NEWS AND COMMENTARY

Small but not forgotten

JN Maloof

New empirical evidence from a study of *Arabidopsis thaliana* biomass helps to answer a long-standing and fundamental question in biology: how genes influence complex phenotypes. How much of the genetic difference between individuals is due to the relatively large allelic effects of a handful of genes and how much is caused by the very small effects of a large number of genes? We know that typically there are several large to medium effect Quantitative Trait Loci (QTL) present in any given cross, but a new study by Kroymann and Mitchell-Olds (2005) provides empirical evidence that small, epistatic loci may be very wide spread.

The last century of breeding experiments has revealed that most phenotypes of ecological and agricultural significance are quantitative: influenced by multiple genes and the environment. Heritable differences within or among populations are predominantly caused by the action of QTL: functionally diverged alleles that cause modest changes along a continuum of trait values (Mackay, 2001). Characterizing variation caused by QTL as well as cloning the underlying genes is important to a diverse range of biological disciplines, from pharmacogenomics to plant and animal breeding. Evolutionary biologists study QTL because they may help to answer the fundamental question of what maintains variation in fitness-related traits within species. For these fields of study, knowing the distribution of QTL, their effects, and the prevalence of nonadditive interactions between loci (epistasis) is critical.

The findings of Kroymann and Mitchell-Olds are reminiscent of the infinitesimal model (Fisher, 1958) and thus not entirely unexpected. Theoretical work on the genetic basis of adaptation suggests that as organisms adapt to new environments, the allelic effects of loci that become fixed will be large at first and then become increasingly small as the fitness optimum is approached (Orr, 2005). Most QTL mapping studies find several large effect and then increasing numbers of smaller effect loci yielding an exponential or L-shaped distribution (see review by Mackay (2004) for *Drosophila*). However, simulation studies show that allelic effects of large effect loci are often overestimated, and that mapping experiments can produce an L-shaped distribution regardless of the true shape of the underlying curve (Beavis, 1994; Bost *et al*, 2001). Furthermore, most traditional QTL mapping studies have inherent limitations. In these studies, a segregating mapping population is phenotyped for traits of interest, genotyped throughout the genome, and then a genome-wide scan is made to find statistical associations between marker allele states and trait values (Doerge, 2002). For practical reasons most studies are limited to several hundred individuals or lines (or fewer); as a result, the number of recombination events separating closely linked loci is small and overall power to detect small effect loci, especially when there are linked epistatic loci, is relatively low. Nevertheless, there are examples of very tightly linked epistatic loci. For example, dissection of a high-temperature growth QTL in yeast revealed three closely linked genes with epistatic interactions (Steinmetz *et al*, 2002).

The design used by Kroymann and Mitchell-Olds overcomes both the power and resolution limitations of traditional studies, although it too has limitations. Instead of performing a genome-wide scan, Kroymann and Mitchell-Olds focused on 210 kb of a single chromosome and asked what effect, if any, segments within those 210 kb had on biomass accumulation.
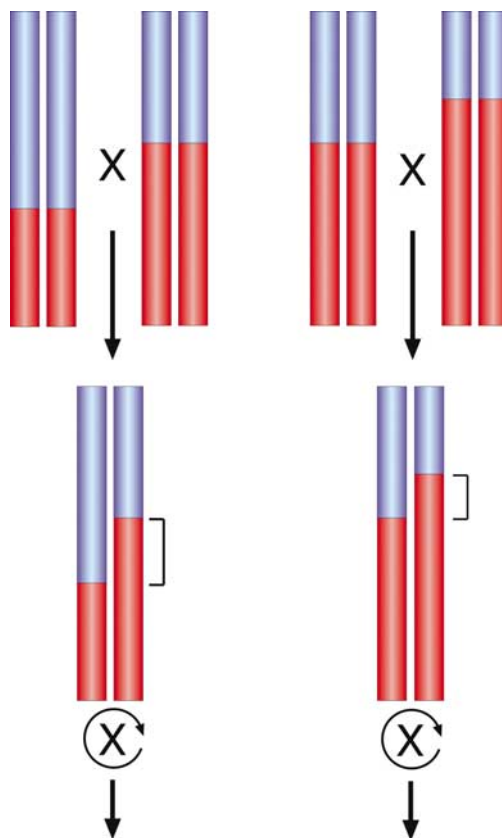
Figure 1 Advanced crosses used for fine-scale QTL mapping. A series of homozygous lines with recombination across the interval of interest (top row) are crossed. The progeny from these crosses are homozygous for all but a small portion of the interval (bracket). These F1 are allowed to self-fertilize and their progeny are genotyped at a marker within the segregating interval and phenotyped to determine if a QTL is segregating in the heterozygous region. Although not shown here, a reciprocal set of crosses (red genome above; blue below) was also performed.

This allowed examination of many recombinants within the interval and many individuals carrying each recombinant chromosome, increasing power and resolution. The region was chosen because prior studies had shown it to harbor genes important for defense against insect herbivory (Kroymann et al, 2003). The present study was designed to ask if there is any cost (measured as reduced biomass) associated with carrying the alleles that confer resistance. An initial survey of recombinants across the region revealed two QTL for biomass in the interval, although neither was associated with the herbivory loci. A series of almost (see below) reciprocal advanced crosses (Figure 1) was then used to both finely map the loci responsible and examine epistasis. For both loci the reciprocal mapping lines show different allelic effects: for one locus the direction of allelic effects is reversed and for the other the allelic effect is nullified. Kroymann and Mitchell-Olds conclude that these discrepancies are best explained by epistatic effects, although other explanations are possible (see below). As a result of the relatively small allelic effects and epistatic interactions, it is unlikely that these loci would have been detected in a genome-wide mapping study. Importantly, there was no prior indication that this region was important for biomass accumulation, and thus it may represent a 'typical' segment of the *Arabidopsis* genome. If so, then the genome may be filled with hundreds of small effect loci that go undetected because of their small effects and epistatic interactions.

Can we conclude from Kroymann and Mitchell-Olds' work that the genome is indeed full of small-effect QTL? Not necessarily. First, we do not really know if the region studied is representative of the whole genome; additional regions need to be sampled before we generalize with confidence. Second, aerial biomass may be influenced by more genetic pathways than typical plant traits, since alterations in almost any aspect of plant development or physiology is likely to affect biomass. Thus, there may be more biomass QTL than would be found for more specific traits. Third, although epistasis is a reasonable explanation for the apparent dependency of allelic effects on the genotype of the flanking region, it is important to remember that the crosses examined were not entirely reciprocal.
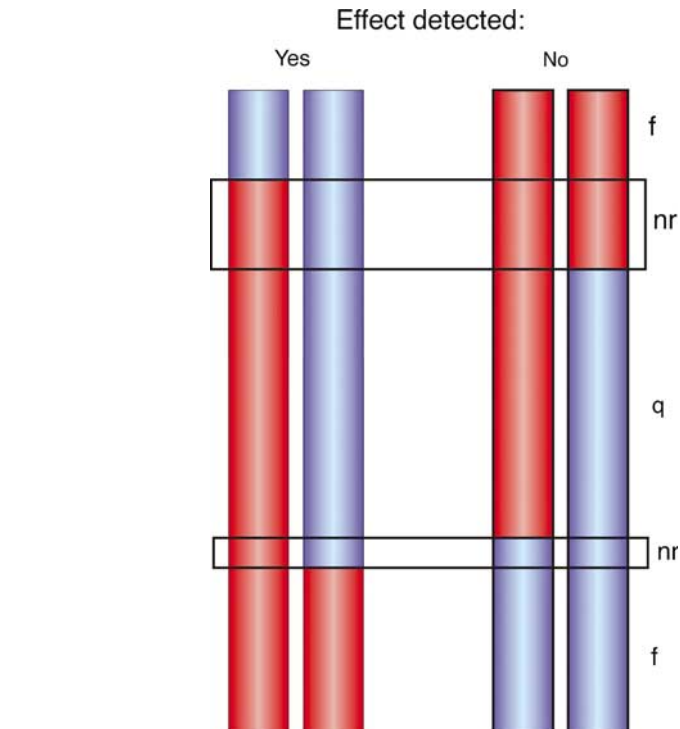
Effect detected:

Figure 2 Epistasis alternatives. For one locus studied, the ability to detect an allelic effect differed for the two reciprocal crosses. The authors suggest that there is a QTL in the central region (q) whose affect depends on the genotype of the flanking sequences (f). Alternatively, the QTL could reside in the non-reciprocal regions (nr; boxed) that are only segregating in one of the two crosses. This alternative requires that the QTL be caused by small intronic or intergenic sequence changes. A QTL in the nonreciprocal region of the other locus studied could similarly explain the apparent epistasis there, although a second QTL in the segregating region, with opposite and stronger effects, must be invoked (not shown).

An alternative explanation is that the QTL maps to the nonreciprocal region (Figure 2). Kroymann and Mitchell-Olds argue against this because the nucleotide changes in these regions are all intronic or intergenic; however, given the prevalence of microRNAs and of transcripts derived from noncoding regions, and our limited knowledge of the molecular basis of small-effect QTL, this possibility should be kept in mind.

In summary, in their interesting paper, Kroymann and Mitchell-Olds have used a clever empirical approach to examine the prevalence of small-effect QTL and epistatic interactions. As the authors state, if the results are typical, then QTL studies are underestimating the number of loci even more than previously thought. A full understanding of complex traits will require sophisticated empirical studies that build upon the theory and methodology of evolutionary quantitative genetics. This paper is an important example of how to proceed.

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Further Reading

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