Testing the correspondence between map positions of quantitative trait loci

PETER D. KEIGHTLEY* AND SARA A. KNOTT
Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

(Received 15 April 1999 and in revised form 9 July 1999)

Summary

There are several instances in which quantitative trait locus (QTL) mapping experiments have been independently carried out for similar traits in different laboratories. We develop a permutation test of the correspondence between the test statistics obtained from genome-wide QTL scans in two such experiments to test whether the same QTLs are segregating in the experimental pair. In simulations, we show that the permutation test has the desired properties if chromosomes are of equal length, but bias can occur if chromosomes are of unequal length, a problem connected with autocorrelation of test statistic values. We apply the test to data from three recent mouse body weight QTL mapping experiments. The results from the test are non-significant, and imply a lack of overall concordance between the QTLs that were segregating in these experiments.

1. Introduction

One of the most active areas of research in quantitative genetics is the analysis of quantitative traits with genetic markers. The aims of these quantitative trait locus mapping experiments are several-fold. In model organisms such as Drosophila, one aim is to obtain an understanding of the genetics of quantitative variation within natural populations. A detailed description of the effects, frequencies and interactions between the alleles at the loci which contribute to quantitative variation in a model trait should lead to an improved understanding of the genetic basis and maintenance of genetic variation for quantitative traits in general. Mice are less well suited to this kind of investigation, so research with natural populations has been focused on the genetics of between-species differences (Zechner et al., 1996). In mice, much effort is also aimed at finding novel genes for specific traits, such as obesity or growth rate, and at locating genes that contribute to responses to artificial selection for traits like these. The first experiment to use a set of genetic markers to locate QTLs in mice was carried out by Ian Garnett and Douglas Falconer (Garnett & Falconer, 1975). They measured allele frequency differences between replicated lines selected high and low for 6-week body weight to test for direct effects of the marker loci themselves or for the presence of linked QTLs for body weight. Since DNA-based markers were not available at this time, electrophoretic differences at nine enzyme loci plus two visible genetic marker loci that segregated in the cross were used. A significant QTL linked to the Hbb locus was found, but Garnett and Falconer could not carry their investigation further due to a lack of genetic markers. The intervening period has seen a revolution in molecular marker technology. There are currently around 6000 microsatellite markers available for mice (Dietrich et al., 1996). QTL mapping experiments in mice and other experimental and commercial organisms have become commonplace, and in many cases independent experiments have been carried out in different populations with similar traits. The issue we address in this paper is whether it is possible to tell if the same QTLs are being discovered in independent experiments.

The basic results from analysis of genome-wide QTL mapping experiments are test statistic values (usually F-ratios or log likelihood ratios) that have been computed at a series of points throughout the genome. The presence of a statistically significant QTL is inferred if the test statistic exceeds a threshold value, usually obtained by permutation of the marker data. One way to test the concordance between two QTL mapping experiments would be to calculate the probability that the test statistic peaks coincide more frequently than expected under a null hypothesis of a
random scatter of test statistic peaks. This method was used by Paterson et al. (1995) to test for the concordance between QTLs for agronomically important traits in various independently domesticated cereal species. Using the test suggested by Lin et al. (1995), Paterson et al. concluded that QTLs in different species affecting a given trait (e.g. seed mass) are often at homologous positions of the genomes in such diverse species as rice, sorghum and maize. However, the definition of a match (when the peaks from different experiments coincide) is somewhat arbitrary, as is the maximum number of identifiable QTL locations possible in the genome. Similarly, Mackay (1995) has also noted a tendency for QTLs for bristle number in Drosophila identified in different experiments to cluster together, although the hypothesis has not been tested formally.

Mackay (1995, 1996) has also presented experimental evidence that QTLs for Drosophila bristles often map to locations near ‘candidate genes’ (loci at which alleles with major mutant phenotypes arise), and there is evidence from quantitative complementation tests that the QTL variants are alleles at these loci or loci that interact with them (Long et al., 1996). To test in a formal manner for the coincidence between mapped QTLs for Drosophila bristle and candidate genes, Nuzhdin et al. (1998) used a resampling strategy. No evidence was found for an overall association in the sternopleural bristle mapping experiment reported by Nuzhdin et al., but there was a significant association in data from an abdominal bristle mapping experiment by Long et al. (1995). A similar test with five candidate loci for obesity was applied by Keightley et al. (1998) to data on QTLs for fatness in mice. The test was negative for overall fat content, suggesting no coincidence between detected QTLs and candidate loci, and was only weakly positive for gonadal fat pad weight in males.

Here, we develop the resampling tests proposed by Keightley et al. (1998) and Nuzhdin et al. (1998) to test for concordance between the positions of QTLs in pairs of experiments. Rather than taking the approach of Paterson et al. (1995), who compared the map positions of significant QTLs only, we produce an overall correlation between the test statistics of two complete genome scans, and test its significance by permuting the data while maintaining the autocorrelation between linked test statistic values and to use this distribution to obtain suitable significance thresholds. This study focuses on QTLs in mice, which have 19 autosomes, so we chose to permute the data by randomizing the order of chromosomes for each experiment. The reordered chromosomes are lined up and the correlation between the test statistics obtained. This process is repeated many times to give the distribution of \( r \) under the null hypothesis.

If all chromosomes are of equal length, discontinuities in the autocorrelation at chromosome boundaries appear at the same place in both experiments in all permutations. However, if chromosomes are of unequal length (the situation with the real data), discontinuities appear at different places in the reordered data of the two experiments and this can lead to a biased sampling distribution for the correlation between the test statistics. The extent of this bias is investigated by simulation.

(ii) Simulation experiments

To investigate the performance of the permutation test, data were simulated under a range of different genetic models. Our first set of simulations was carried out to investigate the performance of the proposed test when all chromosomes were identical in length. A marker map was generated for each population with 19 equal-length chromosomes and 16 randomly located markers per chromosome. Every third marker was selected to give six markers per chromosome to be simulated in the F2. Phenotypes were generated for 500 F2 individuals for each experiment with no genetic component and a normally distributed residual. In our second set of simulations, two typical mouse mapping experiments, each with 500 F2 individuals, were simulated. Two marker maps were generated, one for each experiment. Markers were randomly located within a given chromosome and, as before, every third marker selected for use in the simulation and analysis of the F2 to give 80 markers in the genome. These two marker maps were used for all subsequent analyses with 80 markers. The mapped regions of each genome covered approximately 12 morgans (M), with about 9-5 M mapped in both experiments. One set of simulations was performed
with no genetic variance. In other simulations, the heritability was assumed to be approximately 0.5 in both experiments, which was explained by a number of QTLs of equal, additive effect. Models with 4, 8 and 100 QTLs affecting each trait were simulated. The 4-QTL model had all QTLs in association and gave a difference between the original lines of 5.6 residual standard deviations. The other models had a proportion of QTLs in dispersion in order to give approximately the same heritability and line difference. Three situations were considered with regard to the covariance between experiments. In the first, the same QTLs were contributing to the variance in both experiments; in the second, 50% of the QTLs were segregating in both experiments and 50% were unique; and in the last, no QTLs were in common in the two experiments. The QTLs were randomly located in the genome with the constraint that they were within the mapped regions common to both experiments. In the third set of simulations we examined the effect of the number of F2 (decreasing it to 100 or increasing it to 1000), and increasing the number of markers to 160 (with approximately 13 M mapped in each experiment and 12 M in common).

The data were analysed using a least squares approach (Haley & Knott, 1992), fitting both an additive and dominance effect at 2 cM intervals throughout the genome (the same locations in the two experiments). F-ratios within the regions mapped in both experiments were selected and the correlation between these calculated. The permutation test as described above was performed with 1000 permutations to obtain the distribution of r under the null hypothesis. We were interested in both positive and negative values of r (i.e. both concordance and discordance between the results of the mapping experiments) and, hence, used a two-tailed test. The observed r, therefore, was compared with the 2.5 and 97.5 percentiles from the distribution obtained by permutation giving a 5% significance level. For the first set of simulations, after the QTL analysis, chromosomes were truncated to be equal in length, taking the F-ratios from the central portion of the regions common to the two experiments. Each set of simulations was repeated 100 times.

(iii) Mouse body weight QTL mapping experiments

There are three genome-wide QTL mapping experiments for body weight in mice that could be used for a concordance test:

(a) Cheverud et al. (1996) (CHE96) crossed two inbreds derived from outbred lines that had been divergently selected on body weight, and mapped QTLs in an F2 population of 535 individuals with 75 microsatellite markers. Several growth traits were measured, including body weight at various ages. Many QTLs were detected, often with age-specific effects. For example, a total of 15 QTLs affecting 6-week weight (6 wkwt) were detected, although some of these were linked on the same chromosome.

(b) Brockmann et al. (1998) (BRO98) crossed an extremely high body weight selection line (DU6) with its control line to produce an F2 population of 341 individuals, and mapped QTLs with 94 microsatellites. A total of 9 QTLs for 6 wkwt were detected. One-eighth of the genome of the DU6 ancestral population was derived from a C57BL inbred strain (cf. (c)).

(c) Morris et al. (1999) (MOR99) mapped QTLs with 80 microsatellites in an F2 of 927 animals from a cross between the C57BL/6J and DBA/2J inbred strains. Selective genotyping was practised, with 173 individuals typed at all loci, although the complete F2 was typed with a subset of 19 markers. Results from the analysis of the full marker data set are used here. In contrast to CHE96 and BRO98, the parental lines differ little for body weight. QTLs for body weight at 3, 6 and 10 weeks were mapped. A total of 3 QTLs for 6 wkwt were detected. As for CHE96, there was evidence of age-specific QTL effects.

The data used in the concordance test were log likelihood ratios (CHE96, MOR99) or F-ratio statistics (BRO98) from interval mapping analyses with 1 cM (BRO98) or 2 cM (CHE96, MOR99) steps between evaluations. In order to align the genetic maps and their corresponding test statistics we assumed the Mouse Genome Database (MGD) map (Blake et al., 1998) as a consensus map. BRO98 assumed this map in their analysis, so no rescaling of their data is needed. However, CHE96 and MOR99 constructed maps from the linkage data of their respective experiments with mapping software, so a rescaling is necessary. We scaled the data from the published interval mapping analyses by setting the positions of the makers typed to the corresponding MGD positions, and then calculated new map positions for the positions of each test statistic evaluation on the basis of the expanded or contracted map distances between the flanking markers. Linear interpolation between locations of test statistic evaluations was used to compare F-ratios or log likelihoods at the same positions in pairs of experiments.

3. Results and discussion

(i) Simulation experiments

The mean value for r, the mean 5% significance thresholds derived by permutation (2.5 and 97.5 percentiles), and the proportion of replicates significant are shown for experiments with F2 populations of size 500, 80 markers, and various QTL models in Table 1. We performed an initial check of the permutation test by simulating experiments with chromosomes of equal length and no QTLs in either
Table 1. Simulation results: effect of changing number of QTLs simulated and degree of concordance in experiments with 500 F2 individuals and heritability of 50%

| Number of QTLs | QTL effects (SD units) | Concordance (%) | Mean 5% thresholds$^a$ | Proportion significant |
|---------------|------------------------|-----------------|------------------------|-----------------------|
| 0$^b$         | 0                      | 0               | 0.30                   | 0.044                 |
| 0             | 0                      | 0               | 0.31                   | 0.076                 |
| 4             | 0.7                    | 0               | 0.46                   | 0.13                  |
| 4             | 0.7                    | 50              | 0.47                   | 0.57                  |
| 4             | 0.7                    | 100             | 0.51                   | 1.0                   |
| 8             | 0.5                    | 0               | 0.48                   | 0.11                  |
| 8             | 0.5                    | 50              | 0.46                   | 0.36                  |
| 8             | 0.5                    | 100             | 0.48                   | 1.0                   |
| 100           | 0.14                   | 0               | 0.45                   | 0.12                  |
| 100           | 0.14                   | 50              | 0.44                   | 0.29                  |
| 100           | 0.14                   | 100             | 0.46                   | 1.00                  |

One hundred replicates were carried out.

$^a$ Mean 2.5 and 97.5 percentiles of the distribution of $r$ obtained by permutation.

$^b$ Equal chromosome lengths; otherwise chromosomes were of variable length.

As expected, the mean value of $r$ over experiments is close to zero and the proportion of significant $r$ values (i.e. type I errors) is close to 5%. The mean significance thresholds are not symmetrical about zero, however, an effect caused by a degree of non-randomness in the positions of markers in the simulated experiments, coupled with the tendency for the test statistic values to be highest at positions of markers when there is no QTL. With chromosomes of unequal length and no QTLs, the mean value of $r$ is also close to zero but the type I error rate based on permutation is slightly higher than 0.05. The reason for this bias is connected with discontinuities that occur at chromosome boundaries. The unpermuted data from the two experiments align perfectly, because only regions of chromosomes that coincide are compared. In the permuted data, however, the chromosome boundaries, which break the autocorrelation between linked sites, occur in different locations in the two data sets, giving more breaks when considering the two experiments together and, hence, the $r$ values obtained are lower.

For simulations incorporating QTLs, if the QTLs are in exactly the same places in both experiments, $r$ is always significant, and its mean value is > 0.8 (Table 1). If the simulation is set up with zero concordance between the locations of the QTLs in the two experiments, the mean value of $r$ is close to zero and around 12% of runs were significant (compared with 8% when no QTLs were simulated). There is therefore a higher level of bias than for the case of no QTLs, and this is reflected as greater asymmetry in the mean significance thresholds. Results for the intermediate levels of concordance between the experiments are more likely to reflect real experimental situations. In the cases with 4 QTLs of large effect, the mean $r$ value is about 50%, and is significant more than 50% of the time. However, with increasing numbers of QTLs, $r$ and the power to detect correspondence between QTLs decreases substantially. Note that the mean upper significance threshold approaches 50% in the cases shown in Table 1; hence the empirical $r$ would have to exceed this value to be significant. Table 2 shows the results when the marker density and the number of F2 individuals were altered for the case of 50% of the QTLs in common. Decreasing the number of F2 to 100 decreased both the mean $r$ and the proportion significant, presumably because the F-ratio plots become noisier. Although individually increasing the number of F2 and marker density had only a small effect, increasing both together had a large effect on power. This is presumably due to an increased sharpness of the F-ratio peaks near the QTLs, and leads to a small increase in $r$, but surprisingly little change in the average significance thresholds.

(ii) Correspondence between body weight QTLs in mice

We computed $r$ values for each of the three pairs of mouse QTL experiments, and tested the significance of the observed $r$ values by permutation (Table 3). It was necessary to truncate the data sets in regions of the genome that were not typed in both members of a given pair of studies. The results show no evidence of correspondence between the positions of mapped QTLs in any pair of experiments. The most extreme $r$ value is −0.22, from the comparison of CHE96 and BRO98 (i.e. the QTLs are in different positions in these experiments); the correspondence between
Mor99 and Bro98, which had genes in common in
their base populations, is only weakly positive, and
non-significant. It seems that the QTLs segregating
in the different mouse body weight mapping experi-
ments are, on the whole, different from one other, al-
though the empirical r values could have been quite
high, but non-significant (see significance thresholds,
Table 3).

Unsurprisingly, r values computed for pairs of
traits within an experiment are highly significant
and positive: e.g. for Che96, values are 0.81 (3 wkwt,
6 wkwt), 0.94 (6 wkwt, 10 wkwt) and 0.71 (3 wkwt,
10 wkwt).

(iii) Concluding remarks
Our simulation results suggest that obtaining a
statistically significant correlation between two QTL
mapping experiments would be unlikely if the var-
iation in the trait is explained by large numbers of
QTLs and the experimental populations are not closely
related. This seems to be the case with the mouse body
weight selection experiments investigated here. The
most likely situation in which a significant result
might be obtained with unrelated strains is where
there is one or more QTL in common that explains a
very high fraction of the variance in both experiments.
If there are several minor-effect concordant QTLs, the
test will be swamped by non-concordance between
major effect QTLs. It seems more likely that significant
correspondence would be found for traits such as
Drosophila bristle number, for which QTLs with quite
large effects are often picked up (e.g. Shrimpton &
Robertson, 1988; Long et al., 1995) and the number
of genes capable of having a large effect is likely to be

**Table 2. Simulation results: effect of changing number of QTLs
simulated, F2 population size (n), and number of markers for the case of
50% concordance between the QTL locations, and heritability = 50%**

| n   | Number of QTLs | Number of markers | Mean 5% thresholds | Proportion significant |
|-----|----------------|------------------|--------------------|-----------------------|
| 100 | 4              | 80               | 0.30               | -0.24 0.44 0.31       |
| 500 | 4              | 80               | 0.47               | -0.21 0.47 0.57       |
| 1000| 4              | 80               | 0.46               | -0.21 0.45 0.57       |
| 500 | 4              | 160              | 0.47               | -0.19 0.43 0.59       |
| 1000| 4              | 160              | 0.49               | -0.19 0.43 0.70       |
| 100 | 8              | 80               | 0.23               | -0.26 0.42 0.21       |
| 500 | 8              | 80               | 0.36               | -0.29 0.46 0.36       |
| 1000| 8              | 80               | 0.38               | -0.25 0.50 0.43       |
| 500 | 8              | 160              | 0.38               | -0.23 0.45 0.41       |
| 1000| 8              | 160              | 0.41               | -0.23 0.43 0.58       |
| 100 | 100            | 80               | 0.11               | -0.28 0.39 0.17       |
| 500 | 100            | 80               | 0.27               | -0.28 0.44 0.29       |
| 1000| 100            | 80               | 0.29               | -0.28 0.45 0.31       |
| 500 | 100            | 160              | 0.24               | -0.26 0.41 0.31       |
| 1000| 100            | 160              | 0.31               | -0.26 0.43 0.39       |

One hundred replicates were carried out.
a Mean 2.5 and 97.5 percentiles of the distribution of r obtained by permutation.

**Table 3. Values of r and corresponding probabilities obtained by
permutation for three pairs of mouse body weight QTL mapping
experiments**

| Trait | Experimental pair | r     | Probability* | Significance thresholds# |
|-------|-------------------|-------|--------------|--------------------------|
| 3 wkwt| MOR99–CHE96       | -0.15 | 0.39         | -0.25 0.41                |
| 6 wkwt| MOR99–CHE96       | -0.06 | 0.89         | -0.26 0.37                |
| 6 wkwt| MOR99–BRO98       | 0.14  | 0.41         | -0.26 0.39                |
| 6 wkwt| CHE96–BRO98       | -0.22 | 0.09         | -0.24 0.71                |
| 10 wkwt| MOR99–CHE96      | -0.06 | 0.79         | -0.25 0.43                |

a Probability of the observed r based on a two-tailed test.
# The significance thresholds are limits outside which an observed r value would be judged significant at the 5% level.
lower than for growth rate, which can be considered as a life history trait. However, our permutation test has been tailored to an organism with many chromosomes, for which randomization of the chromosome order would generate a very large number of combinations. In a species with few chromosomes, such as Drosophila which has only three major chromosomes, too few combinations would be generated by simply randomizing the chromosome order. An appropriate strategy could therefore be to randomize the chromosome orders of the two experiments, then to join the two linear arrays of chromosomes to make circles, followed by sampling of different alignments between two genomes by rotating them past each other. In models of the mouse genome we did not achieve a wholly satisfactory test of correspondence, due to the problem of discontinuities at chromosome boundaries. In species with fewer chromosomes the problem is likely to be more severe, although this would have to be tested for specific cases by simulations.

The approach taken in this paper is applicable to experiments which can be quite different in structure, as the only requirements are that a common marker map can be obtained and that test statistics throughout the genome are available. In fact, problems may arise if all the same markers are used in the experiments. This is because the markers will cause the test statistics to be more similar in the original orientation when marker locations coincide, compared with all permutations when the locations will be different. If experiments are similar in structure and the raw data are available, an alternative approach would be to perform a joint analysis of the experiments together. This would require a consensus map and markers could be identical in the experiments. A joint analysis would enable direct tests of whether a given QTL is acting in each population (for example Walling et al., 1998). This is in contrast to the correlation approach here which considers the whole genome.

We thank J. Cheverud and G. Brockmann for kindly providing the data from their mouse growth rate QTL mapping experiments, and for comments on the manuscript, and the Royal Society for support.

References

Blake, J. A., Eppig, J. T., Richardson, J. E., Davisson, M. T. & the Mouse Genome Informatics Group. (1998). The Mouse Genome Database (MGD): a community resource. Status and enhancements. Nucleic Acids Research 26, 130–137.

Brockmann, G. A., Haley, C. S., Renne, U., Knott, S. A. & Schwerin, M. (1998). Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. Genetics 150, 369–381.

Cheverud, J. M., Routman, E. J., Duarte, F. A. M., van Swinderen, B., Cothran, K. & Perel, C. (1996). Quantitative trait loci for murine growth. Genetics 142, 1305–1319.

Dietrich, W. F., Miller, J., Steen, R., Merchant, M. A., Damronboles, D., Husain, Z., et al. (1996). A comprehensive genetic map of the mouse genome. Nature 380, 149–152.

Garnett, I. & Falconer, D. S. (1975). Protein variation in strains of mice differing in body size. Genetical Research 25, 45–57.

Haley, C. S. & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69, 315–324.

Keightley, P. D., Morris, K. H., Ishikawa, A., Falconer, V. M. & Oliver, F. (1998). Test of candidate gene–quantitative trait locus association applied to fatness in mice. Heredity 81, 630–637.

Lin, Y. R., Schertz, K. F. & Paterson, A. H. (1995). Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetcs 141, 391–411.

Long, A. D., Mullaney, S. L., Reid, L. A., Frey, J. D., Langley, C. H. & Mackay, T. F. C. (1995). High resolution mapping of genetic factors affecting abdominal bristle number in Drosophila melanogaster. Genetics 139, 1273–1291.

Long, A. D., Mullaney, S. L., Mackay, T. F. C. & Langley, C. H. (1996). Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in Drosophila melanogaster. Genetics 144, 1497–1510.

Mackay, T. F. C. (1995). The genetic basis of quantitative variation: numbers of sensory bristles of Drosophila melanogaster as a model system. Trends in Genetics 11, 464–470.

Mackay, T. F. C. (1996). The nature of quantitative genetic variation revisited: lessons from Drosophila bristles. Bioessays 18, 113–121.

Mouse Genome Database (MGD), Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine. World Wide Web (URL: http://www.informatics.jax.org/) (2 February 1999).

Morris, K. H., Ishikawa, A. & Keightley, P. D. (1999). Quantitative trait loci for growth traits in C57BL/6 x DBA/2J mice. Mammalian Genome 10, 225–228.

Nuzhdin, S. V., Keightley, P. D., Pasyukova, E. G. & Morozova, E. A. (1998). Mapping quantitative trait loci affecting Drosophila melanogaster sternopleural bristle number using changes of marker allele frequencies in divergently selected lines. Genetical Research 72, 79–91.

Paterson, A. H., Lin, Y. R., Li, Z. K., Schertz, K. F., Doebley, J. F., Pinson, S. R. M., Liu, S. C., Stansel, J. W. & Irvine, J. E. (1995). Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269, 1714–1718.

Shrimpton, A. E. & Robertson, A. (1988). The isolation of polygenic factors controlling bristle score in Drosophila melanogaster. II. Distribution of third chromosome bristle effects within chromosome sections. Genetics 118, 445–459.

Walling, G. A., Archibald, A. L., Cattermole, J. A., Downing, A. C., Finlayson, H. A., Nicholson, D., Visscher, P. M., Walker, C. A. & Haley, C. S. (1998). Mapping of quantitative trait loci on porcine chromosome 4. Animal Genetics 29, 415–424.

Zechnner, U., Reule, M., Orth, A., Bonhomme, F., Strack, B., Gueuet, J. L., Hameister, H. & Fundele, R. (1996). An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrids. Nature Genetics 12, 398–403.
The correspondence between map positions of QTLs