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# The genetic architecture of quantitative traits: lessons from *Drosophila*

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Understanding the genetic architecture of quantitative traits begins with identifying the genes regulating these traits, mapping the subset of genetically varying quantitative trait loci (QTLs) in natural populations, and pinpointing the molecular polymorphisms defining QTL alleles. Studies in *Drosophila* have revealed large numbers of pleiotropic genes that interact epistatically to regulate quantitative traits, and large numbers of QTLs with sex-, environment- and genotype-specific effects. Multiple molecular polymorphisms in regulatory regions of candidate genes are often associated with variation for complex traits. These observations offer valuable lessons for understanding the genetic basis of variation for complex traits in other organisms, including humans.

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Current Opinion in Genetics & Development 2004, 14:253–257

This review comes from a themed issue on  
Genetics of disease

0959-437X/\$ – see front matter  
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DOI 10.1016/j.gde.2004.04.003

## Abbreviations

**Ddc** *Dopa decarboxylase*  
**LD** linkage disequilibrium  
**QTL** quantitative trait locus  
**QTN** quantitative trait nucleotide  
**RI** recombinant inbred  
**smi** *smell-impaired*

## Introduction

Understanding the genetic architecture of any quantitative trait breaks down to a number of issues: the genes (quantitative trait loci [QTLs]) regulating the trait; the subset of QTLs affecting naturally occurring variation in the trait; the homozygous, heterozygous, epistatic, and pleiotropic effects of QTL alleles, in a wide range of environments; the molecular basis of the allelic variation; and finally, the evolutionary forces responsible for maintaining genetic variation for quantitative traits in nature. Understanding all of these is a tall order, likely only to be delivered using model systems with excellent genetic and genomic resources, such as *Drosophila melanogaster*.

The list of quantitative trait phenotypes that can be investigated using *Drosophila* is only limited by the imagination of the investigator. In addition to obvious aspects of morphology, including the classic numbers of sensory bristles [1], one can quantify *Drosophila* behaviors: stress resistance and drug tolerance; enzyme activities and metabolic traits; and fertility and longevity. Given the evolutionary conservation of genes affecting important biological processes, it is likely that genes affecting homologous traits will affect the same traits in other species, including humans.

*Drosophila* brings an impressive toolkit to the challenge of genetically dissecting the multiple interacting loci, with individually small and environmentally sensitive effects, that affect complex traits. This includes mutagenesis using *P* transposable elements [2] and RNA interference [3••] to identify genes regulating quantitative traits. *Drosophila* collected from nature can be screened for genotypes with extreme trait phenotypes, either by inbreeding whole genomes to homozygosity or cloning single chromosomes using balancer stocks [2]. More deviant genotypes can be created by artificial selection [4]. Mapping the QTLs responsible for naturally occurring variation is facilitated by the ability to rear large numbers of flies, and to construct recombinant inbred and near-isoallelic lines. Complementation tests to deficiency stocks enables rapid high-resolution QTL mapping, and complementation to mutations identifies positional candidate QTLs for further study. Linkage disequilibrium decays rapidly in *Drosophila* regions of normal recombination [5], enabling identification of molecular polymorphism(s) that functionally define QTL alleles. Finally, the genome sequence [6] and multiple platforms for whole genome transcriptional profiling facilitate genomic approaches for identifying genes affecting quantitative traits, and variation in quantitative traits. Here, I review recent progress that has been made towards understanding the complex genetic architecture of quantitative traits in *Drosophila*, and outline directions for future research.

## *P*-element mutagenesis

The first question, ‘what genes affect quantitative traits?’, is best addressed by mutagenesis. Lines containing independent insertions of single *P*-elements are readily produced by simple crosses, and the exact insertion sites of the *P*-elements are easy to determine. Assessing subtle, quantitative effects of new mutations derived in an isogenic background is a highly efficient method for discovering novel loci affecting quantitative traits [7,8,9••,10]. Bristles are external sensory organs of the

peripheral nervous system, and a large number of loci involved in neural development have been identified by traditional screens for mutations with large effects [11]. Screening for quantitative mutational effects of *P*-element insertions on bristle number [8,9\*\*] has identified several insertions in known loci with well-characterized roles in neurodevelopment, many insertions in known genes with previously unknown and pleiotropic effects on bristle number, and many putative first mutations in predicted genes [9\*\*]. Quantitative screens for effects of *P*-element mutations on olfactory behavior [7] and resistance to starvation stress [12] have provided new insights to the genetic architecture of these less-understood complex traits. *Smell-impaired* (*smi*) insertional mutations [7] with subtle effects on olfactory behavior interact epistatically [13]. The *smi60E* mutation is an insertion in the gene encoding the NaCP60E cation channel [14], and *smi97B* is an allele of *scribble* [15], which is essential for early development [16]. The large numbers of insertions affecting bristle number, olfactory behavior and starvation resistance found in all of the screens implies extensive pleiotropy, and implicates roles for key developmental loci in adult morphology, behavior and physiology.

### Whole genome expression analysis

Co-isogenic *P*-element insertional mutations affecting the same trait afford the opportunity to ask to what extent single mutations alter expression of other genes, whether the same genes are either up- or down-regulated in the backgrounds of different mutations, and whether genes exhibiting transcriptional co-regulation also exhibit epistasis for the trait phenotype. Analysis of variation in transcript abundance between five co-isogenic *smi* mutations revealed significant *trans*-regulation in transcription for >500 genes [17\*]. Most (61%) transcripts showed altered regulation in response to only a single *P*-element insertion, but 39% were altered in two or more *smi* lines. Evaluation of epistasis for olfactory behavior using quantitative complementation tests to mutations in genes with altered transcript levels in one or more *smi* lines revealed that epistatic interactions for olfactory behavior often mirrored *trans*-regulation at the level of transcription, thus identifying new candidate QTLs regulating olfactory behavior. Mutations affecting the same complex trait induced in the same isogenic background facilitate a systems-biology approach for further elucidating networks of interacting QTLs. Similar logic was used to identify candidate genes affecting geotactic behavior from differences in gene expression between a line selected for increased and one selected for decreased geotactic behavior [18\*].

### QTL mapping: genome scans

To understand the evolutionary forces responsible for maintaining genetic variation for quantitative traits in nature, we need to map the genes affecting natural variation in the trait, and determine the molecular poly-

morphisms defining QTL alleles. This is an iterative procedure, beginning with a genome scan to map regions containing one or more QTLs affecting the trait. *D. melanogaster* is a natural outbreeder, but inbred lines can be created in the laboratory. Thus, mapping populations derived from segregating backcross, F2, advanced intercross or recombinant inbred (RI) lines derived from two genetically divergent inbred strains are the most powerful designs for mapping *Drosophila* QTLs [4]. QTLs are mapped in the usual manner by linkage to polymorphic molecular markers, utilizing statistical methods designed to account for multiple QTLs [19] and appropriate control for the experiment-wise false-positive error rate [20].

RI lines are a particularly useful resource for QTL mapping: marker genotypes need only be assessed once for each line; replicated phenotypic measurements of each genotype give a more accurate measure of the mean genotypic effect; and the same lines can be examined for multiple traits, and for the same trait in multiple environments. A panel of 98 RI lines derived from *Oregon-R* and *2b* have been used to map QTLs for longevity [21–24]; abdominal and sternopleural bristle number [25]; ovariole number [26]; olfactory behavior [27\*]; courtship signal [28]; flight [29] and measures of metabolism [29]. Other studies have utilized RI chromosomes, in a standard inbred background, to map QTLs for sensory bristle number [30–32,33\*] and wing shape [34,35].

General principles emerging from studies of *Drosophila* QTLs are likely to apply to quantitative traits in other species. First, the distribution of homozygous QTL effects is exponential, with a large number of QTLs with small effects, and a smaller number with large effects; the latter contribute most of the variation between the parental lines [33\*]. Second, most QTLs are not strictly additive [33\*], and often interact epistatically with other QTLs [23,24,33\*,34,35]. Third, QTL effects are often sex-specific [21–25,30,31,33\*] and exhibit QTL by environment interaction [22–25,33\*]. Some QTL effects are even more complicated, with epistasis contingent on the external environment [23]. If pervasive in other species where it is not realistic to take both sexes and multiple environments into account, these features complicate QTL mapping efforts by reducing the marginal effects of the QTLs (and hence the power to detect QTLs) averaged over males and females, and uncontrolled environmental variation. Evolutionary arguments implicate QTL by sex and QTL by environment interaction in the maintenance of variation for complex traits [36], suggesting that these features may indeed be general.

### QTL mapping: deficiency and mutant complementation tests

On average, QTLs identified by genome scans in *Drosophila* encompass ~4300kb and 500 genes. The challenge

for QTL mapping in *Drosophila* is the same as for all other species: resolving the QTLs into individual contributing genes. *Drosophila* geneticists can short-circuit the usual laborious process of high-resolution recombination mapping by utilizing the set of overlapping deficiencies that together uncover 70–80% of the genome. Briefly, one crosses the two parental lines used to map the QTLs to the set of deficiencies spanning each QTL region. Failure of a deficiency to complement the QTL is inferred if the difference in trait phenotype between deficiency hemizygous progeny is greater than that between heterozygous progeny [37]. Comparison of the breakpoints of the deficiencies that fail to complement with those of overlapping complementing deficiencies can map QTLs to sub-cM intervals. Studies utilizing this method have found that single QTLs tend to fractionate into multiple linked QTLs [12,27,37,38]. For example, two QTLs affecting variation in longevity between *Oregon R* and *2b* have been resolved into at least eight linked QTLs [37,38]. If closely linked QTLs, sometimes with opposite and sex-specific effects, is a general hallmark of the genetic architecture of complex traits, the level of difficulty for genetic dissection of QTLs in less genetically tractable organisms will increase considerably.

The *Drosophila* genome sequence enables listing all positional candidate genes in the intervals to which the QTLs map. Mutations are available for a large number of loci, and will ultimately be produced in every *Drosophila* gene [39]. Thus, one can perform quantitative complementation tests for the two parental lines and a mutant and wild-type allele of each of the positional candidate genes, exactly as done for deficiency complementation mapping. Many candidate genes affecting nervous system development have been implicated as corresponding to QTLs affecting sensory bristle numbers using this method [30,31]. A novel gene, *Vanaso*, is a candidate gene affecting variation in olfactory behavior [27]; *Dopa decarboxylase (Ddc)* fails to complement QTL alleles for longevity [38]; and 12 candidate genes have been identified that affect variation in resistance to starvation stress [12]. Analysis of differences between the two parental lines in expression of positional candidate genes can also nominate candidate genes corresponding to QTLs [40].

Loci implicated as corresponding to QTLs using these methods remain ‘candidate genes’. Proof that the candidate gene indeed causes the difference in quantitative trait phenotype ultimately requires transformation of the parental alleles into a strain containing a null mutation of the candidate gene and recovering the difference in trait phenotype [41] — a major effort if the magnitude of the effect of the allelic substitution is of the same order as the variation induced by different insertion sites of the transgenes. This problem can be alleviated by precise replacement of alternative alleles at the site of the endogenous locus, in the same genetic background [42], which has

been used to show that a naturally occurring allele of *desaturase 2* affects stress resistance [43]. In the absence of formal proof, supporting evidence for causality includes expression of the candidate gene in appropriate tissues, functional differences in mRNA and/or protein expression levels, potentially functional DNA sequence differences between the two alleles, and demonstration that naturally occurring molecular polymorphisms in the candidate gene are also associated with phenotypic variation in the trait.

### Linkage disequilibrium mapping

Linkage disequilibrium (LD) mapping capitalizes on historical recombination to identify candidate genes affecting complex traits [4]. The resolution of the method depends on local recombination rates, number of generations following the mutation event, population demography, and the density of polymorphic markers surveyed. In regions of normal recombination in *Drosophila*, LD decays rapidly within several hundred bp [5], creating a highly favorable scenario for mapping quantitative trait nucleotides (QTNs) defining QTL alleles. LD mapping requires large sample sizes, but in *Drosophila* the ability to substitute homozygous wild-derived chromosomes into a common inbred background [5,38,44–47] and to test whether near-isoallelic lines of the wild alleles fail to complement mutations of the candidate genes [5,48,49] considerably increases the power to detect associations with small effects. LD mapping has been used in *Drosophila* to show that molecular polymorphisms in candidate genes are associated with quantitative variation in enzyme activity [44], numbers of sensory bristles [5,45–49] and longevity [38]. Most of the polymorphisms associated with quantitative variation are in putative regulatory regions. Further, LD mapping has uncovered epistatic interactions between multiple polymorphisms within candidate genes [38,50], revealing an unprecedented degree of complexity in the genetic architecture of quantitative traits.

Demonstrating that molecular polymorphisms in a candidate gene identified via linkage analysis and mutant complementation tests are associated with naturally occurring phenotypic variation for the trait is excellent corroborating evidence that the gene indeed corresponds to the QTL. However, we can rarely conclude that the polymorphisms exhibiting LD with the trait actually cause the trait variation, because LD could arise from recent admixture, and utilizing only a subset of polymorphic markers within the candidate genes leaves open the possibility that the true causal polymorphism is in LD with the significant site. The latter problem can be alleviated by fully sequencing each candidate gene allele, and ultimately transformation of alternate alleles into a null background [50] or homologous replacement into the endogenous gene [43] will be required to prove that particular polymorphisms cause the differences in trait phenotypes.

## Conclusions

Less than a decade ago, studies of the genetic architecture of numbers of *Drosophila* sensory bristles [1] painted the optimistic picture that natural variation in bristle number could be attributed to polymorphisms in relatively few candidate genes involved in neural development. However, even these early studies hinted at complications arising from sex-specific QTL effects and interactions between QTLs. We now know that there are large numbers of epistatically interacting loci regulating quantitative traits (including numbers of sensory bristles), and that any one subtle mutational perturbation can induce hundreds of epigenetic co-regulated transcriptional interactions. The challenge for the future will be to assess pleiotropic mutational effects on multiple quantitative traits, and to link networks of transcriptional interactions to genetic interactions. Higher resolution recombination and deficiency complementation mapping has revealed that single QTLs fractionate into multiple linked QTLs, giving large numbers of QTLs and candidate genes affecting naturally occurring variation. QTL effects are strongly conditional on the sexual and external environment, and genetic background, even for largely 'additive' traits. Even within a single candidate gene, multiple polymorphisms interact to affect trait variation.

In the future, combining QTL mapping with whole genome expression analyses of the mapping population to identify transcripts corresponding to QTLs will give a more complete picture of the complex genetic architecture of quantitative traits. Utilizing complete DNA sequences of large numbers of candidate gene alleles in LD mapping studies has the potential to identify actual QTNs affecting trait variation, and to evaluate evolutionary mechanisms for maintenance of quantitative genetic variation. Finally, functional analyses are required to understand how molecular variation in non-coding regions translates to phenotypic variation.

## Acknowledgements

I thank Robert Anholt for comments on this manuscript. Work in the author's laboratory is supported by grants GM45146, GM45344 and GM59469 from the National Institutes of Health.

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