# STRATEGIES FOR MAPPING AND CLONING QUANTITATIVE TRAIT GENES IN RODENTS

*Jonathan Flint, William Valdar, Sagiv Shifman and Richard Mott*

Abstract | Over the past 15 years, more than 2,000 quantitative trait loci (QTLs) have been identified in crosses between inbred strains of mice and rats, but less than 1% have been characterized at a molecular level. However, new resources, such as chromosome substitution strains and the proposed Collaborative Cross, together with new analytical tools, including probabilistic ancestral haplotype reconstruction in outbred mice, Yin–Yang crosses and *in silico* analysis of sequence variants in many inbred strains, could make QTL cloning tractable. We review the potential of these strategies to identify genes that underlie QTLs in rodents.

In the 1990s, when it first became possible to map the chromosomal locations of quantitative trait loci (QTLs) in rodents, it looked as if QTL discovery would soon lead to the identification of genes involved in any medically important phenotype that could be modelled in mice or rats[1–4].

More than 10 years later, the field of QTL analysis is heading towards a crisis. The National Center for Biotechnology Information (NCBI) lists 700 QTLs that are found in rats (see the Entrez Gene web site in the Online links box), and the mouse genome database currently contains 2,050 mouse QTLs (see the Mouse Genome Informatics web site in the Online links box). Compare these figures with the number of candidate genes that are proposed to underlie QTLs in rodents (TABLES 1,2). If we uncritically accept all the claims as correct, only about 20 genes have been identified. Even if no more QTLs are mapped, at the present rate of progress (20 genes identified in 15 years) it will take 1,500 years to find all the genes that underlie known QTLs.

Several recent developments have been published that might make what is currently almost impossible more tractable. These include new genomic resources (for example, the availability of sequences and sequence variants), animal resources (for example, chromosome substitution strains and the proposed Collaborative Cross), techniques (such as *in silico* mapping and whole-genome expression studies) and analytical tools (such as quantitative complementation tests and Yin–Yang crosses, see below for more information).

In this review, we discuss the realized or potential success of new approaches to identify genes that underlie QTLs. We focus on methods to identify genetic variants that have only a small effect on the phenotype (by which we mean that the variants account for 10% or less of the phenotypic variation of a quantitative trait). In the past, robust and reliable detection of small-effect QTLs has been a problem[5]; but, as should be evident from the number of QTLs that have been reported, difficulties in QTL detection are not impeding research. The methods might not be the most efficient, but there is no doubt that QTLs can be detected at high levels of significance and that the findings can be replicated, even for behavioural phenotypes[6–9]. Although it is likely that only a small fraction of the total number of QTLs that segregate in inbred strain crosses are currently known to us (not to mention those in outbred stocks), without the ability to determine the genes they represent, their detection is of limited interest. We urgently need advances in gene, not QTL, identification.

*Wellcome Trust Centre for Human Genetics, Oxford University, Roosevelt Drive, Oxford OX3 7BN, United Kingdom.*
*Correspondence to J.F.*
*e-mail: jf@well.ox.ac.uk*
doi:10.1038/nrg1576



© 2005 Nature Publishing Group

Table 1 | **Cloned quantitative trait loci**

| Gene symbol | Gene name | Phenotype/function | Variation (%) | Gene identification method | Reference* |
|---|---|---|---|---|---|
| *Alox15* | Arachidonate 12/15 lipoxygenase | Abnormal bone mass | 4% | Expression differences, analysis of knockout alleles, pharmacological inhibition | 107 |
| *Rgs2* | Regulator of G-protein signalling 2 | Fear-related behaviour | 5% | Outbred mice, QTL–knockout interaction | 31 |
| *Cyp11b1* | Cytochrome P450, subfamily 11B, polypeptide 1 | Abnormal blood pressure | 5% | Congenics, sequence variants | 154 |
| *Lipc* | Hepatic lipase | Obesity | 7% | Multiple crosses, knockout interaction | 155 |
| *C5* | Complement component 5 | Experimental allergic asthma | 8% | Expression differences, loss-of-function mutation | 106 |
| *Ace2* | Angiotensin 1 converting enzyme 2 (peptidyl-dipeptidase A) | Abnormal blood pressure | 12% | Knockout phenotype | 156 |
| *Apoa2* | Apolipoprotein A2 | HDL cholesterol | 23% | Multiple-strain haplotypes, coding sequence variants | 88 |
| *Mpdz* | Multiple PDZ domain protein | Pentobarbital drug withdrawal with seizures | 25% | Genotype-dependent differences in coding sequence and expression | 16 |
| *Ncf1* | Neutrophil cytosolic factor 1 | Severe arthritis | 25% | Functional sequence change, pharmacological treatment | 157 |
| *Kcnj10* | Potassium inwardly-rectifying channel, subfamily J, member 10 | Susceptibility to seizure | 25% | Coding sequence variants, multiple-strain haplotypes | 15 |
| *Ptprj* (*Scc1*) | Protein tyrosine phosphatase receptor type, J polypeptide | Susceptibility to colon cancer | 29% | Recombinant haplotypes, sequence differences, loss of heterozygosity in human cancer | 158 |
| *Tas1r3* | Taste receptor, type 1, member 4 | Saccharin response | 30% | Multiple-strain haplotypes, coding sequence variants | 159 |
| *Nppa* | Natriuretic peptide precursor A | Cardiac hypertrophy | 44% | Promoter variants increase gene expression | 160 |
| *Cd36* | Fatty acid translocase | Abnormal fatty acid metabolism | 50% | Expression differences, loss-of-function mutation, transgene complementation | 161 |
| *Pla2g2a* | Phospholipase A2, group IIA (platelets, synovial fluid) | Modifier of tumours in intestinal cancer | 50% | Frameshift loss of function and transgene complementation (cosmid) | 162 |
| *Mtap1a* | Microtubule-associated protein 1a | Modifier of hearing loss | 57% | Coding sequence variants, transgene complementation (cosmid) | 163 |

*The first publication of this material. HDL, high density lipoprotein.

Before proceeding, we should also be clear about what we exclude from our discussion. In this paper, we do not consider artificially induced mutations that present as QTLs, such as those that are due to mutagenesis, although it has been suggested that mutagenesis in the mouse can be used to determine the genetic basis of quantitative traits instead of QTL mapping[10]. Screening mice for the quantitative effects of induced mutations is doubtless an efficient method for the identification of genes that are involved in the expression of a phenotype, quantitative or otherwise. However, our concern here is with the subset of polymorphic loci that give rise to phenotypic variation in natural populations (although we admit that laboratory mouse populations are in many respects unnatural). Mutagenesis does not distinguish between these loci and the large number of loci that are functionally invariant in nature, but in which mutations can be induced.

#### Lessons from previous success stories

Are there any lessons from success stories in the field of QTL mapping that might allow us to determine the probable effectiveness of the new approaches? To answer this question, we need to define success, which is not so straightforward. Two recent reviews that attempt this task identify 21 genes in total, but agree about the identification of only four genes in mice and five in rats[11,12]. As many have pointed out, there is no single proof that confirms a gene at a QTL and, to a large extent, each case has to be evaluated on its own strengths[13,14]. Replacing one allele with another at a locus through targeted mutagenesis with subsequent functional analysis provides a stringent test, as does complementation, but these procedures might not be possible (or even necessary) in every case: alternative evidence can provide sufficient proof that the gene has been found.

There are also issues about what to include as a QTL. Strictly speaking, a QTL is any locus that contributes to a phenotype that is measured quantitatively. However, some investigators use a quantitative measure for a phenotype that others assess qualitatively (for example, susceptibility to cancer can be assessed as affected or not affected, or by a quantitative measure, such as the number of tumours). To be comprehensive, we have summarized data on candidate genes in



© 2005 Nature Publishing Group

Table 2 | **Cloned susceptibility loci**

| Gene symbol | Gene | Phenotype | Penetrance | Method of detection | Reference* |
|---|---|---|---|---|---|
| *Cblb* | Casitas B-lineage lymphoma b | Type 1 diabetes | High | Nonsense mutation, transgene complementation (cDNA) | 164 |
| *Stk6* | Serine–threonine kinase 6 | Skin tumour susceptibility | Low | Multiple-strain haplotypes, outbred mice, expression difference | 74 |
| *Ctla4* | Cytotoxic T-lymphocyte-associated protein 4 | Autoimmune disease | Low | Sequence variants, association studies in humans | 165 |
| *Il2* | Interleukin 2 | Type 1 diabetes | Low | Coding sequence variants, differences in electrophoretic patterns | 166 |
| *Pctr1* | Plasmacytoma resistance 1 | Susceptibility to plasmacytoma | Low | Coding sequence variants, protein variant less active | 167 |
| *B2m* | β-2-Microglobulin | Type 1 diabetes | Low | Coding sequence variant, transgene complementation (plasmid) | 168 |

*The first publication of this material.

TABLES 1,2. TABLE 1 lists candidate genes at loci where the effects are expressed as the percentage of the total phenotypic variance. TABLE 2 lists candidate genes at susceptibility loci, the effects of which are expressed as differences in penetrance.

The weight of evidence in favour of each gene varies considerably. For example, Ferraro and colleagues have proposed *Kcnj10* as a candidate gene that is involved at a seizure-susceptibility locus on the basis of finding sequence variants in coding regions and gene expression in the relevant tissue, but had to consider data for 120 genes within the region of study[15]. Shirley and colleagues applied similar criteria for the candidacy of *Mpdz*, but had a much smaller interval, with only three known and three predicted genes to validate, and so were able to make a more convincing case for gene identification[16]. Although TABLES 1,2 should not be interpreted as definitive lists, they do allow us to draw two conclusions of general importance.

***Effect size.*** First, the QTLs that have yielded genes have had exceptionally large EFFECT SIZES. To appreciate how large, we ideally need an unbiased estimate of the typical QTL effect size. Unfortunately this is difficult to obtain because, in cases of low statistical power to detect effect sizes, the contributions of detected QTLs are

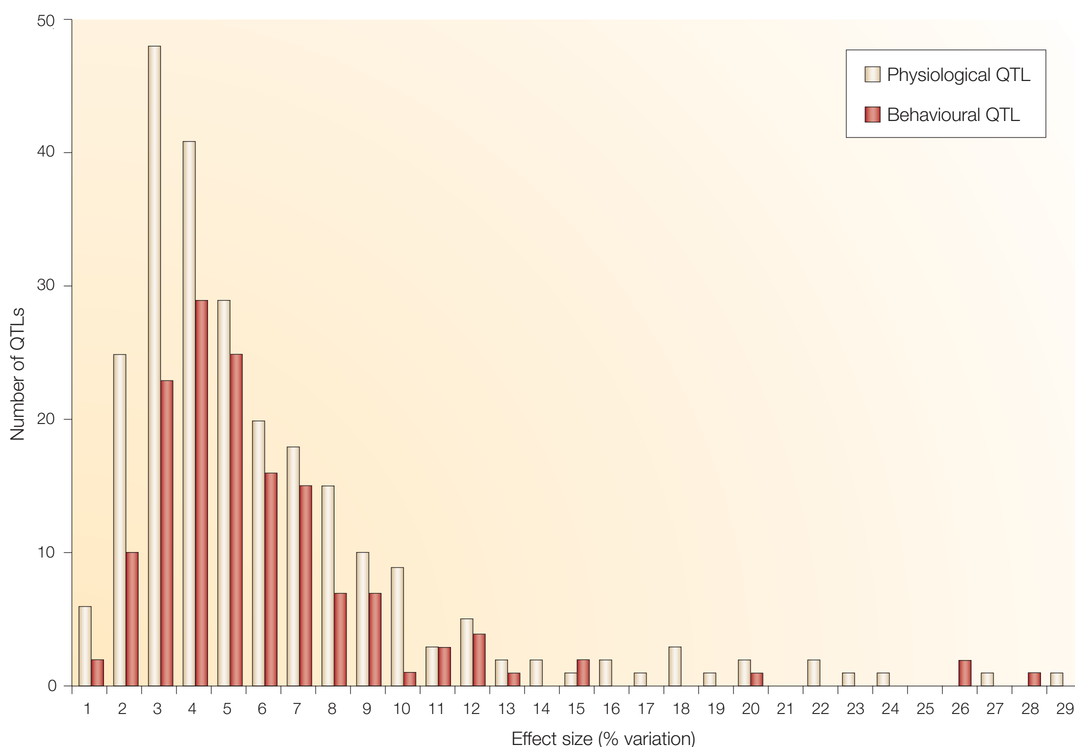


Figure 1 | **The distribution of QTL effect sizes.** Data are from 14 recent quantitative trait loci (QTL) mapping experiments (including 244 significant QTLs). Behavioural QTL data are from REF. 116. Data for physiological QTLs are from REFS 140–153.

EFFECT SIZE
The percentage of the total phenotypic variation that is attributable to a QTL.



© 2005 Nature Publishing Group

### Box 1 | How QTL effect size depends on the mapping population

The effect size of a quantitative trait locus (QTL) depends on the population in which the QTL alleles segregate. Here, we give a quantitative treatment and show that a genetic effect that produces a QTL with a 5% effect in a recombinant inbred (RI) population will produce a QTL that explains ~2.6% of the phenotypic variance in an F2 generation and ~1.3% in a backcross (BC). Consider a diallelic QTL at which a major allele has frequency $p$ and physiological effect $g$, and a minor allele has frequency $q = (1 - p)$. Let $G$ be the genetic effect of the QTL on the phenotype for a given animal. The genetic variance, $Var(G)$, in each population is $4g^2pq$ for RI, $2g^2pq$ for F2, and $g^2pq$ for BC (REF. 5). This shows that the genetic variance coming from the QTL — that is, the proportion of the phenotypic variance it explains — decreases in the ratio 4:2:1 for RI:F2:BC (FIG. 2). What does this do to the effect size of the QTL in these populations? In the simplest case where there are no other genetic components, the environmental variance $Var(E)$ is constant and the effect size, $\theta$, is shown in equation 1.

$$\theta = \frac{Var(G)}{(Var(G)+Var(E))} \qquad (1)$$

The top part of the table shows $\theta$ for each population and how the effect of a QTL in an RI becomes smaller in F2 and BC populations[132]. (Note that '|' in the table means 'conditional on'.)

| Measure | RI | F2 | BC |
|---|---|---|---|
| ***Single QTL system*** | | | |
| $\theta$ | $\frac{4g^2pq}{4g^2pq+Var(E)}$ | $\frac{2g^2pq}{2g^2pq+Var(E)}$ | $\frac{g^2pq}{g^2pq+Var(E)}$ |
| $\theta \vert \theta_{RI}$ | $\theta_{RI}$ | $\frac{\theta_{RI}}{2-\theta_{RI}}$ | $\frac{\theta_{RI}}{4-3\theta_{RI}}$ |
| $\theta \vert \{\theta_{RI}=5\%\}$ | 5% | 2.56% | 1.30% |
| ***Many QTLs system*** | | | |
| $\theta_1$ | $\frac{4\lambda\Gamma}{4\Gamma+Var(E)}$ | $\frac{2\lambda\Gamma}{2\Gamma+Var(E)}$ | $\frac{\lambda\Gamma}{\Gamma+Var(E)}$ |
| $\theta_1 \vert \theta_{1,RI}$ | $\theta_{1,RI}$ | $\frac{\lambda\theta_{1,RI}}{2\lambda-\theta_{1,RI}}$ | $\frac{\lambda\theta_{RI}}{4\lambda-3\theta_{RI}}$ |

But what if there are $k$ QTLs that affect the trait? We take the simple case of $k$ — unlinked, diallelic, independent, additive QTLs. The genetic variance coming from the $i$th QTL in, for example, an RI, will be $4g_i^2p_iq_i$, and the total genetic variance $Var(G)$ in the RI will be

$$Var(G) = \sum_i^k Var(G_i) = 4\sum_i^k g_i^2 p_i q_i \qquad (2)$$

The bottom part of the table gives the effect size, $\theta_1$, of QTL 1, (which accounts for a fraction $\lambda$ of the genetic variance) in RI, F2 and BC populations, as well as conditional effect sizes. In the table, we make the substitution defined in equation 3 for brevity.

$$\Gamma = \sum_i^k g_i^2 p_i q_i \qquad (3)$$

The drop in effect size moving from RI–F2–BC is shallower when other genetic components have a large role in the phenotype. In the extreme case of no environmental variance (when other QTLs account for all the remaining phenotypic variance), there is no drop at all. At the other extreme, when $\lambda = 1$, the formulae are the same as in the top part of the table.

over-estimated[17,18]. With this caveat in mind, FIG. 1 shows a comparison of effect sizes from a reasonably complete survey of behavioural QTLs (94 significant QTLs) and a compendium of physiological phenotypes (such as growth, weight, blood pressure and gall stones; note that we are using data from uncloned QTLs). It is important to realize that effect sizes depend on the mapping population in which they are measured (see BOX 1 for a detailed discussion; see also FIG. 2). Here, and in the rest of this review, we use effect size to mean the percentage of the total phenotypic variance that is explained by the QTL segregating in an F2 intercross.

FIGURE 1 shows that the distribution of effect sizes for both behavioural and physiological phenotypes are almost identical, as are the average effects of the uncloned QTLs for these phenotypes, which are 5.8% and 5.3%, respectively. The average effect size of a cloned QTL is 26% (TABLE 1). If we restrict our attention to the QTL genes that have been most stringently confirmed (by complementation), then the average effect size is 50%. Clearly, it is easier to identify genes when the QTL effect sizes are large.

In fact, the true effect sizes of most uncloned QTLs are almost certainly much smaller than the 5–6% average mentioned above, and this is not just because of



© 2005 Nature Publishing Group

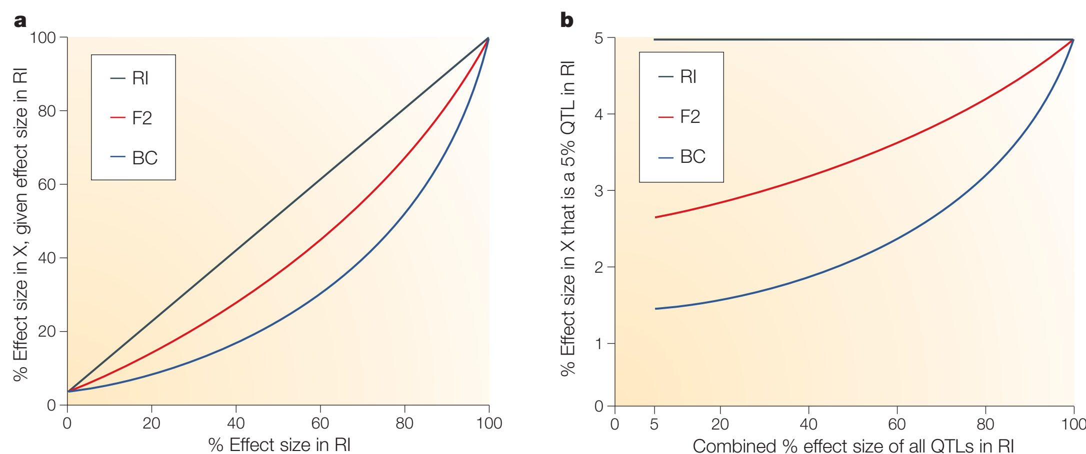


Figure 2 | **Effect size in F2 and BC relative to RI. a** | Illustrates how a QTL's effect size in F2 and BC populations relate to its effect size for a monogenic trait in an RI population. **b** | Shows the effect sizes in F2 and BC populations of what, in RI populations, is a 5% QTL for a single QTL that affects a multigenic trait (heritability is shown on the horizontal axis). Epistatic and gene–environment interactions require a more sophisticated treatment than is presented here. BC, backcross; RI, recombinant inbred.

statistical problems in their estimation. Perhaps the most important finding to emerge from attempts to clone genes that underlie rodent QTLs is that a single genetic effect, as detected in an inbred strain cross, turns out in most cases to be due to several physically linked small effects. There are many examples of this phenomenon, including QTLs that influence seizures[19], obesity[20], growth[21], blood pressure[22–26], diabetes[27], antibody production[28] and infection[29]. Classical approaches to gene identification (BOX 2) generally assume that a single QTL is just that, a single genetic effect. By not allowing for the fractionation of the QTL, gene identification becomes much more frustrating.

Unfortunately, we still do not have a precise picture of the genetic architecture of QTLs in rodents, or indeed in any organism[30]. Just how small are the effects likely to be? At the rarely achieved level of mapping where genetic effects can be resolved into intervals of a hundred kilobases or less, QTLs might further disintegrate. This has been found in studies of a behavioural QTLs in mice[31], and has been seen in similar work in other species[32,33]. Further high-resolution information at other QTLs might drive the estimate of average effect size below 5%.

In short, QTLs are predominantly loci with small effects, so the problem of identifying the genes that underlie them is a problem of identifying the molecular basis of small genetic effects. Whether we call the locus a QTL or not is of little importance; at the moment, the distinction that makes cloning tractable is between large and small effect sizes. Unless we have methods that can identify the genes at QTLs that contribute 1 or 2% to the phenotypic variation, the backlog of uncloned QTLs will remain.

*Molecular signature.* The second lesson from the success stories in TABLE 2 is that finding a recognizable molecular signature at a QTL, such as a sequence variant that introduces a stop codon or that changes protein function, makes QTL cloning tractable[34,35]. In such cases, not only does the sequence tell us which gene is the most likely candidate, it also indicates what experiments are needed to confirm the candidacy. For example, complementation will rescue the function of a null allele, or a biochemical or pharmacological intervention will determine whether an aberrant protein is involved. Unfortunately, possessing a recognizable molecular signature might be the exception rather than the rule for QTLs (although, admittedly, we have only a small sample of cloned QTLs to assess).

Although SNPs that cause monogenic disease have a strong tendency to occur at highly conserved amino acids, this does not seem to be true of variants in complex diseases. At least in human studies, the pattern of disease-associated coding variants is so far indistinguishable from the distribution that is found in the normal population[36]. Moreover, in many cases, no coding-sequence variant is found; instead many sequence variants are detected in non-coding DNA in regions, the function of which (if any) is unclear[31,37]. In these cases, we do not yet have an easy method to prove the involvement of a gene at a QTL.

It is important to emphasize that identifying a gene is not the same as isolating a sequence variant (or quantitative trait nucleotide; QTN). Discovering a QTN does not guarantee gene identification because the position of sequence variants does not necessarily coincide with the position of the genes on which the QTNs have an effect. For example, a QTN could lie within a regulatory region that is megabases away from its cognate gene[38]. Note also that it is a QTN, not a gene, that segregates in a cross, so genetic strategies for QTL dissection focus on sequence variants, not genes.

*The measure of success.* Before we look at new methods, we should be clear about how well the old methods perform (BOX 2). The relevant points (taken from a theoretical literature that is almost as extensive as the reports on QTL mapping) are as follows: 300 F2 animals can be used to map a QTL with an effect size of 5% onto a 40-cM

CONGENIC
A strain produced by a breeding strategy that delineates a genomic region containing a trait locus. Recombinants between two inbred strains are backcrossed to produce a strain that carries a single segment from one strain on the genetic background of the other.



© 2005 Nature Publishing Group

## Box 2 | Existing strategies for QTL mapping based on inbred strain crosses

**F2 intercrosses and backcrosses**

Two parental inbred strains are crossed to produce an F1, the starting point for the two most common mapping strategies. Intercrossing the F1 generates an F2 and backcrossing to one or other of the parental strains produces a backcross. The number of animals ($n$) needed to map an additive QTL in an F2 can be calculated using equation 1 (REFS 5,42).

$$n = \left(\frac{1 - V_{QTL}}{V_{QTL}}\right)\left(\frac{Z_{1-\alpha/2}}{(1 - V_{QTL})^{1/2}} + Z_{1-\beta}\right)^2 \qquad (1)$$

$Z_{1-\alpha/2}$ is the cut-off point in a standard normal distribution for a probability of $1 - \alpha/2$ and $\alpha$ is the significance threshold. If we use a genome-wide significance threshold of $\log P$ 4.3 (REF. 133) from normal tables, the cut-off point is 3.89. If we set the power at 90% (in other words, 10% chance of missing a true association) then we need a $Z_{1-\beta}$ of 1.28 (from normal tables). $V_{QTL}$ is the proportion of variance that is attributable to the QTL. For a purely additive QTL that explains 5% of the variance, the calculation yields equation 2.

$$\left(\frac{0.96}{0.05}\right)\left(\frac{3.89}{0.97} + 1.28\right)^2 = 527 \qquad (2)$$

**Recombinant inbred (RI) lines**

A pair of inbred strains is intercrossed and their progeny is inbred to generate a panel of inbred animals, each with a different combination of the progenitor genomes. Once a set of RI lines has been genotyped, the marker data are available for all subsequent mapping experiments, which take place by determining the strains that are present at a marker and by correlating this strain distribution pattern with phenotypic differences. Using the terminology given above for power in an F2 intercross, the relationship between the number of animals ($n$) and the effect size of the QTL is given by equation 3 (REF. 134).

$$n - 2 = \frac{(1 - V_{QTL})(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(V_{QTL})} \qquad (3)$$

**Congenics and interval-specific congenics**

Congenics are still the mainstay of fine-mapping QTLs in rodents. By repeatedly backcrossing one strain onto another, it is possible to create animals that have a particular genomic region from one strain and the remainder of their genome from the other; subsequent intercrossing makes the genomic segment homozygous and the mouse fully inbred. However, inadequate or incorrect assumptions about the distribution of chromosome segments, the population structure, the marker spacing and the selection strategy might mean that the breeding does not go as predicted[59]. Flaherty and colleagues point out that transgenic mice, which are routinely created on a 129 background and then backcrossed onto another strain (usually C57BL/6), can be used as congenics for the interval around the engineered mutation[135]. An interval-specific congenic is made by first identifying an animal with a recombinant chromosome in a region of interest and then backcrossing it for several generations to remove other QTLs. Resultant animals are intercrossed, and homozygotes for the recombinant haplotype are selected.

**Recombinant congenics and genome-tagged mice**

Recombinant congenics are a type of RI line made by backcrossing and randomly fixing parts of the genome by inbreeding, so that each contains an average of 87.5% genes of a common background strain and 12.5% of a common donor[136]. Genome-tagged mice are sets of overlapping congenics with sufficient material from one strain crossed onto the other so that the whole genome is represented[137]. The 60 strains created by West and colleagues contain segments of about 23 cM that are INTROGRESSED from either DBA/2 or CAST/Ei onto C57BL/6 (REF. 137).

**Recombinant progeny testing**

Animals that have a recombinant chromosome in a region of interest are backcrossed to a parental strain to determine the location of the QTL relative to the recombination point.

**Advanced intercross lines**

Two parental strains are crossed to produce an F1 that is intercrossed to produce an F2. Subsequent generations are produced by intercrossing, so that animals in an advanced intercross line accumulate new recombinants. Animals are maintained according to a pseudo-random breeding protocol to reduce loss of genetic variation in the population. An appropriate advanced intercross line could take at least 5 years to make, but it can be used to map many loci.

**Heterogeneous stocks**

Genetically, heterogeneous stocks are derived from inbred strains through a series of progressive intercrosses. Existing heterogeneous stocks are derived from eight strains, but any number can be used (an advanced intercross line is a heterogeneous stock derived from two strains). By subsequently maintaining the stock for many generations, using a pseudo-random breeding protocol, recombinants are introduced that allow high-resolution mapping. Traits are mapped by associating allelic variants with a phenotype, so that genotyping must be carried out for each experiment. Two heterogeneous stocks have been used for mouse mapping experiments: the older of the two (the Boulder heterogeneous stock), has been breeding for more than 60 generations and is derived from the C57BL/6, BALB/c, RIII, AKR, DBA/2, I, A/J and C3H strains[138]. The second heterogeneous stock (the Northport heterogeneous stock) has passed its fortieth generation[139], and is derived from the A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, CBA/J, DBA/2J and LP/J strains. Genetic variation from many strains complicates analysis, but robust and powerful QTL detection is possible by estimating the probability that an allele descends from each progenitor strain[70] (BOX 3).

INTROGRESSION
Introduction of a chromosomal segment from one strain into another by interbreeding.



© 2005 Nature Publishing Group

interval with 50% power, using markers that are spaced every 20 cM across the genome[39]. Increasing the marker density does not appreciably increase mapping resolution for small-effect QTLs[40]. Increasing the number of animals increases the likelihood of QTL detection (so that with 800 animals there is a greater than 99% chance of detecting a QTL with an effect size of 5%; BOX 2), but does not increase resolution sufficiently to identify genes — even 1,000 animals will only map a 5% QTL onto an interval of about 10 cM (REF. 41). In summary, a standard inbred-strain mapping experiment will need about 400 animals and will use 100 markers.

Starting from a cross between two inbred animals, it could take less than 6 months to map a QTL to a 40-cM region. It will take a few more years to isolate a QTL by creating CONGENICS[42] (BOX 2). Alternatively, by continuing to intercross beyond the F2 generation, fine mapping can be carried out in an advanced intercross line or a heterogeneous stock[43] (BOX 2). After 6 years, we can reasonably expect traditional strategies to have resolved a 5% QTL into a region of about 1 cM, which in the mouse corresponds to 2 Mb on average. The average gene density in the mouse is about 10 genes per megabase, so a 1 cM QTL interval will contain about 20 genes (note that gene density varies significantly across the genome). Keeping this in mind, we now turn to the new strategies to see how they fare in comparison.

### Chromosome substitution strains

Chromosome substitution strains (CSSs) consist of a set of animals in which one chromosome is derived from one strain and all the rest are derived from another. For mice, a panel of 22 CSS strains is required (for 19 autosomes, 2 sex chromosomes and mitochondria), or 44 if a reciprocal set (with both parents as progenitor donors) is used. QTL mapping is carried out through the relatively simple process of comparing the phenotypes of each strain with the parental background strain. CSS strains were first used to map QTLs in mice in 1999 (REF. 44); theoretical aspects of the mapping process were subsequently described for mice[45] and rats[46,47]. The method has a long history in plant[48] and *Drosophila* genetics[49]. The first complete CSS set, created from A/J and C57BL/6 strains, was produced in 2004 and was used to detect QTLs across the mouse genome[50,51].

The ease of QTL detection using CSS strains results from two features: first, the background genetic variance is reduced so that each QTL explains a greater proportion of the total phenotypic variation (BOX 1). Second, a lower significance level is needed for QTL detection because, compared with the ~100 markers tested in an F2 generation, only 21 comparisons need to be made. Singer and colleagues point out that the F2 intercross requires at least 35% more animals for QTL detection[51]. Nevertheless, Belknap estimates that to detect a QTL with an effect size of 6% and with 50% power will require 20 CSS strains and 20 parental animals for each comparison, or between 3 and 400 animals (depending on how many background animals are used) for a genome scan[52]. Belknap's estimate of 3 to 400 animals agrees with the actual figure of 435 animals used to map QTLs in the first mouse CSS experiment[51]. Notably, this is not a substantial saving on the numbers used for an F2 intercross (BOX 2).

A comparison between parental and CSS strains will only map a QTL to a chromosome. For higher-resolution mapping, CSS strains allow the rapid creation of a congenic strain, either by using interval-specific congenic strains or by recombinant progeny testing[42] (BOX 2). Because of the relative increase in effect size, congenic construction and recombinant progeny testing will require 3–4 generations to reduce the interval to 1 cM, rather than the 9–10 generations that are required when starting from an F2 intercross[52].

QTL mapping in a CSS delivers researchers to the same point faster than classical strategies have led them, but no farther. The main drawback of the method is that it makes no allowances for the fractionation of a large QTL effect into many loci with smaller effects. This is the problem that has for so long beset the use of congenics for QTL dissection and gene identification. CSS mapping is a powerful method for the identification of small-effect QTLs, but it does not offer advantages over other methods for the identification of genes.

### Collaborative Cross: a proposal

Would the difficulties of QTL cloning be overcome if we had 1,000 recombinant inbred (RI) lines, as suggested by the Complex Trait Consortium[53]? Although the Collaborative Cross — a panel of 1,000 RIs derived from 8 parental strains — is only a proposal, not an existing resource, we discuss it here because it might provide a tool for gene identification (although this is not the sole reason for its creation[53]).

The use of RI lines for QTL mapping in rodents has so far been limited by the relatively small size of each set. The BXD RI set (which is derived from C57BL/6 and DBA/2 strains) consisting (until recently) of 26 lines has 90% power to detect a QTL that explains half the phenotypic variance in a trait[54]. The comparable effect of a QTL segregating in an F2 generation is half this value (because the F2 animals are not inbred), or 25% of the phenotypic variance (BOX 1). What happens to power and mapping resolution if we have 100 lines?

We have investigated this question using a simulation[55]. Fine mapping of a QTL with a 5% effect is efficient: 100 RI lines provide 80% power for QTL detection at a 5% significance level. Mapping resolution is also good: the 95% confidence interval is 7 cM. Fortunately, RI panels approaching this size are now available: the LXS panel consists of 77 lines[56] and the expanded BXD set consists of 90 lines[57]. Therefore, the new RI sets can deliver high resolution, although this is still insufficiently high to identify genes at small-effect QTLs.

The proposed Collaborative Cross differs in two ways from standard RI lines. It is created from eight progenitors, not two as is the case with RI lines, so that the set would contain more genetic diversity than a standard RI. Second, the proposal is to create 1,000 lines, ten times more than the largest available set. What would be the advantage, for gene identification, in having such an RI set? Mapping resolution improves to



© 2005 Nature Publishing Group

**Box 3 | Probabilistic ancestral haplotype reconstruction**

Single-marker association mapping is an inefficient method when the allele frequencies of the variant and the quantitative trait locus (QTL) do not coincide and when contrasting QTL alleles are in linkage disequilibrium (LD) with the same marker allele, as illustrated in the figure. In this example, the population from which all chromosomes descended consisted of four inbred strains. Inbred strains A and C contain a QTL allele that increases the phenotype, and strains B and D contain an allele that decreases the phenotype. A polymorphic marker, m, that is in complete LD with the QTL possessing four distinct alleles (labelled 1,2,3,4 in the figure) will show evidence of association. Consider what happens with a diallelic marker, n, that, in the ancestral population, has one allele '1' that is physically linked to strains A, B and C and the other allele '0' linked to strain D. Evidence of association will now be reduced because the marker fails to partition the genotypes into two equally contrasting groups. Things are even worse at markers p and q where both alleles 0 and 1 are linked to both increaser and decreaser QTL alleles; there is no detectable association at all. But the haplotypes that are defined by the markers p and q distinguish all four strains and so are surrogates for the efficient marker m. Therefore, multi-point haplotype-based mapping is generally more powerful than single-point mapping.

Heterogeneous stocks are mosaics of eight inbred founder strains, and QTL mapping in these stocks is best approached by reconstructing the ancestral haplotype mosaic. However, the strain distribution patterns of neighbouring markers rarely distinguish between all founder strains, so instead we compute the probability that an animal is descended from a given pair of strains at a particular locus. A useful statistical model of the genome mosaic of a diploid heterogeneous stock animal is a pair of HIDDEN MARKOV MODELS, in which the hidden states of the model are the ancestral strains, and the observed data are the marker genotypes. A DYNAMIC PROGRAMMING ALGORITHM is then used to compute the probabilities of descent using all the available information. The marker alleles must be known in the founder strains for the method to work. Recently, we have shown that other outbred mice of unknown ancestry can be modelled and analysed as mosaics of standard inbred strains in the same way[31].

about 4 cM when the number of lines increases to 1,000 and further lines make it possible to map effects that are smaller than 5%. As we have seen, QTLs have a habit of disintegrating when they are dissected and we need the ability to find the pieces.

If the Collaborative Cross were to be created, it would enable us to map many of the 2,000 uncloned QTLs into small genetic intervals. This is an attractive proposition but it must be balanced against two important disadvantages. First, maintaining and distributing an RI set of 1,000 lines poses significant problems. Even a laboratory devoted to providing animals to the research community, such as the Jackson Laboratory, is able to maintain only a few hundred strains as live stock. Second, the resolution of the Collaborative Cross is not sufficient to identify genes: a 4-cM interval is roughly equivalent to 8 Mb of DNA, which will contain approximately 80 genes. Unfortunately, the Collaborative Cross would give us no help with the final goal of gene discovery. However, one solution might lie in the application of recombination inbred segregation tests (RISTs) and its generalization, Yin-Yang crosses (YYCs).

HIDDEN MARKOV MODEL
A probabilistic description of a system in which the observed data depends on the hidden internal state of the system. The objective is usually to infer the likelihood that the system is in a particular hidden state, given the observed data.

DYNAMIC PROGRAMMING ALGORITHM
An algorithm that finds the optimum solution to a problem involving *N* objects in terms of the solutions to a series of smaller problems that involve subsets of the objects.

### Yin–Yang crosses

RISTs and YYCs, applied to a large number of RIs, will increase mapping resolution to the point where individual genes can be identified. YYCs take advantage of the abundance of recombination events in RI or inbred strains to improve mapping resolution[58]. To understand the principle, we first explain RISTs, which are a particular example of YYCs[42].

Assume that a QTL has been mapped in an inbred-strain cross. RI strains that have recombinants within the QTL interval are crossed with both parental inbred strains to produce two segregating populations. In one cross, the RI and the parental line share the QTL allele, so no QTL segregates; in the other cross, the RI and the parental line have a different allele at the QTL, and the QTL segregates (hence the name segregation test). Consequently, the QTL will be mapped to a position either above or below the recombination point in the RI that is used for the two crosses. The overall results from a selected set of RIs will locate the QTL to a small interval.

Resolution is determined solely by the number of recombinations in the interval. For example, with 100 RI lines, each having approximately 3.3 recombinations per 100 cM (REF. 56), the total number of recombinations is 3.3 multiplied by the number of centimorgans in the genome (~1,400) to give 4,620 recombinations; so, the expected resolution is 1,400/4,620 = 0.30 cM. This corresponds to an interval of 0.6 Mb. With 1,000 RI, the expected accuracy is 0.03cM (potentially a few hundred kilobases). However, recombinations are not distributed evenly in the genome, making the above calculation an approximation only[59].

RIST, as with congenic construction and other strategies that seek to treat QTLs as Mendelian genetic factors, will have problems with multiple linked QTLs;



© 2005 Nature Publishing Group

but unlike the construction of a congenic line, a RIST analysis is quick to carry out, so if multiple QTLs are suspected, it will be possible to test that hypothesis with further RISTs (assuming appropriate recombinant lines are available). Indeed, with 1,000 RI lines, it would be possible to resolve most QTLs onto an interval that contains a few genes in just two generations; an efficient and relatively economical strategy. In some instances, the resolution will be good enough to identify a gene underlying a QTL.

The YYC approach is a generalization of the RIST method that treats inbred strains as if they were RI lines, on the grounds that inbred strains descend from a relatively small number of founders. In this respect, YYCs make the same assumption as *in silico* mapping, which is discussed below. The large number of recombination events that have accumulated in a set of inbred strains since their origin can be used to map the QTL to a small region[58]. Similar to RISTs, YYCs are used as a fine mapping tool that is applied after the initial mapping of the QTL. The allelic state of the QTL in multiple strains is revealed by crossing the two strains that were used initially to map the QTL and any new strain. YYCs establish the strain distribution pattern of the QTL, which is used to identify the list of variants in the region that follows this distribution.

The YYC method is based on the assumption of a single biallelic variant or haplotype that affects the trait in the different strains. Therefore, as for the RIST approach, the main limitation of YYC analysis with inbred strains is that it is not suitable for multiple QTLs in a region. Furthermore, QTLs that primarily have epistatic effects will not be suitable for this kind of analysis. They will result in inconclusive results of the Yin–Yang analysis, but this is unlikely to produce false negative results.

## Outbred stocks

Outbred stocks accumulate recombinants over time, so that they offer high mapping resolution; potentially high enough to identify candidate genes. The effects of individual genes can be mapped by association, as has been successfully shown in human populations[60–67]. In a few cases, QTLs have been mapped in mouse populations: small-effect QTLs have been mapped to sub-centimorgan resolution using heterogeneous stock mice[68–71] (BOX 2); crosses using outbred *Mus spretus* have been used first to map[72,73] and then to identify serine–threonine kinase 6 (*Stk6*) as a candidate skin-tumour susceptibility gene[74]; outbred CD1 mice have been used to map a susceptibility locus for pulmonary adenoma[75]; and outbred MF1 strains were used to identify regulator of G-protein signalling 2 (*Rgs2*) as a gene that influences anxiety in mice[31].

Mapping in outbreds is not as straightforward as mapping in inbred crosses: it requires higher density genotyping and larger numbers of animals. At first sight, the method seems to have not only the advantages, but also to suffer from the same drawbacks, as human genetic association studies, where robust, replicable results have been hard to obtain[76] and sample sizes of many thousands are required[77]. However, QTL analysis in outbred mouse populations is likely to be easier than its human equivalent. In fact, it would be as simple and powerful as mapping in inbreds if each allele at every locus could be traced back to a unique progenitor strain, so that ANALYSIS OF VARIANCE (ANOVA) at a marker extracted maximal information for QTL detection (BOX 3).

In contrast to human populations, in which progenitor alleles are unknown (although there are a few exceptions, such as ADMIXTURE MAPPING[78]), one type of outbred mouse (heterogeneous stock) is derived from known ancestral inbred strains. This makes it possible, with the help of some mathematics, to derive the origin of each allele and map QTLs at sub-centimorgan resolution (BOX 3). Unfortunately, mapping a QTL to a megabase interval is still insufficient to identify a single gene. Other outbred stocks might offer better resolution, but they lack the advantages of progenitor information from the heterogeneous stock.

Several methods have been developed that deal with this problem in its various incarnations[79–84]. So far, there has been only one application of this method in mouse genetics. We have shown that sufficient recombinants have accumulated in a commercially available outbred stock (known as MF1) to map genetic effects onto regions of only a few hundred kilobases or less[31]. Sequence analysis showed that variants in the MF1 strain were identical to those found in inbred strains, and haplotype reconstruction revealed that more than 95% of the haplotypes could be derived from known inbred strains. Therefore, we were able to map the MF1 strain by probabilistic ancestral haplotype reconstruction (BOX 3). It is important to realize that our approach neither required us to recover the correct haplotypes, nor to identify the progenitor strains correctly. We found that a QTL for anxiety-like behaviour, previously mapped to an interval of less than 1 cM, resolved into three smaller independent QTLs, each about 100–200 kb wide. It would also be possible to combine pedigree and LINKAGE DISEQUILIBRIUM (LD) data for fine mapping in the MF1 strain and, indeed, in other outbred mice[79,81,85].

Potentially, outbred animals could deliver a resolution that would be sufficient to guarantee candidature of a single gene. The disadvantages of the method lie in the complexities of the analysis and the need for large numbers of animals and high-density genotyping. For example, genome-wide mapping in heterogeneous stocks (BOX 2) requires at least 6,000 SNP markers. To reduce false-positive results to acceptable levels with this number of markers means imposing stringent significance thresholds: by permutation, we estimate that the 5% and 1% significance levels correspond to *P*-values, expressed as negative logarithms, of 4.4 and 5.1 (REF. 86), and will require about 1,000 animals to provide 80% power to detect a 5% QTL[87]. More markers, and more animals, will be needed for whole-genome analysis of more complex outbred stocks. These features militate against whole-genome analyses using outbreds, but developments in high-throughput genotyping will make it feasible.

ANALYSIS OF VARIANCE
A statistical method to test the null hypothesis that the mean values of two or more groups are equal. The variance around the mean in groups is compared with the variance of the group mean. In genetic applications, the variance between families is compared with the variance within families. A significant *F*-ratio implies that variance between families is larger than within families.

ADMIXTURE MAPPING
Genetic mapping using individuals whose genomes are mosaics of fragments that are descended from genetically distinct populations. This method exploits differences in allele frequencies in the founders to determine ancestry at a locus in order to map traits, in a way that is broadly similar to an advanced intercross.

LINKAGE DISEQUILIBRIUM
The tendency for markers to have correlated genotypes when they are physically close together. Over several generations, recombination will break down linkage between markers and a QTL, so that linkage disequilibrium will only occur between markers that are close to a QTL. This explains why outbred animals can provide high-mapping resolution.



© 2005 Nature Publishing Group

### Box 4 | QTL mapping using recombinant inbred lines versus *in silico* mapping

Because recombinant inbred (RI) panels are descended from (usually) two inbred strains, their genomes are random mosaics of these founder haplotypes. Once established, an RI panel need only be genotyped once, using markers that distinguish between the founder strains. At a given marker, the pattern of genotypes across the panel is called a strain distribution pattern. For example, in a panel of eight RI strains, the SDP 0100111 means that the first, third and fourth strains carry the allele '0' and the remainder the allele '1' at the marker in question. To map a quantitative trait locus, RI animals across the panel are phenotyped (if possible, in replicate, to reduce non-genetic variance), and the values are correlated with the strain distribution patterns at each marker (see the Gene Network web site in the Online links box) to identify markers with statistically significant associations. Mapping resolution depends on the size of the panel, but is roughly equivalent to that of an intercross.

Superficially, *in silico* mapping looks similar. A set of inbred strains (that have an unclear ancestry) replaces the panel of RI strains of known ancestry. An important difference is that regions of HAPLOTYPE SHARING between the inbred strains must first be predicted from runs of contiguous markers with shared genotypes. The inbred strains are phenotyped and correlated with the predicted haplotype distribution patterns as before. Because the blocks of haplotype sharing between inbreds are smaller than in RI lines, theoretically *in silico* mapping resolution should be greater for the same number of strains. The key difference is that mapping using RI lines is by descent, but is by inferred state for *in silico* mapping. RI mapping is uncontroversial; by contrast, the merits of *in silico* mapping are hotly debated.

### Haplotype analysis and *in silico* mapping

Many have pointed out that a QTL must be contained in a region where sequence divergence corresponds to genetic action. So, when QTLs have been mapped in different combinations of inbred-strain crosses, the strain distribution pattern can be combined with mapping data to refine the region that contains the functional variant[68,88–90]. For example, a QTL identified in a cross between strain A and strain B that does not segregate in a cross between strains A and C will be located where sequences from strains A and C are the same, but those from strains A and B are different. This approach has been used to carry out mapping at an extremely high resolution and to identify candidate genes[68,88–90].

A development of this idea is *in silico* mapping (BOX 4). It relies on known phenotypic differences between inbred strains (such as those being collected as part of the Mouse Phenome Project[91]; see the Mouse Phenome Database in the Online links box) and uses a high density of markers to derive a strain distribution pattern that can be used for mapping[92]. The proponents of this method argue that it reduces the time required for analysis of genetic models of complex disease "from many months to milliseconds"[92].

In principle, *in silico* mapping is similar to mapping using RI lines — by establishing phylogenetic relationships between inbred strains from sequence information it should be possible to identify regions that are identical by descent. The problem is that in contrast to a set of RI strains (or indeed all mapping experiments that are derived from crosses between inbreds), in which genotyping unambiguously defines descent so that genetic identities can be associated with phenotypic similarities, finding sequence variants that are shared by a set of inbreds does not guarantee their descent from a common ancestor.

Nevertheless, several observations support the treatment of inbred strains as if they were derived from a set of founder strains. For example, the genealogies of many inbred strains can be traced back to a relatively small number of animals[93,94]. Moreover, the pattern of sequence variation between inbred strains initially indicated that the distribution of polymorphisms has a mosaic structure, which is consistent with their derivation from a relatively small number of Asian and European stocks[95–97].

There is little doubt that *in silico* mapping is effective for monogenic, highly penetrant mutations[95,98,99]. The crucial issue is whether *in silico* mapping performs well in detecting small-effect loci. Wiltshire and colleagues have gone the furthest towards this goal. They used a dense map (of 10,990 SNPs) that was typed in 48 strains and applied a permutation method to determine the significance of their results[99]. They demonstrated the effectiveness of the method for localizing large effects (on coat colour and taste preference) and then showed that for two multigenic complex traits (high-density lipoprotein cholesterol levels and gallstone formation) they could find loci that corresponded to some previously identified QTLs. These results are encouraging, although further work is required to distinguish between true- and false-positive findings.

At present, two difficulties confront *in silico* mapping: low power and the complex structure of the genomes of laboratory strains. At first sight, the problem of power seems to be insuperable: how can the genetic basis of complex traits be dissected with just a few dozen animals, or even a few 100, when we know that successful association studies in humans requires thousands of individuals[77]? The truth of this analogy depends on the extent to which the inbred strains are related by descent. If the inbred strains are completely unrelated, the analogy is correct and thousands of animals will be needed for genetic mapping. This is partly because there will be less LD between loci in unrelated individuals, so that more markers are needed to detect the QTL[77]. More unlinked markers will require an increase in the stringency of the significance threshold to avoid false-positive results, which in turn requires an increase in the number of animals to maintain power for QTL detection. However, more animals are also needed because, in fully outbred populations, more loci are likely to contribute to the genetic variance. Therefore, the relative contribution to the phenotypic variance that is attributable to a single locus will be less than for the same locus in a population

HAPLOTYPE SHARING
Sets of closely linked genetic variants in different individuals that are identical by descent around a locus.



© 2005 Nature Publishing Group

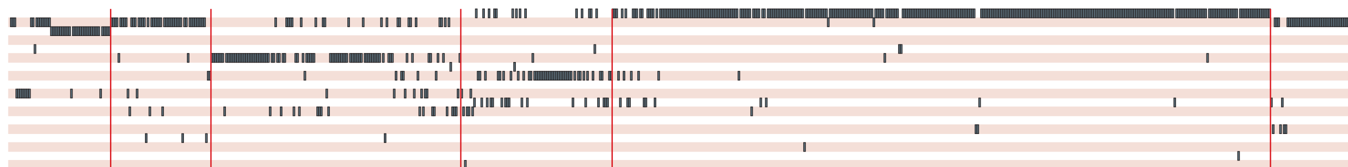

Figure 3 | **Haplotype complexity in inbred strains of mice.** 700 variants with a strain distribution pattern frequency >1% between eight inbred strains across a 2-Mb region of mouse chromosome 1 are shown. The region is represented along the horizontal axis and scaled so that the *n*th coordinate from the left edge corresponds to the *n*th variant. The alternating grey and white tracks show the spatial arrangement of the 13 most common strain distribution patterns. Each track represents one strain distribution pattern, a bar on the track shows where the corresponding variant has that strain distribution pattern. The strains are A/J, AKR, BALB/cJ, C3H, C57BL/6J, DBA/2J, I and RIII. The data presented are from REF. 37.

of individuals who have common ancestors (BOX 1). However, as we have seen, inbred strains do share ancestors, so their degree of genetic relatedness is not equivalent to that in outbred humans.

To obtain a lower limit on the number of animals needed to detect a genetic effect by *in silico* mapping, assume that all inbreds are derived from just two progenitors; in other words, assume the inbreds are a set of RI lines. Using a standard equation to calculate the number of RI lines required to map a QTL, Darvasi argued that between 40 and 150 inbred strains would provide 50% power to detect a QTL that explains 5–20% of phenotypic variation in an F2 cross[42]. So, the smallest number needed to obtain sufficient power will be about 50 animals. However, it will almost certainly be more than that, because inbreds are not as closely related to each other as a set of RI lines.

Nevertheless, Peltz and colleagues claimed that they could detect QTLs using data from just eight inbred strains. They make the point that by phenotyping more animals it is possible to increase the heritability by reducing measurement error[100]. The drawback to this argument is that, even if all environmental noise could be removed in this way, the individual effect of each QTL will still be limited by the number of other QTLs that contribute to phenotypic variation. Good estimates of the average number of QTLs and their relative contributions to a typical complex trait are hard to come by, but assuming an exponential distribution of effect sizes, our own data on behavioural measures yield a mean of 7 QTLs (with an upper limit of 14 using a 95% confidence interval (REF. 101)). So, given the known distribution and effect sizes of QTLs, it is extremely unlikely that a single effect accounting for more than 20% of the phenotypic variance will occur in a comparison of inbred strains. Therefore, we can be reasonably certain that to tackle genetically complex phenotypes successfully, *in silico* mapping will require much larger numbers of strains than have so far been analysed.

The second difficulty for *in silico* mapping concerns the structure of the genomes of inbred strains. Is the degree of mosaicism sufficient to satisfy the assumptions of the mapping method? The idea that large segments of the genomes of inbred mice maintained their sequence identity over many megabases has recently been modified by several publications that describe a much more complex picture[37,102,103].

The power of *in silico* mapping will vary along the genome depending on the complexity of the pattern of sequence variation. There will be effects on both power and resolution, depending on the number of progenitor genomes that are assumed to give rise to the inbred strains and on the size of the blocks of sequence identity. Although initial surveys of polymorphisms have revealed an apparently simple pattern of relatively large blocks of sequence similarity and difference, detailed analyses of multiple strains show that contiguous strain distribution patterns are different, but consistent with the same phylogenetic tree.

FIGURE 3 shows a representative example of the complexities that have been discovered in a single 2-Mb region on mouse chromosome 1 (REF. 38). In the right half of the figure, the structure is relatively simple; without too much loss of information, the distribution of sequence variants can be modelled as if the genomes were descended from just two progenitor strains, with one strain distribution pattern accounting for 93% of the variants. In this case, *in silico* mapping could detect QTLs (assuming that an adequate number of strains has been analysed). However, the method cannot resolve QTLs into an interval that is smaller than the block (which in this case is about 1 Mb). By contrast, the left half of the figure shows a much more complex structure, with several strain distribution patterns that alternate in an unpredictable fashion, but are still consistent with the same phylogenetic tree. Much higher mapping resolution will be obtained in this area, but with some loss in the power to detect QTLs.

The analysis of sequence variation described above was carried out using just eight strains, but we expect that the results can be extrapolated to accommodate more strains. Importantly, the full complexity of the structure was only revealed when near-complete sequence information became available. If the structure is mis-specified, as is likely with a low density of markers (which in this particular region would be more than about 10 kb apart), differences between strains will not be identified and so the power to detect QTLs will be reduced.

*In silico* mapping is an intriguing and attractive method, but it does require the ability to infer identical sequences unequivocally when neighbouring genotypes are the same. The recent announcement that 15 inbred mouse strains are to be resequenced



© 2005 Nature Publishing Group

means that questions about appropriate marker density will soon be addressed (see the US National Institute of Environmental Health Sciences Press Releases web site), but the potential of *in silico* mapping will only materialize when we have the complete sequences of more than 50 inbred strains.

**Gene-expression profiling**

Few strategies even attempt to turn a QTL from a locus into a molecular variant, which highlights the difficulty in confirming the role of a gene in a complex phenotype. Gene-expression profiling is not a method for QTL detection by itself, but when combined with genetic mapping data it can help to identify candidates, even for behavioural phenotypes[104].

In most examples, researchers have compared the gene-expression profiles of inbreds and congenics, to reduce the number of background strain differences and to leave just a small number of genes for further consideration. The good news is that relatively few differences are found, even when a large chromosome segment is derived from two genetically distant strains[105]. Gene-expression profiling identified the fatty-acid translocase gene *CD36* as a QTL that affects insulin fatty-acid metabolism, an example that other researchers have attempted to emulate, with varying degrees of success: complement factor 5 has been suggested as a gene at a QTL that influences susceptibility in a model of asthma[106], lipoxygenase (*Alox15*) has been proposed as a candidate gene for bone mass[107], an interferon-inducible gene for susceptibility to an autoimmune condition (systemic lupus)[108] and glutathione *S*-transferase M2 has been identified as a gene that might be involved in hypertension[109].

Although this method has strengths, there are also several caveats. First, differential gene expression is not always a marker of a QTL. Variants that alter protein structure might not alter expression levels, or there could be compensatory mechanisms that obscure the effect of a QTL on expression. Todd and colleagues used microarray analysis to study a model of type I diabetes and reported that disease protection that is conferred by each of three known loci was not reflected in global effects on the non-induced immune system, and that gene-regulatory systems seemed to be remarkably robust to genetic variation[105].

Second, expression differences might be restricted to certain tissues or developmental stages. For example, expression of 5HT1a receptors (Htr1a) in the forebrain is required to modulate anxiety during embryonic and fetal life, but it is not required for the same task in adult animals[110]. Therefore, expression differences for genes that encode the serotonin receptor that are relevant to anxiety would not be detected in the adult brain, although the phenotype is assessed in the adult animal. Gene-expression analyses will have to be carried out across a large number of tissues at different developmental times to determine whether the gene is differentially expressed.

Third, finding a gene-expression difference within a relevant tissue in a relevant biochemical pathway does not prove the gene's candidacy at the QTL. There is no guarantee that the physical coincidence of gene expression and QTL mapping data mean the two are causally related. This is a problem for Peltz and colleagues, who claim to have identified an allele-specific functional genomic element that regulates the expression of the histocompatibility 2, class II antigen E-α gene (*H2-Ea*)[98]. *In silico* mapping was used to map *H2-Ea* expression variation to a 1-kb block that lies within the first intron of the gene, followed by *in vitro* assays to identify a putative 45-bp regulatory element. But differential expression of *H2-Ea* is caused by several mechanisms, including a loss-of-function deletion of 600 bp in the promoter and first exon of the *H2-Ea* gene[111,112].

**Quantitative complementation**

Quantitative complementation, or QTL–knockout interaction, which was designed originally for QTL work in *Drosophila* by Trudy MacKay[113], is a method for testing the candidacy of a gene at a QTL. It is oblivious to the nature and position of the responsible sequence variant, a feature that is both its strength and weakness, because whereas one interpretation of a positive result is allelism to the QTL, another is epistasis. Strictly speaking, the test is not complementation in its classical form because it is testing for an interaction between the null allele and the QTL, rather than for a main effect of either. For this reason, the name QTL–knockout interaction test is preferable, as suggested by Darvasi[114].

The experimental design is simple — it requires offspring from four crosses. An inbred animal bearing one QTL allele (for example 'high') is mated to an inbred animal with a null allele of the gene of interest ('m') and also to the co-isogenic wild-type animal ('wt'). A similar pair of crosses is established, but this time using an inbred strain with the alternative QTL allele ('low'). If the difference in mean phenotype between the high/m and low/m genotypes is greater than that between the high/wt and low/wt genotypes then we have evidence of quantitative failure of the mutation to complement the QTL alleles. This is detected as a statistical 'cross' (m or wt) by 'line' (high or low) interaction in a two-way analysis of variance. One biological interpretation of a significant interaction is that the expression of the wild-type (that is, functional) gene is modulated by a QTL allele on the homologous chromosome. The test does not implicate any particular QTL, which could be anywhere on the genome where the high and low strains differ.

QTL–knockout interaction tests require co-isogenic wild types, which can be difficult to obtain in mice. Knockouts created in a 129 strain are usually backcrossed onto a different strain (typically C57BL/6) so that often, no pure co-isogenic wild type is available. However, when the experimenter has only the hybrid to work with, the problem of mixed background can sometimes be overcome by taking advantage of the mosaic nature of the mouse genome: some regions of the 129 strain will be identical to the strain onto which it has been backcrossed. Where the targeted gene occurs in such a region (or in a region that is known from genetic crosses not to carry QTLs that



© 2005 Nature Publishing Group

influence the trait of interest), and the rest of the 129 strain has been removed by repeated backcrossing, then it should be possible to find an appropriate co-isogenic wild type. For example, by extensive resequencing of the 129, C57BL/6J and DBA/2J strains, we showed that inbred C57BL/6 could be combined with a targeted mutation of the *Rgs2* gene in a quantitative complementation test[31]. This arduous task can be avoided in the future once the relevant strains have been fully resequenced. Alternatively, knockouts could be made and maintained on a single background or obtained by screening the DNA of mutagenized inbred mice[115].

## Conclusion

Throughout this review we have emphasized two points: the importance of being able to resolve QTLs that contribute to less than 5% of the phenotypic variance into small regions and the need for methods that identify genes, not sequence variants, at the QTL.

We stress the first point because success stories in QTL cloning have identified genetic loci with atypically large effects that have obviously functional sequence changes. Therefore, an expectation has arisen that loci with smaller effects can be characterized using extensions of the same strategies: more mice, more congenics and more expression arrays. However, methods that work for large-effect QTLs are not well suited to finding genes that underlie most QTLs. Not only do most QTLs typically have small effects[116], but the type of sequence variant responsible, even when it occurs in a coding region, is also hard to recognize[36], and the expression differences, if present, are subtle[105].

Given its importance for determining the success of QTL cloning, it is perhaps surprising that so little attention has been paid to emerging clues that reveal considerable variation in the genetic architecture of phenotypes. It seems that the number of loci, their relative effects, and how they interact with each other and the environment, might vary considerably between phenotypes, indicating that some phenotypes might be more tractable to QTL cloning than others. For example, it is striking that susceptibility to infectious disease in inbred strains has frequently turned out to be due to large genetic effects, and, where characterized, to null alleles or coding-sequence mutations[117–122]. Why this should be the case, when other apparently equally complex phenotypes (such as hypertension, drug responses and obesity) have much more complex genetic architecture, is unclear, but it might be a consequence of the nature and extent of the selective pressure that is exerted by infectious agents. There are also differences in the extent to which gene-by-gene interactions (epistasis) shape a phenotype. Pervasive epistatic effects have been documented in some autoimmune conditions[123], morphology[124] and in susceptibility to some cancers[125,126], but despite intensive searches, the genetic architecture underlying fear-related phenotypes consists almost entirely of additive effects[127]. QTL cloning strategies need to accommodate all these possible types of genetic architecture, rather than considering whether there is a strategy that can reduce the genetic basis of any phenotype to a monogenic condition.

High-resolution mapping of small-effect loci will undoubtedly benefit from some of the new resources we have reviewed. Although some, such as the use of CSS, do little more than accelerate QTL discovery, and others, such as *in silico* mapping, make claims that have yet to be realized, there are grounds for optimism. The use of high-resolution mapping in outbred mice and the construction of a large set of RI strains (the Collaborative Cross), combined with RISTs, will make high-resolution mapping routine; and once the sequences of many inbred strains are available, *in silico* mapping could become a powerful tool. Nevertheless, even the ability to map down to under a kilobase will not always deliver genes into the hands of researchers. The availability of whole-genome analyses, at the level of both sequence variation and gene expression, can induce a hubris which blinds us to the fact that complex phenotypes continue to frustrate genetic analysis.

This is one reason why we have stressed gene identification as a goal for QTL mapping — we lack methods that deliver genes. Almost all the methods we discuss are genetic mapping strategies that chase the segregating sequence variants that are responsible for a QTL; they do not directly find genes. It is not our intention to diminish the importance of characterizing sequence variants, as there are cases where we will need to know the causal SNP (or whatever other form the molecular pathology takes). Instead, we wish to make it clear that the two goals are not necessarily the same. Stated simply, finding the QTN does not necessarily give us the quantitative trait gene. One explanation for the subtle effects of QTLs is that they lie in regulatory regions (the sequence features of which are poorly understood) that can lie at a considerable distance from cognate transcriptional units. For example, in the polydactylous mouse mutant Sasquatch, sonic hedgehog (*Shh*) is expressed at an ectopic site. Characterization of the mutant led to the identification of an *Shh* enhancer element that lies within intron 5 of a novel gene, and is also involved in limb development (limb region 1 (*Lmbr1*)[128]), that is situated 1 Mb from *Shh*[129,130]. Consider what could happen if a QTL had been found at this regulatory region: the obvious, but erroneous, conclusion would be that the *Lmbr1* gene was the quantitative trait gene.

The transition from locus to gene remains a formidable task. None of the strategies we have reviewed here provide a comprehensive solution to gene identification after QTL mapping. Simple approaches, such as investigating the phenotype of the knockout, will add weight to the candidature of the gene, but will not prove it. Definitive studies, such as the targeted interchange of nucleotides between two strains, will prove that a sequence variant operates as a QTL, but will not unambiguously identify a gene. For that, we still need functional assays that make few assumptions about the biology of candidate genes (for example, where and when they are expressed, and at what levels).



© 2005 Nature Publishing Group

Among the approaches described here, only the gene–knockout interaction test comes close to meeting the necessary requirements. Where a null allele of the relevant gene can be found, and an appropriate co-isogenic strain is available, the test provides a simple test of candidacy and interpretation of a positive result, allelism or epistasis provides important information. The current drawback is the lack of appropriate mutants, but this might change with plans to knock out all mouse genes[131]. Furthermore, developments in RNAi technology might aid functional investigation of those cases in which QTLs operate by altering expression. It might soon be possible to reproduce naturally occurring variation, to abrogate the expression of either parental mRNA, or to generate null alleles on the appropriate background for gene–knockout interaction testing. The challenge for the coming years is to develop new technologies that will accelerate gene identification after detection of a QTL.


1. Hilbert, P. *et al.* Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. *Nature* **353**, 521–529 (1991).
2. Jacob, H. *et al.* Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* **67**, 213–224 (1991).
3. Lander, E. S. & Botstein, D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199 (1989).
4. Todd, J. A. *et al.* Genetic analysis of autoimmune type 1 diabetes mellitus in mice. *Nature* **351**, 542–547 (1991).
5. Lynch, M. & Walsh, B. *Genetics and Analysis of Quantitative Traits* (Sinauer Associates, Sunderland, Massachusetts, 1998).
6. Turri, M. G., Henderson, N. D., DeFries, J. C. & Flint, J. Quantitative trait locus mapping in laboratory mice derived from a replicated selection experiment for open-field activity. *Genetics* **158**, 1217–1226 (2001).
7. Turri, M. G., Datta, S. R., DeFries, J., Henderson, N. D. & Flint, J. QTL analysis identifies multiple behavioral dimensions in ethological tests of anxiety in laboratory mice. *Curr. Biol.* **11**, 725–734 (2001).
8. Henderson, N. D., Turri, M. G., DeFries, J. C. & Flint, J. QTL Analysis of multiple behavioral measures of anxiety in mice. *Behav. Genet.* **34**, 267–293 (2004).
9. Belknap, J. K. & Atkins, A. L. The replicability of QTLs for murine alcohol preference drinking behavior across eight independent studies. *Mamm. Genome* **12**, 893–899 (2001).
10. Nadeau, J. H. & Frankel, W. N. The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. *Nature Genet.* **25**, 381–384 (2000).
**A discussion of the relative advantages of QTL mapping and mutagenesis for investigating the molecular basis of complex traits.**
11. Korstanje, R. & Paigen, B. From QTL to gene: the harvest begins. *Nature Genet.* **31**, 235–236 (2002).
12. Glazier, A. M., Nadeau, J. H. & Aitman, T. J. Finding genes that underlie complex traits. *Science* **298**, 2345–2349 (2002).
13. Page, G. P., George, V., Go, R. C., Page, P. Z. & Allison, D. B. "Are we there yet?": Deciding when one has demonstrated specific genetic causation in complex diseases and quantitative traits. *Am. J. Hum. Genet.* **73**, 711–719 (2003).
14. Abiola, O. *et al.* The nature and identification of quantitative trait loci: a community's view. *Nature Rev. Genet.* **4**, 911–916 (2003).
15. Ferraro, T. N. *et al.* Fine mapping of a seizure susceptibility locus on mouse chromosome 1: nomination of *Kcnj10* as a causative gene. *Mamm. Genome* **15**, 239–251 (2004).
16. Shirley, R. L., Walter, N. A., Reilly, M. T., Fehr, C. & Buck, K. J. *Mpdz* is a quantitative trait gene for drug withdrawal seizures. *Nature Neurosci.* **7**, 699–700 (2004).
17. Beavis, W. D. in *Molecular Analysis of Complex Traits* (ed. Paterson, A. H.) 123–150 (CRC, Boca Raton, Florida, 1998).
18. Beavis, W. D. in *49th Annual Corn and Sorghum Research Conference* 252–268 (American Seed Trade Association, Washington DC, 1994).
19. Legare, M. E., Bartlett, F. S. & Frankel, W. N. A major effect QTL determined by multiple genes in epileptic EL mice. *Genome Res.* **10**, 42–48 (2000).
20. Stylianou, I. M. *et al.* Genetic complexity of an obesity QTL (*Fob3*) revealed by detailed genetic mapping. *Mamm. Genome* **15**, 472–481 (2004).
21. Christians, J. K. & Keightley, P. D. Fine mapping of a murine growth locus to a 1.4-cM region and resolution of linked QTL. *Mamm. Genome* **15**, 482–491 (2004).
22. Ariyarajah, A. *et al.* Dissecting quantitative trait loci into opposite blood pressure effects on Dahl rat chromosome 8 by congenic strains. *J. Hypertens.* **22**, 1495–1502 (2004).
23. Alemayehu, A., Breen, L., Krenova, D. & Printz, M. P. Reciprocal rat chromosome 2 congenic strains reveal contrasting blood pressure and heart rate QTL. *Physiol. Genomics* **10**, 199–210 (2002).
24. Garrett, M. R. & Rapp, J. P. Two closely linked interactive blood pressure QTL on rat chromosome 5 defined using congenic Dahl rats. *Physiol. Genomics* **8**, 81–86 (2002).
25. Garrett, M. R. & Rapp, J. P. Multiple blood pressure QTL on rat chromosome 2 defined by congenic Dahl rats. *Mamm. Genome* **13**, 41–44 (2002).
26. Frantz, S., Clemitson, J. R., Bihoreau, M. T., Gauguier, D. & Samani, N. J. Genetic dissection of region around the Sa gene on rat chromosome 1: evidence for multiple loci affecting blood pressure. *Hypertension* **38**, 216–221 (2001).
27. Podolin, P. L. *et al.* Localization of two insulin-dependent diabetes (Idd) genes to the *Idd10* region on mouse Chromosome 3. *Mamm. Genome* **9**, 283–286 (1998).
28. Puel, A. *et al.* Identification of two quantitative trait loci involved in antibody production on mouse chromosome 8. *Immunogenetics* **47**, 326–331 (1998).
29. Bihl, F., Brahic, M. & Bureau, J. F. Two loci, *Tmevp2* and *Tmevp3*, located on the telomeric region of chromosome 10, control the persistence of Theiler's virus in the central nervous system of mice. *Genetics* **152**, 385–392 (1999).
30. Mackay, T. F. The genetic architecture of quantitative traits. *Annu. Rev. Genet.* **35**, 303–339 (2001).
31. Yalcin, B. *et al.* Genetic dissection of a behavioral quantitative trait locus shows that *Rgs2* modulates anxiety in mice. *Nature Genet.* **36**, 1197–1202 (2004).
**The complexity of QTL architecture in mice becomes apparent in this paper, which used probabilistic ancestral haplotype reconstruction in outbred mice and a knockout interaction test to identify a candidate gene.**
32. Mackay, T. F. The genetic architecture of quantitative traits: lessons from *Drosophila*. *Curr. Opin. Genet. Dev.* **14**, 253–257 (2004).
**An excellent review of the genetic basis of complex traits, from the view point of *Drosophila* genetics.**
33. Steinmetz, L. M. *et al.* Dissecting the architecture of a quantitative trait locus in yeast. *Nature* **416**, 326–330 (2002).
**Even yeast have QTLs, and the formidable power of yeast genetics was used here to show that neither expression differences nor sequence variation are enough to identify their molecular basis. This paper introduced reciprocal hemizygosity for gene identification.**
34. Aitman, T. J. *et al.* Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. *Nature Genet.* **16**, 197–201 (1997).
35. Vingsbo-Lundberg, C. *et al.* Genetic control of arthritis onset, severity and chronicity in a model for rheumatoid arthritis in rats. *Nature Genet.* **20**, 401–404 (1998).
36. Thomas, P. D. & Kejariwal, A. Coding single-nucleotide polymorphisms associated with complex vs. Mendelian disease: evolutionary evidence for differences in molecular effects. *Proc. Natl Acad. Sci. USA* **101**, 15398–15403 (2004).
**The molecular basis of complex traits in humans is shown to differ from the molecular basis of disorders owing to highly penetrant mutations.**
37. Yalcin, B. *et al.* Unexpected complexity in the haplotypes of commonly used inbred strains of laboratory mice. *Proc. Natl Acad. Sci. USA* **101**, 9734–9739 (2004).
**The first of a series of papers that showed that the DNA-sequence relationship between inbred mouse strains is remarkably complex, a finding with important implications for *in silico* mapping strategies (see also references 102 and 103).**
38. Nobrega, M. A., Ovcharenko, I., Afzal, V. & Rubin, E. M. Scanning human gene deserts for long-range enhancers. *Science* **302**, 413 (2003).
39. van Ooijen, J. W. Accuracy of mapping quantitative trait loci in autogamous species. *Theor. Appl. Genet.* **84**, 803–811 (1992).
40. Darvasi, A., Weinreb, A., Minke, V., Weller, J. I. & Soller, M. Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. *Genetics* **134**, 943–951 (1993).
41. Darvasi, A. & Soller, M. A simple method to calculate resolving power and confidence interval of QTL map location. *Behav. Genet.* **27**, 125–132 (1997).
42. Darvasi, A. Experimental strategies for the genetic dissection of complex traits in animal models. *Nature Genet.* **18**, 19–24 (1998).
**An excellent review of QTL detection and fine-mapping methods in rodents, and the first description of the recombination inbred segregation test, which was then extended to Yin–Yang crosses in reference 58.**
43. Darvasi, A. & Soller, M. Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* **141**, 1199–1207 (1995).
**The first description of advanced intercross lines for QTL mapping in rodents.**
44. Matin, A., Collin, G. B., Asada, Y., Varnum, D. & Nadeau, J. H. Susceptibility to testicular germ-cell tumours in a 129.MOLF-Chr 19 chromosome substitution strain. *Nature Genet.* **23**, 237–240 (1999).
**The first use of a chromosome-substitution strain for QTL mapping.**
45. Nadeau, J. H., Singer, J. B., Matin, A. & Lander, E. S. Analysing complex genetic traits with chromosome substitution strains. *Nature Genet.* **24**, 221–225 (2000).
46. Roman, R. J. *et al.* in *Cold Spring Harbor Symposia on Quantitative Biology* Vol. LXVII 309–315 (Cold Spring Harbor Laboratory, New York, 2002).
47. Cowley, A. W. Jr, Liang, M., Roman, R. J., Greene, A. S. & Jacob, H. J. Consomic rat model systems for physiological genomics. *Acta Physiol. Scand.* **181**, 585–592 (2004).
48. Law, C. N. The location of genetic factors affecting a quantitative character in wheat. *Genetics* **53**, 487–498 (1966).
49. Caligari, P. D. & Mather, K. Genotype–environment interaction. III. Interactions in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* **191**, 387–411 (1975).
50. Singer, J. B., Hill, A. E., Nadeau, J. H. & Lander, E. S. Mapping quantitative trait loci for anxiety in chromosome substitution strains of mice. *Genetics* 15 September 2004 (doi:10.1534/genetics.104.031492).
51. Singer, J. B. *et al.* Genetic dissection of complex traits with chromosome substitution strains of mice. *Science* **304**, 445–448 (2004).
**This paper describes the construction of the first complete set of chromosome-substitution strains and their application in genome-wide QTL mapping.**
52. Belknap, J. K. Chromosome substitution strains: some quantitative considerations for genome scans and fine mapping. *Mamm. Genome* **14**, 723–732 (2003).
53. Churchill, G. A. *et al.* The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nature Genet.* **36**, 1133–1137 (2004).
**This paper describes the Collaborative Cross and explains what the proposed resource would provide for complex trait analysis.**
54. Belknap, J. K., Mitchell, S. R., O'Toole, L. A., Helms, M. L. & Crabbe, J. C. Type I and type II error rates for quantitative trait loci (QTL) mapping studies using recombinant inbred mouse strains. *Behav. Genet.* **26**, 149–160 (1996).
55. Valdar, W., Flint, J. & Mott, R. Simulating the collaborative cross: power of QTL detection and mapping resolution afforded by a large set of recombinant inbred strains. *Genetics* (in the press).
56. Williams, R. W. *et al.* Genetic structure of the LXS panel of recombinant inbred mouse strains: a powerful resource for complex trait analysis. *Mamm. Genome* **15**, 637–647 (2004).




© 2005 Nature Publishing Group

57. Peirce, J. L., Lu, L., Gu, J., Silver, L. M. & Williams, R. W. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet.* **5**, 7 (2004).
58. Shifman, S. & Darvasi, A. Mouse inbred strain sequence information and Yin–Yang crosses for QTL fine mapping. *Genetics* 1 November 2004 (doi:10.1534/genetics.104.032474).
59. Visscher, P. M. Speed congenics: accelerated genome recovery using genetic markers. *Genet. Res.* **74**, 81–85 (1999).
60. Zhang, Y. *et al.* Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nature Genet.* **34**, 181–186 (2003).
61. Lipkin, S. M. *et al.* The MLH1 D132H variant is associated with susceptibility to sporadic colorectal cancer. *Nature Genet.* **36**, 694–699 (2004).
62. Guo, D. *et al.* A functional variant of SUMO4, a new IκBα modifier, is associated with type 1 diabetes. *Nature Genet.* **36**, 837–841 (2004).
63. Stoll, M. *et al.* Genetic variation in *DLG5* is associated with inflammatory bowel disease. *Nature Genet.* **36**, 476–480 (2004).
64. Gretarsdottir, S. *et al.* The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nature Genet.* **35**, 131–138 (2003).
65. Prokunina, L. *et al.* A regulatory polymorphism in *PDCD1* is associated with susceptibility to systemic lupus erythematosus in humans. *Nature Genet.* **32**, 666–669 (2002).
66. Rioux, J. D. *et al.* Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nature Genet.* **29**, 223–228 (2001).
67. Altshuler, D. *et al.* The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nature Genet.* **26**, 76–80 (2000).
68. Hitzemann, R. *et al.* Multiple cross mapping (MCM) markedly improves the localization of a QTL for ethanol-induced activation. *Genes Brain Behav.* **1**, 214–222 (2002).
69. Talbot, C. J. *et al.* Fine scale mapping of a genetic locus for conditioned fear. *Mamm. Genome* **14**, 223–230 (2003).
70. Mott, R., Talbot, C. J., Turri, M. G., Collins, A. C. & Flint, J. A method for fine mapping quantitative trait loci in outbred animal stocks. *Proc. Natl Acad. Sci. USA* **97**, 12649–12654 (2000).
**The introduction of probabilistic ancestral haplotype reconstruction for mapping QTL using heterogeneous stocks of mice.**
71. Talbot, C. J. *et al.* High-resolution mapping of quantitative trait loci in outbred mice. *Nature Genet.* **21**, 305–308 (1999).
**The first use of heterogeneous stock mice for QTL mapping**
72. Nagase, H. *et al.* Distinct genetic loci control development of benign and malignant skin tumours in mice. *Nature Genet.* **10**, 424–429 (1995).
73. Nagase, H., Mao, J. H. & Balmain, A. A subset of skin tumor modifier loci determines survival time of tumor-bearing mice. *Proc. Natl Acad. Sci. USA* **96**, 15032–15037 (1999).
74. Ewart-Toland, A. *et al.* Identification of *Stk6/STK15* as a candidate low-penetrance tumor-susceptibility gene in mouse and human. *Nature Genet.* **34**, 403–412 (2003).
75. Manenti, G., Galbiati, F., Noci, S. & Dragani, T. A. Outbred CD-1 mice carry the susceptibility allele at the pulmonary adenoma susceptibility 1 (*Pas1*) locus. *Carcinogenesis* **24**, 1143–1148 (2003).
76. Neale, B. M. & Sham, P. C. The future of association studies: gene-based analysis and replication. *Am. J. Hum. Genet.* **75**, 353–362 (2004).
77. Zondervan, K. T. & Cardon, L. R. The complex interplay among factors that influence allelic association. *Nature Rev. Genet.* **5**, 89–100 (2004).
78. McKeigue, P. M. Prospects for admixture mapping of complex traits. *Am. J. Hum. Genet.* **76**, 1–7 (2005).
79. Perez-Enciso, M. Fine mapping of complex trait genes combining pedigree and linkage disequilibrium information: a Bayesian unified framework. *Genetics* **163**, 1497–1510 (2003).
80. Meuwissen, T. H. & Goddard, M. E. Prediction of identity by descent probabilities from marker-haplotypes. *Genet. Sel. Evol.* **33**, 605–634 (2001).
81. Meuwissen, T. H. & Goddard, M. E. Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. *Genetics* **155**, 421–430 (2000).
82. Farnir, F. *et al.* Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. *Genetics* **161**, 275–287 (2002).
83. McPeek, M. S. & Strahs, A. Assessment of linkage disequilibrium by the decay of haplotype sharing, with application to fine-scale genetic mapping. *Am. J. Hum. Genet.* **65**, 858–875 (1999).
84. Morris, A. P., Whittaker, J. C., Xu, C. F., Hosking, L. K. & Balding, D. J. Multipoint linkage-disequilibrium mapping narrows location interval and identifies mutation heterogeneity. *Proc. Natl Acad. Sci. USA* **100**, 13442–13446 (2003).
85. Meuwissen, T. H. & Goddard, M. E. Mapping multiple QTL using linkage disequilibrium and linkage analysis information and multitrait data. *Genet. Sel. Evol.* **36**, 261–279 (2004).
86. Churchill, G. A. & Doerge, R. W. Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971 (1994).
87. Mott, R. & Flint, J. Simultaneous detection and fine mapping of quantitative trait Loci in mice using heterogeneous stocks. *Genetics* **160**, 1609–1618 (2002).
88. Wang, X., Korstanje, R., Higgins, D. & Paigen, B. Haplotype analysis in multiple crosses to identify a QTL gene. *Genome Res.* **14**, 1767–1772 (2004).
89. Manenti, G. *et al.* Haplotype sharing suggests that a genomic segment containing six genes accounts for the pulmonary adenoma susceptibility 1 (*Pas1*) locus activity in mice. *Oncogene* **23**, 4495–4504 (2004).
90. Park, Y. G., Clifford, R., Buetow, K. H. & Hunter, K. W. Multiple cross and inbred strain haplotype mapping of complex-trait candidate genes. *Genome Res.* **13**, 118–121 (2003).
91. Grubb, S. C., Churchill, G. A. & Bogue, M. A. A collaborative database of inbred mouse strain characteristics. *Bioinformatics* **20**, 2857–2859 (2004).
92. Grupe, A. *et al. In silico* mapping of complex disease-related traits in mice. *Science* **292**, 1915–1918 (2001).
**The paper that introduced *in silico* mapping to the world of mouse genetics.**
93. Ferris, S. D., Sage, R. D. & Wilson, A. C. Evidence from mtDNA sequences that common laboratory strains of inbred mice are descended from a single female. *Nature* **295**, 163–165 (1982).
94. Beck, J. A. *et al.* Genealogies of mouse inbred strains. *Nature Genet.* **24**, 23–25 (2000).
95. Wade, C. M. *et al.* The mosaic structure of variation in the laboratory mouse genome. *Nature* **420**, 574–578 (2002).
96. Lindblad-Toh, K. *et al.* Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. *Nature Genet.* **24**, 381–386 (2000).
97. Wiltshire, T. *et al.* Genome-wide single-nucleotide polymorphism analysis defines haplotype patterns in mouse. *Proc. Natl Acad. Sci. USA* **100**, 3380–3385 (2003).
**References 95, 96 and 97 presented the first description of genome-wide distribution of genetic variation in the mouse, and suggested that the observed mosaic structure was due to common descent from a relatively few progenitors strains.**
98. Liao, G. *et al. In silico* genetics: identification of a functional element regulating *H2-Eα* gene expression. *Science* **306**, 690–695 (2004).
99. Pletcher, M. T. *et al.* Use of a dense single nucleotide polymorphism map for *in silico* mapping in the mouse. *PLoS Biol.* **2**, e393 (2004).
100. Usuka, J. *et al. In silico* mapping of mouse quantitative trait loci. *Science* **5551**, 2423 (2001).
101. Turri, M. G., De Fries, J. C., Henderson, N. D. & Flint, J. Multivariate analysis of quantitative trait loci influencing variation in anxiety-related behavior in laboratory mice. *Mamm. Genome* **15**, 69–76 (2004).
102. Frazer, K. A. *et al.* Segmental phylogenetic relationships of inbred mouse strains revealed by fine-scale analysis of sequence variation across 4.6 Mb of mouse genome. *Genome Res.* **14**, 1493–1500 (2004).
103. Ideraabdullah, F. Y. *et al.* Genetic and haplotype diversity among wild-derived mouse inbred strains. *Genome Res.* **14**, 1880–1887 (2004).
104. Sandberg, R. *et al.* Regional and strain-specific gene expression mapping in the adult mouse brain. *Proc. Natl Acad. Sci. USA* **97**, 11038–11043 (2000).
105. Eaves, I. A. *et al.* Combining mouse congenic strains and microarray gene expression analyses to study a complex trait: the NOD model of type 1 diabetes. *Genome Res.* **12**, 232–243 (2002).
**An example of how gene-expression profiling might not be of help in gene identification, and a careful consideration of the limitations of this approach for gene location.**
106. Karp, C. L. *et al.* Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. *Nature Immunol.* **1**, 221–226 (2000).
107. Klein, R. F. *et al.* Regulation of bone mass in mice by the lipoxygenase gene *Alox15*. *Science* **303**, 229–232 (2004).
**In contrast to reference 105, references 106 and 107 are demonstrations of the power of gene-expression profiling for gene location.**
108. Rozzo, S. J. *et al.* Evidence for an interferon-inducible gene, *Ifi202*, in the susceptibility to systemic lupus. *Immunity* **15**, 435–443 (2001).
109. McBride, M. W. *et al.* Microarray analysis of rat chromosome 2 congenic strains. *Hypertension* **41**, 847–853 (2003).
110. Gross, C. *et al.* Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**, 396–400 (2002).
**A salutary lesson in the use of gene-expression methods: this paper is an example of a mutation in which the phenotype depends on where and when the mutation occurs.**
111. Mathis, D. J., Benoist, C., Williams, V. E. 2nd, Kanter, M. & McDevitt, H. O. Several mechanisms can account for defective E α gene expression in different mouse haplotypes. *Proc. Natl Acad. Sci. USA* **80**, 273–277 (1983).
112. Jones, P. P., Murphy, D. B. & McDevitt, H. O. Variable synthesis and expression of E α and Ae (E β) Ia polypeptide chains in mice of different H-2 haplotypes. *Immunogenetics* **12**, 321–337 (1981).
113. Long, A. D., Mullaney, S. L., Mackay, T. F. C. & Langley, C. H. 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 (1996).
**The first description of quantitative complementation testing for the investigation of candidate genes at QTL, carried out in *Drosophila melanogaster*.**
114. Darvasi, A. Dissecting complex traits: the geneticists' "around the world in 80 days". *Trends Genet.* (in the press).
115. Coghill, E. L. *et al.* A gene driven approach to the identification of ENU mutants in the mouse. *Nature Genet.* **30**, 255–256 (1999).
116. Flint, J. Analysis of quantitative trait loci that influence animal behavior. *J. Neurobiol.* **54**, 46–77 (2003).
117. Min-Oo, G. *et al.* Pyruvate kinase deficiency in mice protects against malaria. *Nature Genet.* **35**, 357–362 (2003).
118. Mitsos, L. M. *et al.* Susceptibility to tuberculosis: a locus on mouse chromosome 19 (*Trl-4*) regulates *Mycobacterium tuberculosis* replication in the lungs. *Proc. Natl Acad. Sci. USA* **100**, 6610–6615 (2003).
119. Diez, E. *et al. Birc1e* is the gene within the *Lgn1* locus associated with resistance to *Legionella pneumophila*. *Nature Genet.* **33**, 55–60 (2003).
120. Mitsos, L. M. *et al.* Genetic control of susceptibility to infection with *Mycobacterium tuberculosis* in mice. *Genes Immunol.* **1**, 467–477 (2000).
121. Vidal, S. M., Malo, D., Vogan, K., Skamene, E. & Gros, P. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell* **73**, 469–485 (1993).
122. Poltorak, A. *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088 (1998).
123. Wanstrat, A. & Wakeland, E. The genetics of complex autoimmune diseases: non-MHC susceptibility genes. *Nature Immunol.* **2**, 802–809 (2001).
124. Leamy, L. J., Routman, E. J. & Cheverud, J. M. An epistatic genetic basis for fluctuating asymmetry of mandible size in mice. *Evolution* **56**, 642–653 (2002).
125. van Wezel, T., Ruivenkamp, C. A., Stassen, A. P., Moen, C. J. & Demant, P. Four new colon cancer susceptibility loci, *Scc6* to *Scc9* in the mouse. *Cancer Res.* **59**, 4216–4218 (1999).
126. Fijneman, R. J., de Vries, S. S., Jansen, R. C. & Demant, P. Complex interactions of new quantitative trait loci, *Sluc1*, *Sluc2*, *Sluc3*, and *Sluc4*, that influence the susceptibility to lung cancer in the mouse. *Nature Genet.* **14**, 465–467 (1996).
127. Flint, J., De Fries, J. C. & Henderson, N. D. Little epistasis for anxiety-related measures in the DeFries strains of laboratory mice. *Mamm. Genome* **15**, 77–82 (2004).
128. Clark, R. M., Marker, P. C. & Kingsley, D. M. A novel candidate gene for mouse and human preaxial polydactyly with altered expression in limbs of Hemimelic extra-toes mutant mice. *Genomics* **67**, 19–27 (2000).
129. Lettice, L. A. *et al.* A long-range *Shh* enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum. Mol. Genet.* **12**, 1725–1735 (2003).
130. Lettice, L. A. *et al.* Disruption of a long-range *cis*-acting regulator for *Shh* causes preaxial polydactyly. *Proc. Natl Acad. Sci. USA* **99**, 7548–7553 (2002).
131. Austin, C. P. *et al.* The knockout mouse project. *Nature Genet.* **36**, 921–924 (2004).
132. Belknap, J. K. Effect of within-strain sample size on QTL detection and mapping using recombinant inbred mouse strains. *Behav. Genet.* **28**, 29–38 (1998).
133. Lander, E. & Kruglyak, L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genet.* **11**, 241–247 (1995).
134. Belknap, J. K., Mitchell, S. R., Otoole, L. A., Helms, M. L. & Crabbe, J. C. Type-I and type-II error rates for quantitative trait loci (Qtl) mapping studies using recombinant inbred mouse strains. *Behav. Genet.* **26**, 149–160 (1996).




© 2005 Nature Publishing Group

135. Bolivar, V. J., Cook, M. N. & Flaherty, L. Mapping of quantitative trait loci with knockout/congenic strains. *Genome Res.* **11**, 1549–1552 (2001).
136. Moen, C. J. *et al.* The recombinant congenic strains — a novel genetic tool applied to the study of colon tumor development in the mouse. *Mamm. Genome* **1**, 217–227 (1991).
137. Iakoubova, O. A. *et al.* Genome-tagged mice (GTM): two sets of genome-wide congenic strains. *Genomics* **74**, 89–104 (2001).
138. McClearn, G. E., Wilson, J. R. & Meredith, W. in *Contributions to Behavior-Genetic Analysis: the Mouse as a Prototype* (eds Lindzey, G. & Thiessen, D.) 3–22 (Appleton Century Crofts, New York, 1970).
139. Demarest, K., Koyner, J., McCaughran, J. Jr, Cipp, L. & Hitzemann, R. Further characterization and high-resolution mapping of quantitative trait loci for ethanol-induced locomotor activity. *Behav. Genet.* **31**, 79–91 (2001).
140. Rocha, J. L., Eisen, E. J., Van Vleck, L. D. & Pomp, D. A large-sample QTL study in mice: I. Growth. *Mamm. Genome* **15**, 83–99 (2004).
141. Rocha, J. L., Eisen, E. J., Van Vleck, L. D. & Pomp, D. A large-sample QTL study in mice: II. Body composition. *Mamm. Genome* **15**, 100–113 (2004).
142. Brockmann, G. A. *et al.* QTLs for pre- and postweaning body weight and body composition in selected mice. *Mamm. Genome* **15**, 593–609 (2004).
143. Brockmann, G. A. *et al.* Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic $F_2$ variance of growth and obesity in DU6i x DBA/2 mice. *Genome Res.* **10**, 1941–1957 (2000).
144. Taylor, B. A., Wnek, C., Schroeder, D. & Phillips, S. J. Multiple obesity QTLs identified in an intercross between the NZO (New Zealand obese) and the SM (small) mouse strains. *Mamm. Genome* **12**, 95–103 (2001).
145. Pitman, W. A. *et al.* Quantitative trait locus mapping of genes that regulate HDL cholesterol in SM/J and NZB/B1NJ inbred mice. *Physiol. Genomics* **9**, 93–102 (2002).
146. Paigen, B. *et al.* Quantitative trait loci mapping for cholesterol gallstones in AKR/J and C57L/J strains of mice. *Physiol. Genomics* **4**, 59–65 (2000).
147. Anunciado, R. V. *et al.* Quantitative trait locus analysis of serum insulin, triglyceride, total cholesterol and phospholipid levels in the (SM/J x A/J)$F_2$ mice. *Exp. Anim.* **52**, 37–42 (2003).
148. Colinayo, V. V. *et al.* Genetic loci for diet-induced atherosclerotic lesions and plasma lipids in mice. *Mamm. Genome* **14**, 464–471 (2003).
149. Vaughn, T. T. *et al.* Mapping quantitative trait loci for murine growth: a closer look at genetic architecture. *Genet. Res.* **74**, 313–322 (1999).
150. Cheverud, J. M. *et al.* Genetic architecture of adiposity in the cross of LG/J and SM/J inbred mice. *Mamm. Genome* **12**, 3–12 (2001).
151. Workman, M. S., Leamy, L. J., Routman, E. J. & Cheverud, J. M. Analysis of quantitative trait locus effects on the size and shape of mandibular molars in mice. *Genetics* **160**, 1573–1586 (2002).
152. Reed, D. R. *et al.* Loci on chromosomes 2, 4, 9, and 16 for body weight, body length, and adiposity identified in a genome scan of an F2 intercross between the 129P3/J and C57BL/6ByJ mouse strains. *Mamm. Genome* **14**, 302–313 (2003).
153. Lammert, F., Carey, M. C. & Paigen, B. Chromosomal organization of candidate genes involved in cholesterol gallstone formation: a murine gallstone map. *Gastroenterology* **120**, 221–238 (2001).
154. Cicila, G. T. *et al.* High-resolution mapping of the blood pressure QTL on chromosome 7 using Dahl rat congenic strains. *Genomics* **72**, 51–60 (2001).
155. Farahani, P. *et al.* Reciprocal hemizygosity analysis of mouse hepatic lipase reveals influence on obesity. *Obes. Res.* **12**, 292–305 (2004).
156. Crackower, M. A. *et al.* Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* **417**, 822–828 (2002).
157. Olofsson, P. *et al.* Positional identification of *Ncf1* as a gene that regulates arthritis severity in rats. *Nature Genet.* **33**, 25–32 (2003).
158. Ruivenkamp, C. A. *et al. Ptprj* is a candidate for the mouse colon-cancer susceptibility locus *Scc1* and is frequently deleted in human cancers. *Nature Genet.* **31**, 295–300 (2002).
159. Bachmanov, A. A. *et al.* Positional cloning of the mouse saccharin preference (*Sac*) locus. *Chem. Senses* **26**, 925–933 (2001).
160. Deschepper, C. F. *et al.* Functional alterations of the *Nppa* promoter are linked to cardiac ventricular hypertrophy in WKY/WKHA rat crosses. *Circ. Res.* **88**, 223–228 (2001).
161. Aitman, T. J. *et al.* Identification of *Cd36* (*Fat*) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nature Genet.* **21**, 76–83 (1999).
**The first demonstration that gene-expression profiling could be used to find genes that underlie QTLs.**
162. Cormier, R. T. *et al.* Secretory phospholipase Pla2g2a confers resistance to intestinal tumorigenesis. *Nature Genet.* **17**, 88–91 (1997).
163. Ikeda, A. *et al.* Microtubule-associated protein 1A is a modifier of tubby hearing (*moth1*). *Nature Genet.* **30**, 401–405 (2002).
164. Yokoi, N. *et al. Cblb* is a major susceptibility gene for rat type 1 diabetes mellitus. *Nature Genet.* **31**, 391–394 (2002).
165. Ueda, H. *et al.* Association of the T-cell regulatory gene *CTLA4* with susceptibility to autoimmune disease. *Nature* **423**, 506–511 (2003).
166. Podolin, P. L. *et al.* Differential glycosylation of interleukin 2, the molecular basis for the NOD *Idd3* type 1 diabetes gene? *Cytokine* **12**, 477–482 (2000).
167. Zhang, S. L. *et al.* Efficiency alleles of the *Pctr1* modifier locus for plasmacytoma susceptibility. *Mol. Cell Biol.* **21**, 310–318 (2001).
168. Hamilton-Williams, E. E. *et al.* Transgenic rescue implicates β2-microglobulin as a diabetes susceptibility gene in nonobese diabetic (NOD) mice. *Proc. Natl Acad. Sci. USA* **98**, 11533–11538 (2001).

## Acknowledgements
The authors are supported by the Wellcome Trust. We would like to thank C. Benoist for comments on the manuscript.

## Competing interests statement
The authors declare no competing financial interests.

## Online links

**DATABASES**
**The following terms in this article are linked online to:**
**Entrez:** http://www.ncbi.nih.gov/Entrez
*Rgs2* | *Stk6*

**FURTHER INFORMATION**
**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Pager&DB=gene
**Gene Network:** http://www.webqtl.org
**Mouse Genome Informatics:** http://www.informatics.jax.org
**Mouse Phenome Database:** http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home
**US National Institute of Environmental Health Sciences Press Releases:** http://www.niehs.nih.gov/oc/news/micedna.htm
**Access to this interactive links box is free online.**



© 2005 Nature Publishing Group