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Comparison of interspecific to intersubspecific backcrosses demonstrates species and sex differences in recombination frequency on mouse Chromosome 16

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Abstract. One hundred fourteen progeny from an interspecific backcross between laboratory mice and *M. spretus* were typed for six markers spanning most of mouse Chromosome (Chr) 16. Additional maps of 9–10 markers of this chromosome were derived from analysis of over 500 progeny from four backcrosses between inbred laboratory strains and members of the *Mus musculus* group, *M.m. musculus* and *M.m. molossinus* (subspecies). The results of these analyses confirmed the gene order: *(CEN)–Prm-1/Prm-2–Igl-1–Smst–Mtv-6–Gap43–Pit-1(dw)–D21S16h–App–Sod-1–Ets-2–Mx*. Maps produced from these five crosses were of similar lengths, but recombination in several regions was affected by sex of the F_1 parent or by the combination of strains used in the cross. As reported previously, recombination frequencies were elevated significantly at the distal end of the chromosome in a cross using F_1 males. The male map showed significant compression in the interval *Smst* to *Gap43*. Both male and female intersubspecific maps were expanded near the proximal and distal ends of the chromosome relative to the interspecific cross. The *spretus* cross was compressed in the proximal interval, *Prm-1–Igl-1–Smst,* and was slightly expanded in the *Smst–Gap43* interval, relative to intersubspecific crosses using F_1 females. Female intersubspecific maps were expanded about 50% near the distal end of the chromosome when compared to the interspecific cross. The expansion or compression of maps using different strain or sex combinations has implications for the efficient production of high resolution recombinational maps of the mouse genome.

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Introduction

Recombination frequencies in mammals are generally higher in females than in males. In human beings, the female recombinational map is twice as long as that of males (Donis-Keller et al. 1987). Sex-related differences in recombination are less pronounced in mice. Nonetheless, the 1700 centiMorgan (cM) female map is 13% larger than the male map, which spans about 1500 cM (Roderick and Hilyard 1989). However, the expansion of the female map is not universal; a number of regions have been reported which show sex-neutral or male-enhanced recombination. Recombination frequencies are also affected by the strains or species of mice used in a particular cross (Seldin et al. 1989; Crosby et al. 1990; Reeves et al. 1990). In recent years, many backcrosses have been made using different species or subspecies of mice (Robert et al. 1985; Callahan et al. 1984). Such crosses are advantageous because the parental strains are highly polymorphic at the DNA level, so molecular markers can be typed with a minimum of effort. This provides the possibility of making very high resolution maps of the murine genome because virtually any DNA probe will provide an informative marker, and because large numbers of informative meioses can be readily generated.

Map resolution is limited by the number of backcross progeny and the number of available markers. Although large numbers of progeny are easily obtained, typing hundreds of animals is labor-intensive. Fortunately, this effort can be greatly reduced in the mouse, where every meiosis is informative and completely phase-known. Typing a set of reference loci across a chromosome defines a small number of intervals and identifies the subset of animals that demonstrate recombination within each interval. A small scale preliminary analysis can be used to localize a gene to a specific interval, after which only those animals which show recombination in the interval need

be typed to obtain all relevant information from the cross (Avner et al. 1987; Chamberlain et al. 1987; Mock et al. 1987). This strategy has been variously referred to as "interval," "pedigree" and "tiered" mapping.

Backcross analysis using inbred mice is inherently different from other mapping strategies because the genetic background in which recombination takes place is identical in every individual analyzed. This situation is unique in mammals. In most instances, genetic maps are derived from pedigree analysis which averages the genetic effects of the population being studied. For recombinant inbred (RI) strains, the sources of genetic variability are limited to the two founding haplotypes, but meiosis always takes place on different genetic backgrounds during inbreeding, and the final analysis of strain distribution patterns is averaged across genetically and phenotypically distinct strains. Thus, the effects of different genetic backgrounds on recombination frequency can only be measured precisely in a backcross between inbred strains. Comparison of such maps can provide an indication of the contribution of individual variation to maps built by pedigree analysis. To date there are only a few examples of comparisons between long-range, high density maps generated using different strain combinations. In two cases where direct comparisons have been made, interspecific maps of Chr 12 and distal Chr 16 were found to be significantly compressed relative to intraspecific crosses measuring recombination frequency between the same markers (Seldin et al. 1989; Reeves et al. 1990).

In this study, recombination frequencies in five intervals spanning most of Chr 16 were determined on five different interspecific or intersubspecific backcrosses. Comparison of these maps confirmed a previously detected expansion of the male map of distal Chr 16, identified increased female-specific recombination in the *Smst–Gap 43* interval, and demonstrated compression of the proximal Chr 16 map in the interspecific cross.

Materials and methods

Mice

Crosses are listed in Table 1 and referred to by number in the text. All crosses have been previously described (see Reeves et al. 1990; Seldin et al. 1988).

DNA analysis

DNA was prepared by phenol extraction (Reeves et al. 1987; Seldin et al. 1988) or by a modification of a "high salt extraction" technique (Miller et al. 1988). For the latter procedure, tissue extracts were digested with 0.6 mg/ml of Proteinase K in 50 mM Tris HCl, pH 7.5, 400 mM NaCl, 100 mM Na_2EDTA, 0.5% SDS at 55°C for 8–14 h. The extract was adjusted to 1.5 M NaCl, shaken vigorously for 15 seconds and subjected to centrifugation at 12,000 × g for 5 min. An equal volume of EtOH at 20°C was added to the supernatant. DNA was recovered by centrifugation and resuspended in 10 mM Tris/1 mM EDTA, pH 7.5. Restriction digestion and electrophoresis were performed as previously described (Reeves et al. 1987).

Table 1. Backcrosses utilized for mapping studies. Female parent is indicated first. Crosses are referred to by these numbers in the text.

No.	Backcross	Reference
1	(C3H/HeJ-*gld/gld* × *M. spretus*)F_1 × C3H/HeJ-*gld/gld*	[26]
2	(Czech II × BALB/cPt)F_1 × Czech II	[20]
3	(MOLD/Rk × DW/J)F_1 × DW/J	[17]
4	(BALB/cJ × MOLD/Rk)F_1 × BALB/cJ	[19]
5	BALB/cJ × (BALB/cJ × MOLD/Rk)F_1	[19]

Molecular probes used in this analysis are given in Table 2 with restriction endonucleases used to identify restriction fragment length variants (RFLVs) in crosses 1, 4 and 5. Use of these probes to map crosses 2 and 3 and RFLVs used in those analyses have been previously reported, as have detailed maps of the distal portion of Chr 16 determined on crosses 2, 3, 4 and 5 (Table 1). Radiolabeled probes were synthesized by primer extension as described by Feinberg and Vogelstein (1984) and backcross animals were analyzed by Southern blot analysis using standard procedures. Data for crosses 2–5 were compiled and analyzed in a Lotus 1-2-3™ database. Order was determined by minimizing double crossovers ("direct method") using the Genmacro program (Reeves 1988) and confirmed by maximum likelihood analysis (Bishop 1985). As an example of the robust nature of the direct method for determining gene order on a large phase-known backcross, the Lod likelihood for the three point order, *Prm–Igl-1–Smst*, was 4×10^{21} times greater than the next most likely alternative. This was the least certain three point order for the genes mapped in this study; the additional information gained from maximum likelihood analysis of this large data set was minimal. Chi square analysis was used to determine the significance of differences in recombination frequency (Snedecore and Cochran 1967).

Results

Six genes were typed on cross 1 to define five intervals spanning most of Chr 16 (Table 3 and Fig. 1). RFLVs for these markers were readily detected on all crosses (Fig. 2 and Table 2). Localization of *Pit-1* on cross 3 has been reported (Camper et al. 1990) and is confirmed using crosses 4 and 5 in this study. *D21S16h, App, Sod-1* and *Ets-2* were mapped previously to the human Chr 21-homologous segment of distal Chr 16 on crosses 2, 3 and a subset of the progeny of crosses 4 and 5 reported here (Reeves et al. 1990).

Gene order was determined by minimizing double crossovers. Analysis of the five backcrosses showed complete agreement with gene orders reported previously (Reeves et al. 1987, 1990; Cheng et al. 1988; O'Hara et al. 1988; Camper et al. 1990), *Prm-1/Prm-2–Igl-1–Smst–Gap43–Pit-1–D21S16h–App–Sod-1–Ets-2–Mx-1. Prm-1* and *Prm-2* do not recombine on 220 intraspecific and intersubspecific backcross progeny (Reeves et al. 1989, unpublished data). The Prm clones identify the most proximal locus detected with a molecular marker on Chr 16 (with the exception of an alphoid repeat that recognizes centromeric regions of *M. musculus* chromosomes). Based on the position of the *md* coat color gene relative to the centromere and the positions of *md* and the protamines relative to *Igl-1*, the *Prm* locus is approximately 7 cM from the centromere (see Reeves et al. 1987).

Table 2. Restriction endonucleases and molecular clones used to identify RFLVs on crosses 1, 3, 4 and 5. RFLVs for Czech II were described previously [20]. Only polymorphic fragment sizes are shown for *Igl-1* and *Mx1*.

Locus	Clone (Ref.)	Restriction Endonuclease	Fragment Size			
			MOLD/Rk	BALB/cJ, DW/J	C3H/HeJ	*M. spretus*
Prm-1	pMP1 (12)	*Msp* I	—	—	9.4, 6.8	7.0
		*Bst*E II	6.8	17	—	—
Prm-2	pMP2 (12)	*Pvu* II	3.4	3.1		
Igl-1	MOPC104E (24)	*Msp* I	—	—	18, 7.2, 5	15, 3.6
		Pvu II	6.6	9.4, 1.9	—	—
Smst	pMST1.4 (17)	*Eco*R I	—	—	20	9.8
		Hae III	1.1	2.1, 1.8, .65	—	—
Gap43	GA11b (13)	*Taq* I	—	—	21, 3.3	3.8, 1.5
		Pvu II	6.0, 4.8, 1.6	5.5, 4.8, 1.6	—	—
Pit-1	mPitc (4)	*Pvu* II	2.8	3.0	—	—
D21S16h	pGSE9 (30)	*Eco*R I	—	—	6	5.8
		Pvu II	3.2	5.2	—	—
Ets-2	PH1.03 (31)	*Bam*H I	—	—	9	2.8
		Hinc II	5.9, 5.1	11	—	—
Mx-1	pMx2.3 (29)	*Hind* III	6.4	5.9	—	—

Fifty DNAs from cross 2 were typed for *Gap43* to determine its position relative to *Mtv-6*, an endogenous mammary tumor virus previously mapped on this cross. Two recombinations were observed indicating that *Mtv-6* is 4.0 ± 2.8 cM proximal to *Gap43*.

A mutation in the *Pit-1* gene is responsible for the Snell *dw* mutation (Camper et al. 1990; Li et al. 1990). A *Pit-1* cDNA probe was used to determine the position of this gene on crosses 4 and 5 using the interval mapping procedure. A total of 73 out of 451 animals from these crosses showed recombination between *Gap43* and *D21S16h*, the interval containing *Pit-1* (Table 3). Typing of the subset of animals from crosses 4 and 5 that showed recombination in this interval was used to determine the relative order of the three markers and to provide an estimate of the distances between them (Table 4). This approach is effective even if all informative animals from the interval are not typed. On cross 4, for example, analysis of 201 animals identified 32 crossovers between *Gap43* and *D21S16h*, or one recombination event for 6.28 animals typed. Twenty-nine of the 32 informative animals were typed for *Pit-1*, which represents the equivalent of 29 × 6.28 or 182 meioses analyzed. An estimate of the recombination frequencies between *Pit-1* and the markers defining the interval was obtained by dividing the number of recombinants with each flanking marker by 182. This analysis of crosses 3, 4 and 5 produced the order, *Gap43*–.199 ± .019–*Pit-1*–.045 ± .010–*D21S16h*. A total of 78 animals from the three crosses were typed to provide the information from 442 meioses. Recombination across the *Gap43* to *D21S16h* interval was elevated in cross 3, which is segregating the *dw* allele of *Pit-1* (Table 3).

The total length of the Chr 16 maps generated from the different crosses was similar. The cumulative recombination frequency from *Prm-1*/*Prm-2* to *Ets-2* ranged from 0.517 in cross 1 to 0.588 in cross 3. No segregation distortion was observed and all crosses displayed a high level of positive interference. Although the most proximal and distal markers typed were separated by more than 50 cM, no double crossover was observed among the 299 chromosomes analyzed from crosses 1, 2 and 3. Double crossovers occurred in only eight out of 207 animals from cross 4 that were typed for six or more markers over the 56.9 cM interval examined. Nine of the 197 animals from cross 5 showed double crossovers over 59.3 cM. In two cases, double crossovers occurred in the same litter (once in cross 4 and once in cross 5). The two littermates from cross 4 had identical Chr 16 haplotypes including the same double crossover in the 13 cM interval between *Prm-2* and *Smst*, suggesting that these animals might be twins.

Recombination frequencies across five intervals were compared to determine whether recombination was affected by the sex of the F_1 parent or by the combination of strains used in a particular cross (Table 3). The *D21S16h* to *Ets-2* interval was previously shown to be expanded in intersubspecific crosses made with F_1 males compared to crosses 3, 4 and 63 progeny of an interspecific cross using a female (*M. spretus* × laboratory inbred strain) F_1 parent (Reeves et al. 1990). This finding was duplicated here with a second interspecific backcross which used a different laboratory strain. The 22.8% recombination measured in F_1 males on cross 5 was significantly greater than 14.1% seen in F_1 females of the reciprocal cross (cross 4) and was greater than the average female recombination frequency of 11.1% measured on crosses 1, 3 and 4 ($p < 0.005$, $df = 1$). Recombination across this interval in crosses using F_1 females ranged from 7% in cross 1 to 14.1% in cross 4.

A second effect of sex on recombination frequency was demonstrated in the *Smst* to *Gap43* interval, where 6.6% male recombination was significantly less than the average 15.6% in females ($p < 0.005$, $df = 1$) (Table 3). Recombination differences in this interval were also significant between reciprocal crosses 4 and 5. Interspecific cross 1 showed more recombination than other crosses using F_1 females, but the difference was not significant. Low recombination frequencies were measured on cross 1 in the adjacent proximal intervals, *Prm* to *Igl-1* and *Igl-1* to *Smst*.

Table 3. Recombination fractions (frequency) in intervals defined by markers spanning MMU16. Each interval of closest neighbors includes all animals typed for both markers. Crosses 1–4 were made with F_1 females; cross 5 used F_1 males.

Cross	*Prm*[a]*/Igl-1*	*Igl-1/Smst*	*Smst/Gap43*	*Gap43/D21S16h*	*D21S16h/Ets-2*[b]	No. of Crossovers
1	3/114 (.026)	3/114 (.026)	24/114 (.211)	21/114 (.184)	8/114 (.070)	59
2	6/93 (.065)	5/93 (.054)	9/50 (.180)	—	—	44[c]
3	6/92 (.065)	3/92 (.033)	13/85 (.153)	20/83 (.241)	8/83 (.096)	50
4	16/198 (.081)	10/207 (.048)	33/259 (.127)	32/201 (.159)	27/192 (.141)	113
5	12/193 (.062)	9/200 (.045)	16/241 (.066)	41/250 (.164)	55/241 (.228)	133
Totals	43/690 (.062)	30/706 (.043)	95/749 (.127)	114/648 (.176)	93/628 (.140)	399

[a] No recombination was detected between *Prm-1* and *Prm-2* on 220 backcross progeny [21 and unpublished data]. Data presented here include animals typed with one or both protamine probes.
[b] Only animals typed for *D21S16h, App, Sod-1* and *Ets-2* are included for crosses 3, 4 and 5, and recombination frequency is corrected for double crossovers.
[c] Total crossovers calculated from *Prm-1–Igl-1–Smst–Gap43–Mtv-6–App–Sod-1–Ets-2.*

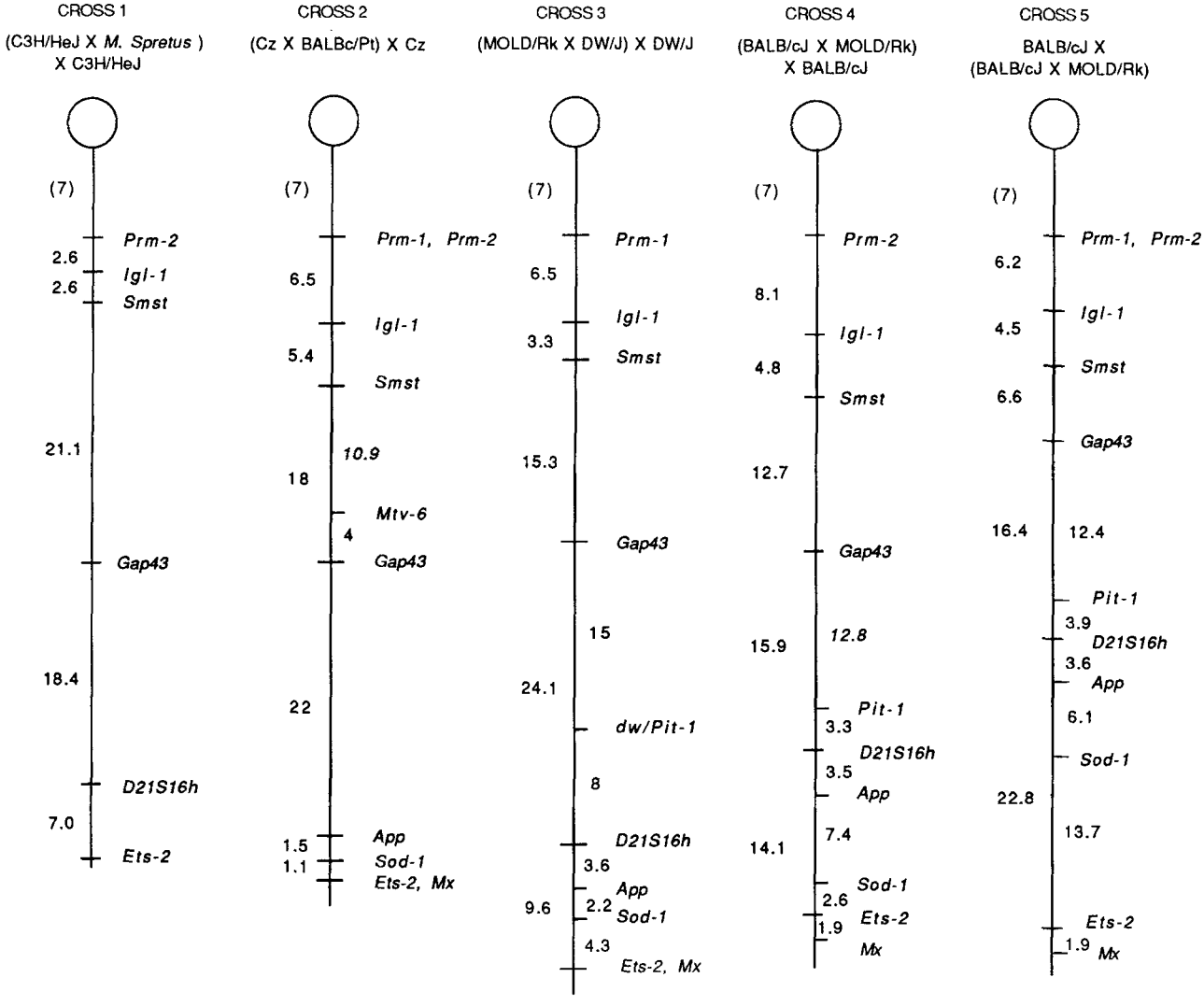

Fig. 1. Recombinational maps of murine Chr 16 determined on five different interspecific or intersubspecific backcrosses. Percent recombination in reference intervals (from Table 3) is shown to the left of each chromosome. Recombination percentages on the right indicate positions of additional markers previously mapped on these crosses. Crosses 1–4 were made with F_1 females, cross 5 used an F_1 male.

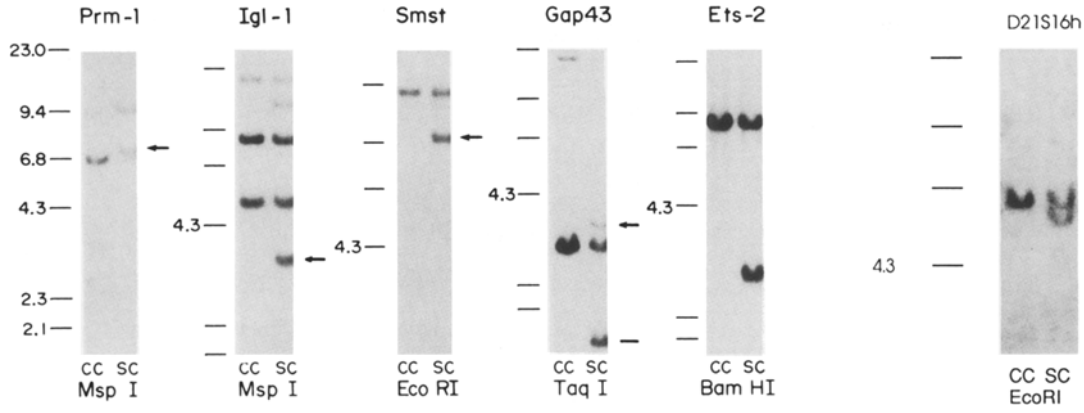

Fig. 2. Detection of RFLVs between C3H/HeJ *gld/gld* (CC) and (C3H/HeJ *gld/gld* × *Mus spretus*)F_1 (SC). Variants were detected using the probes and restriction endonucleases described in Table 2. Positions of molecular weight markers are indicated.

Discussion

Effects of sex on recombination frequency are well-documented, but the basis for these differences is unknown. Two regions affected by sex are now identified on Chr 16: expansion of the female map in the *Smst–Gap43* interval and a distal expansion of the male map. In addition to effects of sex, the particular combination of strains also affects recombination frequency on this chromosome. In this study, the interspecific cross demonstrated increased recombination between *Smst* and *Gap43* and compression in the *Prm–Igl-1–Smst* interval. This same pattern has been observed in another interspecific cross (Irving et al. 1991), and combined data from the two crosses demonstrates significantly reduced recombination between *Prm-1* and *Igl-1* relative to intersubspecific crosses 2–5 ($p < .05$, $df = 1$). Compression of the distal Chr 16 map is seen in three interspecific crosses (Cheng et al. 1988; Irving et al. 1991; present study) relative to intersubspecific crosses 3 and 4. However, this compression is not necessarily due to the fact that the parental strains derive from different species. Intersubspecific cross 2 also shows compression in this region (Fig. 1), and Chr 5 maps made on interspecific backcrosses with *M. spretus* and either of two laboratory strains show significant differences in recombination frequency between the two crosses (Seldin et al. 1990). Suppression of recombination on proximal Chr 17 occurs due to a series of inversions in specific *t* haplotypes (Hammer et al. 1989). Fine scale recombinational and physical mapping will demonstrate whether different species' chromosomes are frequently rearranged relative to each other, or indicate regions in which suppressed recombination is a function of a higher order biological property.

Differences in recombination frequency can be used to advantage in preparing high resolution maps of the mouse genome. Given the same number of animals from the different crosses analyzed in this study, a gene mapping to the *Smst–Gap43* region would be mapped most accurately on the interspecific cross, since recombination frequencies in this region are higher than those in intersubspecific crosses and more

Table 4. Determination of the position of *Pit-1* by interval mapping.

Cross	No. of recombinants per total No. typed *Gap43* to *D21S16h*	No. typed for *Pit-1*	No. typed per one recombinant	Equivalent No. typed
3[a]	20/83	20	83/20 = 4.15	20 × 4.15 = 83
4	32/201	29	201/32 = 6.28	29 × 6.28 = 182
5	41/250	29	250/41 = 6.1	29 × 6.1 = 177

	Cross 3		Cross 4		Cross 5	
	No. of recombinants	RF	No. of recombinants	RF	No. of recombinants	RF
Gap43 to *Pit-1*	13/83	.157 ± .040	23/182	.128 ± .025	22/177	.124 ± .025
Pit-1 to *D21S16h*	7/83	.084 ± .030	6/182	.033 ± .013	7/177	.040 ± .015
Gap43 to *D21S16h*	20/83	.241 ± .047	32/201	.159 ± .026	41/250	.164 ± .023

[a] All animals on cross 3 were typed for *Pit-1*.

than twice as high as those made with F_1 males. An intersubspecific cross using F_1 males would be the obvious choice for mapping markers falling near the distal end of the chromosome; analysis of the *D21S16h* to *Ets-2* interval on 241 animals from cross 5 provides as much information as typing 890 animals from cross 1.

The interval mapping procedure has been used with the intersubspecific crosses described here to provide an efficient means of constructing high resolution maps of Chr 16. RFLVs between laboratory strains and MOLD/Rk have been readily identified for over 30 markers with nine different restriction endonucleases that can be evaluated on a single filter; to date no probe has failed to yield a RFLV between these strains. The 50 recombinant DNAs from cross 3 define eight "reference intervals" on Chr 16. New markers are screened initially against a subset of these DNAs to determine the reference interval to which they map. For any interval in which it does not belong, the unknown marker will always be of the same parental type as the reference marker which is closer to its true location. Once the reference interval is determined, only animals that have crossovers in that interval are typed to accurately determine the position of the marker. On more than 400 DNAs from crosses 4 and 5, 17 markers define 14 intervals with an average size of less than 4 cM and no single interval larger than 10 cM. Thus, mapping with sub-0.5 cM resolution requires typing only 20–50 animals. Since each marker further subdivides the interval, a smaller set of DNAs (on average 50% as many) needs to be typed in each consecutive round. Thus, while the initial effort required to achieve a high number of reference DNAs is high, mapping of any number of markers becomes progressively easier. Any error in typing will stand out as an anomaly as more markers are included, so the map is self-correcting. As demonstrated, a good estimate of recombination frequency can be obtained even if a subset of the informative animals are typed. This is important because in large panels, some DNAs are likely to be used up or degraded.

The high resolution backcross provides a framework for the construction of physical maps since it is in essence an ordered array of physical sites along the chromosome. These recombination sites are readily identified in interspecific and intersubspecific crosses using virtually any DNA segment as a molecular probe, and will be mapped precisely as the maps become more dense. Comparative mapping studies demonstrate clearly that recombination frequency is cross dependent. Further studies of this type and comparison of backcross maps with those produced from analysis of RI strains and outcrosses using the same parental stocks will provide an estimate of the degree to which genetic heterogeneity may contribute to different recombination rates in different families.

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