Mapping quantitative trait loci for body weight on the X chromosome in mice. II. Analysis of congenic backcrosses

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Summary
In a QTL mapping study with an F2 population of mice, we have shown that one or more sex-linked factors account for a large part of the divergence between mouse lines selected for high and low body weight. Here, we describe a study undertaken to map the putative X-linked quantitative trait loci (QTLs) by backcrossing segments of chromosome from the high line onto an inbred line derived from the low line, thereby removing possible contributions from the autosomes and linked segments of the X chromosome. Sublines containing a regional at the proximal end of the X chromosome were found to be associated with large differences in body weight, and to account for almost all the difference between the lines. A Markov chain Monte Carlo based multipoint linkage analysis incorporating the available marker and phenotypic information from the backcross pedigree was used to map the QTL to a region of about 6 cM. There was no evidence for QTLs elsewhere on the chromosome. The estimated QTL effect is approximately 20% of mean body weight in males and females at 10 weeks.

1. Introduction
Previous analysis of the genetic differences between mouse lines selected for growth rate has shown evidence for a large X chromosome effect accounting for approximately 25% of the divergence between the lines (Hastings & Veerkamp, 1993; Veerkamp et al., 1993; Rance et al., 1994). An accompanying study (Rance et al., 1997) has indicated the presence of a major locus for body weight at approximately 23 cM (95% confidence interval) on the X chromosome, with an estimated effect of 5.2 g (about 20%) on 10 week weight in both males and females.

Simulation studies have shown that F2 and first backcross (BC1) experimental designs, often employed in QTL mapping experiments, do not allow the mapping of quantitative trait loci (QTLs) with great precision (van Ooijen, 1992; Darvasi et al., 1993), largely due to genetic noise from linked and unlinked QTLs elsewhere on the genome, and due to the lack of recombination events. Analytical methods for mapping QTLs have been developed to remove genetic noise from linked and unlinked QTLs by fitting linked or unlinked markers as cofactors in the analysis (Jansen, 1993, 1996; Zeng, 1994). These methods require segregating populations to be genotyped at markers spanning the entire genome in order to allow the removal of background genetic noise. An experimental alternative to this analytical procedure, used in the present study, is to progressively backcross regions of the genome of interest onto an inbred background.

A progressive backcross approach has been employed to study the genetic basis of variation in bristle number in Drosophila (Shrimpton & Robertson, 1988a, b), involving the use of morphological markers to maintain specific regions of the third chromosome on a common genetic background, and to generate several congenic lines. This experimental design can be more powerful than a simple F2 or BC1 population, as the congenic lines can be assumed to be genetically identical in all regions of the genome unlinked to the congenic region of interest. The objective of the experiment described in this paper was to backcross
specific regions of the high line X chromosome onto an inbred low line background, as a means of localizing the X-linked QTL, and to provide a basis for fine-scale genetic mapping.

2. Materials and methods

(i) PCR amplification of microsatellites

Genomic DNA was extracted from spleen or ear clip samples by phenol:chloroform extraction. The polymerase chain reaction (PCR) amplification protocol used to genotype animals from BC generations 1–3 was based on the method described by Dietrich et al. (1992). Genotyping of animals in BC generations 4 and 5 was carried out with improvements described by Routman & Cheverud (1994), in which the 55 °C annealing temperature was reduced to 50 °C to improve the signal/noise ratio, and with the addition of 1/5 volume of loading dye (60% sucrose, 1.0 mM cresol red) prior to PCR amplification.

(ii) Backcross population

The mouse lines used in this study (the P6 lines) were divergently selected for body weight for more than 50 generations. At generation 50 of selection, the mean body weights were 49.3 g and 17.3 g in the high and low lines respectively (Mbaga, 1996). The origins of the lines have been described previously by Sharp et al. (1984) and Hastings & Hill (1989). To initiate the backcross lines, females from generation 51 of selection of the P6 high line and males from the P6 low inbred line, at generation 6 of full sib inbreeding (set up at generation 45 of selection), were mated to produce an F1 population. F1 females were mated to inbred low line males (generation 7 of inbreeding) to form generation 1 of the progressive backcross population (BC1):

P6 high line (gen. 45) × P6 low inbred (P6 LI) (gen. 6)

\[ \downarrow \]

F1 × P6 LI (gen. 7)

\[ \downarrow \]

BC1 × P6 LI (gen. 8)

\[ \downarrow \]

BC2 × P6 LI (gen. 9)

\[ \downarrow \]

BC3 × P6 LI (gen. 10)

\[ \downarrow \]

BC4 × P6 LI (gen. 11)

\[ \downarrow \]

BC5 × P6 LI (gen. 12)

Following DNA extraction from all the parents and the BC1 females, these individuals were genotyped at the loci shown in Table 1 (with the exception of DXMit187), which span the length of the X chromosome. The genotypes were used to find marker alleles in the BC1 females which could be traced unequivocally to the high selection line or the inbred low line. Female BC1 individuals were selected to be dams of the BC generation 2, where the markers genotyped were fully informative.

The objective of the mating scheme was to maintain segments of the high line X chromosome in a heterozygous state in the BC population dams (using markers spanning the length of the X chromosome). To do this, all backcross individuals were typed at the loci listed in Table 1. An example of the conservation of an X chromosome segment is shown in Fig. 1. Assuming no double crossovers between flanking markers, the chromosome segment between markers B and D would be inherited from the high selection line, and marker alleles a and e inherited from the inbred low line. The proportion of the high line X chromosome between the markers a and B, and D and e, would also tend to decline with each generation of backcrossing.

The mating scheme was designed to establish a number of lines carrying overlapping segments of the X chromosome inherited from the high selection line, carried on the genetic background of the inbred low line. It should be noted that uniform segments were not obtained until later generations. In BC generations 3, a fully informative marker DXMit187 at 12.2 cM (Table 1) was added to the panel of eight markers used previously. The data collected during the population maintenance are summarized in Table 2.

(iii) Effects associated with chromosome segments

An analysis was carried out to estimate effects associated with carrying segments of the high line X chromosome flanked by known marker loci. The numbers of individuals carrying each segment were very small within each generation, because uniform segments were not established until the final generation

<table>
<thead>
<tr>
<th>Marker</th>
<th>Map position (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXMit55</td>
<td>0.0</td>
</tr>
<tr>
<td>DXMit187</td>
<td>12.2</td>
</tr>
<tr>
<td>DXMit50</td>
<td>19.8</td>
</tr>
<tr>
<td>DXMit25</td>
<td>33.7</td>
</tr>
<tr>
<td>DXMit16</td>
<td>41.9</td>
</tr>
<tr>
<td>DXMit79</td>
<td>52.6</td>
</tr>
<tr>
<td>DXMit38</td>
<td>56.0</td>
</tr>
<tr>
<td>DXMit121</td>
<td>71.4</td>
</tr>
<tr>
<td>DXMit31</td>
<td>72.0</td>
</tr>
</tbody>
</table>

* Map positions are from the accompanying F2 study (Rance et al., 1997).
Fig. 1. An example of the maintenance of a high line X chromosome segment on the inbred low line background, with some examples of the possible backcross progeny.

Table 2. Numbers of successful matings and individuals weaned in each generation of the backcross, and the traits recorded

<table>
<thead>
<tr>
<th>Generation</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of litters</td>
<td>No. weaned</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>BC1</td>
<td>15</td>
<td>62</td>
</tr>
<tr>
<td>BC2</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>BC3</td>
<td>14</td>
<td>90</td>
</tr>
<tr>
<td>BC4</td>
<td>17</td>
<td>96</td>
</tr>
<tr>
<td>BC5</td>
<td>22</td>
<td>123</td>
</tr>
</tbody>
</table>

* DNA available, but no genotypes recorded.

Table 3. Means and variances of 6 and 10 week body weights for the F<sub>1</sub> and BC generations (corrected for the fixed effects and covariates)

<table>
<thead>
<tr>
<th>Generation</th>
<th>6 week weight (g)</th>
<th>10 week weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Variance</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>25.0</td>
<td>5.3</td>
</tr>
<tr>
<td>BC1</td>
<td>18.4</td>
<td>5.3</td>
</tr>
<tr>
<td>BC2</td>
<td>15.1</td>
<td>1.3</td>
</tr>
<tr>
<td>BC3</td>
<td>18.2</td>
<td>4.0</td>
</tr>
<tr>
<td>BC4</td>
<td>17.6</td>
<td>3.1</td>
</tr>
<tr>
<td>BC5</td>
<td>17.3</td>
<td>3.1</td>
</tr>
</tbody>
</table>

An objective of the backcrossing strategy was to obtain an estimate for the X-linked QTL position and effect, which will provide information independent of
that already obtained from the analysis of the F₂ population (Rance et al., 1997). To enable all the information collected in the different generations to be included in the analysis, the pedigree structure of the population needs to be accounted for. In the analysis of an F₂ or a simple first generation backcross population, using maximum likelihood or multiple regression methods (Lander & Botstein, 1989; Haley & Knott, 1992; Martinez & Curnow, 1992), the parental genotypes are assumed to be known, i.e. the founder lines are assumed to be fixed at different QTL alleles, and all the F₁ parents are heterozygous at the QTL. As a result of this assumption, the F₂ or first generation backcross individuals can be considered independently. The pedigree structure of the progressive BC population implies that all animals cannot be considered to be independent (with the exception of animals within the first generation of backcrossing). The individuals within generations are dependent on common ancestors in previous generations, resulting in dependencies across generations, and individuals from different litters may have ancestors in common in the previous generation, leading to dependencies within generations.

Another problem with dealing with more complicated pedigrees than result from F₂ or first generation backcross designs is that the markers are not equally informative. The markers flanking an interval do not provide all the information about the likelihood of a QTL within that interval, so to extract all the information about the position of a QTL on the chromosome a multipoint analysis simultaneously fitting all markers should be performed.

(v) Use of MCMC for genetic analysis

Accounting for missing genotype information in a genetic analysis is done by summation over all possible genotype configurations consistent with the data. There are two known exact algorithms for this summation: the peeling algorithm, proposed by Elston & Stewart (1971) and generalized by Cannings et al. (1978), and the Lander–Green algorithm (Lander & Green, 1987). Both methods are restricted in the types of problems they can be used for. The complexity of peeling is very roughly linear in pedigree size, but exponential in the number of loci being considered, and is thus limited to a relatively small number of loci. The reverse is true for the Lander–Green algorithm, which is roughly linear in the number of loci, but exponential in pedigree size. The number of loci and the pedigree size for the analysis described here precluded either of the above algorithms, and necessitated the use of sampling-based approaches.

The idea of a sampling-based approach is that rather than exact summation over possible genotype configurations, genotype configurations are generated at random, and Monte Carlo estimates of probabilities obtained (Thompson, 1994). For example, to get a Monte Carlo estimate of the probability of our observed data Y (genotypic or phenotypic) given some model parameters θ, we could generate N genotype configurations Gᵢ, i = 1 … N at random and then calculate the Monte Carlo estimate of p(Y|θ) as:

\[
\hat{p}(Y|θ) = \frac{1}{N} \sum_{i=1}^{N} p(Y|Gᵢ, θ)p(Gᵢ, θ).
\]

This can be inefficient for all but simple problems because typically the vast majority of genotype configurations produced have zero or negligible probability given Y, and so contribute nothing to the summation. A more efficient scheme would be to sample the genotype configurations Gᵢ conditional on Y (and θ). Sampling directly from p(G|Y, θ) is not possible without knowing p(Y|θ), the quantity we want to obtain. Markov chain Monte Carlo (MCMC) (Metropolis et al., 1953; Hastings, 1970) methods can be used to produce correlated samples from distributions known only up to a normalizing constant, so can be used to sample from

\[
p(G|Y, θ) \propto p(Y|G, θ)p(G|θ).
\]

The drawback of this approach is that because the genotype samples are correlated, more samples are required to produce a Monte Carlo estimate of a given degree of accuracy than if independent samples were used.

As well as handling missing genotypes, the MCMC approach can also be used in cases where the other model parameters, θ, are also unknown. If we define a joint distribution for all model parameters (treating the genotypes G as parameters), then MCMC can be used to sample from this joint distribution. Estimates of the marginal posterior distribution of any parameter, or group of parameters, of interest can be obtained using this approach. For example, in the context of QTL mapping the model parameters would include the effect and position of the QTL.

(vi) Model for MCMC analysis

A MCMC approach was used in this study to perform the linkage analysis of the progressive BC population. The genetic effect of the trait was modelled as due to a single sex-linked QTL and a polygenic effect. Litter was fitted as a random effect (uncorrelated to either of the genetic effects); parity was fitted as a fixed effect; number weaned from a litter and percentage high line genotype were fitted as linear covariates (this last covariate was used to account for the changes in the means and variances of body weight due to the changing percentage of high line autosomal loci). For the QTL, separate effects were estimated for males and females.

Uniform priors were placed on all model parameters and the MCMC sampler was used to integrate out the ‘unwanted’ parameters. In the case of certain par-
ameters, such as QTL position and effects, there could be prior information available about their distribution; using the MCMC framework, this prior information could be easily incorporated into the analysis. Fitting a uniform prior, while not strictly uninformative, imposes little prior constraint on the parameter values. The existence of strongly peaked posterior distributions for the model parameters (as in this study) indicates that there is a lot of information in the data, and therefore the prior is relatively unimportant in this case.

(vii) MCMC sampling scheme

A typical MCMC sampling scheme is as follows: All model parameters are set to some initial values such that their joint probability is $>0$. Each parameter is updated either singly or in blocks by proposing an update from some known distribution, calculating an acceptance probability $A$ for the update, and then accepting the update with probability $A$. If the update is not accepted then the parameter value remains unchanged. For the current analysis, two types of update steps were used: Metropolis–Hastings steps (Hastings, 1970) and Gibbs steps (Geman & Geman, 1984). With a Metropolis–Hastings step, the proposal distribution for the update can be almost arbitrary. A Gibbs update step is a special case of this in which a parameter is updated by sampling from its distributional conditional on the current values of all the other model parameters. With a Gibbs update step the acceptance probability $A$ is always 1, so the update is always accepted. More details on these update steps, and their use in genetic analysis, is given in Thompson (1994). The sampling scheme used for the analysis was as follows:

1. update complete marker genotypes (including phase) for each marker locus in turn;
2. update QTL effects;
3. update QTL position;
4. update QTL genotypes;
5. update polygenic effects;
6. update variance components (polygenic and environmental);
7. update fixed effects and covariates;
8. repeat.

All these update steps were Gibbs steps, apart from the updating of QTL position which used a Metropolis–Hastings step, with a proposed new position for the QTL being sampled from a uniform distribution along the chromosome. The genotypes (i.e. at QTL and missing marker genotypes) for all individuals were sampled simultaneously for each locus in turn conditional on the marker observations, the trait values, the model parameters, and the genotypes at other loci. This sampling scheme was suggested by Kong (1991) as a means of improving the mixing characteristics of the sampler, and differs from the sampling schemes typically used in genetics applications, where the genotypes at a given locus are updated on an individual-by-individual basis (e.g. Guo & Thompson, 1992; Heath, 1994). The scheme has some similarities to the blocking Gibbs sampler (Jensen & Kong, 1997) where a portion of the pedigree sampler is updated simultaneously. Sampling of the other model parameters such as the QTL effects, polygenic breeding values, covariate effects and variance components was performed in essentially the same way as described by Wang et al. (1993) and Heath (1994). The procedure was extensively tested on simulated data (Heath et al., 1997).

(viii) Estimating posterior distributions

Estimates of the posterior marginal distribution of any parameter of interest can be obtained from the sequence of realizations for that parameter produced by the MCMC scheme. For the analysis presented in this paper, estimates were obtained of the posterior densities of the QTL positions and effects. The confidence intervals for the QTL positions were obtained from the $±2\ SE$ of the mean position as proposed by Durvasi et al. (1993).

3. Results

(i) Changes in the distribution of traits measured over the generations of backcrossing

Means and variances of the body weight traits measured in the BC population, corrected for the fixed effects and covariates, are shown for each generation in Table 3. Mean 6 week and 10 week body weights tended to decline with each generation of backcrossing to the inbred low line, with the exception of generation 2 for which 6 week weights were available only on females. The reduction in mean body weights were also associated with a reduction in the variance of the body weight measurement. This reduction is expected, as the percentage contribution of high line autosomal linked QTLs for body weight decline by an average of 50% with each generation of backcrossing. In the BC5 generation, individuals carry on average 1.56% high line autosomal alleles.

(ii) Effects associated with chromosome segments

An analysis was carried out to estimate the effects associated with carrying specific segments of the high line X chromosome on the inbred low line background. The segment effects estimates (Table 4) represent the difference between the two hemizygous genotypes in males ($2a$) and the difference between the homozygous low and heterozygous genotypes in females ($a + d$). In males, the marker segments at the most distal end of the X chromosome (between $DXMit16$ and $DXMit31$, segments 1–3) are associated with small or negative differences in body weight (these differences are not
Table 4. Mean effect (g) for 10 week weight associated with segments of the X chromosome in males and females from generations 3, 4 and 5 of the progressive backcross population (standard errors, SE, shown in parentheses)

<table>
<thead>
<tr>
<th>Estimated mean effect (g)</th>
<th>Segment no.</th>
<th>DXMit marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (SE)</td>
<td>Females (SE)</td>
<td>55</td>
</tr>
<tr>
<td>0·48 (0·89)</td>
<td>-2·74 (2·00)</td>
<td>1</td>
</tr>
<tr>
<td>-0·15 (0·96)</td>
<td>-1·05 (0·59)</td>
<td>2</td>
</tr>
<tr>
<td>-0·38 (0·72)</td>
<td>-1·80 (0·91)</td>
<td>3</td>
</tr>
<tr>
<td>4·26 (0·95)</td>
<td>2·03 (0·67)</td>
<td>4</td>
</tr>
<tr>
<td>4·41 (0·75)</td>
<td>1·82 (0·59)</td>
<td>5</td>
</tr>
<tr>
<td>3·81 (0·57)</td>
<td>1·09 (0·42)</td>
<td>6</td>
</tr>
<tr>
<td>1·11 (0·83)</td>
<td>-0·12 (0·77)</td>
<td>8</td>
</tr>
<tr>
<td>5·50 (0·84)</td>
<td>1·65 (0·78)</td>
<td>9</td>
</tr>
</tbody>
</table>

The ticks represent markers which can be traced unequivocally to the high selection line. The remaining markers on the X chromosome can be traced to the low inbred line.

significantly different from zero, \( P > 0·05 \). The estimated effects associated with the segments containing markers at the most proximal end of the X chromosome (between \( \text{DXMit55} \) and \( \text{DXMit16} \), segments 4, 5, 8 and 9) are significantly different from zero (\( P < 0·05 \)), and explain approximately 4 g of the difference in mean body weight between the two hemizygote segment groups in males. The estimated effect associated with marker segment 7 (\( \text{DXMit55} \) to \( \text{DXMit187} \)) was not significantly different from zero, however. The same trends are present in the females from the BC generations 3, 4 and 5, although the estimated effects were approximately half of the estimates in males. Similar trends can be seen in the estimates associated with segments for 6 week body weight (data not shown). It should be noted that the origin of the segments of X chromosome between high and low line markers (i.e. where there has been a recombination event) are not precisely known, but with each generation of backcrossing the percentage of high line X chromosome between these markers will tend to decline. The results obtained from the segment analysis show that the distal end of the X chromosome (\( \text{DXMit16} \) to \( \text{DXMit31} \)) is not associated with a QTL for body weight, but there is a significant association between the proximal end of the X chromosome and body weight at 6 and 10 weeks (between markers \( \text{DXMit50} \) to \( \text{DXMit25} \)).

(iii) MCMC sampling

Fig. 2 show the estimated marginal posterior distribution of the QTL position, using a uniform prior over the whole X chromosome for the QTL position. The distribution was obtained from the frequency at which the QTL was estimated as being at each position along the chromosome. The plot shows only the region of the X chromosome of 21 cM to 32 cM, as the probability of the QTL in all sections outside this area was effectively zero. The mean estimated QTL position was 25·4 cM with an estimated 95% confidence interval of \( \pm 2·8 \) cM for 10 week weight, and \( 26·9 \pm 3·0 \) cM for 6 week weight. The analysis therefore points to the QTL position being within the marker interval \( \text{DXMit50} \) (19·8 cM) to \( \text{DXMit25} \) (33·7 cM). The irregular shapes of the curves in Fig. 2 are due to Monte Carlo (sampling) error.

The mean QTL effects estimated using the MCMC method (when the QTL effects were sampled at the mean estimated QTL position) are presented in Table 5. The estimated marginal posterior distribution for

![Relative probability of QTL](image-url)
QTL effects (Fig. 3) shows that the sampled QTL effects are approximately normally distributed. The mean QTL effects are similar in males and females. If we assume no dominance in the females, the estimated QTL effects (representing the difference between the hemizygous males and 2(Aa−aa) in females) were approximately 3 g at 6 weeks and 4 g at 10 weeks in both males and females. These estimated QTL effects are close to those obtained using the segment analysis (Table 4).

4. Discussion

(i) Effects of marked X chromosome segments

The estimated body weight effects associated with segments at the most distal end of the X chromosome (those segments containing markers DXMit79 to DXMit3) are not significantly different from zero in both males and females. However, segments at the proximal end of the X chromosome are associated with estimated effects of approximately 4 g in males and between 1 g and 2 g in females. Segment 8 (Table 4) is not associated with a significant effect, implying that the QTL is not in this segment. Segments 4 and 5 overlap between markers DXMit50 and DXMit25, plus an unknown amount flanking them. Taken together with the significant effects associated with segments 6 and 9, the data are consistent with a QTL in the marker interval DXMit50 to DXMit25, or immediately flanking these markers, with a total effect (2a) of approximately 4 g in males and females, assuming no dominance in females. The results do not allow the conclusion that the X-linked QTL is in the interval DXMit50 to DXMit25, as the points at which the recombination break points occurred in the intervals DXMit187 to DXMit50 and DXMit25 to DXMit16 are not known. The position of the QTL can be estimated more precisely by using information from all individuals in the BC pedigree with the MCMC sampling-based method.

(ii) MCMC analysis of the BC population

The results presented in Fig. 2 show the estimated marginal posterior distribution of the QTL position on the X chromosome for 6 week and 10 week weight data. The frequency at which a QTL was sampled outside the interval 21 cm to 32 cm was close to zero, therefore strongly indicating that the QTL is positioned in the marker interval DXMit50 to DXMit25, with no evidence for a second QTL elsewhere on the X chromosome. The estimated positions of the QTL (+95% CI) for 6 week weight and 10 week weight within this interval were 25.4±2.8 cm and 26.9±3.0 cm respectively. The positions estimated for the two body weight measurements therefore show overlapping CI for the QTL position.

(iii) Combining information from the F2 study

Combining the estimated QTL positions obtained from the F2 population described in the associated study (Rance et al., 1997) and the present study points to a single X-linked QTL (or a number of very closely linked QTLs) positioned in the marker interval

\[
\text{DXMit50 to DXMit25}
\]

Fig. 3. Marginal posterior distributions for QTL effects on 10 week weight, shown as the difference between the hemizygotes in males and the difference between the homozygous low and heterozygous genotypes in females.

Fig. 4. Crossing scheme for progeny test with female recombinants.
DXMit50 to DXMit25. Pooling the estimates for the QTL positions obtained in the two populations by weighting the estimates by the reciprocal of their sampling variance, the estimated QTL position (± SE) was 24.6 ± 1.2 cM for 10 week weight and 25.8 ± 1.3 cM for 6 week weight. It should be noted that the QTL positions were estimated using two different methods in the two experiments: by multiple regression in the F2 population, and by MCMC sampling assuming uniform priors for the QTL parameters in the progressive backcross population. When the estimates are expressed as a percentage of the mean body weight, the QTL appears to have a very similar percentage contribution in both populations, which suggests the X-linked QTL has a multiplicative effect on mean body weight. The single X-linked QTL appears to explain almost the whole contribution of the X chromosome, increasing mean body weight by approximately 18%.

(iv) Strategy for positional cloning

QTL mapping is necessarily a multi-stage process, with a logical end point of map-based cloning, but so far there have been no reports of the positional cloning of QTLs. It is becoming apparent that the effort involved in fine-scale mapping of a QTL exceeds that for an initial screen of the entire genome. Mapping to a sub-centimorgan level is required to positively clone any gene, and ultimately depends on knowing the phenotype of individuals containing rare recombination events. In fine-scale mapping of QTLs, an additional problem is the lack of a one-to-one correspondence between a recombinant individual’s phenotypic value for the trait and its QTL ‘genotype’, so each recombinant provides less information than for mapping a Mendelian locus. As proposed by Lander & Botstein (1989), ‘progeny testing’ of individuals known to be recombinant between markers flanking a region containing a QTL can allow the same resolution as for mapping a Mendelian locus.

A progeny testing scheme suitable for an X-linked QTL is shown in Fig. 4. A suitable starting point for the cross would be a congenic line containing high line markers DXMit50 and DXMit25, which flank the QTL, on the low line inbred background. Females heterozygous for the segment would be crossed with low line inbred males to generate progeny recombinant between the two flanking microsatellite markers, detected by genotyping at the flanking markers. Such recombinant females would then be crossed to inbred low line males, causing the recombinant segment to segregate in their male progeny. If the QTL is in the recombinant segment, a large difference in body weight would be observed between individuals containing or not containing the segment. The exact position of the recombination break point in the recombinant individual, base on further markers, would be the information required to fine-scale map the QTL, and to allow it to be cloned by the same strategies as for a Mendelian locus.

References


