Review

Mutations and quantitative genetic variation: lessons from *Drosophila*

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A central issue in evolutionary quantitative genetics is to understand how genetic variation for quantitative traits is maintained in natural populations. Estimates of genetic variation and of genetic correlations and pleiotropy among multiple traits, inbreeding depression, mutation rates for fitness and quantitative traits and of the strength and nature of selection are all required to evaluate theoretical models of the maintenance of genetic variation. Studies in *Drosophila melanogaster* have shown that a substantial fraction of segregating variation for fitness-related traits in *Drosophila* is due to rare deleterious alleles maintained by mutation–selection balance, with a smaller but significant fraction attributable to intermediate frequency alleles maintained by alleles with antagonistic pleiotropic effects, and late-age-specific effects. However, the nature of segregating variation for traits under stabilizing selection is less clear and requires more detailed knowledge of the loci, mutation rates, allelic effects and frequencies of molecular polymorphisms affecting variation in suites of pleiotropically connected traits. Recent studies in *D. melanogaster* have revealed unexpectedly complex genetic architectures of many quantitative traits, with large numbers of pleiotropic genes and alleles with sex-, environment- and genetic background-specific effects. Future genome wide association analyses of many quantitative traits on a common panel of fully sequenced *Drosophila* strains will provide much needed empirical data on the molecular genetic basis of quantitative traits.

Keywords: maintenance of quantitative genetic variation; mutation–selection balance; balancing selection; pleiotropy; context-dependent effects; genetic architecture

1. MUTATIONS AND QUANTITATIVE GENETIC VARIATION

Most morphological, physiological, behavioural, life history and biochemical phenotypes vary continuously in natural populations. The continuous variation is attributable to the combination of segregating alleles at multiple loci affecting the traits, environmental effects and gene–environment interactions (Falconer & Mackay 1996; Lynch & Walsh 1998). Quantitative genetics theory further enables us to partition genetic variation into additive genetic, dominance and epistatic variance components, which depend on homozygous and heterozygous effects of alleles at individual loci, interaction effects of alleles at two or more loci and allele frequencies. The magnitude and type of segregating genetic variation determines the correlation among relatives, responses to natural and artificial selection and inbreeding depression (and its converse, heterosis) for the trait.

Evolutionary explanations for the magnitude and type of segregating genetic variation for quantitative traits hinge on the relationship of the trait to reproductive fitness, the effects of newly arising mutations on

both the trait and fitness, the mutation rate and the species effective population size. Quantitative traits can be broadly categorized as major components of fitness under directional selection, traits under stabilizing selection for an intermediate optimum and selectively neutral traits (Charlesworth 1994; Falconer & Mackay 1996). The long-term effect of directional or stabilizing selection is to reduce genetic variation (Robertson 1956; Charlesworth 1994; Falconer & Mackay 1996). Thus, for any quantitative trait, segregating genetic variation will be caused by a unique characteristic mixture of (1) low-frequency alleles with deleterious effects on fitness that arose by recent mutations and have not yet been eliminated by selection, (2) selectively neutral alleles that span the range of allele frequencies expected in a population at mutation–drift equilibrium, and (3) alleles at intermediate frequencies because they have opposing effects on major components of fitness (i.e. exhibit antagonistic pleiotropy) or are expressed late in life, when the force of natural selection weakens or have context-dependent effects such that averaged over all contexts, net selection coefficients are small (Charlesworth 1994; Falconer & Mackay 1996). Understanding the relative contribution of each of these mechanisms for the maintenance of quantitative genetic variation is important for predicting deleterious side effects of selection, evolutionary constraints and the evolution of senescence and late-onset diseases.

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2. *DROSOPHILA* AS A MODEL FOR EVOLUTIONARY QUANTITATIVE GENETICS

The *Drosophila melanogaster* model system provides an impressive arsenal of genetic tools and resources that can be used for the genetic analysis of quantitative traits. First and foremost, *Drosophila* has a rich repertoire of traits that can be rigorously quantified, including the major components of fitness (viability and fertility), measures of fitness itself and life-history traits (e.g. mating behaviour, lifespan and resistance to environmental stressors). Other traits thought to be under stabilizing selection include aspects of morphology (body size, numbers of sensory bristles, wing shape) and various behaviours (locomotor and aggressive behaviour, learning and memory, sensitivity to the inebriating effects of ethanol). The rapid generation time and large number of progeny facilitate the construction of inbred lines and long-term artificial selection lines, and balancer chromosomes that suppress recombination for each of the three major chromosomes enable the cloning of entire chromosomes by genetic crosses in a few generations. *Drosophila* geneticists can use standard methods for estimating genetic components of segregating variation for quantitative traits from resemblance between parents and offspring and sibs or responses to selection (Rose & Charlesworth 1981a,b; Falconer & Mackay 1996). In addition, it is possible to estimate homozygous genetic variation from variation among inbred lines and perform crosses among inbred lines (Hughes & Charlesworth 1994; Charlesworth & Hughes 1996) to directly estimate inbreeding depression. The ability to measure large numbers of individuals of the same genotype in controlled environments is especially important for fitness and related traits that are exquisitely sensitive to small changes in the environment and that typically have low heritabilities. The ability to construct chromosome substitution lines facilitates the detection of inter-chromosomal epistatic interactions. The short generation time also facilitates the study of spontaneous mutations by creating replicate lines from an inbred base population that accumulate mutations. Such lines can be sheltered from natural selection by maintaining the mutation accumulation (MA) lines as heterozygotes over a balancer chromosome (Mukai 1979; Houle *et al.* 1992, 1994), by maintaining the lines in unselected small populations (Mackay *et al.* 1995) or by subjecting lines to artificial selection for a quantitative trait (Caballero *et al.* 1991; Mackay *et al.* 1994). Thus, using *Drosophila*, we can directly estimate spontaneous mutational variance as well as segregating genetic variance for fitness and quantitative traits.

In addition to assessing the genetic architecture of quantitative traits at the level of the trait, *Drosophila* is also a favourable system for identifying the actual loci at which de novo mutations and segregating alleles affect the trait and for characterizing the properties of mutations and polymorphisms. *P* transposable element mutagenesis is the main workhorse for the former endeavour, since single genetically engineered *P*-elements can be induced to hop into new genomic locations while maintaining a common co-isogenic

background by simple crosses to a stock containing a chromosomally stable source of transposase (Mackay 2001; Venken & Bellen 2005). Eliminating the chromosome containing the transposase in the following generation creates a stable stock with a new insertion, the exact genomic location of which can be readily determined. If the quantitative trait phenotype of a *P*-element insert line is significantly different from the co-isogenic *P*-element free control strain, the gene into or near which the *P*-element has inserted is a candidate gene affecting the trait. Collections of mutations in a common isogenic background also facilitate the detection of epistatic interactions among them (Fedorowicz *et al.* 1998; van Swinderen & Greenspan 2005; Sambandan *et al.* 2006; Yamamoto *et al.* 2008). This is done by measuring the trait phenotypes for all possible double heterozygous genotypes (a classic quantitative genetic half-diallel cross design) and determining whether particular double heterozygotes are more (enhancing epistasis) or less (diminishing epistasis) deviant from wild-type than predicted from the additive heterozygous effects of the two parental genotypes.

Identifying the loci harboring naturally segregating variation affecting quantitative traits is the province of quantitative trait locus (QTL) mapping, which can be done using linkage or association mapping. The two common designs used for linkage mapping in *Drosophila* capitalize on the ability to create replicated genotypes, such as recombinant inbred lines (RILs) derived by inbreeding the F₂ generation of a cross of two inbred lines to near-homozygosity (Nuzhdin *et al.* 1997; Kopp *et al.* 2003) and recombinant isogenic chromosomes derived by substituting single recombinant chromosomes between two inbred strains into a common isogenic background (Long *et al.* 1995; Gurganus *et al.* 1999; Weber *et al.* 1999, 2001). *Drosophila* geneticists can circumvent the problem of restricted genetic sampling in QTL mapping efforts based on two parental lines by deriving the inbred lines used to generate the mapping population from lines created by long-term artificial selection from a large heterogeneous base population (Long *et al.* 1995; Gurganus *et al.* 1999; Weber *et al.* 1999, 2001; Forbes *et al.* 2004) or by constructing RILs (Kopp *et al.* 2003) or segregating populations (Macdonald & Long 2007) from more than two haploid genomes. Linkage mapping of QTLs in *Drosophila* suffers the same problems as in other organisms: the QTL regions inferred from initial genome scans with typical sample sizes and marker densities are usually very broad, encompassing on average 500 genes (approx. 4000 kb). In *D. melanogaster*, however, we can perform quantitative complementation tests to overlapping deficiency stocks to map QTLs to sub-centimorgan regions without recombination and to mutations to define candidate genes (Mackay 2001). Alternatively, we can increase the resolution of recombination by selecting individuals from the extreme phenotypic tails of a large (tens of thousands of flies) F₂ population derived from two inbred lines that vary for the trait. We can then hybridize DNA from pools of multiple individuals from the phenotypic extremes to short-oligonucleotide microarrays designed for whole

genome expression profiling to map QTLs linked to marker loci that exhibit changes in allele frequency between the selected tails of the distribution (Lai *et al.* 2007).

While linkage mapping can identify genes affecting quantitative traits, it provides no information as to which of the many polymorphisms distinguishing the parental alleles are the functional polymorphisms or whether they were common or rare in the population from which the parental lines were derived. However, association mapping in *Drosophila* offers the possibility of identifying the causal quantitative trait nucleotides (QTNs) affecting the traits and estimating their population frequencies. This is because the structure of linkage disequilibrium (LD) in natural populations of *Drosophila* is exceptionally fine-grained, and decays rapidly in regions of normal recombination—so rapidly, in fact, that polymorphic sites adjacent to each other can be in linkage equilibrium (Carbone *et al.* 2006)! While this means that association mapping in *Drosophila* is best performed for particular candidate genes for which complete DNA sequences have been obtained (Dworkin *et al.* 2003; Nikoh *et al.* 2004; Palsson & Gibson 2004; Carbone *et al.* 2006; Wang *et al.* 2007), it also means that individual polymorphic sites associated with the trait in these studies are likely to be the causal QTNs. Association mapping requires large sample sizes, which increase as the fraction of the phenotypic variance contributed by segregation at the QTN decreases (Long & Langley 1999). In *Drosophila*, however, the ability to obtain replicate measures of the same genotype by creating inbred lines, chromosome substitution strains and congenic introgression lines greatly increases the accuracy of estimating the mean genotypic value of each line (Long *et al.* 1998; Robin *et al.* 2002; De Luca *et al.* 2003).

3. LESSONS FROM *DROSOPHILA*: QUANTITATIVE GENETIC ANALYSES

To what extent is segregating quantitative genetic variation (V_G) due to a balance between the continuous rain of new mutations on the genome each generation and their removal from the population by selection? The input of genetic variance of the trait from new mutations each generation is V_M . For traits under directional selection, such as components of fitness and life-history traits, we assume that new mutations have a largely deleterious effect on fitness and that the equilibrium genetic variance from a balance between mutation and selection will occur when the mutational variance is of the same order as the selection coefficients (Barton 1990). The mutational variance for viability, a major component of fitness, has been estimated in several experiments by maintaining populations of an initially isogenic chromosome for many generations, sheltered from natural selection by a balancer chromosome (Houle *et al.* 1996). Comparisons of estimates of V_M with estimates of V_G and the average selection coefficient against heterozygous mutations are consistent with a large fraction of segregating variation for viability from deleterious alleles maintained at low frequencies by mutation–selection

balance (Houle *et al.* 1996). A similar conclusion holds for life-history traits, such as longevity, fecundity, male mating success and productivity (Houle *et al.* 1994, 1996).

To what extent is some variation for life-history traits due to alleles at higher frequencies? This could be due to alleles with antagonistic pleiotropic effects on two fitness components or alleles that affect the trait at later ages, after reproductive maturity (Medawar 1952; Williams 1957; Charlesworth 1994). This question has been extensively investigated with respect to longevity and senescence. If alleles with antagonistic pleiotropic effects between lifetime viability and fertility segregate, there should be a negative genetic correlation between lifespan and early fertility. This negative genetic correlation should be estimable in classic sib designs assessing genetic variation in early fertility and longevity. In addition, this model predicts that there should be genetic variation in longevity such that artificial selection for increased lifespan is successful, with a correlated response in increased fertility at late age (postponed senescence) and a correlated response in decreased fertility at early ages. These predictions have been experimentally validated in some experiments (Rose & Charlesworth 1980, 1981a,b; Luckinbill *et al.* 1984; Rose 1984; Partridge & Fowler 1992; Zwaan *et al.* 1995), but not all studies (Roper *et al.* 1993; Houle *et al.* 1994; Hughes 1995; Tatar *et al.* 1996). It should be noted that the concept of antagonistic pleiotropy could be extended to opposite fitness effects of alleles affecting different life-history traits from viability and fertility (including beneficial early and detrimental late effects of the same trait), between males and females, different environments or different genetic backgrounds. If alleles with late-age-specific expression accumulate in natural populations and cause variation in senescence, we expect an increase in genetic variation with age. Again, this has been observed in some studies (Hughes & Charlesworth 1994; Hughes 1995; Charlesworth & Hughes 1996; Lesser *et al.* 2006; Swindell & Bouzat 2006; Borash *et al.* 2007), but not all (Rose & Charlesworth 1980, 1981a; Promislow *et al.* 1996; Tatar *et al.* 1996). Other evidence for the existence of alleles at intermediate frequency affecting segregating variation for life-history traits comes from comparison of the change in mean from short term artificial selection of the trait to the change of mean on inbreeding. If rare, partially recessive alleles maintained by mutation–selection balance largely affect the standing variation, the change in mean due to inbreeding will be much greater than the change in mean from selection (Kelly 1999). Application of this test to female fecundity indicated the contribution of intermediate frequency alleles to natural variation of this trait (Charlesworth *et al.* 2007). In summary, a substantial fraction of segregating variation for fitness-related traits is due to rare deleterious alleles maintained by mutation–selection balance, with a smaller but significant fraction attributable to intermediate-frequency alleles maintained by alleles with antagonistic pleiotropic effects and late-age-specific effects.

Many quantitative traits appear to be under stabilizing selection in natural populations. The strength of

stabilizing selection, V_S , is $-1/2\gamma$, where γ is the standardized stabilizing selection gradient, measured as the regression of relative fitness on the square of the deviation of the trait value from the mean (Falconer & Mackay 1996; Johnson & Barton 2005). Johnson & Barton's (2005) analysis of a comprehensive survey of studies of natural selection in the wild (Kingsolver *et al.* 2001) indicated a median value of $\gamma = -0.1$, which corresponds to values of V_S typically used in theoretical models. The exact expressions for the expected genetic variance at mutation-stabilizing selection equilibrium depend on whether stabilizing selection acts directly on the trait (real stabilizing selection), or acts on the trait indirectly, through pleiotropic effects on other traits that are under selection (apparent stabilizing selection) (Johnson & Barton 2005; Zhang & Hill 2005). Models of real stabilizing selection cannot simultaneously account for observed levels of genetic variance, per locus and per trait mutation rates, mutational variance and stabilizing selection (Johnson & Barton 2005; Zhang & Hill 2005). Further, the concept of real stabilizing selection assumes that natural selection perceives organisms as subdivided into as many compartments as there are traits, each of which causally affects fitness (Robertson 1967). Genetic load arguments indicate that real stabilizing selection cannot operate independently on many traits (Robertson 1967; Barton 1990). Models of apparent stabilizing selection use an assumed bivariate distribution of mutational effects on the focal trait and on fitness. While these models can maintain appreciable genetic variance at equilibrium, the genetic variance is due to the segregation of neutral mutations with large effects on the trait, and therefore these models do not generate the observed amount of stabilizing selection (Johnson & Barton 2005; Zhang & Hill 2005). Apparent stabilizing selection models also predict levels of segregating genetic variance that are dependent on the effective population size, which is also contrary to observation (Johnson & Barton 2005; Zhang & Hill 2005). More recent models (Zhang *et al.* 2004) have combined real and apparent stabilizing selection into a joint model of stabilizing selection–mutation balance that suggests that most variation for the trait is due to alleles that have nearly neutral effects on fitness and that most of stabilizing selection is due to alleles with large effects on the trait.

It is, of course, possible that estimates of mutational variance are biased and/or that more complex genetics should be incorporated into the theoretical models. We know estimates of V_M from MA experiments performed without balancer chromosomes are biased downward, since natural selection cannot be completely prevented (Keightley *et al.* 1993; Houle *et al.* 1996), but this bias is unlikely to be by an order of magnitude. It is also possible that there is suppressing epistasis between mutations (i.e. the effect of the multiple mutant genotype on the trait is less than predicted from the sum of the effects of the individual mutations), leading to an overestimate of the strength of stabilizing selection and/or an underestimate of mutational variation. There is some support for this hypothesis. Long-term MA experiments on sensory

bristle number show that the divergence among inbred lines (Mackay *et al.* 1995) and among lines selected for divergent trait values from an inbred base population (Mackay *et al.* 1994) attenuate over time, and the effects of mapped mutations exceed the divergence among lines (Mackay *et al.* 2005), as would be expected if new mutations exhibit suppressing epistasis.

Further progress towards understanding the mechanisms responsible for maintenance of quantitative genetic variation in natural populations will depend on our ability to identify the loci and molecular polymorphisms affecting variation in any focal trait. We also need to understand how these polymorphisms interact to affect variation in the focal trait, their pleiotropic effects on other traits (including fitness) and the extent to which the effects of the variants vary in different ecologically relevant environments.

4. LESSONS FROM *DROSOPHILA*: GENE MAPPING STUDIES

Studies of the effects of *P*-element mutations on a variety of quantitative traits indicate that the mutational target size of most traits is surprisingly large. Approximately 22 per cent of *P*-element mutations screened affected abdominal bristle number (Norga *et al.* 2003), 23 per cent affected sternopleural bristle number (Norga *et al.* 2003), 41 per cent affected starvation stress resistance (Harbison *et al.* 2004), 5.6 per cent affected olfactory behaviour in response to a single odorant (Anholt *et al.* 1996; Sambandan *et al.* 2006), 22 per cent affected wing shape (Weber *et al.* 2005), 37 per cent affected locomotor startle response (Yamamoto *et al.* 2008) and 35 per cent affected aggressive behaviour (Edwards *et al.* 2009). The vast majority of the genes tagged by the *P*-elements were novel and not previously annotated to affect adult quantitative traits. Many of the loci had well-characterized roles in early development, while others were computationally predicted with no previous annotation. These studies identified loci that could potentially affect natural variation in complex traits. To what extent is natural variation similarly complex? Are the loci harbouring segregating polymorphisms a subset of the loci identified via mutagenesis, as would be expected if the mutation screens are approaching saturation?

The early *Drosophila* QTL mapping studies were encouraging as they revealed a simple genetic basis for variation of quantitative traits, with a few QTLs with relatively large effects affecting most traits (Mackay 2001; Flint & Mackay 2009). However, the majority of these studies had limited power to detect QTLs due to the small size of the mapping populations and the relatively low marker densities. Studies using larger samples and more markers revealed larger numbers of QTLs (Weber *et al.* 1999, 2001; Lai *et al.* 2007). Subsequent efforts to map QTLs with higher resolution by generating more informative recombinants (Dilda & Mackay 2002) or by performing quantitative complementation tests to deficiencies (Pasyukova *et al.* 2000; De Luca *et al.* 2003; Wilson *et al.* 2006) typically reveal more

complex genetic architectures than implicated from the initial genome scans, in which single QTLs fractionated into multiple, closely linked QTLs, often with opposite effects. The loci implicated to affect natural variation for adult quantitative traits are also largely unexpected, and include genes previously annotated to affect early development as well as novel computationally predicted genes. Naturally occurring genetic variation at loci with mutational effects on the development of sensory bristles and wings was indeed associated with variation in bristle number and wing shape (Long *et al.* 1996, 1998, 2000; Lyman & Mackay 1998; Palsson & Gibson 2000, 2004; Robin *et al.* 2002); however, even for these phenotypes, many QTL intervals contain novel loci (Dilda & Mackay 2002; Macdonald & Long 2007). Further, genes affecting natural variation in adult life-history and behavioural traits (Fanara *et al.* 2002; Harbison *et al.* 2004; Jordan *et al.* 2006; Edwards & Mackay 2009) do not overlap the loci affecting these traits from *P*-element mutagenesis (Anholt *et al.* 2003; Harbison *et al.* 2004; Dierick & Greenspan 2006; Edwards *et al.* 2006, 2009; Sambandan *et al.* 2006; Jordan *et al.* 2007; Rollmann *et al.* 2008; Yamamoto *et al.* 2008). Thus, neither approach has reached saturation in terms of understanding the genetic basis of quantitative genetic variation for most traits. Clearly, polymorphic alleles at hundreds rather than tens of loci affect variation for quantitative traits in natural populations of *Drosophila*. These results highlight how little we know about 'candidate genes' affecting quantitative traits: the majority of the genome is uncharted territory with respect to phenotypic effects of naturally segregating alleles affecting even extensively studied phenotypes in a genetic model organism. The allelic effects of new mutations and QTLs tend to follow an exponential distribution, as proposed by Robertson (1967), whereby a few loci have large effects and increasingly larger numbers of loci with increasingly smaller effects make up the remainder of the distribution (Shrimpton & Robertson 1988; Dilda & Mackay 2002).

A corollary of the observation that a substantial fraction of the genome can affect any single trait is that most genes must be pleiotropic and affect multiple traits. Examples of *P*-element mutations with unexpected pleiotropic effects include the developmental loci *neuralized*, *Semaphorin 5c* and *Calreticulin*, which affect both numbers of sensory bristles, olfactory behaviour and locomotor behaviour (Norga *et al.* 2003; Sambandan *et al.* 2006; Rollmann *et al.* 2007; Yamamoto *et al.* 2008). Mutations in the intergenic region between *Tre1* (which affects transepithelial migration of germ cells) and *Gr5a* (a trehalose taste receptor) affect not only trehalose sensitivity, but also lifespan and resistance to starvation and heat stress (Rollmann *et al.* 2006). A mutation in *muscleblind* is associated with increased aggression (Edwards *et al.* 2006) and increased resistance to the inebriating effects of ethanol (Morozova *et al.* 2007), but reduced locomotor activity in response to a mechanical stress (Jordan *et al.* 2007). However, different alleles of pleiotropic genes do not have the same constellation of pleiotropic effects on quantitative traits. The

P-element mutations in the *Tre1/Gr5a* intergenic region, generated in the same co-isogenic genetic background, are associated with both increased and decreased longevity and resistance to starvation and heat stress (Rollmann *et al.* 2006). Similarly, three *P*-element insertions in *neuralized* collectively affect sternopleural bristle number, abdominal bristle number, olfactory behaviour, locomotor startle response, aggression and the morphology of two brain structures, the ellipsoid and mushroom bodies (Rollmann *et al.* 2008). However, none of the three mutations affect all traits. Finally, several studies have evaluated associations of polymorphisms in candidate genes with more than one quantitative trait. Polymorphisms in the *achaete-scute* complex (Mackay & Langley 1990; Long *et al.* 2000), *scabrous* (Lai *et al.* 1994; Lyman *et al.* 1999), *Delta* (Long *et al.* 1998) and *hairy* (Robin *et al.* 2002) were tested for associations with both abdominal and sternopleural bristle number. Polymorphisms in the *Epidermal growth factor receptor* were tested for associations with wing shape (Palsson & Gibson 2004) and cryptic variation for photoreceptor determination (Dworkin *et al.* 2003). Polymorphisms in *Dopa decarboxylase* were tested for associations with longevity (De Luca *et al.* 2003) and locomotor behaviour (Jordan *et al.* 2006); and polymorphisms in *Catecholamines up* were tested for associations with locomotor behaviour, longevity, starvation resistance, abdominal and sternopleural bristle number (Carbone *et al.* 2006) and sleep traits (Harbison *et al.* 2009). In each case, different polymorphic sites were independently associated with the different traits. These observations indicate that pervasive pleiotropy does not necessarily impose evolutionary constraints in the form of strong genetic correlations between traits. The wide range of pleiotropic effects of new mutations is the first condition for maintenance of quantitative genetic variation by antagonistic pleiotropy. In the future, examination of fitness effects of mutations with pleiotropic effects on quantitative traits will be necessary to evaluate whether the empirical properties match theoretical conditions for the maintenance of genetic variation by antagonistic pleiotropy.

Opposing effects of alleles on fitness in males and females, different environments and different genetic backgrounds can, under some conditions (Turelli & Barton 2004), be responsible for maintaining genetic variation for traits under strong directional or stabilizing selection. How often are such context-dependent effects observed? Sex-specific effects are very common for both common *P*-element mutations and QTLs and have been reported for sensory bristle number (Mackay & Lyman 2005), olfactory behaviour (Fanara *et al.* 2002; Anholt *et al.* 2003; Sambandan *et al.* 2006), longevity (Mackay *et al.* 2006; Lai *et al.* 2007), locomotor startle response (Jordan *et al.* 2006) and resistance to starvation stress (Vieira *et al.* 2000; Harbison *et al.* 2004; Rollmann *et al.* 2006), cold stress (Morgan & Mackay 2006) and heat stress (Morgan & Mackay 2006; Rollmann *et al.* 2006). In most cases, the sex-specific effects are due to a difference in the magnitude of the allelic effects between the sexes. However, opposite effects in males and females

have been noted for mutations/QTLs affecting longevity (Vieira *et al.* 2000) and starvation stress resistance (Harbison *et al.* 2004)—these alleles could potentially lead to the maintenance of genetic variation in these traits. Although there have been fewer studies mapping QTLs in different environments, in all cases genotype by environment interaction was observed—for numbers of sensory bristles (Gurganus *et al.* 1998; Dilda & Mackay 2002; Geiger-Thornsberry & Mackay 2002), longevity (Leips & Mackay 2000, 2002; Vieira *et al.* 2000), competitive fitness (Fry *et al.* 1998), immune response to different bacteria (Lazzaro *et al.* 2006) and olfactory behaviour (Sambandan *et al.* 2008). Again, genotype by environment interaction is mostly attributable to environment-specific expression of QTL alleles. However, for lifespan, there are clear indications of alleles with opposite effects in different environments (Vieira *et al.* 2000), which could lead to the maintenance of genetic variation for lifespan if the environments were equally frequent. Formal evaluation of theoretical models for the maintenance of quantitative genetic variation by sex- and environment-dependent effects is difficult, however, since it requires estimating fitness effects in a range of ecologically relevant environments.

Classical variance component analyses generally show little, if any, contribution of epistatic interactions to the total genetic variance for most quantitative traits (Falconer & Mackay 1996; Lynch & Walsh 1998; Hill *et al.* 2008). However, epistasis is difficult to detect using these designs, since even strong epistatic interactions contribute little epistatic variance; all possible interactions among loci affecting variation in the trait are lumped together; and the epistatic interaction term has a high sampling variance, requiring huge sample sizes for detection. Detecting epistasis is also difficult in QTL mapping studies, because the large number of pair-wise tests for marker-marker interactions imposes a low experiment-wise significance threshold and large mapping populations are required to sample individuals in the rarer two-locus genotype classes. Further, segregation of other QTLs can interfere with detecting epistasis between the pair of loci under consideration. Epistasis is more readily detectable in crosses among lines in which genetic heterogeneity is reduced, the genetic background is controlled precisely, and allele frequencies are intermediate.

Diallel crosses have revealed extensive epistasis between *Drosophila* *P*-element mutations in a common isogenic background affecting olfactory (Fedorowicz *et al.* 1998; Sambandan *et al.* 2006), climbing (van Swinderen & Greenspan 2005), and startle (Yamamoto *et al.* 2008, 2009) behaviour. Clark & Wang (1997) constructed all nine possible two-locus genotypes for several pairs of *P*-elements and identified extensive epistasis affecting *Drosophila* metabolic activity. Another powerful design for detecting epistatic interactions is to construct a panel of chromosome substitution lines, in which single chromosomes from one strain are each substituted into the homozygous genetic background of a second strain; or even more precisely, to construct segmental introgression lines, in which small genomic segments

from one strain are introgressed into the background of a second strain, such that the entire collection of introgression lines tile across the genome. In the presence of epistasis, the sum of the effects of all chromosome substitution lines or introgression lines is not equal to the difference in the mean value of the trait between the two parental strains. Using these designs, substantial epistasis has been revealed for *Drosophila* locomotor startle response (Yamamoto *et al.* 2009) and aggressive behaviour (Edwards & Mackay 2009). In both of the latter cases, the direction of the epistatic interactions was to suppress the effects of mutations or introgressed QTLs. Waddington (1957) suggested that traits under strong stabilising selection would be strongly genetically canalized; i.e. the phenotype would be robust to mutations, and the mechanism underlying genetic canalization is suppressing epistasis. If common, suppressing epistasis has implications for QTL mapping studies, since the main effects of individual loci will be underestimated if suppressing epistasis exists but is not accounted for in a mapping study. Suppressing epistasis could also lead to an overestimate of the strength of stabilizing selection and underestimate of mutational variance, which would lead to a revision of the inference that mutation–selection balance does not account for much segregating variation for traits under stabilizing selection (Yamamoto *et al.* 2009).

A full accounting of the nature and frequency of molecular polymorphisms causally affecting quantitative traits is required if we are to solve the long-standing puzzle of why there is so much segregating variation for complex traits in natural populations (Barton & Turelli 1989; Falconer & Mackay 1996; Barton & Keightley 2002; Johnson & Barton 2005). In particular, we need to know whether causal polymorphisms are rare, as would be consistent with maintenance in the population by mutation–selection balance; or at intermediate frequencies, as would be consistent with maintenance by a balancing selection mechanism, or selective neutrality. The few studies in *Drosophila* to date performing association analyses with full genome sequence of candidate genes indicate both intermediate and low frequency alleles are associated with complex traits (Dworkin *et al.* 2003; Nikoh *et al.* 2004; Palsson & Gibson 2004; Carbone *et al.* 2006; Wang *et al.* 2007).

It is clear that simple balances between the evolutionary forces of mutation and drift (Hill 1982) or mutation and real or apparent stabilizing selection (Johnson & Barton 2005; Zhang & Hill 2005) cannot simultaneously account for empirically observed levels of segregating and mutational variance and stabilizing selection. Thus, multiple evolutionary mechanisms must be in play, and application of molecular population genetics analyses to DNA sequences of genes associated with quantitative traits can detect signatures of purifying selection, selective sweeps, balancing selection and neutrally evolving polymorphisms (Sabeti *et al.* 2006). Molecular signatures of balancing selection have been observed for genes associated with variation in longevity (De Luca *et al.* 2003; Carbone *et al.* 2006), locomotor behaviour and bristle number (Carbone *et al.* 2006), sperm

precedence (Begun *et al.* 2000), immune function (Lazzaro & Clark 2003) and olfactory behaviour (Wang *et al.* 2007).

5. *DROSOPHILA* EVOLUTIONARY QUANTITATIVE GENETICS: FUTURE PROSPECTS

Recent and imminent advances in high-resolution genotyping and DNA sequencing technologies (Fan *et al.* 2006) will revolutionize our ability to achieve a deeper understanding of the effects of spontaneous mutations and to rapidly map polymorphisms affecting naturally segregating variation in quantitative traits, particularly in the *Drosophila* model system. The major limitation to interpreting MA experiments has been the absence of knowledge of what mutations actually occurred in the lines and which of them affected the traits. With the advent of next generation sequencing technology, it will be possible to directly characterize all of the actual mutations that occurred in each line and test their effects on quantitative traits (Haag-Liautard *et al.* 2007, 2008; Keightley *et al.* 2009). Since the extent and pattern of MA may vary according to the starting genotype, it is desirable to perform these experiments with several different genetic backgrounds. Technical advances have also revolutionized our ability to rapidly map QTLs with high resolution, by combining genotyping using short oligonucleotide arrays with genotyping phenotypically extreme individuals selected from a huge mapping population (Lai *et al.* 2007). Sequence capture arrays (Albert *et al.* 2007; Okou *et al.* 2007) spanning QTL intervals will enable massively parallel sequencing of thousands of individuals with informative recombinations in QTL intervals to map the QTLs to the level of single genes. This method can also be used to identify molecular variants in candidate gene regions affecting quantitative traits in large samples of individuals from natural populations. Such studies are essential if we are to understand what loci affect variation in quantitative traits and whether causal polymorphisms are common or rare.

However, DNA sequence variation does not affect quantitative traits directly, but does so through networks of intermediate molecular phenotypes. Understanding the relationship between DNA sequence variation, transcriptional, protein and metabolite networks and organismal-level phenotypes is the main challenge for the future and will add the missing biological context to genotype–phenotype associations (Sieberts & Schadt 2007; Ayroles *et al.* 2009; Harbison *et al.* 2009; Loewe 2009; Mackay *et al.* 2009). This ‘systems genetics’ approach to understanding the quantitative genetic variation is best applied to a reference panel of inbred lines, newly derived from nature, that can be used by the entire *Drosophila* research community. The *Drosophila* Genetic Reference Panel (DGRP) consists of 192 *D. melanogaster* lines derived from the Raleigh, USA population by 20 generations of full sib mating. These lines are publicly available to the *Drosophila* community and are currently being fully sequenced by Baylor College of Medicine Sequencing Center, using both 454 long-read technology and Illumina 75

base pair, paired end short-read technology. This sample of 192 inbred lines will capture most variants segregating with a minor allele frequency greater than 2 per cent and a representative sample of rarer alleles. Each inbred line is a defined genotype that can be scored for multiple quantitative traits, including intermediate molecular phenotypes, in a range of social and physical environments, and be used as a starting point for MA experiments. Whole genome association tests will capitalize on the fine-grained LD in *Drosophila* to identify candidate genes associated with a wide range of organismal and molecular phenotypes. Combining information on DNA sequence variation with transcriptional co-expression networks in these lines will enable us to infer causal transcriptional networks associated with quantitative traits (Sieberts & Schadt 2007; Mackay *et al.* 2009). Since the traits and environments to which the lines can be exposed are limited only by the creativity of the *Drosophila* community, we will shortly have unprecedented insight regarding distributions of allelic effects, pleiotropy, epistasis and gene–environment interaction that can only be achieved in a model organism. The DGRP lines can also be crossed to form outbred populations with known allelic composition from which we can predict variance components, inbreeding depression and response to artificial selection and laboratory evolution and compare the predictions with actual estimates to test quantitative genetics theory.

This manuscript is dedicated to Professor Brian Charlesworth on the occasion of his 65th birthday, in honour of his many seminal contributions to *Drosophila* evolutionary and quantitative genetics. This work was supported by grants GM45146, GM076083 and AA016560 from the National Institutes of Health. This is a publication of the W. M. Keck Center for Behavioral Biology.

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