MAPPING AND ANALYSIS OF QUANTITATIVE TRAIT LOCI IN EXPERIMENTAL POPULATIONS

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Simple statistical methods for the study of quantitative trait loci (QTL), such as analysis of variance, have given way to methods that involve several markers and high-resolution genetic maps. As a result, the mapping community has been provided with statistical and computational tools that have much greater power than ever before for studying and locating multiple and interacting QTL. Apart from their immediate practical applications, the lessons learnt from this evolution of QTL methodology might also be generally relevant to other types of functional genomics approach that are aimed at the dissection of complex phenotypes, such as microarray assessment of gene expression.

MULTIFACTORIAL GENETICS

COMPLEX TRAIT
A trait determined by many genes, almost always interacting with environmental influences.

QUANTITATIVE TRAIT LOCUS
A genetic locus identified through the statistical analysis of complex traits (such as plant height or body weight). These traits are typically affected by more than one gene, and also by the environment.

The vision of the early population geneticists Sax[1], Thoday[2] and Fisher[3], combined with the scientific and technological breakthroughs in molecular genetics of Watson and Crick[4], Southern[5], Sanger *et al.*[6] and Saiki *et al.*[7], to name only a few, have brought us to a revolutionary time in science. These advances and ideas have empowered science to the point of turning biology from a once observational perspective into that of prediction (BOX 1), whereby the connection between genotype and phenotype can be more completely understood. Monogenic traits supplied the early and encouraging successes in associating genotype with phenotype in humans[8–10], and domesticated animal[11] and plant[12] populations. By contrast, COMPLEX TRAITS have proved to be more challenging, simply because it is impossible to follow all genomic regions that are responsible for the complex variation of the trait without some further idea of how these regions segregate.

A key development in the field of complex trait analysis was the establishment of large collections of molecular/genetic markers, which could be used to construct detailed genetic maps of both experimental and domesticated species. These maps provided the foundation for the modern-day QUANTITATIVE TRAIT LOCUS (QTL) mapping methodologies[13–36] that are the focus of this article. These techniques include single-marker mapping[13–15], interval mapping[16], composite interval mapping[24,28,30,31] and multiple trait mapping[32–36], which allow statistical analyses of the associations between phenotype and genotype for the purpose of understanding and dissecting the regions of a genome that affect complex traits.

However, even when statistically significant associations between the complex quantitative trait(s) and molecular markers are identified, the genomic regions are usually so large that subsequent experiments, used to zero in on these regions, are often expensive. Through the limitations of the technology, this results in the loss of at least one of the regions of interest. In the event of success, and the genomic region containing genes that are responsible for the complex trait variation[37], the expense and time from the beginning to the end of this process is often too great for widespread application to problems of scientific, economic or medical importance. For years, access to knowing which genes are responsible for the genetic variation of complex traits has held scientists back. Furthermore, the subset of genes that are thought to contribute to variation has often provided only a limited and disappointing percentage of the total variation associated with the trait.

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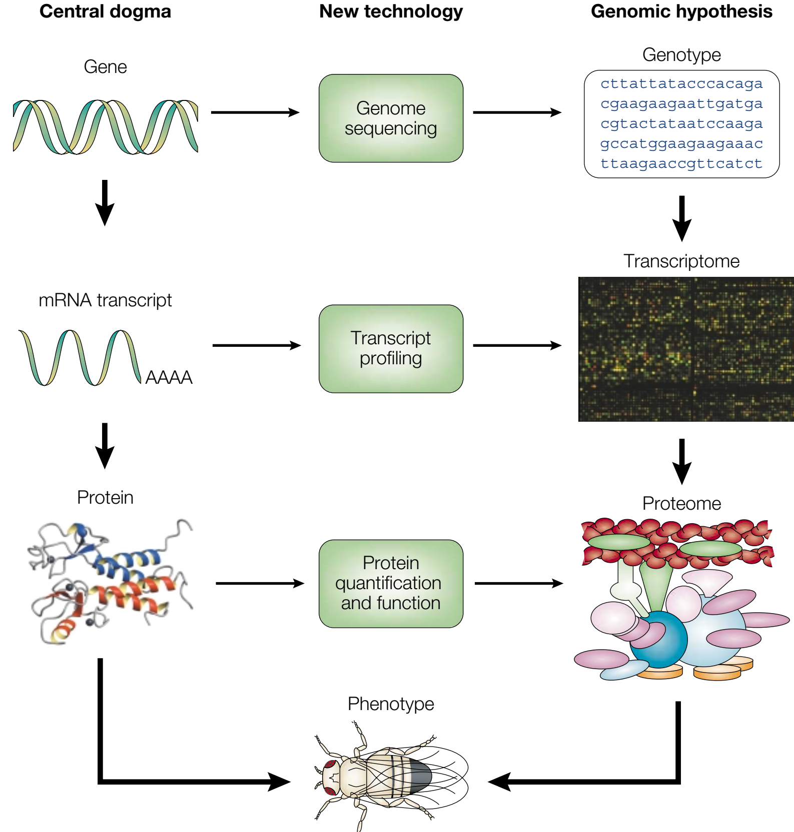
Box 1 | **Evolution of the central dogma**

The central dogma outlines the flow of information that is stored in a gene, transcribed into RNA and finally translated into protein. The ultimate expression of this information is the phenotype of the organism. Each step of the central dogma is accompanied by recent technological innovations that allow genome-wide analysis. Although the central dogma once presented a view that was essentially descriptive, and limited to gene-by-gene studies, it can now be coupled with technology and viewed as experimental and testable. Hypotheses can be formulated and revised for the purpose of elucidating the detailed connections between genotype and phenotype, therefore unravelling the inner molecular biology of an organism.



Genomics has brought a new level of hope to unravelling the secrets of complex traits. Genome sequences themselves have the potential to provide a comprehensive list of the genes in an organism. Functional genomics approaches can then be used to generate information about gene function, as well as data on genetic interactions, not only among and between gene complexes, but also in response to environmental stimuli. At present, microarray, or array, technology[38] is providing the most comprehensive assessment of gene function and variation. Our ability to view the transcription of the genome is improving rapidly and, as a result, the potential to dissect complex traits is also developing. Already, array technology has been instrumental in identifying groups of co-expressed genes in various physiological states, including stages of development[39,40] and disease[41,42]. Although array technology is valuable, these data are not conclusive or comprehensive as regards gene function, and only provide one more piece (that is, transcriptional profile) of the puzzle. The translation of genes into proteins is another key step in gene action and it will be essential to subject protein synthesis, as well as protein interaction, to the same genome-wide analysis[43,44], to understand how genotype can influence a complex phenotype.

EPISTASIS
In the broad sense used here, it refers to any genetic interaction in which the combined phenotypic effect of two or more loci exceeds the sum of effects at individual loci.

There are many reviews and texts on QTL analysis[45–49], and on the present status of array technology[38,50,51]. How the growing collections of data at the DNA, RNA and protein levels can be combined to dissect complex traits and diseases remains to be seen. It has been proposed that the power available through the merger of genetics and genomics (called genetical genomics by Jansen and Nap[52]) might lead to further unravelling of metabolic, regulatory and developmental pathways, but rigorous investigations still need to be completed. What is clear, however, is that technology (BOX 1) is emerging that will supply quantities of data that require detailed statistical and mathematical analyses. With this as the motivation, this review summarizes the principal steps and statistical contributions made in the past 20 years towards the detection and location of QTL in experimental populations. It also previews the potential that these methods have in associating modifications in gene expression to genetic maps for any diploid, and potentially polyploid[53,54], species under investigation. Borrowing from what has been learned in developing statistical methodology for quantitative trait mapping, the hope is that the statistical design of genome-wide experiments and analyses of the resulting data might culminate in the complete dissection of complex traits.

Quantitative trait locus mapping

A QTL is a region of any genome that is responsible for variation in the quantitative trait of interest. The goal of identifying all such regions that are associated with a specific complex phenotype might, at first, seem quite simple, especially with all the genomic and computational tools available to help us. Unfortunately, the task is difficult because of the sheer number of QTL, and the possible EPISTASIS or interactions between QTL, and because of the many additional sources of variation[55]. To combat this, QTL experiments can be designed with the aim of containing the sources of variation to a limited number, so that dissection of a complex phenotype might be possible. In general, a large sample of individuals has to be collected to represent the total population, to provide an observable number of recombinants and to allow a thorough assessment of the trait under investigation. Using this information, coupled with one of several methodologies to detect or locate QTL (see below), associations between quantitative traits and genetic markers are made as a step towards understanding the genetic basis of complex traits.

The first step in any QTL-mapping experiment is usually to construct populations that originate from homozygous, inbred parental lines (FIG. 1). The resulting F_1 lines will tend to be heterozygous at all markers and QTL. From the F_1 population, crosses are made (for

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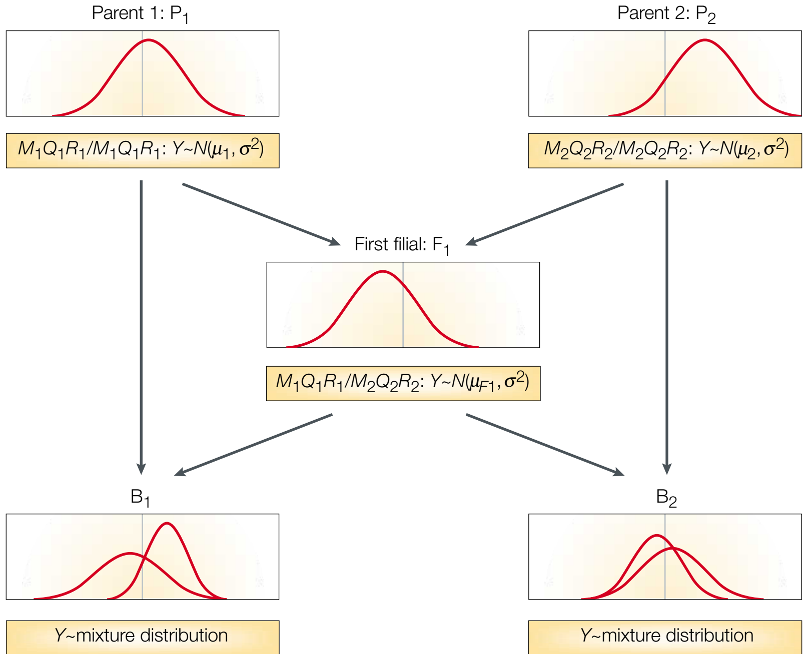

Figure 1 | **Experimental design for quantitative trait loci mapping.** Standard backcross mating design for markers *M* (with alleles M_1 and M_2) and *R* (with alleles R_1 and R_2). The hypothetical quantitative trait locus (QTL) *Q*, with alleles Q_1 and Q_2, is also illustrated. The haplotypes are separated by a solidus. The trait value (*Y*) is assumed to have a normal distribution (*N*) with mean μ and variance σ^2 in the parental populations P_1 and P_2. B_1 and B_2 represent the reciprocal backcross progeny. The trait value in the backcross progeny has a distribution that represents the mixture of the F_1 trait distribution and the respective (recurrent) backcrossed parental line. Statistical tools are applied to test whether there is an association between the genotypic and quantitative trait information collected from either backcross population.

example, backcross, F_2 intercross and crosses to generate RECOMBINANT INBRED LINES), and the segregation of markers and QTL are statistically modelled. In general, experimenters assume that markers are segregating randomly, but if, in fact, markers are subject to SEGREGATION DISTORTION, it is not possible to anticipate how the resulting estimates of recombination will be affected, as well as any potential QTL locations. Once the data are collected on each individual, statistical associations between the markers and quantitative trait are established through statistical approaches that range from simple techniques, such as analysis of variance (ANOVA), to models that include multiple markers and interactions. The simpler statistical approaches tend to be methods of QTL detection that assess differences in the phenotypic means for single-marker genotypic classes. The actual location of QTL involves an estimated genetic map with known distances between markers, and evaluations of a likelihood function that is maximized over the established parameter space.

Single-marker tests

Simple, single-marker tests (for example, using *t*-test, ANOVA and simple linear regression statistics; BOX 2) that assess the segregation of a phenotype with respect to a marker genotype (FIG. 1), indicate which markers are associated with the quantitative trait of interest and, therefore, point to the existence of potential QTL. Typically, the null hypothesis tested is that the mean of the trait value is independent of the genotype at a particular marker. The null hypothesis is rejected when the test statistic is larger than a crucial value (to be discussed later), and the implication is that a QTL is linked to the marker under investigation. Although the *t*-test, ANOVA and simple linear regression approach are all equivalent to each other when their hypotheses are testing for differences in the phenotypic means, they fail to provide a CLOSED FORM ESTIMATE of QTL location, or recombination frequency between the marker and the QTL. This is because the QTL effect and the location are confounded, or are unable to be estimated separately. The effect of this phenomenon has recently been highlighted in the context of human genetics[56]. Confounding, in these situations, is addressed by (incrementally) fixing the location of the QTL and estimating the QTL effect between intervals of markers. These intervals of markers lead naturally to a method that estimates both QTL effect and the location, known as 'interval mapping'[16] (see below).

Detecting QTL by this type of single-marker approach is a simple procedure that can be accomplished with any standard statistical analysis software package, and has the potential to identify numerous significant markers. Two important issues should be considered when assessing these statistical results. The first consideration is sample size. The number of individuals studied provides information for the estimation of phenotypic means and variances. A large sample of individuals provides the opportunity to observe recombinant events and to estimate parameters with greater accuracy and, therefore, a greater ability to detect QTL through a single-marker test. The second issue concerns the problem of multiple testing and arises when many markers are investigated through independent statistical tests. This problem is coupled with the level of statistical significance that is set by the investigator and can lead to detection of false-positive QTL. Typically, an investigator is willing to tolerate incorrectly detecting a QTL in, for example, 5% of cases. Therefore, given a 5% level of significance, and 100 positive, unique marker tests, five of the 100 markers would detect QTL incorrectly. This problem can be accounted for through a multiple test[57] adjustment, such as Bonferroni, Scheffe or Tukey, that will correct the SIGNIFICANCE LEVEL according to how many independent statistical tests are made.

Single-marker analyses are still used as a means to identify markers that are segregating with a trait. Most of these applications[58–60] deal primarily with detecting individual markers, rather than genomic regions, and are a quick and efficient means to screen large populations for specific traits, such as disease resistance. Typically, when investigations focus on questions of genomic location, then more sophisticated methods of QTL analysis, which rely on the estimated order of markers, are used. The added information that is gained from knowing the relationships between markers is essential to QTL methodologies that aim to locate QTL.

RECOMBINANT INBRED LINES
A population of fully homozygous individuals that is obtained by repeated selfing from an F_1 hybrid, and that comprises ~50% of each parental genome in different combinations.

SEGREGATION DISTORTION
The non-random segregation of alleles. Apparent segregation distortion can result from incorrect genotype classification.

CLOSED FORM ESTIMATE
An estimate of a population parameter that can be calculated directly from an equation to obtain an exact solution.

SIGNIFICANCE LEVEL
A probability for a test statistic that gives the maximum acceptable value of rejecting a 'true' null hypothesis.

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Box 2 | **Statistical and genetic mapping software**

Statistical software

- SAS
  A comprehensive set of statistical analysis procedures that can be used to model and test for various quantitative trait loci (QTL) effects.
- Minitab
  A user-friendly statistical software package that provides simple analyses, often used for single-marker QTL mapping, as well as regression analysis.
- Splus
  Powerful suite of mathematical and statistical functions that provide a programming language environment (similar to the C programming language), in which unique QTL analyses and graphical summaries can be calculated.

Genetic mapping software

- An alphabetical list of genetic analysis software
  A comprehensive list of gene and QTL mapping, and linkage, software (with links and updates) that covers the vast range of applications, with no limitations due to species or lack of population structure.
- Quantitative genetics resources
  General information on mapping quantitative traits.
- QTL mapping software
  Additional QTL mapping software information for experimental populations.
- QTL references
  A very-detailed reference list of influential QTL methodological publications.

The URLs for these resources are provided in the links box at the end of this article.

Genetic maps

Single-marker analyses investigate individual markers independently, and without reference to their position or order. When markers are placed in genetic (linear) map order, so that the relationships between markers are understood, the additional genetic information gained from knowing these relationships provides the necessary setting to address confounding between QTL effect and location. A genetic map also provides a genetic representation of the chromosome on which the markers and QTL reside (FIG. 2). Pairwise information, or recombination, is first estimated for all markers that are segregating as expected, and then any marker that is linked to any other marker is placed in the same linkage group. The linear arrangement of markers into linkage groups, or chromosomes, provides the genetic map for locating QTL that are relative to intervals of markers (or statistically related sets of markers). In addition to supplying the structure in which to search for QTL, the estimated genetic map benefits the estimation of missing marker information by using the surrounding marker genotypes to infer knowledge of the missing marker genotypes.

When using genetic maps in this way, it is important to distinguish between recombinational events and genetic distance. The essential difference is that genetic distances are additive, whereas recombination units are not because they are probabilities and because of GENETIC INTERFERENCE. Recombinational units and genetic distance can be translated between by using a map function (such as the Haldane and Kosambi map functions). The practical value of a genetic map is that the QTL can be mapped more easily in an interval of defined genetic distance. The methods for linearly ordering the molecular markers rely on minimizing the recombination between pairs of markers. As the estimated genetic distance between markers is a function of the average number of observed recombination events between them, minimizing these values best represents the frequency of recombination. The unit for expressing the genetic distance between markers on a chromosome is the Morgan (or, more usually, the centiMorgan, cM), and is defined as the distance along which one recombinational event is expected to occur per gamete per generation. When several markers are considered, they are ordered simultaneously on the basis of minimized recombination — an approach called multipoint linkage mapping[61–64] (BOX 2).

The accuracy of locating QTL is limited by the information, in particular the number of recombinants, that is gained from observing the genotypic states of the markers. These observed recombinants can be limited by both small sample size and missing genotypic data. With this in mind, a commonly asked question is: 'Should I genotype more markers on fewer individuals, or score more individuals (for genotype and phenotype) on fewer markers?' Because observed recombinants provide the information, scoring more individuals addresses both previously mentioned concerns. Lynch and Walsh[47] and Lui[48] present standard closed form calculations for the purpose of evaluating how many markers to genotype relative to the desired QTL location accuracy, and how many individuals to phenotype given a particular significance level, QTL effect and location.

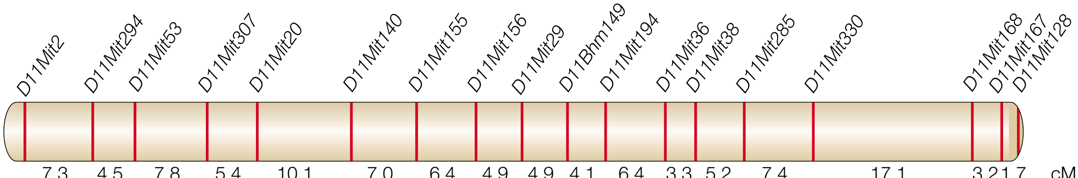

Figure 2 | **A genetic map of mouse chromosome 11.** The first step in quantitative trait locus (QTL) identification and location is to estimate the linear ordering, or genetic map, of markers in a linkage group. These markers are linearly ordered (this map was estimated with MAPMAKER/EXP[21,64]) across the chromosome to minimize the genetic distance between markers, and therefore across the chromosome[99]. Genetic distances (cM) are given relative to chromosome 11. In the second stage of QTL mapping, the estimated genetic map becomes the *x* axis of the LOD (logarithm of the odds) score profile (*y* axis) for QTL mapping (FIG. 3).

GENETIC INTERFERENCE
The presence of a recombinational event in one region affects the occurrence of recombinational events in adjacent regions.

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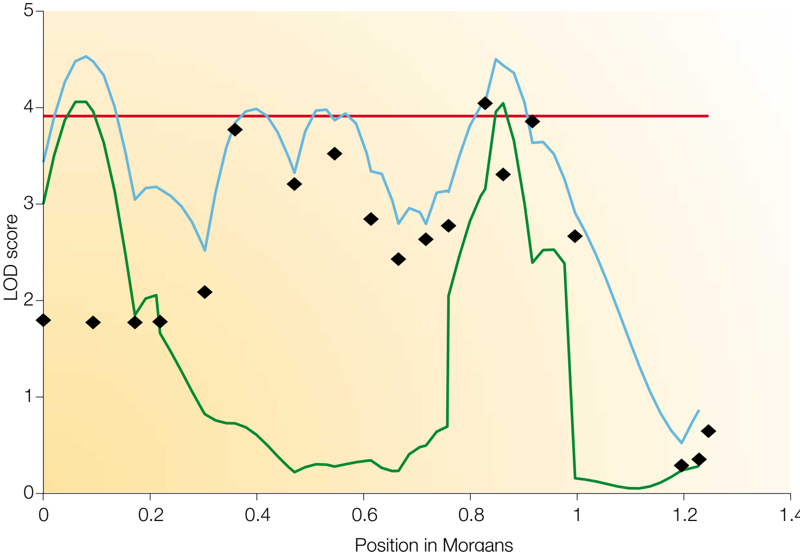

Figure 3 | **Choices of analysis for quantitative trait locus mapping.** The data shown are from an analysis of mouse chromosome 11 for the quantitative trait called 'severity' in a study of experimental allergic encephalomyelitus (EAE)[99]. EAE is the principal animal model for multiple sclerosis in humans. Microsatellite markers (FIG. 2) were genotyped in 633 F_2 mice that were followed for this study. Severity of the disease was quantified from the average of several assessments of 'tail droopiness' per mouse. Quantitative trait locus (QTL) analysis was carried out using QTL-Cartographer and several different approaches: single-marker analysis using a *t*-test (black diamonds); interval mapping (blue line); and composite interval mapping (green line). The red line represents the 95% significance level on the basis of 1,000 permutations of the phenotypic data. The single-marker *t*-tests identify one significant marker (D11Mit36), interval mapping locates four maximum QTL locations on the LOD (logarithm of the odds) profile, and composite interval mapping finds two significant QTL. The differences seen between the single-marker analysis, and interval and composite interval mapping, are the result of information gained from the estimated genetic map. The difference between interval mapping and composite interval mapping is the result of composite interval mapping's use of a 'window' or genomic region that allows other effects that are outside the window, but associated with the quantitative trait, to be eliminated from the analysis point under consideration. The benefit of defining a window is that the variation associated with the point of analysis is confined to the QTL effects within the window and not outside the window, thereby reducing the effects of linked and ghost QTL. The result of composite interval mapping is illustrated by elimination of the two central (ghost) QTL.

GHOST QTL
Quantitative trait locus (QTL) effects that occur as artefacts due to real QTL in surrounding intervals.

Interval mapping

Interval mapping[65,66], made popular by Eric Lander and David Botstein[16] (BOX 2), uses an estimated genetic map as the framework for the location of QTL. The intervals that are defined by ordered pairs of markers (FIG. 2) are searched in increments (for example, 2 cM), and statistical methods are used to test whether a QTL is likely to be present at the location within the interval or not. It is important to realize that interval mapping, as defined by Lander and Botstein, statistically tests for a single QTL at each increment across the ordered markers in the genome. The results of the tests are expressed as LOD (logarithm of the odds) scores, which compare the evaluation of the likelihood function under the null hypothesis (no QTL) with the alternative hypothesis (QTL at the testing position) for the purpose of locating probable QTL.

Interval mapping searches through the ordered genetic markers in a systematic, linear (also referred to as one-dimensional) fashion, testing the same null hypothesis and using the same form of likelihood at each increment. In addition, as the LOD scores taken together represent a LOD profile across the genetic map, the locations of the maximum LOD profile have the potential to indicate multiple or GHOST QTL[67] incorrectly when a single QTL model is used (FIG. 3). Determining which of the many peaks indicates a single QTL leads to issues of determining statistically significant results. Because the likelihood is usually a function of mixtures of (normal) distributions[46] (FIG. 1) and, when maximized under both the null and alternative hypotheses, leads to test statistics that fail to follow standard statistical distributions, it is difficult to declare a QTL with confidence. This happens even when the previously noted issues of multiple testing are taken into consideration. Both multiple testing and distributional assumptions of the test statistic can be accounted for through an application of resampling methodology[68] that will be discussed later. Nevertheless, although interval mapping is certainly more powerful than single-marker approaches to detect QTL (because of the structure and additional genotypic information supplied by the genetic map), it is limited by both the model that defines it as a single QTL method, and by the one-dimensional search that does not allow interactions between multiple QTL to be considered.

Multiple quantitative trait loci

Statistical approaches for locating multiple QTL are more powerful than single QTL approaches because they can potentially differentiate between linked and/or interacting QTL. When the alleles of two or more QTL interact (epistasis), this has great potential to alter the quantitative trait in a manner that is difficult to predict. One of the most extreme (and simplest) cases is the complete loss of trait expression in the presence of a particular combination of alleles at multiple QTL. The ultimate challenge in the search for multiple QTL is to consider every position in the genome simultaneously, for the location of a potential QTL that might act independently, be linked to another QTL, or interact epistatically with other QTL. Interacting QTL are of particular interest as they indicate regions of the genome that might not otherwise be associated with the quantitative trait using a one-dimensional search.

Although the concept of locating multiple, interacting QTL is straightforward, implementation is quite difficult due to the tremendous number of potential QTL and their interactions, which lead to innumerable statistical models and heavy computational demand. One heuristic approach that has been taken[69] is to first locate all single QTL, then to build a statistical model with these QTL and their interactions and, finally, search in one dimension for significant interactions. Kao *et al.*[33] made such a proposal through a direct extension of interval mapping to include a simultaneous search for multiple epistatic QTL. Owing to the computational intensity of a multidimensional search, a simultaneous investigation is not possible, and the search is referred to as a quasi-simultaneous investigation. Approaches like

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this have the potential to work in many situations, but are limited to the pool of QTL that resulted from the first-pass QTL analyses, and have little hope of establishing true epistatic effects for QTL that are not individually significant. Searching through all potential models is a problem known as model selection, and remains an active area of research in theoretical statistics.

Composite interval mapping[24,28] as introduced by Zhao-Bang Zeng in 1993 and, similarly, multiple QTL mapping[30,31,49,70] as introduced by Ritsert Jansen in the same year, achieve the same result by reducing the number of potential models under consideration. Both methods extend the ideas of interval mapping to include additional markers as cofactors — outside a defined window of analysis — for the purpose of removing the variation that is associated with other (linked) QTL in the genome. The limitations of both approaches are that they are restricted to one-dimensional searches across the genetic map, and are challenged at times by the multiplicity of epistatic QTL effects. There is also a risk of putting too many markers in the model as cofactors[71], and care should be taken to preserve the amount of information that is available for estimation of the QTL effect. The benefits of composite interval mapping are seen in FIG. 3.

The importance of developing models with multiple QTL is well understood for linked QTL, and has an even greater role in the estimation and location of epistatic QTL. The limiting feature in successfully using multiple QTL models is not our inability to write an equation for a model, it is our inability to identify the best model, or subset of models (from potentially millions). Enumeration of all possible QTL models that consider the appropriate genetic architecture for the experiment, as well as linkage and epistasis, is a daunting task. Accurate and fast simultaneous multidimensional searches through the most likely models, and their comparisons, are required to determine the most feasible models that warrant further investigation. As we have seen, one-dimensional searches (for example, interval mapping and composite interval mapping) have benefited the mapping community, but are limited in their inability to accommodate multiple linked QTL. Because a stepwise linear approach to model building[57], by adding and deleting every combination of multiple (linked) QTL and their interactions, is not computationally feasible, many investigators[72–74] have proposed solutions by addressing the computational issues rather than the QTL-mapping method itself. One approach is to globally search for the optimum multiple QTL genotype using GENETIC ALGORITHMS[75–78]. The application of genetic algorithm(s) to multiple QTL problems is one of many beneficial approaches because it allows a sampling of the QTL models across unequal QTL numbers to be considered, and because it can be used in conjunction with any QTL-mapping methodology that is implemented for a multidimensional search of a genome.

GENETIC ALGORITHM
Numerical optimization procedures based on evolutionary principles, such as mutation, deletion and selection.

An inclusive computational framework for addressing many of the previously mentioned challenges, namely, covariates, non-normal trait distributions, epistatic QTL and the issues of multiple simultaneous searches, has recently been put forward by Sen and Churchill[73]. The approach breaks the QTL problem into two distinct parts: the relationship between the QTL and the quantitative trait, and the location of the QTL. Disjoining these two independent relationships allows the initial focus to be placed on estimation of the unknown QTL genotypes, and then on allowing the search for different models and their comparisons with the information gained from completing the QTL genotype information. The power in breaking a problem into two independent parts is not new as it was dealt with by Jansen in 1993, and lies in the fact that information is gained in the first part that can be used in the second part. Once the QTL genotypes are estimated, Sen and Churchill explore all possible models using an approach that allows distinct models of different QTL numbers to be considered. As the QTL genotypes are calculated independently from the QTL effect and location, previous issues of epistasis and linked QTL are eliminated because the state of the QTL genotype and QTL number are known before the estimation of their effects and interactions.

Multi-trait QTL mapping can also benefit from the computational framework of Sen and Churchill by simply extending from a single phenotype to multiple correlated phenotypes, and by dissecting the problem in a similar manner. The additional information gained from knowing the covariation between multiple traits is the same as the treatment originally detailed by Jiang and Zeng[32], but the computational mechanics of the solution follow the Sen and Churchill approach. Although the Sen and Churchill view has been shown to benefit QTL mapping, it might have an even larger potential for accommodating other types of problem and data structure. The power of this computational approach[30,73] lies in its ability to dissect the problem into two unique and independent parts, and might prove to have an even greater potential for the dissection of complex traits using microarray technology (see below).

Statistical significance

Regardless of the method used to estimate and locate single or multiple QTL, once the test statistics are calculated the likelihood of the event is assessed. The statistical basis of these comparisons relies on model assumptions, the most common of which requires the quantitative trait values to be normally distributed. If in fact these assumptions hold, many wonderful statistical properties follow, and valid conclusions can be drawn from a range of powerful statistical tests. In reality (FIG. 1), however, the distribution of the trait values is not normal, and needs to be considered as a mixture of (normal) distributions[46]. Violating the normality assumption has an impact on the distribution of the statistic used to test for a QTL[79–81], which makes standard statistical procedures potentially inaccurate.

One approach to obtaining the distribution (or behaviour, in the long term) of the test statistic is to use a computer simulation to produce the data[16,30,82]. Thousands of data sets, taken from the same statistical model, are simulated and the test statistics calculated.

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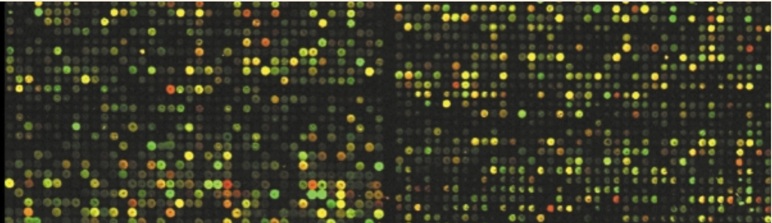
Box 3 | **Transcriptional profiling with microarrays**

A microarray comprises a set of hundreds or thousands of nucleic-acid spots, or features, on a solid support — often a glass slide. Each spot represents an expressed sequence, and consists of an oligonucleotide or cDNA probe. When a labelled cDNA is hybridized to the array, the amount of hybridization to each feature is quantified as an intensity reading that represents individual gene activity. This provides a global picture of gene transcript levels, and has become a widely used technique that is being used to study gene expression during development, in particular disease states and so on. Various statistical methods[50,92–96] can be used to identify statistically significant changes and groupings of gene expression. At present, these statistical methods are viewed as either linear-model-based approaches[93,94] that account for both biological variation and technological variation, or multivariate approaches, such as clustering[95,96], that allow the relationships among gene profiles (across time or treatments) to group the genes according to similar behaviour. Model-based inference is formulated around a scientific hypothesis that can be tested through a statistical calculation (or statistical test), and from which statistically significant conclusions can be drawn. Conversely, multivariate analyses are clustering techniques that are viewed as summary/exploratory in that they are typically not hypothesis based, and the resulting DENDROGRAMS are not testable. However, the testability of gene-expression dendrograms has recently been addressed[97,98] to assess statistical significance.



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Together, these test statistics show the behaviour of the test in the long run and, therefore, represent the statistical distribution of the particular test statistic. From this distribution, one chooses the level of statistical significance or threshold above which results are considered statistically significant (valid). This approach is indeed useful if the model used to simulate the data is the true model. However, the model rarely describes the complicated relationships that occur in the genome. For example, epistasis is difficult to model unless the interacting QTL are known in advance of modelling. When a detailed model accurately describes complex relationships between multiple (interacting) QTL, it is often the case that simulation-based thresholds are the only practical way to assess statistical significance because alternative approaches are so computationally demanding (see below).

NON-PARAMETRIC resampling methods[83] have provided a useful alternative to simulation-based thresholds. Permutation resampling[3,68] and bootstrap resampling[84,85] have been applied as a means of randomizing the phenotypic (trait) data for the purpose of evaluating any test statistic under a null hypothesis that tests for a QTL (FIG. 3). A permutation randomization of the data samples phenotypes without replacement. In other words, traits are randomly assigned to individuals in the data set with no single trait value being assigned to more than one individual. A bootstrap randomization of the data samples phenotypes with replacement such that after an individual receives a random trait assignment, some other individual might receive the same random trait assignment. The debate about permutation or bootstrap randomization is continuing, and is based on the argument that a permutation retains the summary information of the trait, whereas the bootstrap changes the mean and variance of the bootstrap sample. In both resampling approaches, the genotypic (marker) assignments remain as in the original data and, therefore, the genetic map does not change. An additional implication of not changing the genetic map is that all genotypic and population information are retained (such as segregation distortion, missing data and recombination fractions). Both resampling methods have been noted as being computationally demanding techniques that require more than 1,000 resamples, and each potentially leads to different results. Additionally, when the models are very complex, the extension of resampling methods to these situations quickly becomes computationally too demanding, as one would have to provide up to 1,000 resamples for every model considered.

Motivated by the computational intensity of the resampling-based methods, Piepho[86] suggests a quick method for calculating approximate QTL thresholds that are based on the work of Davies[87,88]. Because the Piepho thresholds are theoretically based and do not retain the previously mentioned genetic specifications, they remain constant across experiments, even though it is well known that the environment has a large role in the variation of a quantitative trait and, therefore, the accuracy of QTL location. In situations in which the biological and statistical effects are minimized (for example, segregation distortion, environmental variation, small sample size and incomplete data), the theoretical and resampling-based thresholds are generally the same[89,90]. An example of this can be seen in FIG. 3 in which the permutation threshold (based on 1,000 permutations) is 3.9, and Van Ooijen's[89] simulation-based threshold has a value of 4.0.

Dissecting quantitative traits using arrays

Advances in technology are providing tools that allow the simultaneous quantification of both gene expression and protein expression (BOX 1). Specifically, gene-expression arrays have become more accessible in recent years, and are providing new and abundant levels of information on genome-wide patterns of transcription. It is expected that protein arrays[43,44] will evolve in a similar manner and will provide the same challenges as incurred by microarray experimentation. Realizing that routine application of high-quality protein arrays is yet to be established, this discussion will focus on gene-expression arrays in which experiments can be designed to accommodate various treatments in various situations, and for data to be collected. How these data are examined is relevant to the dissection of quantitative traits.

If taken in its simplest setting, expression data for a single gene can be viewed as a quantitative assessment of the activity of a gene and how it changes. So, we can think of change in a particular gene as an expression-level polymorphism (ELP; D. St Clair and

DENDROGRAM
A branching 'tree' diagram that represents a hierarchy of categories on the basis of degree of similarity or number of shared characteristics, especially in biological taxonomy. The results of hierarchical clustering are presented as dendrograms, in which the distance along the tree from one element to the next represents their relative degree of similarity in terms of gene expression.

NON-PARAMETRIC
Statistical procedures that are not based on models, or assumptions pertaining to the distribution of the quantitative trait.

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R. Michelmore, personal communication), which, in part, reflects the underlying genetic variation. ELPs can be associated with regions of the genome, much like QTL. As there are thousands of genes, some of which are functionally related, this approach seems to have great potential for dissecting the complex relationship among and between genes across some condition or set of conditions. Essentially, the same framework and machinery that exists for QTL mapping can now be superimposed on the gene-expression data, and can be used as a means to identify regions of a genome associated with the expression of groups of (potentially, interacting) genes. (See BOX 3 for basic information on microarray analysis, and REFS 38,50 for further reading.)

Although gene-expression analysis and its results provide valuable information towards understanding gene function, these results are not helpful in relating gene function to complex traits, because they do not address anything other than the relative change in gene expression across treatments (for example, condition or time). Total genomic approaches, although powerful in their ability to provide an understanding of complete metabolic, regulatory and developmental pathways, will most likely be limited in their explanation of the complexities of segregating populations. Towards this end, Jansen and Nap[52] have recently outlined a strategy called 'genetical genomics' that couples the power of traditional genetics with the recent technological successes of genomics. Although they point out that genetical genomics is likely to be most successful in self-compatible plants, it should be applicable to any segregating population and should benefit directly from more advanced QTL frameworks, such as those detailed by Sen and Churchill. The details of how ELP mapping can be accomplished are in the process of being worked through, and therefore must be considered premature. My purpose here is to expand on the vision of Jansen and Nap to provide a forecast of how genomic regions that are associated with variation in gene expression could be identified using existing QTL methods and software.

We have seen that QTL methodology is powerful for identifying the genomic regions that are associated with quantitative traits. The same QTL methodology has great potential to associate changes in gene expression with a genetic map for the purpose of pursuing QTL at the molecular level and determine gene function. QTL experiments continue to widen our understanding of quantitative variation and complex interactions, but they are failing to identify anything more than large genomic segments. Those QTL experiments that have shown progress have been refined investigations[37,55,91], which use fine mapping, and have delivered precise results that account for a great proportion of the quantitative variation. However, so far, no one has identified all QTL (major and minor) that explain epistasis, let alone total variation in a quantitative trait. With this as motivation, one can look to the future and realize that gene-expression data are quantitative assessments of gene-related variation that can benefit from the tools developed for QTL analysis.

Genes might be considered one at a time, in groups, or as the total in the genome. The relationships between the ELP and genetic map can be achieved by using the framework of Sen and Churchill to allow the dissection of genomic state and location. The expression variation is explained through a statistical relationship (akin to the multidimensional model searches discussed earlier) and associated with the genome through genetic markers that are aligned on a genetic map. A single expression analysis has the potential to identify significant regions of the genome, but, more interestingly, when groups of genes are taken together, their coordinated gene expression (that is, co-variation) has the potential to uncover associated regulatory segments of the genome. To accomplish this, it is expected that the multi-trait analyses that have been developed for QTL mapping might be useful. The results of ELP analyses might turn out to be a key factor in understanding the role of epistasis in genetic variation, as the co-variation and relationships between the genes are an inherent part of the data. If the full sequence of an organism is available, this approach will be even more powerful, and not necessarily limited to the level at which the gene activity is measured (for example, mRNA, protein or metabolic).

As exciting as these combined approaches might seem, some caution should be expressed, because the issues that haunt every statistical analysis remain. And, although the technological advances are supplying information at increasingly impressive scales and levels of molecular detail, we should not forget the many statistical issues that have come before us in QTL mapping. Most notable are the statistical issues that are related to sample size, statistical design and modelling, multiple testing and statistical significance. It can only be expected that these statistical issues will continue to provide challenges to the scientific community, but, if dealt with appropriately, are not insurmountable.

Conclusion

Not long ago, detecting a QTL was the motivation for many scientific investigations and was an achievable goal. Now, locating the multiple interacting QTL that are associated with multiple traits, by continually evolving sophisticated statistical analyses, is the goal and the jumping-off point for further investigations that are supported by technology and result in even more data. What this technological and statistical progression has hopefully shown is that new levels of data are being collected that explain the flow of information through the central dogma (BOX 1). Quantitative variation is evident at each of these stages, and is supplying us with the knowledge that, when combined with proper statistical attention, is unprecedented in its power to unravel the inner workings of a genome. As we look ahead to developing new technologies, methodologies and training new minds, we must remember that no single technological advance or statistical method will unravel the genomic mystery. Instead, it will be the conglomeration of ideas, techniques and analyses that provide the end to this endeavour.

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Online links

DATABASES

The following terms in this article are linked online to:

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
multiple sclerosis

FURTHER INFORMATION

An alphabetical list of genetic analysis software:
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Minitab: http://www.minitab.com
QTL mapping software:
http://dendrome.ucdavis.edu/qtl/software.html
QTL references:
http://www.stat.wisc.edu/~yandell./statgen/reference/qtl.html
Quantitative genetics resources:
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