.

RESULTS

Cross-Hybridization of Human VNTR Probes. Fourteen (48%) of the 29 probes tested exhibited multiple band patterns in the mouse under the cross-hybridization conditions designed to favor interspecific cross-hybridization. Nine (31%) additional probes detected some cross-hybridization to the mouse in the form of either single band pattern or multiple faint band patterns, compatible with the recognition of a single locus, or multiple more distantly related loci, that could yield some information for linkage studies (i.e., at least one interpretable band) in high-resolution conditions.

Minisatellite Polymorphisms. Ten VNTR probes that had exhibited multiple band patterns in the cross-hybridization experiments described above were chosen as candidate sequences to test for the detection of minisatellite, or fingerprint, polymorphisms. The mouse probe pSP.2.5.EI was also tested because of previous findings that it detects multiple bands in mice (21) and cattle (6).

The probes were hybridized to test membranes containing DNA from eight inbred strains of mice, digested as described above. An example of the pattern obtained is shown in Fig. 1. As shown in Table 1, the number of polymorphic differences detected between strains ranged from 115 to 453 (mean, 239.6), and the mean number of bands present in one strain and absent in another is 119.8 bands (range, 49–237). SPE/Pas exhibits an average of more than twice as many polymorphisms than as seen between other laboratory strains in the pairwise comparisons. Otherwise, the degree of observed polymorphism does not appear to be strongly correlated to the degree of known filiation between the strains, as given in ref. 27.

Table 1. Polymorphic differences between eight mouse strains studied with 11 probes

	+ strain							
− strain	DBA/2J	C3H/He	129/Sv	C57BL/6	BALB/c	SJL/J	NOD/Lt	SPE/Pas
DBA/2J	—	49	112	84	131	104	107	193
C3H/He	66	—	88	67	98	78	70	154
129/Sv	97	65	—	82	125	109	99	201
C57BL/6	101	65	119	—	141	114	111	197
BALB/c	79	50	98	73	—	89	89	192
SJL/J	99	56	125	91	133	—	97	199
NOD/Lt	96	51	105	79	123	91	—	199
SPE/Pas	209	144	237	184	269	211	233	—

The following probes were used: pSP.2.5.EI, pYNZ2, pEFD134.7, pYNH24, pEWRB2-3, pAW101, p68RS32.0, pINS310, pYNH37.3, pYNZ22, pRMU3. Entries are the total number of bands present in the strain indicated on the horizontal line and absent in the strain indicated on the vertical line.

Segregation and Linkage Studies. Characterization of the 11 probes in the 26 B × D RI lines (examples shown in Fig. 2) revealed several new mutations, in which a band present in one or more RI lines was not found in DNA from the parental lines. Bands seen in one of the parental strains, but in none of the RI lines, were also considered as possible new mutations. The latter patterns could also have arisen due to chance loss of the band or to selection against genetic factors in the region of the band when the RI lines were established. After removal of possible new mutations, 346 polymorphic bands could be interpreted for segregation and linkage studies. This is greater than the sum of the number of polymorphic differences between C57BL/6J and DBA/2J from Table 1 because DNA of higher molecular weight was used in the segregation experiments, and this allowed detection of larger fragments that had not previously been seen.

The data were examined for segregation distortion that could be due to undetected new mutations or the presence of comigrating bands. The χ^2 test for deviations from the 50% segregation ratio exceeded 3.8 ($P < 0.05$) for 35 (10%) of the bands. Markers in the public data base showed a similar pattern, with a significant χ^2 statistic for 15 (11%) of the loci. Therefore, the inheritance of the minisatellite bands appears to be similar to that of other loci examined in the RI panel.

Pairwise recombination estimates and lod scores were calculated for the 346 polymorphic bands detected by the 11 minisatellite sequences in the B × D RI panel. After bands that exhibited complete linkage (no recombinants in 26 RI lines) were grouped, we obtained 166 systems, each representing a single locus, or a cluster of closely linked loci. These include 98 (59%) systems that are represented by a single band (or singleton). We also allowed for recombination between tightly linked bands by assigning systems to the same linkage group if they contained bands that were linked with probability ≥0.99. This led to the formation of 59 groups containing ≥2 bands, and 42 singletons (a total of 101 linkage groups). Linkage groups containing multiple systems were often formed by bands detected by a single probe (40%), and bands from both parental lines were present in 46 (46%) of the linkage groups.

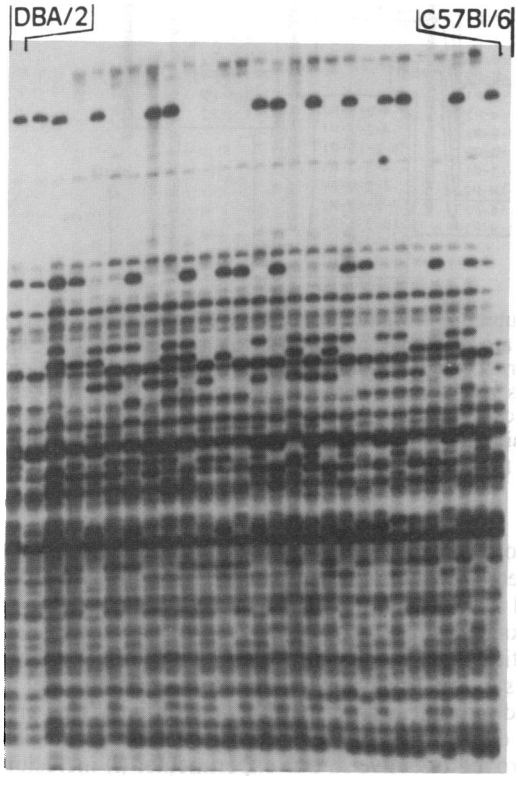

FIG. 2. Hybridization of a human VNTR probe (pYNZ2) to DNA from the 26 RI B × D strains (numbered 1–26) and the parental strains (C57BL/6J on the right and DBA/2J on the left). The DNA was digested with *Hin*fI and studied under fingerprint conditions.

The mean number of linkage groups detected with a single probe was 14.9 (range, 9–22). Although linkage is observed between bands detected by different probes, all of the probes contributed some independent information. For example, if a random subset of 10 probes is characterized, the 11th probe introduces a mean of 6.1 new linkage groups. The most effective single enzyme was *Hae* III (146 polymorphisms in 73 linkage groups), followed by *Hin*fI (125 polymorphisms in 61 linkage groups), and *Taq* I (75 polymorphisms in 41 linkage groups). The combination of *Hae* III and *Hin*fI detected polymorphic bands in 90 (90%) of the linkage groups.

A data base of 142 polymorphic marker loci with known chromosomal localizations was examined to obtain a preliminary linkage map of the minisatellite bands (Fig. 3). Thirty-eight (38%) of the minisatellite linkage groups contain bands that show significant pairwise linkage to one or more markers in the data base; this provides chromosome assignments for 181 (52%) of the 346 polymorphic bands studied. Thirty-four (56%) of the 61 linkage groups from the public data base contain one or more of the minisatellite bands. The combination of markers in the public data base and minisatellite bands produced a total of 124 linkage groups.

DISCUSSION

The results of this study show that human VNTR probes provide an abundant source of polymorphism for linkage studies in the mouse. Under the conditions employed here, 80% of human VNTR probes cross-hybridized to mouse DNA, and 48% produced complex fingerprint patterns compatible with the detection of many minisatellite loci. The 11 probes that we characterized in detail provide a useful set of markers for rapid screening of a large portion of the mouse genome in linkage studies of inbred mouse lines. These probes detected between 115 and 453 polymorphisms in pairwise comparisons of different mouse strains; *M. spretus* was the most divergent of the strains examined.

Linkage studies of the B × D panel were undertaken to examine the distribution of the polymorphisms and to provide preliminary minisatellite maps that could be applied to other crosses involving one of these strains. Similar minisatellite linkage maps could be derived for other mouse strains; backcross panels involving *M. spretus* could be ideal for this because of its allelic divergence (29, 30), although the backcross must be made to *M. spretus* in order to map bands in the laboratory strain.

The 346 polymorphic bands scored in the B × D panel were reduced to 166 systems when grouped by complete linkage, and 101 systems when grouped on the criterion ≥0.99 probability of linkage (3 or fewer recombinants when all 26 RI lines are typed for both loci, equivalent to $\theta < 0.04$). Loci are often detected in a single parental strain under the conditions of migration and hybridization employed here, as bands from both parental lines are present only in 46/166 of the groups under complete linkage and 46/101 of the groups under the criterion ≥0.99 linkage.

Assignment of 38 of the 101 minisatellite linkage groups by linkage to other markers characterized in the RI panel confirmed the wide distribution of these polymorphisms in the mouse genome. Minisatellite linkage groups were localized to all autosomal chromosomes except 10, 16, 18, and 19; the public data base contained no chromosome markers on the first three of these chromosomes and only a single linkage group composed of two markers on chromosome 19. No markers were available to obtain linkage to the X chromo-

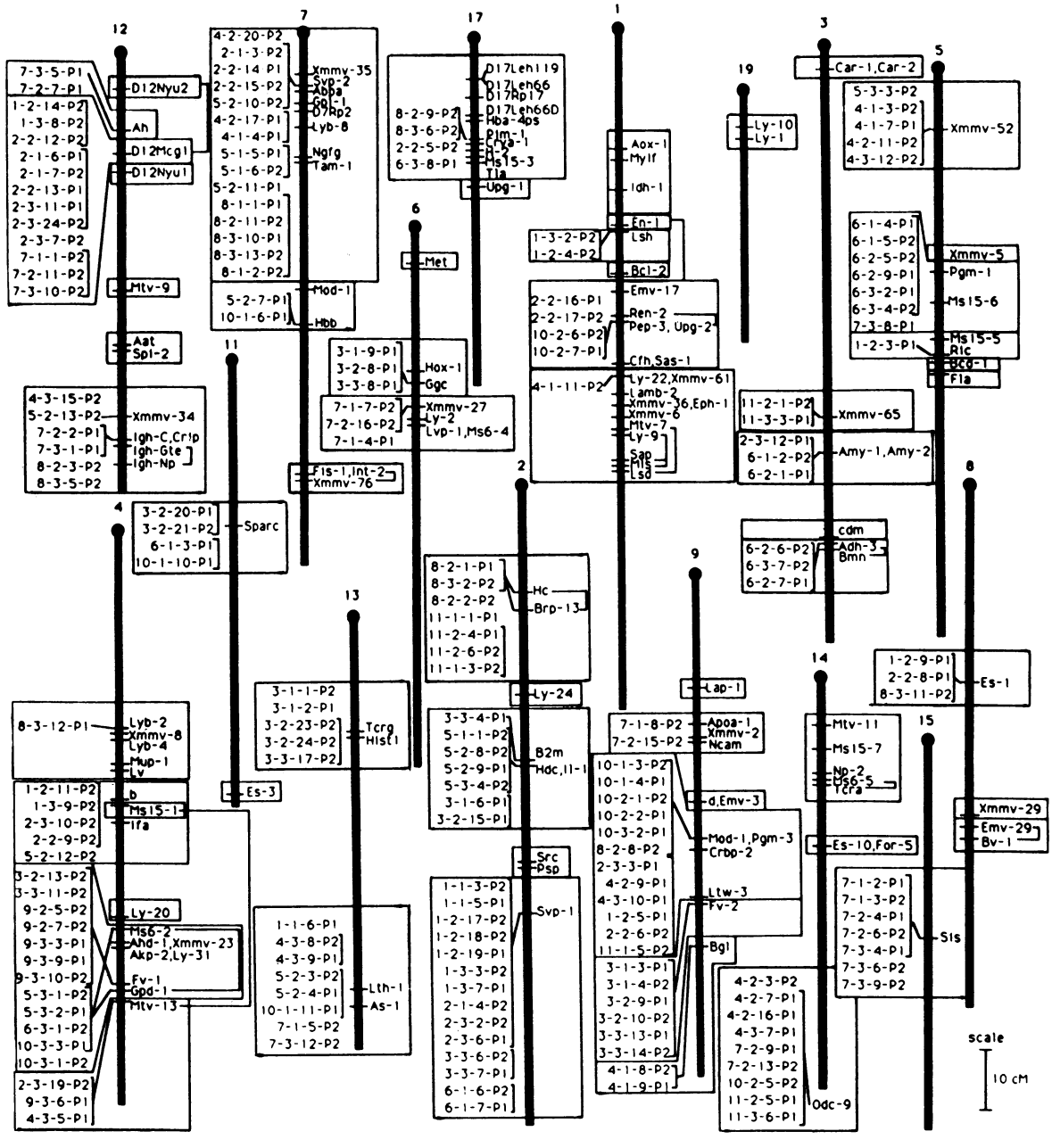

FIG. 3. Mouse autosomal chromosomes showing the regional locations for a subset of polymorphic bands studied in the B × D RI panel. The chromosome drawings and localizations of markers from the public data base are adapted from Hillyard *et al.* (28). Selected polymorphic markers from the data base are shown to the right of each chromosome when their locations are known; distances are scaled in centimorgans (cM). Chromosomes 10, 16, and 18 are not included, as none of the public markers was localized to these chromosomes. Other markers were not included if they exhibited complete linkage to a locus already in the map. Public markers are not indicated if their regional localization was unknown, except ODC9, which is the only marker on chromosome 14. Polymorphic bands that could be localized by linkage are indicated on the left. The system of nomenclature is explained in the text. Boxes indicate linkage groups ($P \geq 0.99$) of polymorphic minisatellite bands and/or public markers. Complete linkage is indicated by solid brackets.

some. Presumably, the other minisatellite linkage groups could not be localized because coverage of the genome with markers from the public data base is incomplete, and only tight linkage can be detected in RI lines. For these reasons, it is also difficult to address the problem of conservation of loci detected by VNTR probes in the human and mouse genomes from these data.

Although other human VNTR probes provide an additional source of markers for linkage studies in the mouse, one advantage of the minisatellite polymorphism described here is that large sections of the genome can be investigated with a small number of probes. A set of six probes (pSP.2.5.EI, pYNZ2, pEFD134.7, pYNH24, p68RS2.0, pINS310) provides coverage of 80% of the minisatellite linkage groups that we detected in the RI strains. Combination of two enzymes, *Hae* III and *Hin*fI, provides polymorphic markers for 90% of the linkage groups.

Identification of equivalent bands in different experiments requires internal controls to allow for variation in gel migration and hybridization or washing conditions. This problem can be dealt with by a combination of three strategies that have proved effective in our experiments: (*i*) inclusion of a molecular size marker that is visible with each probe hybridization; (*ii*) hybridization of the blots with a probe or probe mixture that yields an unvariant multiband pattern that is insensitive to minor variations in hybridization/washing con-

ditions; and (*iii*) incorporation of at least one duplicate sample on each blot, so that the patterns can be aligned. Ideally, images of the multiband patterns could be digitalized, so that the band information would be automatically entered into a computer to minimize errors and speed interpretations.

Two potential problems must be addressed to use minisatellite polymorphisms in linkage studies. First, some hypervariable loci have been shown to be unstable in humans (31) and inbred mice strains (5, 32). The overall mutation rate at minisatellite loci has been estimated to be on the order of 1×10^{-4} per gamete from studies in humans (4) and cattle (10). The VNTR probes used in this study were previously characterized in a panel of 59 human reference families containing ≈1200 meioses; they exhibited mutation rates less than, or consistent with, this figure. However, Jeffreys *et al.* (31) have described higher rates of new mutation associated with extremely polymorphic VNTR loci in humans. A family of unstable minisatellite loci has also been described in the C57BL/6J mouse strain (32); these unstable polymorphisms were first identified in linkage studies in the B × D panel. An unstable minisatellite has also been observed in a cattle pedigree, in which several new mutations were observed in the offspring of a single bull; these could be attributed to germ-line mosaicism from a study of sperm DNA (10). A second difficulty that may be encountered with minisatellite polymorphisms is the presence of comigrating bands from different loci; potentially, comigrating bands could introduce apparent heterogeneity in linkage studies.

In practice, neither problem has created serious difficulties in previous applications to domestic animals or inbred laboratory lines (5, 10). Our results show that after elimination of possible new mutations, segregation distortion for the remaining minisatellite bands was similar to that observed with other marker loci. Examination of the linkage results showed that 35/50 (68%) of the marker loci and minisatellite bands that exhibited segregation distortion were contained in only seven linkage groups. This suggests that segregation distortion in certain chromosome regions may be a feature of the B × D RI lines. However, 9/35 (26%) of the minisatellite bands with significant segregation distortion were not linked to any other systems, compared to 2/15 (13%) of the markers in the public data base. Therefore, some segregation distortion of the minisatellite bands due to comigrating bands or new mutations cannot be excluded. New mutations are expected to be less frequent when minisatellite polymorphisms are studied in backcross or F_2 panels, as the parental lines and offspring are separated by many fewer generations.

Application of the strategy described in this paper to backcross or F_2 panels would permit the detection of linkage at greater recombination distances and allow minisatellite maps to be extended for wider coverage of the genome. Although interspecific crosses involving *M. spretus* are potentially the most informative for mapping purposes, further studies are necessary to establish the stability of the minisatellite polymorphisms in *M. spretus* [since mutation rates may be strain specific (32)] and the degree of inbreeding in laboratory strains of this species.

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