Simultaneous Detection and Fine Mapping of Quantitative Trait Loci in Mice Using Heterogeneous Stocks

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ABSTRACT

We describe a method to simultaneously detect and fine map quantitative trait loci (QTL) that is especially suited to the mapping of modifier loci in mouse mutant models. The method exploits the high level of historical recombination present in a heterogeneous stock (HS), an outbred population of mice derived from known founder strains. The experimental design is an F_2 cross between the HS and a genetically distinct line, such as one carrying a knockout or transgene. QTL detection is performed by a standard genome scan with ~100 markers and fine mapping by typing the same animals using densely spaced markers over those candidate regions detected by the scan. The analysis uses an extension of the dynamic-programming technique employed previously to fine map QTL in HS mice. We show by simulation that a QTL accounting for 5% of the total variance can be detected and fine mapped with >50% probability to within 3 cM by genotyping ~1500 animals.

IT is relatively straightforward to map quantitative trait loci (QTL) that segregate in crosses between two inbred strains, but identifying the responsible molecular variant is very taxing, largely because of the difficulties of resolving loci into intervals small enough to identify genes (Flint and Mott 2001). Two general strategies have been adopted to fine map QTL. In the first, attempts are made to place a single QTL from one inbred strain on the genetic background of another. For example, by breeding congenic mice derived from a cross of the two inbred strains used to detect the QTL, recombination can be used to reduce the size of the chromosomal region containing the QTL. The second approach exploits large numbers of recombinants across the genome to fine map all QTL simultaneously. One way in which this can be done is by increasing the number of F_2 individuals in the cross used to detect the QTL. However, for QTL with moderate or small effects, many thousands of F_2's are needed to make the interval sufficiently small to attempt positional cloning. This strategy is appropriate for species that are cheap to breed, such as plants (Alpert and Tanksley 1996), but less suited for livestock or laboratory rodents. In any event, the genotyping costs, which scale with the number of markers typed times the number of individuals, are almost prohibitive. Alternatively, many generations of intercrossing may be used to achieve subcentimorgan resolution (Darvasi and Soller 1995; Darvasi 1997). Fewer genotypes are required, but the experiment takes much longer.

Ideally we would like to map QTL to high resolution without exhausting the resources of a single laboratory and within a reasonable period of time (for instance, within 3 years). One solution may be to use outbred rather than inbred crosses, such as a genetically heterogeneous stock (HS) derived from eight inbred strains and outbred for a number of generations prior to the experiment (McClearn and Meredith 1970). Historical recombination turns each HS chromosome into a fine-grained mosaic of the founder strain haplotypes. For example, after 60 generations (the current age of the HS animals we have used) the average distance between recombinants is 1/60 or 1.7 cM. The large number of recombinants means that the HS can map small to moderate effect QTL into subcentimorgan intervals, as we have recently demonstrated (Talbot *et al.* 1999; McPeek 2000; Mott *et al.* 2000).

The HS has the potential to be a general-purpose tool for the genetic dissection of complex traits in model organisms, but it has a number of drawbacks. First, whole genome scans using the HS require a very large number of genotypes. To achieve power of 80% to detect a QTL accounting for 5% of the phenotypic variance, markers spaced ~1 cM apart across the whole genome have to be genotyped on 2000 animals (Mott *et al.* 2000). By contrast, for a similar QTL detection experiment in an F_2 cross, markers spaced every 20 cM and genotyped on a few hundred animals will suffice. Second, the HS can be used only to fine map QTL that segregate in crosses between the eight founder strains. While this represents substantially more genetic diversity than is found in most inbred crosses, it excludes the use of the HS for fine mapping many QTL of great interest, for example, modifiers affecting mouse models of human disease.

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A cross between an inbred and HS animal (which we call an inbred-outbred cross) extends the fine-mapping capacity of the HS, using F_1 and F_2 animals. Here we develop and evaluate a particular type of inbred-outbred cross that requires data collected from the F_2 generation only. The design is much like an F_2 intercross between two inbred lines except that the data analysis means we can both detect and fine map QTL with high probability. The method makes it possible to screen the entire genome of the HS with the same number of markers used in a backcross or F_2 intercross and extends the use of HS animals for fine mapping loci that modify the phenotypes produced by single-gene mutations (Threadgill *et al.* 1995). The latter is of particular interest given the growing number of transgenic mice and the production of large numbers of novel mutants by random mutagenesis (Wells and Brown 2000).

Genetic mapping in HS populations is more complex than mapping in inbred crosses, because at most marker loci there are far fewer alleles than progenitor strains. We find there are frequently only two or three alleles at a locus so that it is impossible to assign unambiguously the strain of origin (of which there are eight) to each allele. The result is that single-marker association analysis, the standard method for detecting QTL in inbred crosses, cannot distinguish between strains having different QTL effects, but identical alleles, and therefore may fail to detect the QTL. We overcame this problem using a multipoint analysis that uses a dynamic-programming (DP) algorithm to assign the probability that an allele descends from each progenitor in the HS. Under an additive model of genetic action, the expected genetic effect for a diploid individual with ancestral founders s and t at the QTL will be the sum of the strain effects for these founders, $T_s + T_t$, say, of the QTL at the locus. A test for a QTL is then equivalent to testing for differences between strain effects by analysis of variance. Using this approach we showed that in one experiment single-marker association analysis detected only two out of five QTL detected by the DP method in the HS (Mott *et al.* 2000).

Analysis of the inbred-outbred cross must not only assign progenitor strains to alleles in the HS, but additionally it must confront the difficulty of distinguishing alleles that descend from inbred and outbred animals in the cross. In this article we describe an extension of our dynamic-programming method suitable for analyzing the inbred-outbred cross. We explore the method's power by simulation, and we show that it has a high probability of both detecting and fine mapping QTL. The method is incorporated into freely available software.

THE INBRED-OUTBRED CROSS: OVERVIEW

The inbred-outbred cross has a number of applications, two of which we discuss here. In the first instance, the objective is to detect and fine map a QTL segregating in the HS. The experimental design is an F_2 cross between the outbred HS, denoted by *O*, and a background line, *I*. For simplicity we assume *I* is an inbred strain, although it is straightforward to extend the method so that *I* is another outbred stock, provided it is genetically distinct from *O*. The F_1 generation of the $I \times O$ cross comprises animals with homologous chromosomes that are either pure *I* or *O*. In the F_2 generation, formed by crossing the F_1's with themselves, each chromosome will contain roughly equal amounts of *I* and *O* with about one recombinant per chromosome. The key point is that the F_2 chromosomes contain *I* segments with no internal recombination and *O* segments dense in recombinants (Figure 1).

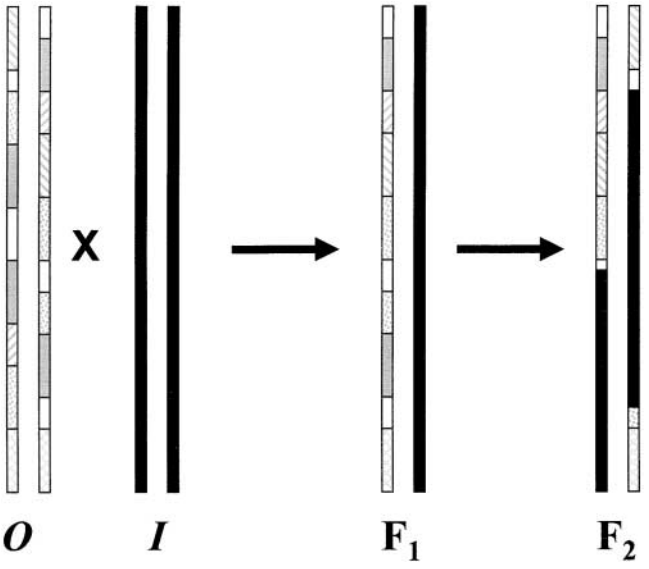

Figure 1.—The inbred-outbred cross. An inbred line, *I* (black chromosomes), is crossed with an outbred population, *O*. Each *O* chromosome is a fine-scale mosaic of known founder strains (represented as shaded segments). The F_1 generation comprises homologous chromosomes that are entirely *I* or *O*. The chromosomes from the F_2 generation contain a mixture of *I* and *O* segments.

The presence of two scales of recombination makes it possible to carry out low resolution QTL detection and high resolution QTL fine mapping in the same animals. A single set of individuals from the F_2 generation is genotyped twice. First, a standard genome scan involving ~80–150 microsatellite markers spaced 10–20 cM apart is performed. The data are analyzed to detect QTL and then any positive candidate region is retyped at 1-cM spacing for fine mapping. The number of markers typed in the fine-mapping phase will depend on how many QTL were detected.

The genetic variance attributable to the QTL in the F_2 generation may be split into V_{I0}, the variance between *I* and *O*, and V_{00}, the variance within *O*. When we analyze differences between *I* and *O*, the data resemble a genome scan of a standard F_2 detection experiment between two inbred lines. The dense set of markers subse-

TABLE 1

Distribution of genotypes and mean traits for QTL detection

Genotype		Mean trait value	Probability
I	*I*	$-2a$	¼
I	*O*	$(2p - 2)a$	½
O	*O*	$(4p - 2)a$	¼

quently typed over regions detected in the scan permits the dissection of the variance within the *O*-derived chromosomes and consequently the fine mapping of the QTL. Clearly, the fine-mapping resolution depends on the extent to which the QTL segregates within the HS founders.

The inbred-outbred design can also be used to map modifiers of mutations, which can be introduced into *O* by crossing the outbreds to either homozygous or heterozygous mutant animals. In the former case the design is identical to that described above. In the latter, where the *I* line carries a mutation that has been maintained as a heterozygote by backcrossing onto wild-type *I* animals, then approximately one-half the F_1's have the mutation. The F_2 generation is then formed by first screening the F_1's for the mutation and either mating each mutant F_1 with a nonmutant, when one-half of the F_2 would carry the mutation and the rest would be normals, or alternatively intercrossing the F_1 mutants, to produce a mix of 25% wild type, 50% mutant heterozygotes, and 25% mutant homozygotes. The choice will depend on the nature of the mutation, in particular on the viability of mutant homozygotes.

ANALYSIS

To detect a QTL that segregates in the inbred-outbred cross we calculate the probability that a chromosomal segment is descended either from *I* or from a founder of *O*, conditional on the observed genotypes. The probability is calculated using an extension of a multipoint dynamic-programming algorithm that we previously developed to calculate *O* founder probabilities for fine mapping (Mott *et al.* 2000). To simplify the analysis, we first describe the ideal situation in which the markers are completely informative, that is, *I* and each *O* founder strain carry distinct alleles. We then show how to compute descent probabilities when the markers are not fully informative, by using information from flanking markers.

QTL detection: For simplicity we assume the presence of an additive diallelic QTL. Alleles descended from *I* have effect $-a$, and those descended from *O* have effect $+a$ with probability p and $-a$ with probability $1 - p$. Thus *O* chromosomes containing the QTL are a mixture of increasers and decreasers with apparent effect $-a(1 - p) + ap = a(2p - 1)$. Table 1 gives the distribution of possible F_2 genotypes, trait values, and probabilities, from which we see the genetic variance between *I* and *O* is $V(p)_{IO} = 2p^2$. The power to detect the QTL increases with the variance and hence with the proportion of *O* chromosomes that differ from *I* at the QTL. Consequently the optimal detection occurs at $p = 1$, when all the HS chromosomes carry the increaser QTL allele. When $p = 0$, *O* is indistinguishable from *I* and there is no power to detect.

TABLE 2

Distribution of genotypes and mean traits for QTL fine mapping

Genotype		Mean trait value	Probability
I	*I*	$-2a$	¼
I	*O*+	0	$p/2$
I	*O*−	$-2a$	$(1 - p)/2$
O−	*O*−	$-2a$	$(1 - p)^2/4$
O−	*O*+	0	$p(1 - p)/2$
O+	*O*+	$+2a$	$p^2/4$

Fine mapping: In the fine-mapping phase, markers are typed densely so the QTL is likely to be linked to a marker even on *O*-descended chromosomes. Thus, in addition to the rather coarse-grained information provided by the *I vs. O* contrast, it is now possible to dissect the differences between the HS founders. We denote an outbred chromosome carrying an increaser allele as *O*+ and a decreaser as *O*−. The possible QTL genotypes, trait values, and probabilities are given in Table 2.

This fully captures all the information about the QTL and corresponds to the situation of typing a dense set of completely informative markers. The total genetic variance from the above trait distribution is therefore

$$V(p)_{\text{TOTAL}} = 2p(2 - p)a^2$$

and the extra genetic variance, V_{OO}, explained by fine mapping over that explained by detection alone is therefore

$$V(p)_{OO} = V(p)_{\text{TOTAL}} - V(p)_{IO} = 2p(2 - p)a^2 - 2p^2a^2 = 4p(1 - p)a^2.$$

Note that the trait variance within the original outbred HS population is

$$V(p)_{\text{HS}} = 8p(1 - p)a^2 = 2V(p)_{OO},$$

consistent with the observation that as the F_2 is 50% *O*, the variance attributable to *O* in the inbred-outbred cross should be one-half that in a pure *O*. These functions are graphed in Figure 2a. The genetic variance available for QTL detection always increases with p, but the extra variance explained by the full model attains a maximum at $p = ½$. When p is close to 0, then *I* and *O* are indistinguishable and there is little power to detect or localize a QTL; when p is close to 1, then *O* resembles an inbred line—the power to detect is high but the localization is poor—the experiment reverts to F_2 detec-

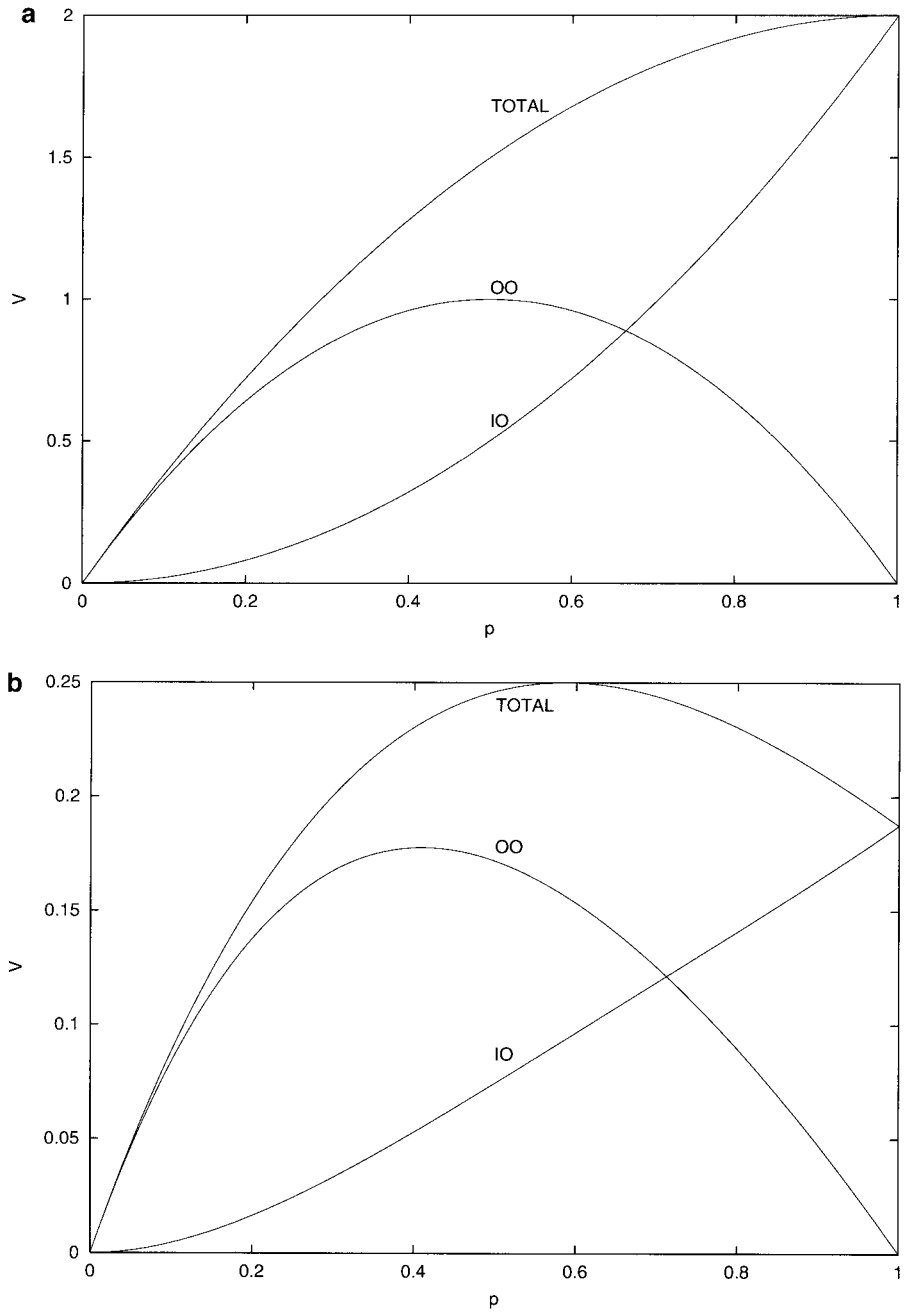

FIGURE 2.—Partitioning of genetic variance in the inbred-outbred cross. The genetic variances $V_{OO}(p)$, $V_{IO}(p)$, $V_{\text{TOTAL}}(p)$ are graphed as functions of the proportion p of O chromosomes carrying (a) an additive increaser QTL with effect +1. All I chromosomes carry decreaser alleles with effect −1. (b) A dominant increaser QTL with effect +1. All I chromosomes carry recessive alleles with effect 0.

tion. Consequently this experimental design is most appropriate when about one-half the founders of O carry QTL alleles that differ from I.

The sample size required for QTL detection will be equivalent to that required in an F_2 cross between two inbred strains with genetic variance equal to $V(p)_{IO}$, rather than $V(p)_{\text{TOTAL}}$ or $V(p)_{\text{HS}}$. The variance available for QTL detection in the F_2 can be significantly less than the genetic variance originally present in the HS. For example, if $p = \frac{1}{2}$ and $V(\frac{1}{2})_{OO} = 5\%$, then $V(\frac{1}{2})_{\text{TOTAL}} = 6.67\%$, but $V(\frac{1}{2})_{IO} = 1.67\%$.

Qualitatively similar behavior holds if the QTL is dominant with effect $+a$ in O with probability p and recessive with effect 0 in I, when

$$V(p)_{\text{HS}} = p(1-p)^2(2-p)a^2$$
$$V(p)_{\text{TOTAL}} = p(2-p)^2(4-p)a^2/16$$
$$V(p)_{IO} = p^2(8-8p+3p^2)a^2/16$$
$$V(p)_{OO} = (1-p)(4-3p+p^2)a^2/4$$

(Figure 2b), and consequently any intermediate between dominance and additivity should also follow the same pattern.

Multipoint mapping: The analysis of real data is complicated by the issue of marker informativity. Not all strains within O or between the O and I are distinguishable at each marker locus, because the number of alleles present is often less than the number of haplotypes. Con-

sequently the variances derived above are idealizations that only give upper bounds on the power of the inbred-outbred cross.

Previously we have shown how to use a multipoint dynamic-programming algorithm, implemented in the program HAPPY, to determine the probability of each founding haplotype at any locus (Mott *et al.* 2000). The analysis requires the phenotypes and marker genotypes of the final generation and the marker alleles of *I* and the founder strains used to make the HS. Pedigree information is not required. Use of flanking marker data in this way alleviates the problem of marker informativity and significantly increases the power to detect and fine map QTL.

We now describe the necessary modifications required to analyze an inbred-outbred cross. The QTL-detection step of the analysis is an F_2 cross between *I* and *O*, in which *O* is treated as if it were a single strain (albeit no longer inbred), so the cross is like a heterogeneous stock formed from two founders *I* and *O* and just two generations old. It can then be analyzed using the original version of HAPPY.

For the fine-mapping step it is necessary to model the different scales of recombination present within and between chromosomal segments descended from *I* or *O*. In Mott *et al.* (2000) the genotypes were modeled as a realization of a hidden Markov chain, in which the ancestral haplotypes were the hidden states and the marker genotypes the observed data. We use a similar formulation for the inbred-outbred cross, except that the priors and transition probabilities between founder states are no longer uniform, but instead depend on whether the states are *I* or *O*. The prior probability that a locus is descended from *I* or *O* is ½. The probability of descent from any particular *O* founder, say O_f, is $S/2$, where *S* is the number of founders. We assume the Haldane model of recombination applies: in an interval of length *d* morgans, the probability that no recombination events occur in a single meiosis is e^{-d}. In an *O* interval intercrossed over *G* generations the probability of no recombination is e^{-Gd}. Consequently, the prior probability $r_m(s|\sigma)$ that the ancestral state on a chromosome at some marker $m + 1$ is *s*, given it is σ at marker *m*, distance *d* apart, and before any genotype information at $m + 1$ is considered, is

$$
\begin{aligned}
r_m(I|I) &= e^{-d} + (1 - e^{-d})/2 \\
r_m(O_f|I) &= (1 - e^{-d})/2S \\
r_m(I|O_f) &= (1 - e^{-d})/2 \\
r_m(O_g|O_f) &= (1 - e^{-d})/2S + e^{-d}(\delta_{gf}e^{-Gd} + 1 - e^{-Gd})/S,
\end{aligned}
$$

where δ_{gf} is the delta function. Apart from these changes, the statistical development is similar to Mott *et al.* (2000).

The test for the presence of a QTL in a given marker interval is by analysis of variance. The additive effect on the trait of *I* and each founder O_f is estimated by least squares. Differences between the estimates are evaluated with an *F*-test. Although the bulk of the fine-mapping information is due to differences between the founders of *O*, there is also some information from the *I*/*O* contrast. Hence we estimate the QTL position by the marker interval with the smallest ANOVA *P* value from fitting a full model with both *I* and *O* present.

The overall efficiency $\Pr(r)$ of the method is defined as the probability that a QTL will be both detected with a genome-wide *P* value <1.5% (*i.e.*, at least one marker interval has an ANOVA *P* value $<10^{-4}$) and fine mapped to within *r* 1-cM intervals of its true position. In this study localization to within 3 cM is deemed to be a success.

SIMULATIONS

We investigated the power of the inbred-outbred cross by computer simulation. First, *O* populations were generated by intercrossing eight inbred founders for 60 generations, keeping 40 mating pairs in each generation and avoiding brother-sister matings. Each individual was simulated as a 100-cM diploid chromosome. Individuals from the final *O* generation were then crossed with a pure inbred line, either identical to one of the eight *O* founders or completely distinct, in the sense that each marker distinguished between *I* and *O*. Real crosses will lie between these extremes.

The 80 *O* animals were mated with *I* to produce 400 F_1 offspring, which were crossed to produce F_2 populations of 1000, 1500, and 2000 subjects. Microsatellite markers spaced 1 cM apart were typed across the chromosome. The distribution of alleles per marker was modeled on the real microsatellites used in Talbot *et al.* (1999) and ranged from 2 to 5 with a mean of 3.80. An additive QTL accounting for 5, 7.5, or 10% of the total phenotypic variance in the F_2 was placed midway between a randomly chosen pair of adjacent markers. One-half of the *O* founders, chosen at random, carried increaser alleles and the remainder carried decreasers. Genetic drift during the breeding protocol meant that the proportion *p* of chromosomes carrying the increaser in the final *O* generation varied considerably between simulations. In ~5% of simulations the QTL drifted to fixation, and these runs were discarded. Environmental variance was normally distributed and uncorrelated with the QTL. A total of 1000 simulations were performed for each combination of parameters.

QTL detection: QTL detection was performed using a subset of 10 markers spaced 10 cM apart across the chromosome, corresponding to a genome scan of 150 markers (taking the mouse genome as 1500 cM). QTL detection was deemed successful if the HAPPY analysis of the 10 markers identified an interval with an ANOVA *P* value $<10^{-4}$, corresponding roughly to a genome-wide *P* value of <1.5%. We chose to use a higher threshold

TABLE 3

Power to detect and fine map QTL: *I, O* not distinct

QTL variance (%)	No. F_2		Pr(detect): $P < 1.5\%$	Pr[fine map to within (cM)]			
	Genotyped	Phenotyped		0	1	2	3
5	1000	1000	0.41	0.05	0.14	0.18	0.23
	1500	1500	0.53	0.08	0.23	0.32	0.38
	2000	2000	0.68	0.13	0.31	0.44	0.52
	500	2000	0.55	0.09	0.24	0.32	0.37
	500	3000	0.62	0.13	0.31	0.42	0.48
	1000	2000	0.69	0.15	0.34	0.47	0.53
	1000	3000	0.78	0.22	0.44	0.55	0.63
7.5	1000	1000	0.68	0.15	0.37	0.48	0.54
	1500	1500	0.82	0.23	0.54	0.64	0.71
	2000	2000	0.88	0.29	0.62	0.75	0.81
	500	2000	0.82	0.21	0.51	0.65	0.71
	500	3000	0.84	0.25	0.58	0.69	0.74
	1000	2000	0.86	0.27	0.60	0.72	0.77
	1000	3000	0.92	0.31	0.68	0.81	0.86
10	1000	1000	0.80	0.22	0.51	0.62	0.69
	1500	1500	0.85	0.25	0.59	0.70	0.76
	2000	2000	0.91	0.30	0.67	0.79	0.85
	500	2000	0.87	0.26	0.56	0.70	0.76
	500	3000	0.91	0.31	0.66	0.78	0.85
	1000	2000	0.89	0.30	0.65	0.76	0.83
	1000	3000	0.94	0.37	0.73	0.82	0.88

Shown is the probability to detect and fine map a QTL at a 1.5% genome-wide significance level, over a range of QTL effects and numbers of F_2 animals genotyped/phenotyped, and when the inbred line *I* has marker alleles indistinguishable from one of the founders of *O*. Each probability is estimated from 1000 simulations of the inbred-outbred cross.

than is normal (5%) because we wished to reduce the risk of false positives. In addition, the threshold is conservative because the tests are not independent.

The power to detect the QTL is given in Tables 3 (*I* is identical to one of the founders of *O*) and 4 (*I* and *O* are completely distinguishable). Power increases with sample size and genetic variance and when *I* is separable from *O*. For example, for a QTL accounting for 5% of the phenotypic variance, genotypes from 2000 animals produce 80% power to detect the QTL with markers that can distinguish *I* from *O* compared with only 68% otherwise.

Tables 3 and 4 also show that power is increased significantly by selective genotyping, *i.e.*, phenotyping a larger number of F_2's and then genotyping only the extremes. Simulations were performed with populations of 1000, 2000, and 3000 F_2 individuals, from which only the extremes (500/3000 = 17%, 500/2000 = 25%, 1000/2000 = 50%, and 1000/3000 = 33%) were genotyped. A 5% QTL can be detected with 77% power in a selected sample of 1000 out of 2000 individuals.

In Figure 3 the detection *P* value is plotted against p, the proportion of chromosomes in the final HS generation that carry the modifier, for 5% QTL effect, 1500 animals, and informative markers. Figure 3 shows that the probability of detecting the QTL increases with p as expected, but that there is considerable variation in the significance level achieved at a given p, due perhaps to variation in the information content of markers near the QTL. The effect of genetic drift on the QTL is also shown; starting from $p = \frac{1}{2}$ in the founders, p is roughly uniformly distributed in the final generation of the HS.

Fine mapping: QTL fine mapping using 100 markers spaced 1 cM apart across the chromosome was performed only when the QTL detection was significant. The position of the QTL was estimated as the fine-map interval with the smallest ANOVA *P* value, using a version of HAPPY modified to analyze the nonhomogeneous chromosome structure. The fine-map error was defined as the number of marker intervals separating the QTL's true and predicted positions. A fine-map error of n intervals implies that the distance between the true and predicted positions lies between $n - \frac{1}{2}$ and $n + \frac{1}{2}$ cM.

The resolution of the fine-mapping step over a range of QTL effects, sample sizes, and selection regimes is summarized in Tables 3 and 4. The resolution is expressed in terms of the probability that the QTL is both detected and fine mapped to a 1-cM interval 0, 1, 2, or 3 cM from the correct interval. In general, fine-mapping

TABLE 4

Power to detect and fine map QTL: *I*, *O* distinct

QTL variance (%)	No. F_2		Pr(detect): $P < 1.5\%$	Pr[fine map to within (cM)]			
	Genotyped	Phenotyped		0	1	2	3
5	1000	1000	0.59	0.10	0.25	0.34	0.40
	1500	1500	0.69	0.15	0.38	0.47	0.52
	2000	2000	0.80	0.22	0.51	0.63	0.69
	500	2000	0.70	0.15	0.39	0.49	0.55
	500	3000	0.77	0.19	0.42	0.56	0.63
	1000	2000	0.77	0.20	0.46	0.58	0.65
	1000	3000	0.86	0.25	0.55	0.68	0.76
7.5	1000	1000	0.60	0.12	0.30	0.40	0.44
	1500	1500	0.71	0.18	0.40	0.50	0.56
	2000	2000	0.81	0.20	0.48	0.62	0.69
	500	2000	0.71	0.16	0.40	0.50	0.57
	500	3000	0.79	0.20	0.46	0.58	0.66
	1000	2000	0.78	0.21	0.42	0.57	0.65
	1000	3000	0.86	0.21	0.54	0.67	0.75
10	1000	1000	0.69	0.16	0.39	0.49	0.54
	1500	1500	0.79	0.20	0.47	0.61	0.67
	2000	2000	0.85	0.27	0.55	0.70	0.76
	500	2000	0.77	0.17	0.45	0.58	0.65
	500	3000	0.85	0.22	0.49	0.64	0.72
	1000	2000	0.83	0.22	0.52	0.65	0.73
	1000	3000	0.90	0.27	0.58	0.73	0.80

Shown is the probability to detect and fine map a QTL at a 1.5% genome-wide significance level, over a range of QTL effects and numbers of F_2 animals genotyped/phenotyped, and when the inbred line *I* has marker alleles distinguishable from all of the founders of *O*. Each probability is estimated from 1000 simulations of the inbred-outbred cross.

efficiency increases with QTL effect and sample size, marker informativity, and when extremes are genotyped. Thus for a 5% effect QTL with 2000 animals and *I* and *O* separable there is a 69% probability of fine mapping the QTL to within 3 cM and a 65% probability if only the extreme 1000/2000 are genotyped. Table 4 shows

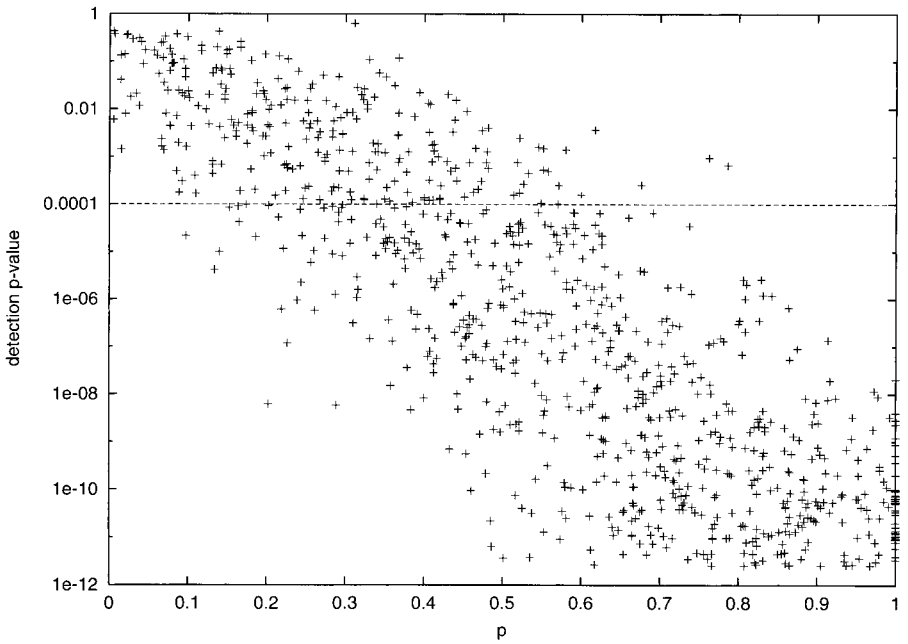

FIGURE 3.—QTL detection depends on the separation between *I* and *O* chromosomes. The *P* value for QTL detection from 1000 simulations of the inbred-outbred cross for an additive 5% QTL effect size, 1500 F_2 animals, and markers that can distinguish *I* from *O* is shown. *p* is the proportion of *O* chromosomes that differ from the inbred line *I* at the QTL. The horizontal line indicates the approximate threshold for <1.5% genome-wide significance.

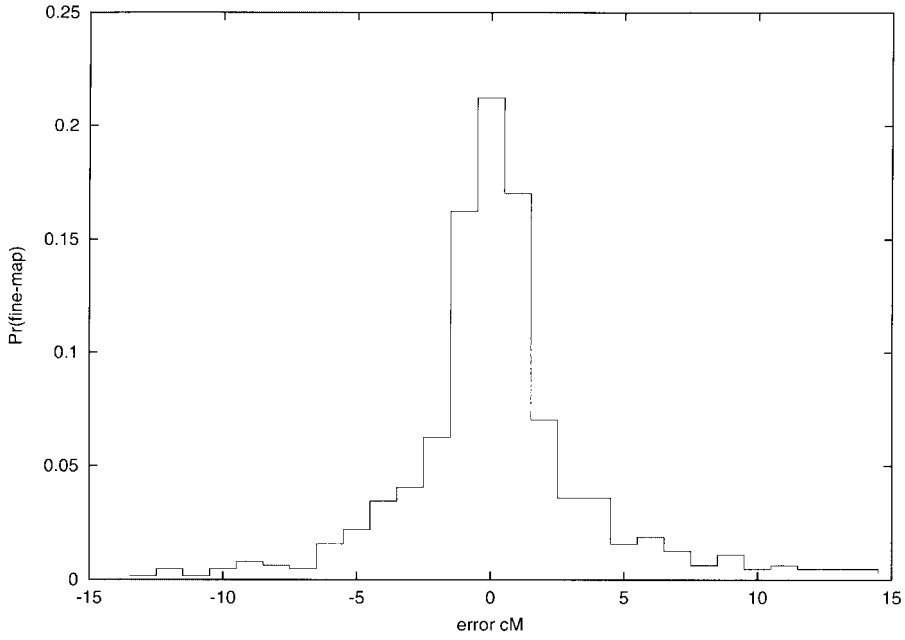

FIGURE 4.—Spatial distribution of QTL fine-map precision. Data are from 1000 simulations of the inbred-outbred cross for an additive 5% QTL effect size, 1500 F_2 animals, and markers that can distinguish *I* from *O*, expressed as the probability that the QTL was fine mapped, given it was detected.

that there is only a small reduction in power with selective genotyping, yet at a significant reduction in genotyping cost. Figure 4 shows the distribution of the fine-mapping error for one simulation set.

In a few instances the fine-mapping step completely failed to localize the QTL. In these cases usually the fine-mapping *P* value was almost flat over a wide block of intervals, indicating behavior akin to a detection experiment. Consequently it should be possible to identify likely failures from the shape of the fine-map curve or by bootstrapping.

DISCUSSION

We have presented an experimental design to detect and fine map QTL segregating in HS outbred mice. The method overcomes a potential limitation of using the HS for fine mapping, namely the prohibitively large number of genotypes required for a genome scan, and in addition enables the simultaneous detection and fine mapping of modifier loci when the inbred line used in the cross carries a mutation. Depending on the effect size of the QTL, in the order of 1000–2000 animals are required to detect a QTL with 80% power. Selective genotyping reduces this number to nearer 1000. In most cases there is at least a 50% probability that the QTL will be both detected and fine mapped to within 3 cM.

In general we expect to find multiple loci influencing a trait. For instance, in our previous work (MOTT *et al.* 2000) we fine mapped five loci that had been identified previously in F_2 intercrosses. More QTL should segregate in the HS than in an intercross between only two inbred lines, so we expect a genome scan using the eight founders of the HS to detect perhaps double this number of loci. Applying the inbred-outbred cross, we would be able to fine map over one-half of these. Consequently the method should yield a sufficient number of fine-mapped candidate regions for further study in an average-sized laboratory.

The analysis relies on knowing the probability of whether the inbred line or one of the founders of the HS was present at any position on the genome, conditional upon the observed marker data. We implemented the calculation as a dynamic-programming algorithm in the HAPPY package, which is able to carry out a complete analysis of an inbred-outbred cross, including an analysis of variance to test for QTL significance in each marker interval. The required input data are the phenotypes and marker genotypes from the F_2 animals, the genotypes of the inbred progenitor strains of the HS and of the inbred line, and the genetic distances between the markers. No pedigree data are used. The package is available free from http://www.well.ox.ac.uk/happy.

The major advantage of the inbred-outbred cross over other experimental designs is that it provides high QTL genome-wide mapping resolution in a short period of time. One obvious application will be in mapping modifiers that influence the phenotype either of transgenic models of human disease or of mutants produced by mouse mutagenesis projects. A disadvantage is that unless the QTL segregates within the HS, the ability of the inbred-outbred cross to fine map the locus will be no better than a standard F_2 or backcross.

This strategy is within the reach of most laboratories. The expense of performing the inbred-outbred cross, in the form described here, is divided between animal and genotyping costs. The strategy requires 400 F_1's as 200 breeding pairs and phenotypes from $\sim$1500 F_2's. The genome scan, without selection, using 150 markers, will need 225,000 genotypes. At the fine-mapping stage,

a further ~50 markers are needed for each QTL identified or 75,000 genotypes for the 1500 mice. If there are 10 QTL in the genome and 80% are detected, then ~750,000 genotypes will be required in total. Selection significantly reduces the amount of genotyping required (by up to 50%), at the cost of approximately doubling the phenotyping effort and F_2 animal costs. Alternatively, genotyping could be reduced by one-quarter without selection, once the QTL has been localized to ~10–20 cM, by noting that ~25% of the animals should be inbred line homozygotes *I-I* over the region and hence cannot contribute to the fine mapping. These individuals are identifiable from the genome scan and need not be genotyped further. Hence the effort could be easily reduced to about half a million genotypes.

Our simulations point to some important considerations for the experimental design. First, obtaining adequate power to detect a QTL in the inbred-outbred cross requires more animals than for an F_2 intercross. However, it should be noted that the QTL effect sizes are not directly comparable between the two designs. All the QTL variance in the F_2 intercross is available for QTL detection, whereas only a part contributes to detection in the inbred-outbred cross, depending on the proportion p of HS chromosomes carrying a different allele from the inbred. We cannot know or control that proportion. The variance available for QTL detection in the F_2 can be significantly less than the genetic variance originally present in the HS. For example, if $p = \frac{1}{2}$ and the QTL accounts for 5% of the total variance in the F_2 generation, then the variance available for detection is <2%. It should be recognized that there is some ambiguity in the definition of genetic variance: different results might be obtained if, for instance, the genetic variance were fixed in the initial HS generation rather than in the F_2. We deliberately ignored simulation runs that had drifted to fixation at the QTL; were they taken into account, the power might be reduced slightly.

Second, fine-mapping resolution can be increased further by spacing markers closer together, although there is a limit to the density of markers that can be usefully employed. Theoretically, resolution depends on the structure of the haplotypes present in a region surrounding the QTL in the HS population, itself a function of the number of generations G since the HS was founded and the effective HS population size. This must be $<100/GN$ cM, where N is the number of HS chromosomes. Using 40 mating pairs over 60 generations gives a lower bound of 100/2400 or 0.02 cM. Genetic drift, operating to decrease heterozygosity over time, makes this limit highly improbable. Our experience of mapping QTL in the HS suggests that markers spaced closer than 0.1 cM do not help to increase resolution. Furthermore the current mouse genetic and radiation-hybrid maps cannot resolve the order of very closely spaced markers, although this will change when the mouse genome is sequenced.

Third, we stress the advantages of using markers whose alleles are different in the inbred line and the outbreds. In practice, most mouse knockout mutants originate on a 129 strain, which is not a founder in any current HS population. Consequently it should be possible to choose, at least in the detection phase, informative markers for mapping crosses between knockout lines and the HS. Microsatellite markers are preferable to SNPs because of their cost and information content. Because a SNP has only two alleles, compared with about four in a microsatellite in the HS, we would need to double the number of markers in the fine-mapping phase, but the ease and accuracy of SNP typing may make them the marker of choice in the future.

We have not explored all possible extensions of this strategy and there are likely to be experimental and analytical modifications that will improve the efficiency of both detection and high resolution mapping. These include variations in the breeding design and the use of variance components approaches (Searle *et al.* 1992) to extract information from families (McPeek 2000). Furthermore, genotyping the F_1 generation may enable us to detect genotype errors and determine the phase of the F_2 genotypes, which should increase power. Mixture models, fitted using Markov chain Monte Carlo algorithms, may also be useful, for instance, when a number of linked QTL are present (Lange 1997; Sillanpaa and Arjas 1998; Xu and Yi 2000; Balding *et al.* 2001).

An alternative breeding strategy would be to use F_1 animals for fine mapping, instead of the F_2 generation (as discussed briefly in Mott *et al.* 2000). However, for an additive QTL the genetic variance in the F_1 will be less than in the F_2, so more animals will be required to obtain the same accuracy as the F_2 strategy. Furthermore the detection step still requires genotypes from the F_2 generation. On the other hand, typing F_1's may be more appropriate when mapping modifiers with dominant or epistatic effects (Abney *et al.* 2000). Another possibility would be to use an advanced intercross line, such as an F_3 or F_4 in place of the F_2; more of the genetic variance would be then available for fine mapping, but at the cost of additional breeding and a higher density of markers required during the genome scan.

In this study we used variants of one experimental design, namely 40 HS mating pairs and 400 F_1 animals. This is appropriate for a modifier screen in which a litter of 10 F_1's is bred from each HS animal, of which one-half carry the mutation. However, if the mutant reduces fertility it may be necessary to breed the F_2's from a smaller number of F_1's or to produce more litters of F_1's. These may alter the power to detect and fine map QTL. Simulations (not shown) indicate it is important to maintain the genetic heterogeneity in the F_1 generation. Since there are 160 HS chromosomes present, 320 F_1's are required to give at least the possibility that all this genetic material is still present in the F_1 generation.

The ability to fine map QTL to a resolution approach-

ing 1 cM throughout the genome represents a substantial advance over competing methodologies. However, investigations in Drosophila suggest that, rather than being caused by a single molecular change, a QTL can comprise a tight cluster of interacting polymorphisms (Long *et al.* 1996) and it is likely that mammalian QTL will exhibit a similar hidden complexity. Therefore, in addition to fine-scale genetic mapping, QTL analysis calls for an integrated repertoire of tools, including the completed and fully annotated sequences of the human and mouse genomes, gene expression data (Aitman *et al.* 1999), and chromosomal engineering techniques (Ramirez-Solis *et al.* 1995). Determining the molecular basis of the QTL remains a formidable challenge and will occupy researchers for some time to come.

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