PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2005) **360**, 1513–1527
doi:10.1098/rstb.2005.1672
Published online 13 July 2005

Drosophila bristles and the nature of quantitative genetic variation

Trudy F. C. Mackay* **and Richard F. Lyman**

Department of Genetics, North Carolina State University, Campus Box 7614, Raleigh, NC 27612, USA

Numbers of *Drosophila* sensory bristles present an ideal model system to elucidate the genetic basis of variation for quantitative traits. Here, we review recent evidence that the genetic architecture of variation for bristle numbers is surprisingly complex. A substantial fraction of the *Drosophila* genome affects bristle number, indicating pervasive pleiotropy of genes that affect quantitative traits. Further, a large number of loci, often with sex- and environment-specific effects that are also conditional on background genotype, affect natural variation in bristle number. Despite this complexity, an understanding of the molecular basis of natural variation in bristle number is emerging from linkage disequilibrium mapping studies of individual candidate genes that affect the development of sensory bristles. We show that there is naturally segregating genetic variance for environmental plasticity of abdominal and sternopleural bristle number. For abdominal bristle number this variance can be attributed in part to an *abnormal abdomen*-like phenotype that resembles the phenotype of mutants defective in catecholamine biosynthesis. *Dopa decarboxylase* (*Ddc*) encodes the enzyme that catalyses the final step in the synthesis of dopamine, a major *Drosophila* catecholamine and neurotransmitter. We found that molecular polymorphisms at *Ddc* are indeed associated with variation in environmental plasticity of abdominal bristle number.

Keywords: *P*-element mutagenesis; quantitative trait loci mapping; linkage disequilibrium mapping; genetic variance of environmental plasticity

1. INTRODUCTION

Most variation between individuals in physiology, behaviour, morphology, disease susceptibility and reproductive fitness can be attributed to the segregation of multiple quantitative trait loci (QTLs) with individually small effects, whose expression is conditional on the environment (Falconer & Mackay 1996). Understanding the genetic and environmental factors that cause this variation is of fundamental importance for medicine, agriculture, evolution and the emerging discipline of functional genomics. A comprehensive understanding of the 'genetic architecture' of any quantitative trait would include knowledge of: (i) the numbers and identities of all genes in the developmental, physiological and/or biochemical pathways that lead to the phenotype; (ii) the subset of these genes that affect naturally occurring variation in the trait; (iii) the distribution of homozygous and heterozygous effects of new mutations and segregating alleles; (iv) the pleiotropic effects of alleles on other traits; (v) the extent to which additive, dominance, epistatic and pleiotropic effects vary between the sexes, and in a range of ecologically relevant environments; and (vi) the molecular polymorphism(s) that functionally define QTL alleles.

This is a tall order, which can most effectively be met by studying a quantitative trait that can be scored rapidly and accurately in a model organism with sophisticated genetic resources. The numbers of mechanosensory bristles on the sternopleural plates and abdominal sternites of *Drosophila melanogaster* (sternopleural and abdominal bristles, respectively) fulfil both criteria. Bristle traits have been studied in experimental quantitative genetics for over 50 years. *Drosophila* bristle numbers have abundant and largely additive naturally segregating variation, with heritabilities of the order of 0.5 (Clayton *et al.* 1957). Bristle numbers have been used to check short-term (Clayton *et al.* 1957; Frankham *et al.* 1968) and long-term (Clayton & Robertson 1957; Jones *et al.* 1968) selection theory and to assess the contribution of new mutations to quantitative variation (Keightley *et al.* 1993). They were also the first traits for which QTLs were localized by introgression (Breese & Mather 1957) and interval mapping combined with progeny testing (Thoday 1979; Shrimpton & Robertson 1988*a*,*b*). Further, the many loci required for peripheral nervous system development (Campos-Ortega 1993; Jan & Jan 1993; Kania *et al.* 1995; Salzberg *et al.* 1997; Norga *et al.* 2003) provide a rich repertoire of candidate genes that could affect variation in these external sensory organs. Here, we discuss recent progress towards understanding the genetic architecture of these classic quantitative traits.

* Author for correspondence (trudy_mackay@ncsu.edu).

One contribution of 16 to a Theme Issue 'Population genetics, quantitative genetics and animal improvement: papers in honour of William (Bill) Hill'.

2. HOW MANY LOCI?

There are two distinct contexts in which we seek to understand the number of loci that affect a quantitative

trait: the number of loci required to produce the trait, and the subset of these loci that harbour naturally occurring allelic variation for the trait. The first endeavour is best carried out by mutational analysis, while the answer to the second is the province of QTL mapping.

(a) *P-element mutagenesis*

Over 100 loci affecting peripheral nervous system development have been identified by screening for major mutations (Campos-Ortega 1993; Jan & Jan 1993; Kania *et al.* 1995; Salzberg *et al.* 1997; Norga *et al.* 2003). Although these are candidate genes for adult sensory bristle number, the majority of these mutations are homozygous lethal, which precludes direct analysis of their effects on adult bristle number. We proposed that analysis of subtle, quantitative effects of adult viable and fertile mutations generated by *P*-element insertions in an isogenic background would be an effective and more direct screen for genes that affect complex traits. Conducting such screens in an isogenic background is critical for detecting subtle mutational effects, since effects of segregating QTLs in an outbred strain will be of the same magnitude as the effects we wish to detect. Further, it is necessary to evaluate multiple individuals bearing the same mutation for the trait phenotype, since mutations with quantitative effects are sensitive to environmental variation.

We performed two independent screens for *P*-element mutations affecting adult sensory bristle numbers. Lyman *et al.* (1996) generated 1094 *P*[*lArB*]-element insertion lines in the homozygous Samarkand; ry^{506} genetic background, and Norga *et al.* (2003) generated 1731 *P*[*GT1*]-element insertions in Canton S; w^{1118} isogenic backgrounds. Remarkably, in both screens, over 20% of the *P*-element inserts had effects on abdominal and sternopleural bristle number that exceeded the 95% confidence limits of the mean (Norga *et al.* 2003). Indeed, 9.9% of the lines in the Lyman *et al.* (1996) screen, and 3.8% of the lines in the Norga *et al.* (2003) screen, had effects on abdominal bristle number that exceeded the 99.9% confidence limits. For sternopleural bristles the corresponding numbers were 6.7% (Lyman *et al.* 1996) and 4.0% (Norga *et al.* 2003). Although these numbers refer to *P*-element insertions and not genes, the unexpected conclusion that a substantial fraction of the *Drosophila* genome affects sensory bristle numbers is inescapable.

Insertion sites were determined for 445 lines in the Norga *et al.* (2003) screen, which tagged 262 unique genes. Of these, 162 insertions were in 84 genes with a previously defined role in neurodevelopment, confirming the sensitivity of the screen. Further, there were 119 insertions in 75 genes that had no previously known function in neurodevelopment, and 164 insertions which represent first mutations in 103 predicted genes. Thus, quantitative screens for subtle effects of *P*-element mutations that have been induced in an isogenic background is a highly efficient method for genome annotation.

(b) *QTL mapping*

The effect of any one QTL affecting naturally occurring variation in bristle number is expected to be a small fraction of the total phenotypic variation, relative to the effects of other segregating QTLs and environmental variation. Therefore, QTLs must be mapped by linkage to polymorphic molecular markers for which genotypes can be distinguished unambiguously. Strategies to improve the accuracy of determining the QTL genotype by measuring replicated progenies improve the precision of mapping. Most of the early efforts to map QTLs were done using *Drosophila* bristles, because of the availability of lines selected for high and low bristle numbers, the ability to manipulate whole chromosomes without recombination, and a marker map of polymorphic morphological mutations. For example, Breese & Mather (1957) introgressed six regions of the third chromosome from a line with high numbers of abdominal bristles into the background of the low strain, and found that all regions contributed to the total divergence in bristle number attributable to this chromosome. Thoday (1961) introduced the technique of interval mapping followed by progeny testing to localize QTLs between flanking markers, and found a total of nine QTLs on all three major chromosomes affecting divergence in sternopleural bristle number between selected strains.

These early studies suggested that natural variation for bristle number is of manageable complexity. However, in all cases the base populations from which the selected lines were derived were small samples of the total variation in nature. Furthermore, the morphological marker maps, although the best available at the time, were still quite sparse, and the markers themselves often affected bristle number. In addition, QTLs with recessive or partly recessive effects would have been missed when examining heterozygous effects against a tester chromosome. Finally, progeny testing only gives accurate estimates of map positions and effects if all loci that increase the value of the trait are fixed on one parental chromosome, and if all loci that decrease the value of the trait are fixed on the other parental chromosome (McMillan & Robertson 1974). A hint of the complexity that would be realized later came from the classic study of Shrimpton & Robertson (1988*b*). They combined the introgression technique of Breese & Mather (1957) with the interval mapping and progeny testing analysis of Thoday (1961) to map a minimum of 17 third chromosome QTLs that affect the divergence in sternopleural bristle number between high and low selected lines derived from a heterogenous base population.

More recent analyses of naturally segregating variation affecting sensory bristle numbers began with lines selected for increased and decreased numbers of abdominal and sternopleural bristles from a large base population recently collected from the wild. QTLs on the X and third chromosomes that affect divergence between the selected lines were mapped by linkage to polymorphic cytological insertion sites of *roo* transposable element markers, giving a 4 cM marker map. The power to detect QTLs with

small effects was increased further by replacing the background genotype with that of the isogenic low scoring parental strains, by constructing isogenic lines for each recombinant chromosome, and by measuring multiple individuals of each recombinant isogenic line. The initial results suggested that a moderate number of QTLs affected natural variation in bristle numbers. Two QTLs on the X chromosome and five on chromosome 3 affected response to selection for abdominal bristle number (Long *et al.* 1995), whereas two QTLs on the X chromosome and six on chromosome 3 affected response to selection for sternopleural bristle number (Gurganus *et al.* 1999).

Does allelic variation at single genes correspond to these QTLs, or do the QTLs result from multiple linked loci? High resolution mapping by generating more recombinants is necessary to address this question. Nuzhdin *et al.* (1999) created six populations of advanced intercross recombinant isogenic chromosomes from the same parental chromosome lines used in the initial experiments. These chromosomes were backcrossed to the inbred Samarkand strain (with wild-type bristle numbers) for three generations to increase the number of informative recombinants. The isogenic advanced intercross chromosomes were substituted into the Samarkand genetic background, and scored for both insertion sites of *roo* element markers, and abdominal and sternopleural bristle numbers. A minimum of 26 QTLs were found to affect one or both bristle traits. These lines were then crossed to both Samarkand and the parental selected chromosomes, facilitating estimates of homozygous and heterozygous effects, and also reared in three different temperature environments (Dilda & Mackay 2002). The number of QTLs detected that affect bristle number increased to 53, with 33 affecting sternopleural bristle number, 31 affecting abdominal bristle number, and 11 affecting both traits. These analyses exclude the contribution of the second chromosome and thus pertain only to 60% of the genome, and even more QTLs could be detected in larger experiments with more recombinants. Thus, it is becoming clear that over 100 loci could potentially contribute to naturally occurring variation in bristle numbers.

Many of the QTLs that affect natural variation in bristle numbers mapped to the same location as candidate genes that affect the development of sensory bristles, as implicated by mutation screens. Quantitative complementation tests of mutations at several of these candidate genes implicated the *achaete–scute* complex (*ASC*), *Notch*, *bobbed*, *extramacrochaetae*, *que mao*, *hairy* (*h*), *abdominal*, *polychaetoid*, *sex combs reduced*, *Delta* (*Dl*), *Hairless* (*H*) and *Enhancer of split* as genes that interact with QTLs associated with variation in bristle number (Long *et al.* 1996; Gurganus *et al.* 1999). As our understanding of the repertoire of genes that affect quantitative variation in sensory bristle numbers grows (Norga *et al.* 2003), we will be able to use these mutations to query genetic interactions with naturally occurring QTLs, and to identify candidate genes that potentially harbour naturally occurring variation.

3. EFFECTS

(a) *Homozygous and heterozygous effects*

In accordance with Robertson's (1967) prediction, the observed distributions of homozygous effects of *P*-element insertions (Lyman *et al.* 1996; Norga *et al.* 2003) and QTLs (Shrimpton & Robertson 1988*b*; Dilda & Mackay 2002) are exponential, with a few genes (QTLs) with major effects and increasingly more with smaller effects, down to the limit of detection imposed by the scale of the experiments. This is of practical importance, since it implies that most of the variation in natural populations could be accounted for by relatively few QTLs with large effects. However, an underlying infinitesimal distribution of gene effects could also lead to an apparent exponential distribution of QTL effects under a number of different models (Bost *et al.* 2001). Under the infinitesimal model, one would expect the magnitude of QTL effects to be directly proportional to the physical size of the intervals containing the QTLs. This is not true for bristle number QTLs (Dilda & Mackay 2002).

On average, effects of *P*-element mutations on sensory bristle numbers are partially recessive (Lyman *et al.* 1996) and QTLs have additive effects (Dilda & Mackay 2002). However, there is great variation in the degree of dominance of mutations and QTLs that affect bristle numbers, spanning the range from strictly recessive, additivity and dominance. Most QTLs are partially dominant or recessive.

(b) *Sex-specific effects*

On average, females have more abdominal and sternopleural bristles than males, most probably as a consequence of their larger size. Frankham (1968) first noted that there was genetic variation in the magnitude of sex dimorphism for abdominal bristle number, and, consequently, the genetic correlation between male and female bristle number was significantly less than unity. More recently, this phenomenon has been extensively documented for *P*-element insertions (Lyman *et al.* 1996; Norga *et al.* 2003), spontaneous mutations (Mackay *et al.* 1995) and QTLs (Long *et al.* 1995; Gurganus *et al.* 1998; 1999; Nuzhdin *et al.* 1999; Dilda & Mackay 2002) affecting sensory bristle numbers. Although sex-specific effects are common for both bristle traits, the reduction in genetic correlation between the sexes is typically much greater for abdominal bristles. Dilda & Mackay (2002) classified sex-specific effects of QTLs as conditionally neutral (the QTL is expressed in only one sex), exhibiting antagonistic pleiotropy (the QTL is expressed in both sexes, but in opposite directions), or having sex-biased expression (the QTL is expressed in both sexes, but the absolute magnitude of the effect differs between males and females). All QTL by sex interactions for sternopleural bristle number were conditionally neutral. Both conditional neutrality (57.9%) and antagonistic pleiotropy (42.1%) were observed for QTL by sex interactions for abdominal bristle number.

(c) *Environment-specific effects*

The extent to which QTL effects are conditional on the external environment affects our ability to extrapolate estimates of effects determined under laboratory

conditions to nature. Detecting genotype by environment interactions requires that the same genotypes are reared in multiple environments. The variance of the trait can then be statistically partitioned into components attributable to genotypes, environments, and the genotype–environment interaction (GEI; Falconer & Mackay 1996). Early studies established that there is GEI for bristle numbers in *D. melanogaster* (Caligari & Mather 1975) and *D. pseudoobscura* (Gupta & Lewontin 1982). More recently, the magnitude of GEI for new mutations and QTLs that affect bristle traits was examined by rearing mutation accumulation lines (Mackay & Lyman 1998) and QTL mapping populations at three temperatures (Gurganus *et al.* 1998; Dilda & Mackay 2002).

Mackay & Lyman (1998) reared lines that had accumulated mutations affecting bristle number for approximately 200 generations at 18, 25 and 29°, and estimated the mutational variance attributable to genotype by environment interaction. For sternopleural bristle number, the mutational variance attributable to GEI was 16% of the mutational variance common to all temperatures. For abdominal bristle number the mutational genotype by environment interaction variance was 43% of the mutational variance common to all temperatures.

Gurganus *et al.* (1998) performed a genome scan for QTLs that affect variation in sternopleural and abdominal bristle number using a mapping population of 98 recombinant inbred lines derived from two isogenic strains that were not selected for bristle number. Each recombinant inbred line was reared at 18, 25 and 29°. The fraction of the total genetic variance attributable to GEI was 0.41 for sternopleural bristle number and 0.34 for abdominal bristle number. Of the nine QTLs that affected sternopleural bristle number, three QTLs were expressed in both sexes and all three environments, while the remainder were sex- and/or environment specific. Similarly, 11 QTLs affected abdominal bristle number, but only two of these were expressed in both sexes and all three environments. Dilda & Mackay (2002) evaluated bristle numbers in the mapping populations described above in the same three temperature environments. After Bonferroni correction for multiple tests, 33.3% of the QTLs that affected sternopleural bristle number and 55.3% of that QTLs that affected abdominal bristle number had significant QTL by temperature interactions. Thus, estimates of QTL effects obtained under standard laboratory conditions may not always be highly correlated with effects that would be expressed in a range of natural conditions.

(d) *Epistasis*

Early estimates of heritability of abdominal bristle number were the same from offspring parent regressions, half-sib correlations, and full sib correlations (Clayton *et al.* 1957). Further, mean abdominal bristle number did not change with inbreeding (Kidwell & Kidwell 1966). These results suggested that variation for bristle number is largely additive, with little (directional) dominance and epistasis (gene by gene interaction). However, epistasis is difficult to detect with these designs, partly because even strong epistatic interactions contribute little to the epistatic variance, and partly because these terms have very high sampling errors, requiring huge sample sizes for detection (Barker 1979). In addition, the candidate genes that affect peripheral nervous system development functionally interact (Campos-Ortega 1993; Jan & Jan 1993). Is it that natural alleles of these putative candidate genes have different properties from the mutations used to determine the genetic networks, or that the observations of additive gene action are a consequence of lower power to detect epistasis? There were some early indications that the latter explanation might be true. Breese & Mather (1957) observed epistasis for abdominal bristle number between their introgression lines, as did Caligari & Mather (1975) in their analysis of sternopleural bristle number in a full diallel cross of all possible chromosome substitution lines between two inbred strains. Kidwell (1969) used balancer chromosomes to produce all 81 possible combinations of homozygous and heterozygous whole chromosomes derived from two isogenic strains, and found that interactions between chromosomes accounted for 26% of the total genetic variance in abdominal bristle number. Further, Spickett & Thoday (1966) documented strong epistasis between QTLs that affect sternopleural bristle number, and Shrimpton & Robertson (1988*a*) showed that a QTL on the right arm of the third chromosome that affects sternopleural bristle number had no effect on its own, but a strong effect in combination with at least two QTLs on the right arm of chromosome 3.

Recent studies in which QTLs affecting bristle number were mapped by linkage to molecular markers afforded the opportunity to test for interactions between QTLs on the same and different chromosomes. Epistasis was evaluated using models that fitted the main effects of all significant QTLs and the specific pair-wise interaction between significant QTLs tested. Not only was epistasis common, but effects were of the same magnitude as the main effects, and often sex-specific (Long *et al.* 1995; Gurganus *et al.* 1999; Dilda & Mackay 2002). Dilda & Mackay (2002) categorized epistatic interactions between QTLs as 'diminishing' (less than additive) or 'synergistic' (greater than additive). Of the 26 significant interactions between QTLs affecting sternopleural bristle number, 22 (84.6%) were less than additive. There were 13 significant interactions between QTLs affecting abdominal bristle number, of which 8 (61.5%) were greater than additive. Diminishing epistasis is expected to occur between loci affecting a trait under stabilizing selection (Robertson 1967; Kondrashov & Turelli 1992), leading to genetic canalization (Wagner *et al.* 1997).

Note that the numbers of epistatic interactions in these studies are minimum numbers, because lack of statistical power precluded examining epistasis between QTLs with main effects and markers that do not themselves have main effects on bristle number, or even between pairs of markers that do not have significant marginal effects. Epistasis confounds QTL mapping. Detecting QTLs by linkage to molecular markers is based on the marginal effects of the markers on the trait, and estimates of the main QTL effects are

biased in the face of epistasis. Sophisticated statistical methods such as multiple interval mapping (Kao *et al.* 1999) are capable of fitting main and interacting QTL effects simultaneously, and iteratively converging on a best-fitting model, but these methods require large sample sizes for successful implementation. Pervasive epistasis also complicates the interpretation of complementation tests to mutations. As for all complementation tests, quantitative or qualitative, failure to complement cannot unambiguously be attributed to an interaction between the mutation and alternative QTL alleles at the locus, as failure to complement could also be due to an interaction between the mutation and alternative QTL alleles at an epistatically interacting locus. This does not invalidate the utility of complementation tests to identify candidate genes. In either case the mutation in the candidate gene interacts with QTLs that affect variation in the trait; one just cannot conclude unambiguously whether the effect is direct, or through interaction with another locus.

(e) *Pleiotropy*

Observations that a substantial fraction of the *Drosophila* genome affects abdominal and/or sternopleural bristle number implies massive pleiotropy (Norga *et al.* 2003). It is well known that genes that affect the development of sensory bristles are also involved in development of the central nervous system, sex determination, embryonic pattern formation and eye and wing development. Pleiotropic effects of genes that affect bristle number also extend to other quantitative traits. For example, *P*-element insertions in transcribed regions of the neurodevelopment loci *extra macrochaetae*, *roundabout*, *tramtrack* and *kekkon-1* had significant effects on bristle number (Norga *et al.* 2003) and starvation tolerance (Harbison *et al.* 2004). A *P*-element insertion in *scribble* (*smi97B*), which is essential for establishing polarity in epithelial cells during embryonic development (Bilder & Perrimon 2000) affects bristle number (Lyman *et al.* 1996; Norga *et al.* 2003) and olfactory behaviour (Anholt *et al.* 1996; Ganguly *et al.* 2003). *P*-element insertions in *scribbler* affect adult bristle number (Norga *et al.* 2003) and larval turning behaviour (Suster *et al.* 2004). The practical implication of pervasive pleiotropy is that nomination of candidate genes that correspond to QTLs for bristle numbers should not be confined to loci currently understood to affect bristle development.

Knowledge of the pleiotropic effects on fitness of QTLs that affect bristle number is important for understanding the balance of evolutionary forces that maintain high levels of segregating variation for bristle traits. Some fraction of the naturally occurring genetic variation must be attributable to that expected at equilibrium between the input of new deleterious alleles by mutation and their elimination by natural selection, which occurs when mutant alleles are rare (Barton 1990). Intermediate allele frequencies are expected at loci at which variation is maintained by a balance of selective forces (Falconer & Mackay 1996; Hartl & Clark 1997), while the classic U-shaped distribution of allele frequencies is expected for selectively neutral loci at mutation–drift balance (Kimura 1983).

There is little doubt that new mutations that affect bristle number are often deleterious. Many cases of homozygous lethal genes with large heterozygous effects on bristle number have been reported in selection lines (Clayton & Robertson 1957; Hollingdale 1971; Yoo 1980; Frankham 1980*a*; Caballero *et al.* 1991). New spontaneous mutations affecting bristle number reduce the fitness of the lines in which they occur (López & López-Fanjul 1993; Nuzhdin *et al.* 1995). Finally, there are significant negative correlations between fitness and the absolute value of the effect on bristle number of new *P*-element insertions (Mackay *et al.* 1992; Lyman *et al.* 1996) and spontaneous mutations (Nuzhdin *et al.* 1995; Garcia-Dorado & Gonzalez 1996), indicating an intermediate optimum relationship between bristle number and fitness.

Evidence for the relationship of naturally occurring variation in bristle number to fitness is far more equivocal. Direct estimates of both fitness and bristle number in chromosome substitution lines reveal no detectable association between bristle number and fitness (Spiers 1974; Mackay 1985). Natural selection for a trait can be inferred indirectly by artificially selecting for increased and decreased values of the trait for a few generations, thus perturbing gene frequencies at the underlying loci away from their equilibrium frequencies. If natural selection acts on the trait, one should observe a return of the mean to that of the original base population on suspension of selection. When such perturbation experiments were conducted on abdominal and sternopleural bristle number, the means only partially returned to the base population levels (Clayton *et al.* 1957; Latter & Robertson 1962), which suggests weak selection. On the other hand, Kearsey & Barnes (1970) documented strong stabilizing selection on sternopleural bristle number.

It therefore seems likely that there is heterogeneity in causal relationships to fitness among loci that affect natural variation in bristle number. For example, limits to artificial selection were not severely reduced in lines derived from single pairs of parents relative to selection limits achieved starting from large base populations (Robertson 1967; Frankham 1980*b*). This suggested that alleles at intermediate frequency affected variation in bristle number. Higher limits to selection in large populations and substantial variation in selection response among long-term selection lines also suggest that rare alleles are present in natural populations (Frankham *et al.* 1968). Further understanding of this unresolved problem must be achieved by analysis of fitness effects of individual loci that affect bristle traits.

(f) *Natural allelic variation*

Linkage disequilibrium (LD) refers to the correlation of allele frequencies at two (or more) polymorphic loci (Falconer & Mackay 1996; Hartl & Clark 1997). When a new mutation occurs at a QTL allele, it is initially in complete LD with all other polymorphic loci in the genome. Recombination quickly dissipates LD between the new mutation and unlinked loci, but equilibrium is restored more slowly between closely linked loci. The length of the genomic fragment containing loci in LD with the original mutation

depends on the average amount of recombination per generation experienced by that region of the genome, the number of generations that have passed since the mutation occurred and the effective population size (Hill & Robertson 1968; Falconer & Mackay 1996; Hartl & Clark 1997). For old mutations in large populations, strong LD is expected to extend over distances of the order of a few kilobases or less, whereas larger tracts of LD are expected in populations derived from a recent founder event or in population isolates with small effective population size. In *Drosophila*, LD decays rapidly with physical distance in *Drosophila* regions of normal recombination (Miyashita & Langley 1990; Long *et al.* 1998), which is a favourable scenario for identifying the actual polymorphisms (quantitative trait nucleotides, or QTNs) that cause the differences in phenotype between QTL alleles.

The requirements for LD mapping are simple and analogous to QTL mapping. First, one needs a sample of alleles from a single random mating population (population admixture generates spurious LD; Falconer & Mackay 1996; Hartl & Clark 1997). Second, one needs to determine genotypes of each allele for polymorphic markers in the candidate gene (or candidate gene region) of interest, and a measure of the trait phenotype for each allele. One then groups the population sample by marker (or haplotype) genotype, and conducts a statistical test to assess whether there is a difference in trait mean between marker genotype classes. If so, the QTN is in LD with the marker.

LD mapping requires large samples—at least 500 individuals are necessary to detect a QTN contributing 5% of the total phenotypic variance with 80% power (Long & Langley 1999). The ability to construct chromosome substitution lines in *Drosophila*, in which single chromosomes sampled from nature are made isogenic (homozygous) and substituted into a highly inbred background, greatly increases the power of LD mapping. First, genetic variance attributable to chromosomally unlinked loci is eliminated. Second, the ability to measure multiple individuals per substitution line increases the accuracy in estimating the genotypic value of each line, which is particularly useful for low heritability traits such as longevity. And third, all markers are homozygous, which circumvents the problem of inferring haplotypes in the presence of heterozygotes in outbred populations. In addition, further increases in power can be achieved by introgressing the candidate gene alleles into a common isogenic chromosome background (Long *et al.* 1998; 2000; Lyman *et al.* 1999; Robin *et al.* 2002).

LD mapping has been applied to four candidate genes that affect the development of sensory bristles: *ASC* (Mackay & Langley 1990; Long *et al.* 2000), *scabrous* (*sca*; Lai *et al.* 1994; Lyman *et al.* 1999), *Dl* (Long *et al.* 1998) and *h* (Robin *et al.* 2002). In all cases, polymorphisms at intermediate frequency, located in non-coding regions, were found to be associated with QTNs with moderately large effects on bristle number. Combined together, insertions of transposable elements in *ASC* were associated with reduced numbers of abdominal bristles, although each insertion was individually rare. These observations are consistent with maintenance of variation for bristle number by both mutation–selection balance and balancing selection.

One caveat regarding LD mapping studies is that the cost of genotyping combined with the requirement for genotyping large numbers of alleles typically constrains studies to sample molecular polymorphisms across the gene, that have been predetermined to be at intermediate frequency. In this case, observed associations may not be causal, but in LD with the true causal polymorphism(s) that were not genotyped in the sample. Further, associations with rare alleles are not tested. In order to exclude the possibility of hidden causal polymorphisms and evaluate the contribution of rare mutations with large effects, it will be necessary in the future to obtain complete sequence data for large samples of alleles of each candidate gene. Such data are also required to detect the signatures of selective sweeps, purifying selection, balancing selection and neutrally evolving polymorphisms (Hartl & Clark 1997). In addition, there are many potentially interacting candidate genes that affect bristle number. This challenges future association studies to incorporate all relevant candidate genes simultaneously in order to evaluate interactions between loci.

4. GENETIC VARIANCE OF ENVIRONMENTAL PLASTICITY

One of the earliest unexpected results to emerge from early long-term selection experiments on *Drosophila* abdominal bristle number was that phenotypic variance in the high and low selection lines dramatically increased relative to the unselected controls, although response to selection for mean bristle number reached a limit (Clayton & Robertson 1957). A similar phenomenon was observed in a spontaneous mutation accumulation line selected for low abdominal bristle number (Mackay *et al.* 1994). Hill & Zhang (2004) have shown that these results could be explained if the environmental variance among loci that affect the trait is not constant, as usually assumed, but is rather heterogenous. In this case, strong directional selection (i.e. a low proportion selected and high selection intensity) can readily cause substantial increases in environmental (and hence total phenotypic) variance. This is because genotypes with higher (or lower) means have a higher probability of selection if they also confer high variability.

What is the magnitude of genetic variance of residual environmental variance, i.e. genetic variance in environmental plasticity? An analysis of Brotherstone & Hill (1986), which documented variation among herds of dairy cattle in the within-herd phenotypic variance of milk yield, hints that this could be large. Sorensen & Waagepetersen (2003) also detected variation among genotypes in residual variation for litter size in pigs. Here, we show that there is substantial naturally segregating variance for environmental plasticity of abdominal and sternopleural bristle number.

(a) *Variation in bristle number*

We derived isofemale lines from the Raleigh, NC population, and used these to construct over 300 chromosome 2 and 3 substitution lines. Each of these

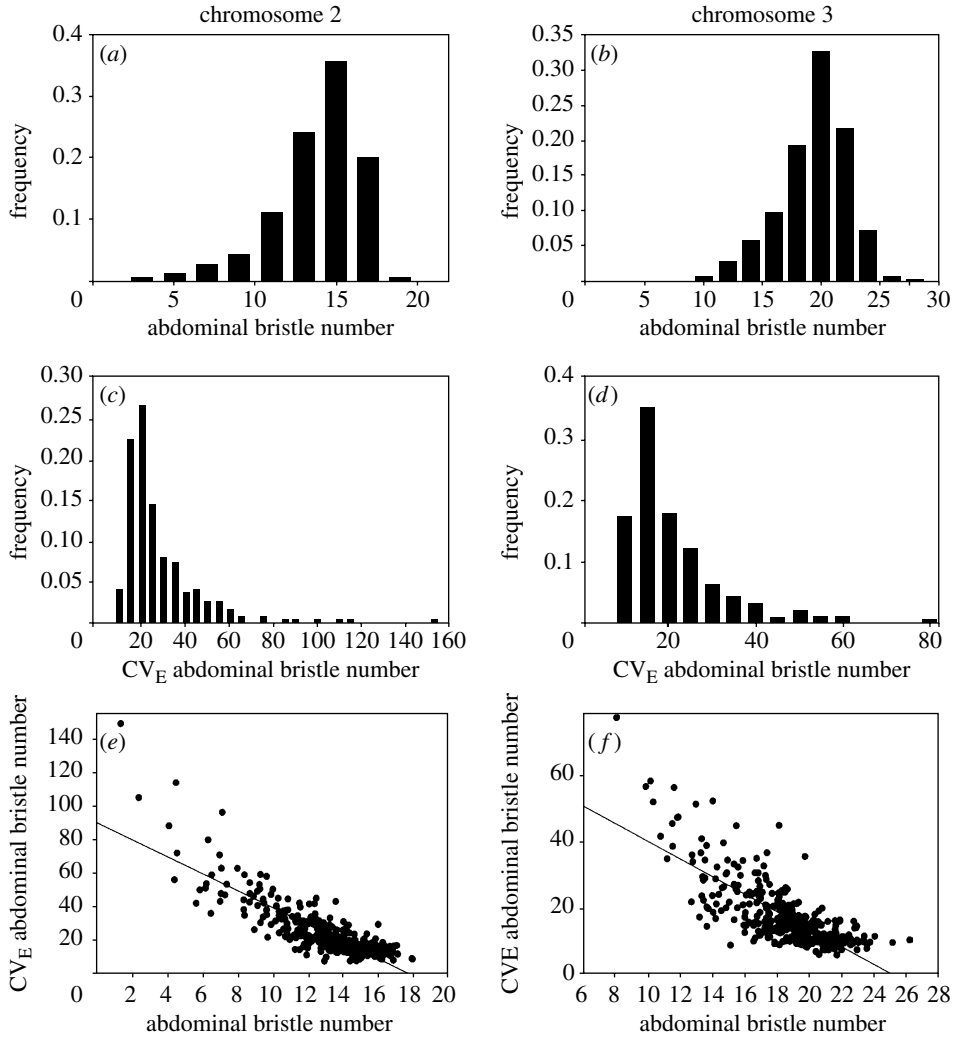

Figure 1. Abdominal bristle number. Variation in mean abdominal bristle number among (*a*) chromosome 2 and (*b*) chromosome 3 substitution lines. Variation in abdominal bristle number CV_E (*c*) among chromosome 2 and (*d*) chromosome 3 substitution lines. Relationship between mean and CV_E for abdominal bristle number (*e*) for chromosome 2 and (*f*) chromosome 3 substitution lines.

lines contained an independent isogenic second or third chromosome, in the background of the homozygous Samarkand (Lyman *et al.* 1996) inbred strain. We scored bristle number for five males and females in each of two replicate vials for each of these lines, and partitioned variance in bristle number using the two-way mixed model analysis of variance (ANOVA) model $Y=\mu+S+L+S\times L+R(L)+S\times R(L)+E$, where μ is the overall mean, S and L denote the cross-classified main effects of sex (fixed) and line (random), respectively, R is replicate vial (random) and E is the environmental variance within vials. As expected, there was considerable segregating variation for both traits on both autosomes (figures 1*a*,*b*, 2*a*,*b*). The effect of line was significant at $p<0.0001$ in all four analyses, and the effect of the line by sex interaction was significant at $p<0.0001$ in all analyses except for sternopleural bristles on chromosome 2, for which the line by sex interaction was less pronounced ($p=0.02$; data not shown).

We estimated the variance components (σ^2) for all random effects in the models, and computed the proportion of the total variance attributable to the total genotypic variance among lines as $[\sigma^2_L+\sigma^2_{SL}]/\sigma^2_T$; where σ^2_L, σ^2_{SL} and σ^2_T are the among-line, sex by line and total variance components, respectively (Falconer & Mackay 1996). For sternopleural bristle number, these estimates were 0.34 for chromosome 2 and 0.53 for chromosome 3. For abdominal bristle number, the estimates were 0.45 for chromosome 2 and 0.41 for chromosome 3. We did not compute the heritabilities of bristle number, since this requires the assumption of strict additivity for completely inbred lines (Falconer & Mackay 1996), which we will show is not true for these data.

We also crossed each of the isogenic chromosome substitution lines to the inbred Samarkand strain, and scored bristle numbers on the heterozygous progeny, using the same experimental design described above. The regressions ($b\pm$s.e.) of mean heterozygous bristle score on mean homozygous bristle score for chromosomes 2 and 3, respectively, were $b=0.35\pm0.027$ and $b=0.35\pm0.021$ for sternopleural bristle number and $b=0.30\pm0.021$ and $b=0.22\pm0.023$ for abdominal bristle number. All estimates were significantly different from 0 and 0.5 ($p<0.0001$). We estimated the average degree of dominance, k, from the regressions as $k=2(b-0.5$; Mackay 1987). k thus ranges from $k=-1$ (homozygous chromosomes recessive) through $k=0$ (additivity) to $k=1$ (homozygous chromosomes

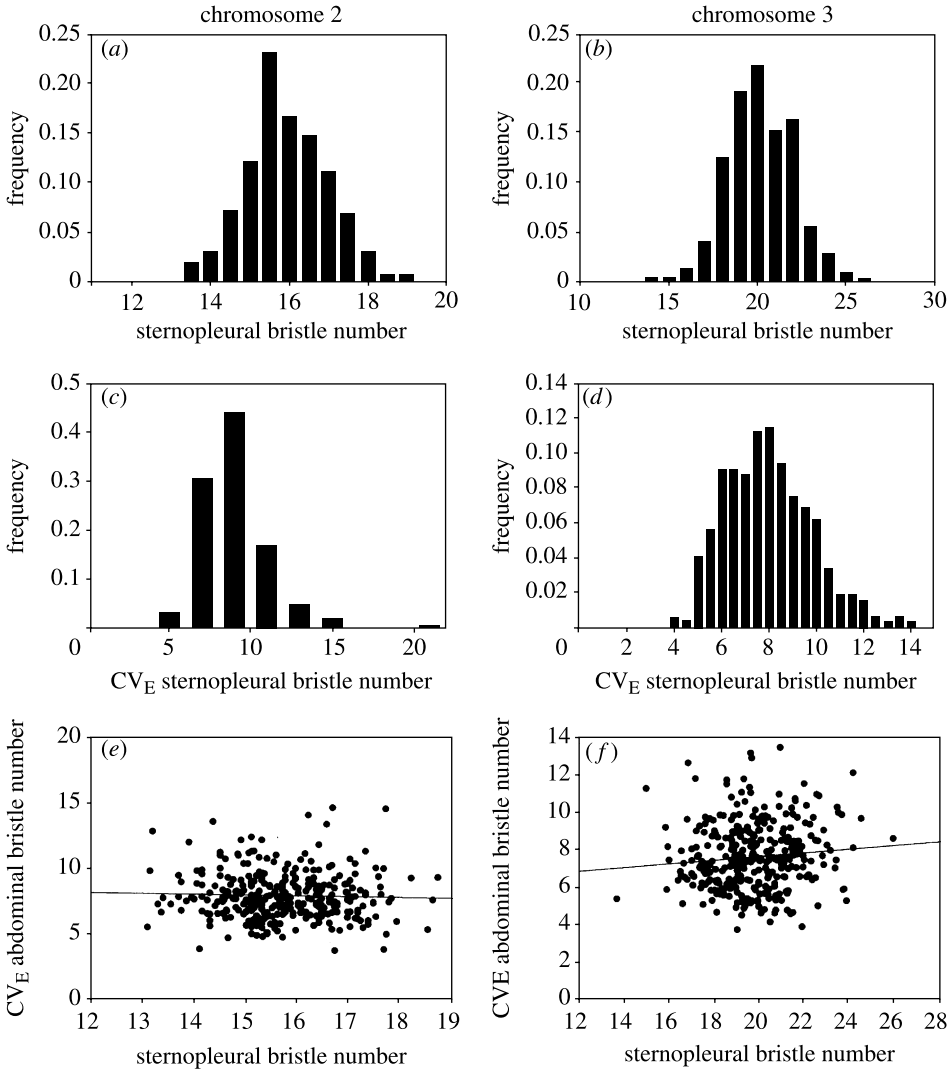

Figure 2. Sternopleural bristle number. Variation in mean sternopleural bristle number among (*a*) chromosome 2 and (*b*) chromosome 3 substitution lines. Variation in sternopleural bristle number CV_E among (*c*) chromosome 2 and (*d*) chromosome 3 substitution lines. Relationship between mean and CV_E for sternopleural bristle number for (*e*) chromosome 2 and (*f*) chromosome 3 substitution lines.

dominant). The effects QTLs that affect bristle number in this population are, on average, partially recessive. Estimates of k for sternopleural bristle number were $k=-0.30$ (both chromosomes). Estimates of k for abdominal bristle number were $k=-0.4$ (chromosome 2) and $k=-0.56$ (chromosome 3).

(b) *Genetic variance of environmental variance*

We computed the environmental coefficient of variation ($CV_E = 100[\sigma_E/\bar{X}]$, where σ_E is the within-vial environmental standard deviation and $\bar{X}$ is the vial mean bristle score), separately for each sex and replicate vial, for each of the chromosome substitution lines. To assess whether or not there was genetic variation between the lines for environmental plasticity, we partitioned the variance in CV_E using the ANOVA model $Y=\mu+S+L+S\times L+E$, where μ, S and L are as defined above, and E is the variance in CV_E between replicate vials. The striking results are that there is highly significant genetic variance in residual variance for both bristle traits, on both chromosomes (tables 1 and 2; figures 1*c*,*d*, 2*c*, *d*); i.e. there is a genetic component for sensitivity to environmental variation. The residual variance of abdominal bristle number for chromosome 2 is highly sex-specific; females are far more variable than males. The estimates of the proportion of the total variance in CV_E attributable to the total genotypic variance in CV_E among lines for sternopleural bristle number were 0.10 for chromosome 2 and 0.16 for chromosome 3. For abdominal bristle number, the estimates were 0.60 for chromosome 2 and 0.48 for chromosome 3.

We performed the same analyses on heterozygous progeny from crosses of the isogenic chromosome substitution lines to Samarkand. The results are given in tables 3 and 4. The first observation to note is that the total residual variance among the heterozygous chromosomes is in all cases less than that of the homozygous chromosomes, but the reduction in variance is far more pronounced for abdominal than for sternopleural bristle number. The total variance in environmental plasticity of abdominal bristle number in second and third chromosome heterozygotes was only 17 and 24% that of the second and third chromosome homozygotes, respectively.

Table 1. Analyses of genetic variance of environmental plasticity for homozygous chromosome 2 substitution lines.

	sternopleural bristle number					abdominal bristle number			
source	d.f.[a]	MS[b]	F	p	σ^{2c}	MS[b]	F	p	σ^{2c}
sex	1	66.030	6.34	0.0122	fixed	44 182.0	167.86	<0.0001	fixed
line	325	14.544	1.40	0.0013	1.089	1117.717	4.23	<0.0001	220.481
sex×line	320	10.403	0.97	0.6117	−0.155	264.288	1.47	<0.0001	43.429
error	623	10.706			10.706	179.235			179.235

[a] Degrees of freedom. The design is unbalanced as some lines produced individuals of only one sex.
[b] Type III mean squares.
[c] Variance component.

Table 2. Analyses of genetic variance of environmental plasticity for homozygous chromosome 3 substitution lines.

	sternopleural bristle number					abdominal bristle number			
source	d.f.[a]	MS[b]	F	p	σ^{2c}	MS[b]	F	p	σ^{2c}
sex	1	75.868	7.84	0.0054	fixed	9031.54	80.69	<0.0001	fixed
line	322	12.863	1.33	0.0056	0.840	425.600	3.80	<0.0001	78.483
sex×line	320	9.693	1.18	0.0451	0.737	112.130	1.19	0.0373	8.936
error	625	8.240			8.240	94.518			94.518

[a] Degrees of freedom. The design is unbalanced as some lines produced individuals of only one sex.
[b] Type III mean squares.
[c] Variance component.

Table 3. Analyses of genetic variance of environmental plasticity for heterozygous chromosome 2 substitution lines.

	sternopleural bristle number					abdominal bristle number			
source	d.f.[a]	MS[b]	F	p	σ^{2c}	MS[b]	F	p	σ^{2c}
sex	1	51.247	7.47	0.0067	fixed	960.980	17.06	<0.0001	fixed
line	277	6.687	0.97	0.5849	−0.044	122.967	2.18	<0.0001	16.723
sex×line	277	6.862	0.98	0.5760	−0.074	56.315	0.96	0.6299	−1.031
error	552	7.009			7.009	58.370			58.370

[a] Degrees of freedom.
[b] Type III mean squares.
[c] Variance component.

Table 4. Analyses of genetic variance of environmental plasticity for heterozygous chromosome 3 substitution lines.

	sternopleural bristle number					abdominal bristle number			
source	d.f.[a]	MS[b]	F	p	σ^{2c}	MS[b]	F	p	σ^{2c}
sex	1	167.688	28.31	<0.0001	fixed	20.383	0.58	0.4451	fixed
line	289	7.853	1.33	0.0083	0.487	63.213	1.81	<0.0001	7.146
sex×line	288	5.919	0.90	0.8418	−0.327	34.852	0.97	0.6240	−0.598
error	571	6.569			6.569	36.040			36.040

[a] Degrees of freedom. The design is unbalanced as some lines produced individuals of only one sex.
[b] Type III mean squares.
[c] Variance component.

For sternopleural bristle number, the reduction of total residual variance in heterozygous second and third chromosomes was 59 and 72% that of the respective homozygous chromosomes. This observation echoes earlier reports that homozygous lines are more sensitive to environmental variation than heterozygous lines, for abdominal bristle number (Rasmuson 1952) and for other quantitative traits (Reeve & Robertson 1953; Robertson & Reeve 1955).

Inspection of the genotypic component of variance of environmental sensitivity for the heterozygous chromosomes reveals that genetic effects on environmental plasticity are largely recessive. For example, the among-line genotypic variance ($\sigma^2_L + \sigma^2_{SL}$) in sensitivity for abdominal bristle number in second and third chromosome heterozygotes is 6.3 and 8.2% for that of the second and third chromosome homozygotes, respectively. The large sex by line effect for second

chromosome homozygotes is recessive and disappears in chromosome 2 heterozygotes.

We quantified the average degree of dominance for CV_E from the regressions of heterozygous on homozygous metrics, as described above. The regressions ($b \pm$ s.e.) of mean heterozygous CV_E on mean homozygous CV_E for sternopleural bristle number were $b = 0.04 \pm 0.04$ and $b = 0.04 \pm 0.05$ for chromosomes 2 and 3, respectively. Since these estimates are not significantly different from 0, the corresponding estimates of k are not significantly different from -1, which indicates completely recessive effects. The respective chromosome 2 and 3 regressions of mean heterozygous CV_E on mean homozygous CV_E for abdominal bristle number were $b = 0.12 \pm 0.01$ and $b = 0.07 \pm 0.02$ for chromosomes 2 and 3, respectively. These estimates are significantly different from both 0 and 0.5, and yield estimates of $k = -0.76$ (chromosome 2) and $k = -0.86$ (chromosome 3); i.e. largely recessive.

(c) *Abnormal abdomen?*

The correlation (r) between mean sternopleural bristle number and the average CV_E for sternopleural bristle number was not significantly different from zero for either the chromosome 2 ($r = -0.04$, $p = 0.45$) or chromosome 3 ($r = 0.10$, $p = 0.08$) homozygous substitution lines (figure 2*e*,*f*). However, there were strong negative correlations between the mean and CV_E for abdominal bristle number of $r = -0.84$ ($p < 0.0001$) and $r = -0.76$ ($p < 0.0001$) for the homozygous chromosome 2 and chromosome 3 substitution lines, respectively (figure 1*e*,*f*).

The strong negative association between CV_E and mean abdominal bristle number is partly attributable to 14 chromosome 2 lines (4.3%) and 15 chromosome 3 lines (4.6%) that exhibit striking developmental abnormalities. In these lines, the repeatability (Falconer & Mackay 1996) of abdominal bristle number between adjacent abdominal sternites within individual flies is very low (data not shown). This indicates dysfunction in developmental homeostasis. Further analysis is necessary to determine the extent to which the observed environmental variation between individuals is associated with within-individual environmental variation in general. However, we note that various disruptions of the sternites and tergites are associated with this phenotype, including hemireduction of one or more sternites and tergites, one-sided lateral reductions of tergites and sternites, disrupted pigmentation, low viability and slow development time. These phenotypes are strikingly similar to the *abnormal abdomen* syndrome described by Sobels (1952) over 50 years ago, and which we have repeatedly observed to segregate in the Raleigh population. We now have the tools available to map the QTLs responsible for this odd phenotype, and elucidate the mechanisms responsible for its persistence at appreciable frequencies in natural populations.

(d) *A candidate gene*

The *abnormal abdomen* phenotype resembles adult visible phenotypes associated with mutations that affect catecholamine biosynthesis, which include incomplete formation of abdominal tergites and loss of bristles (Stathakis *et al.* 1999). We had available data on 36 molecular polymorphisms at *Dopa decarboxylase* (*Ddc*) for 173 of the second chromosome substitution lines (DeLuca *et al.* 2003). *Ddc* encodes the enzyme that catalyses the final step in the synthesis of dopamine, a major *Drosophila* catecholamine and neurotransmitter, as well as serotonin. *Ddc* is required for the production of dopamine and serotonin in the central nervous system (Livingstone & Tempel 1983), and is essential in the hypoderm for the sclerotization and melanization of the cuticle (Lunan & Mitchell 1969).

We assessed associations of each of the molecular polymorphisms at *Ddc* with variation in abdominal bristle number, and CV_E for abdominal bristle number, using the ANOVA model $Y = \mu + S + M + S \times M + E$, where μ and S are as defined above, M is marker genotype and E is the variance between line means within marker genotypes. Two markers were associated with variation in sternopleural bristle number at $p < 0.05$ (figure 3*a*), which is close to the 1.7 associations at this significance level expected by chance given 34 tests (two of the 36 polymorphic sites were in complete LD with neighbouring sites). Perhaps surprisingly, however, one of the markers, T644G, was associated with CV_E for abdominal bristle number at $p = 0.0004$, which is lower than the conservative Bonferroni-corrected significance threshold of $p = 0.0015$ (table 5 and figure 3*a*). In addition, T644G is in strong LD with T440G ($\chi^2_1 = 48.42$, $p < 0.0001$) and with a 12 bp insertion/deletion polymorphism (12pbin, $\chi^2_1 = 59.40$, $p < 0.0001$). These markers are also strongly associated with variation in CV_E for abdominal bristle number (table 5, figure 3*a*).

The difference in mean abdominal bristle number CV_E between homozygous genotypes for the significant markers ranged from 4.14 to 5.18, or 0.33 to 0.47 genetic standard deviation units (table 6). We estimated the genetic variance attributable to each marker by treating the marker term as a random effect in the ANOVA. The significant markers individually accounted for 6.33–9.99% of the total genotypic variance in abdominal bristle number CV_E. Since the three markers are in strong global LD ($\chi^2_4 = 249.36$, $p < 0.0001$; figure 3*b*), we assessed the contribution of the seven observed haplotypes to abdominal bristle number CV_E. There was significant variation in mean abdominal bristle number CV_E among the haplotypes ($F_{6,658} = 2.74$, $p = 0.0122$; figure 3*c*), which accounted for 5.97% of the total genotypic variance in abdominal bristle number CV_E attributable to this sample of second chromosomes. The contribution of the haplotypes to the total genetic variance is somewhat less than the individual markers because the effects of the markers are conditional on the genotype of the other markers. For example, the insertion/deletion polymorphism has no effect in the background of T440, T644; and the 440 polymorphism has no effect in the background of 12bpin, G644 (figure 3*c*). That is, there is apparently diminishing epistasis between the markers associated with this trait.

All three markers associated with variation in abdominal bristle number CV_E are in the promoter region. Normal expression of *Ddc* in the hypoderm requires *cis*-acting elements located in the promoter

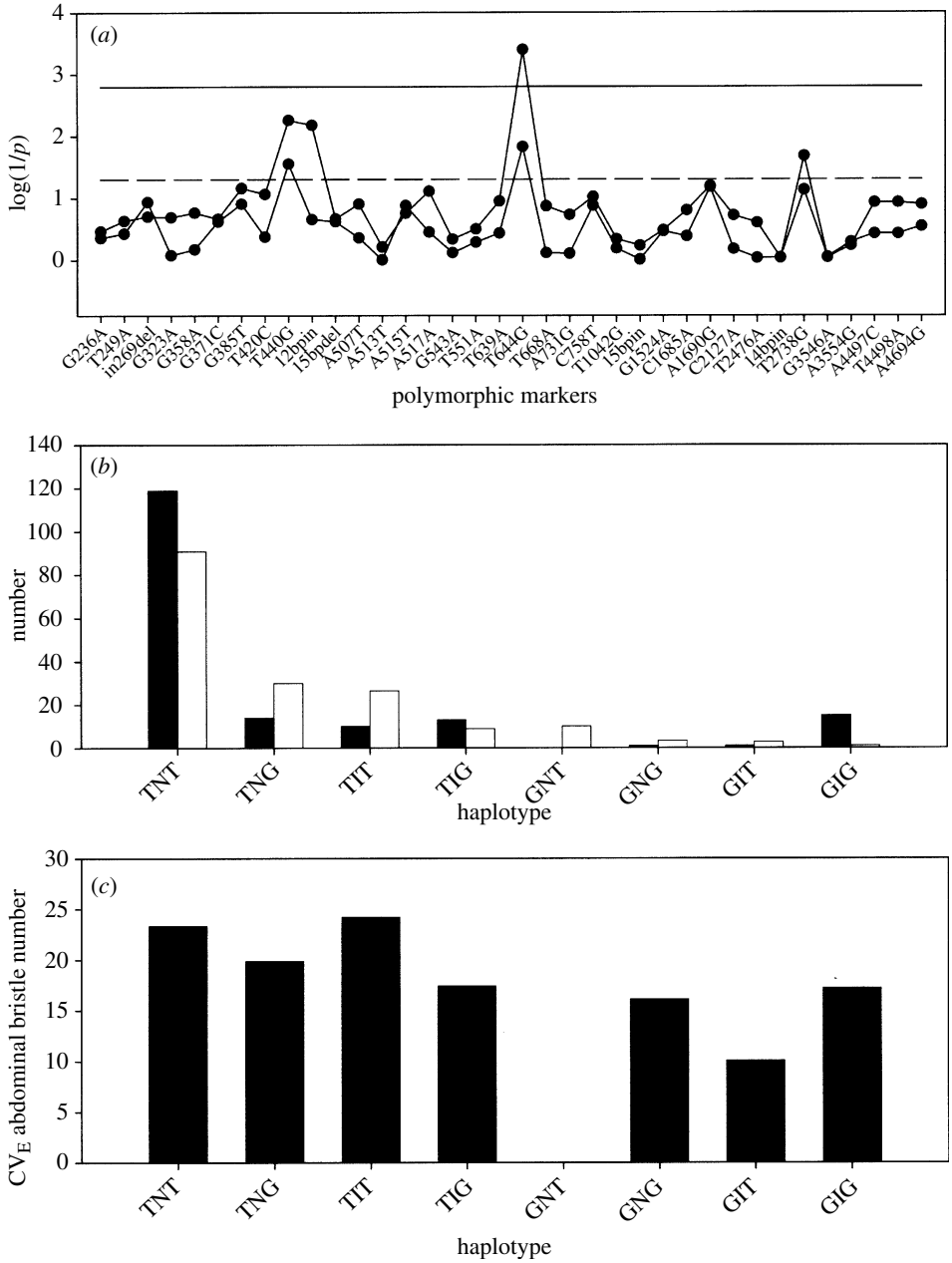

Figure 3. Association of abdominal bristle number CV_E with molecular variation in the *Ddc* gene region. (*a*) *p* values (transformed to log (1/*p*, *y*-axis) from ANOVA tests of association of abdominal bristle number CV_E (solid line) and abdominal bristle number (dotted line) for each of the polymorphic molecular markers in the *Ddc* region (*x*-axis). The dashed horizontal line indicates the nominal $p=0.05$ significance threshold, and the solid horizontal line indicates the experiment-wise $p=0.05$ threshold given by the Bonferroni correction for multiple tests. (*b*) Global LD between polymorphisms at *Ddc* associated with variation in abdominal bristle number CV_E. Haplotype designations refer to genotypes at T440G, 12bpin, and T644G, respectively. Black bars denote observed and white bars indicate expected haplotype frequencies. (*c*) Variation in abdominal bristle number CV_E associated with T440G, 12bpin, and T644G haplotypes.

Table 5. Analysis of variance of molecular marker–abdominal bristle number CV_E associations.

		T440G			12bpin			T644G		
source	d.f.	MS[a]	*F*	*p*	MS[a]	*F*	*p*	MS[a]	*F*	*p*
marker (*M*)	1	2101.94	7.85	0.0052	1991.85	7.457	0.0065	3405.32	12.86	0.0004
sex (*S*)	1	3908.45	14.59	0.0001	8126.00	30.41	<0.0001	9116.85	34.42	<0.0001
$M\times S$	1	316.24	1.18	0.2776	848.26	3.17	0.0752	986.08	3.72	0.0541
error	668	267.82			267.20			264.87		

[a] Type III mean squares.

Table 6. Effects of molecular polymorphisms at *Ddc* on abdominal bristle number CV_E.

	T440G	12bpin	T644G
$2a$[a]	5.87	4.14	5.18
p[b]	0.90	0.78	0.75
a/σ_G[c]	0.47	0.33	0.41
σ^2_M[d]	15.79	10.01	15.34
% V_G[e]	9.99	6.33	9.70

[a] Mean difference in abdominal bristle number CV_E between homozygous genotypes.
[b] Frequency of allele increasing abdominal bristle number CV_E.
[c] Effect in genetic standard deviation units.
[d] Genetic variance attributable to marker.
[e] Percentage of total genetic variance ($\sigma^2_G = 158.14$) attributable to marker.

region 208 bp 5′ of the transcription start site, but not beyond this region (Lundell & Hirsh 1994). The T644G polymorphism, which has the strongest individual association with abdominal bristle number CV_E, is in the region required for hypodermal expression, and is plausibly associated causally with variation in this trait. The usual caveats regarding LD mapping pertain to this observation: the associations could be due to LD with true causal polymorphisms that were not genotyped, and the observation needs to be replicated in an independent sample. Nonetheless, the hypothesis that the *abnormal abdomen* phenotype is associated with catecholamine metabolism is testable by measuring dopamine levels in the affected strains, and performing similar tests for association of the phenotype with molecular polymorphisms at other key genes in this pathway.

5. CONCLUSIONS

Drosophila sensory bristle numbers are archetypical quantitative traits, and there is no reason to assume that insights regarding the genetic architecture of bristle number will not be applicable to other quantitative traits in *Drosophila*, or indeed quantitative traits in any organism. Analysis of subtle changes in quantitative traits induced by co-isogenic *P*-element mutations can be applied to any quantitative trait. Indeed, this technique has been used to identify novel loci affecting olfactory behaviour (Anholt *et al.* 1996) and resistance to starvation stress (Harbison *et al.* 2004). A significant fraction of the genome was implicated as affecting these traits as well, underscoring the ubiquity of pleiotropy. If high resolution mapping reveals that QTLs affecting bristle number detected by an initial genome scan with moderate power tend to fractionate into multiple linked QTLs, increasing complexity should be expected, and is indeed found (Pasyukova *et al.* 2000; Harbison *et al.* 2004; Moehring & Mackay 2004), for other traits. Sex-specific effects were first described for alleles affecting bristle number and have subsequently been documented in *Drosophila* for enzyme activity (Game & Oakeshott 1990), olfactory behaviour (Anholt *et al.* 1996), longevity (Nuzhdin *et al.* 1997; Pasyukova *et al.* 2000; Vieira *et al.* 2000; Leips & Mackay 2000; 2002) and starvation stress resistance (Harbison *et al.* 2004); as well as for body size in mice (Vaughn *et al.* 1999) and even human life span (De Benedictis *et al.* 1998). Genotype by sex interactions are even common at the level of transcription (Jin *et al.* 2001; Anholt *et al.* 2003), although the mechanisms causing this phenomenon remain a mystery. Studies of *Drosophila* bristles have highlighted the importance of environment-specific expression of QTLs, which also figures prominently in the genetic architecture of longevity (Vieira *et al.* 2000; Leips & Mackay 2000). The prevalence of epistatic interactions between QTLs that affect natural variation in traits historically considered to have strictly additive gene action poses the challenge of understanding variation for complex traits in terms of networks of interacting genes. Bristle numbers remain ideal for testing quantitative genetic theory, and our demonstration here of substantial segregating genetic variance for environmental plasticity of abdominal and sternopleural bristle number show that even old traits can display new tricks. Finally, despite the emerging complexity of the genetic architecture of bristle numbers, it is possible to identify single genes that correspond to QTLs that affect bristle number and show that they harbour naturally occurring allelic variants associated with variation in bristle number. This bodes well for eventually describing quantitative genetic variation in terms of complex genetics rather than complex statistics.

We thank R.R.H Anholt for comments on the manuscript. This work was supported by grants GM45344 and GM45146 from the National Institutes of Health. This is a publication of the W.M. Keck Center for Behavioral Biology.

REFERENCES

Anholt, R. R. H., Lyman, R. F. & Mackay, T. F. C. 1996 Effects of single *P* element insertions on olfactory behavior in *Drosophila melanogaster*. *Genetics* **143**, 293–301.

Anholt, R. R. H., Dilda, C. L., Chang, S., Fanara, J. J., Kulkarni, N. H., Ganguly, I., Rollmann, S. M., Kamdar, K. P. & Mackay, T. F. C. 2003 The genetic architecture of odor-guided behavior in *Drosophila*: epistasis and the transcriptome. *Nat. Genet.* **35**, 180–184.

Barker, J. S. F. 1979 Inter-locus interactions: a review of experimental evidence. *Theor. Popul. Biol.* **16**, 323–346.

Barton, N. H. 1990 Pleiotropic models of quantitative variation. *Genetics* **124**, 773–782.

Bilder, D. & Perrimon, N. 2000 Localization of apical epithelial determinants by the basolateral PDZ domain protein Scribble. *Nature* **403**, 676–680.

Bost, B., de Vienne, D., Hospital, F., Moreau, L. & Dilmann, C. 2001 Genetic and nongenetic bases for the L-shaped distribution of quantitative trait loci effects. *Genetics* **157**, 1773–1787.

Breese, E. L. & Mather, K. 1957 The organization of polygenic activity within a chromosome of *Drosophila*. I. Hair characters. *Heredity* **11**, 373–395.

Brotherstone, S. & Hill, W. G. 1986 Heterogeneity of variance amongst herds for milk production. *Anim. Prod.* **42**, 297–303.

Caballero, A., Toro, M. A. & López-Fanjul, C. 1991 The response to artificial selection from new mutations in *Drosophila melanogaster*. *Genetics* **128**, 89–102.

Caligari, P. D. S. & Mather, K. 1975 Genotype–environment interaction. III. Interactions in *Drosophila melanogaster*. *Genetics* **191**, 387–411.

Campos-Ortega, J. A. 1993 Early neurogenesis in *Drosophila melanogaster*. In *The development of Drosophila melanogaster* (ed. M. Bate & A. Martinez Arias), vol. 2, pp. 1091–1129. Plainview, NY: Cold Spring Harbor Laboratory Press.

Clayton, G. A. & Robertson, A. 1957 An experimental check on quantitative genetical theory. II. The long-term effects of selection. *J. Genet.* **55**, 152–170.

Clayton, G. A., Morris, J. A. & Robertson, A. 1957 An experimental check on quantitative genetical theory. Short-term responses to selection. *J. Genet.* **55**, 131–151.

De Benedictis, G., *et al*. 1998 Gene/longevity association studies at four autosomal loci (*REN*, *THO*, *PARP*, *SOD2*). *Eur. J. Hum. Genet.* **6**, 534–541.

De Luca, M., Roshina, N. V., Geiger-Thornsberry, G. L., Lyman, R. F., Pasyukova, E. G. & Mackay, T. F. C. 2003 *Dopa-decarboxylase* affects variation in *Drosophila* longevity. *Nat. Genet.* **34**, 429–433.

Dilda, C. L. & Mackay, T. F. C. 2002 The genetic architecture of *Drosophila* sensory bristle number. *Genetics* **162**, 1655–1674.

Falconer, D. S. F. & Mackay, T. F. C. 1996 *Introduction to quantitative genetics*, 4th edn. Harlow: Addison Wesley Longman.

Frankham, R. 1968 Sex and selection for a quantitative character in *Drosophila*. II. The sex dimorphism. *Aust. J. Biol. Sci.* **21**, 1225–1237.

Frankham, R. 1980 Origin of genetic variation in selection lines. In *Selection experiments in laboratory and domestic animals* (ed. A. Robertson), pp. 56–68. Slough: Commonwealth Agricultural Bureaux.

Frankham, R. 1980 The founder effect and response to artificial selection in *Drosophila*. In *Selection experiments in laboratory and domestic animals* (ed. A. Robertson), pp. 87–90. Slough: Commonwealth Agricultural Bureaux.

Frankham, R., Jones, L. P. & Barker, J. S. F. 1968 The effects of population size and selection intensity for a quantitative character in *Drosophila*. III. Analysis of the lines. *Genet. Res.* **12**, 267–283.

Game, A. Y. & Oakeshott, J. G. 1990 The association between restriction site polymorphism and enzyme activity variation for Esterase 6 in *Drosophila melanogaster*. *Genetics* **139**, 907–920.

Ganguly, I., Mackay, T. F. C. & Anholt, R. R. H. 2003 *Scribble* is essential for olfactory behavior in *Drosophila*. *Genetics* **164**, 1447–1457.

Gupta, A. P. & Lewontin, R. C. 1982 A study of reaction norms in natural populations of *Drosophila pseudoobscura*. *Evolution* **36**, 934–948.

Garcia-Dorado, A. & Gonzalez, J. A. 1996 Stabilizing selection detected for bristle number in *Drosophila melanogaster*. *Evolution* **50**, 1573–1578.

Gurganus, M. C., Fry, J. D., Nuzhdin, S. V., Pasyukova, E. G., Lyman, R. F. & Mackay, T. F. C. 1998 Genotype–environment interaction for quantitative trait loci affecting sensory bristle number in *Drosophila melanogaster*. *Genetics* **149**, 1883–1898.

Gurganus, M. C., Nuzhdin, S. V., Leips, J. W. & Mackay, T. F. C. 1999 High resolution mapping of quantitative trait loci affecting sternopleural bristle number in *Drosophila melanogaster*. *Genetics* **152**, 1585–1604.

Harbison, S. T., Yamamoto, A. H., Fanara, J. J., Norga, K. K. & Mackay, T. F. C. 2004 Quantitative trait loci affecting starvation resistance in *Drosophila melanogaster*. *Genetics* **166**, 1807–1823.

Hartl, D. L. & Clark, A. G. 1997 *Principles of population genetics*, 3rd edn. Sunderland, MA: Sinauer.

Hill, W. G. & Robertson, A. 1968 Linkage disequilibrium in finite populations. *Theor. Appl. Genet.* **38**, 226–231.

Hill, W. G. & Zhang, X.-S. 2004 Effects on phenotypic variability of directional selection arising through genetic differences in residual variability. *Genet. Res.* **83**, 121–132.

Hollingdale, B. 1971 Analyses of some genes from abdominal bristle number selection lines in *Drosophila melanogaster*. *Theor. Appl. Genet.* **41**, 292–301.

Jan, Y. N. & Jan, L. Y. 1993 The peripheral nervous system. In *The Development of Drosophila melanogaster* (ed. M. Bate & A. Martinez Arias), vol. 2, pp. 1207–1244. Plainview, NY: Cold Spring Harbor Laboratory Press.

Jin, W., Riley, R. M., Wolfinger, R. D., White, K. P., Passador-Gurgel, G. & Gibson, G. 2001 The contributions of sex, genotype and age to transcriptional variance in *Drosophila melanogaster*. *Nat. Genet.* **29**, 389–395.

Jones, L. P., Frankham, R. & Barker, J. S. F. 1968 The effects of population size and selection intensity in selection for a quantitative character in *Drosophila*. II. Long-term response to selection. *Genet. Res.* **12**, 249–266.

Kania, A., Salzberg, A., Bhat, M., D'Evelyn, D., He, Y., Kiss, I. & Bellen, H. J. 1995 *P*-element mutations affecting embryonic peripheral nervous system development in *Drosophila melanogaster*. *Genetics* **139**, 1663–1678.

Kao, C. H., Zeng, Z. B. & Teasdale, R. 1999 Multiple interval mapping for quantitative trait loci. *Genetics* **152**, 1203–1216.

Kearsey, M. J. & Barnes, B. W. 1970 Variation for metrical characters in *Drosophila* populations. II. Natural selection. *Heredity* **25**, 11–21.

Keightley, P. D., Mackay, T. F. C. & Caballero, A. 1993 Accounting for bias in estimates of the rate of polygenic mutation. *Proc. R. Soc. B* **253**, 291–296.

Kidwell, J. F. 1969 A chromosomal analysis of egg production and abdominal chaeta number in *Drosophila melanogaster*. *Can. J. Genet. Cytol.* **11**, 547–557.

Kidwell, J. F. & Kidwell, M. M. 1966 The effects of inbreeding on body weight and abdominal chaeta number in *Drosophila melanogaster*. *Can. J. Genet. Cytol.* **8**, 207–215.

Kimura, M. 1983 *The neutral theory of molecular evolution*. Cambridge University Press.

Kondrashov, A. S. & Turelli, M. 1992 Deleterious mutations, apparent stabilizing selection and the maintenance of quantitative variation. *Genetics* **132**, 603–618.

Lai, C., Lyman, R. F., Long, A. D., Langley, C. H. & Mackay, T. F. C. 1994 Naturally occurring variation in bristle number and DNA polymorphisms at the *scabrous* locus in *Drosophila melanogaster*. *Science* **266**, 1697–1702.

Latter, B. D. H. & Robertson, A. 1962 The effects of inbreeding and artificial selection on reproductive fitness. *Genet. Res.* **3**, 110–138.

Leips, J. & Mackay, T. F. C. 2000 Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. *Genetics* **155**, 1773–1788.

Leips, J. & Mackay, T. F. C. 2002 The complex genetic architecture of *Drosophila* life span. *Exp. Aging Res.* **28**, 361–390.

Livingstone, M. S. & Tempel, B. L. 1983 Genetic dissection of monoamine neurotransmitter synthesis in *Drosophila*. *Nature* **303**, 67–70.

Long, A. D. & Langley, C. H. 1999 Power of association studies to detect the contribution of candidate genetic loci to complexly inherited phenotypes. *Genome Res.* **9**, 720–731.

Long, A. D., Mullaney, S. L., Reid, L. A., Fry, J. D., Langley, C. H. & Mackay, T. F. C. 1995 High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. *Genetics* **139**, 1273–1291.

Long, A. D., Mullaney, S. L., Mackay, T. F. C. & Langley, C. H. 1996 Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in *Drosophila melanogaster*. *Genetics* **144**, 1497–1518.

Long, A. D., Lyman, R. F., Langley, C. H. & Mackay, T. F. C. 1998 Two sites in the *Delta* gene region contribute to naturally occurring variation in bristle number in *Drosophila melanogaster*. *Genetics* **149**, 999–1017.

Long, A. D., Lyman, R. F., Morgan, A. H., Langley, C. H. & Mackay, T. F. C. 2000 Both naturally occurring insertions of transposable elements and intermediate frequency polymorphisms at the *achaete–scute* complex are associated with variation in bristle number in *Drosophila melanogaster*. *Genetics* **154**, 1255–1269.

López, M. A. & López-Fanjul, C. 1993 Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. II. Distribution of mutant effects on the trait and fitness. *Genet. Res.* **61**, 117–126.

Lunan, K. D. & Mitchell, H. K. 1969 The metabolism of tyrosine O phosphate in *Drosophila*. *Arch. Biochem. Biophys.* **132**, 450–456.

Lundell, M. J. & Hirsh, J. 1994 Regulation of the *Dopa decarboxylase* gene during *Drosophila* development. *Adv. Dev. Biochem.* **3**, 55–86.

Lyman, R. F., Lawrence, F., Nuzhdin, S. V. & Mackay, T. F. C. 1996 Effects of single *P*-element insertions on bristle number and viability in *Drosophila melanogaster*. *Genetics* **143**, 277–292.

Lyman, R. F., Lai, C. & Mackay, T. F. C. 1999 Linkage disequilibrium mapping of molecular polymorphisms at the *scabrous* locus associated with naturally occurring variation in bristle number in *Drosophila melanogaster*. *Genet. Res.* **74**, 303–311.

Mackay, T. F. C. 1985 A quantitative genetic analysis of fitness and its components in *Drosophila melanogaster*. *Genet. Res.* **47**, 59–70.

Mackay, T. F. C. 1987 Transposable element-induced polygenic mutations in *Drosophila melanogaster*. *Genet. Res.* **49**, 225–233.

Mackay, T. F. C. & Langley, C. H. 1990 Molecular and phenotypic variation in the *achaete–scute* region of *Drosophila melanogaster*. *Nature* **348**, 64–66.

Mackay, T. F. C. & Lyman, R. F. 1998 Polygenic mutation in *Drosophila melanogaster*: genotype–environment interaction for spontaneous mutations affecting bristle number. *Genetica* **102/103**, 199–215.

Mackay, T. F. C., Lyman, R. F. & Jackson, M. S. 1992 Effects of *P* element insertions on quantitative traits in *Drosophila melanogaster*. *Genetics* **130**, 315–332.

Mackay, T. F. C., Fry, J. D., Lyman, R. F. & Nuzhdin, S. V. 1994 Polygenic mutation in *Drosophila melanogaster*: estimates from response to selection of inbred strains. *Genetics* **136**, 937–951.

Mackay, T. F. C., Lyman, R. F. & Hill, W. G. 1995 Polygenic mutation in *Drosophila melanogster*: non-linear divergence among unselected strains. *Genetics* **139**, 849–859.

McMillan, I. & Robertson, A. 1974 The power of methods for the detection of major genes affecting quantitative characters. *Heredity* **32**, 349–356.

Miyashita, N. & Langley, C. H. 1990 Molecular and phenotypic variation of the white locus region in *Drosophila melanogaster*. *Genetics* **120**, 199–212.

Moehring, A. J. & Mackay, T. F. C. 2004 The quantitative genetic basis of male mating behavior in *Drosophila melanogaster*. *Genetics* **167**, 1249–1263.

Norga, K. K., *et al*. 2003 Quantitative analysis of bristle number in *Drosophila* mutants identifies genes involved in neural development. *Curr. Biol.* **13**, 1388–1397.

Nuzhdin, S. V., Fry, J. D. & Mackay, T. F. C. 1995 Polygenic mutation in *Drosophila melanogaster*: the causal relationship of bristle number to fitness. *Genetics* **139**, 861–872.

Nuzhdin, S. V., Pasyukova, E. G., Dilda, C. L., Zeng, Z. B. & Mackay, T. F. C. 1997 Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **94**, 9734–9739.

Nuzhdin, S. V., Dilda, C. L. & Mackay, T. F. C. 1999 The genetic architecture of selection response: Inferences from fine-scale mapping of bristle number quantitative trait loci in *Drosophila melanogaster*. *Genetics* **153**, 1317–1331.

Pasyukova, E. G., Vieira, C. & Mackay, T. F. C. 2000 Deficiency mapping of quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Genetics* **156**, 1129–1146.

Rasmuson, M. 1952 Variation in bristle number of *D. melanogaster*. *Acta Zoolog. Stockh.* **33**, 277–307.

Reeve, E. C. R. & Robertson, F. W. 1953 The analysis of environmental variability in quantitative inheritance. *Nature* **171**, 874.

Robertson, A. 1967 The nature of quantitative genetic variation. In *Heritage from Mendel* (ed. A. Brink), pp. 265–280. Madison, WI: University of Wisconsin.

Robertson, F. W. & Reeve, E. C. R. 1955 Studies in quantitative inheritance. VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster*. *Zeitschridt indukt. Abstammungs Vererbungslehre* **86**, 439–458.

Robin, C., Lyman, R. F., Long, A. D., Langley, C. H. & Mackay, T. F. C. 2002 *Hairy*: a quantitative trait locus for *Drosophila* bristle number. *Genetics* **162**, 155–164.

Salzberg, A., Prokopenko, S. N., He, Y., Tsai, P., Pal, M., Maroy, P., Glover, D. M., Deak, P. & Bellen, H. J. 1997 *P*-element insertion alleles of essential genes on the third chromosome of *Drosophila melanogaster*: mutations affecting embryonic PNS development. *Genetics* **147**, 1723–1741.

Shrimpton, A. E. & Robertson, A. 1988 The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. I. Allocation of third chromosome bristle effects to chromosome sections. *Genetics* **118**, 437–443.

Shrimpton, A. E. & Robertson, A. 1988 The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. II. Distribution of third chromosome bristle effects within chromosome sections. *Genetics* **118**, 445–459.

Sobels, F. H. 1952 Genetics and morphology of the genotype "asymmetric" with special reference to its 'abnormal abdomen' character (*Drosophila melanogaster*). *Genetica* **26**, 117–274.

Sorensen, D. & Waagepetersen, R. 2003 Normal linear models with genetically structured residual variance: a case study. *Genet. Res.* **82**, 202–222.

Spickett, S. G. & Thoday, J. M. 1966 Regular responses to selection. 3. Interactions between located polygenes. *Genet. Res.* **7**, 96–121.

Spiers, J. G. C. 1974 The effects of larval competition on a quantitative character of *Drosophila melanogaster*. Ph.D. thesis, University of Edinburgh, Edinburgh.

Stathakis, D. G., Burton, D. Y., McIvor, W. E., Krishnakumar, S., Wright, T. R. F. & O'Donnell, J. M. 1999 The catecholamines up (Catsup) protein of *Drosophila melanogaster* functions as a negative regulator of tyrosine hydroxylase activity. *Genetics* **153**, 361–382.

Suster, M. L., Karunanithi, S., Atwood, H. L. & Sokolowski, M. B. 2004 Turning behavior in *Drosophila* larvae: a role for the small *scribbler* transcript. *Genes Brain Behav.* **3**, 273–286.

Thoday, J. M. 1961 Location of polygenes. *Nature* **191**, 368–370.

Thoday, J. M. 1979 Polygene mapping: uses and limitations. In *Quantitative genetic variation* (ed. J. N. Thompson & J. M. Thoday), pp. 219–223. New York: Academic Press.

Vaughn, T. T., Pletscher, S., Peripato, A., King-Ellison, K., Adams, E., Erikson, E. & Cheverud, J. 1999 Mapping quantitative trait loci for murine growth: a closer look at genetic architecture. *Genet. Res.* **74**, 313–322.

Vieira, C., Pasyukova, E. G., Zeng, Z. B., Hackett, J. B., Lyman, R. F. & Mackay, T. F. C. 2000 Genotype–environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. *Genetics* **154**, 213–227.

Wagner, G., Booth, G. & Bagheri-Chaichian, H. 1997 A population genetic theory of canalization. *Evolution* **51**, 329–347.

Yoo, B. H. 1980 Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. II. Lethals and visible mutants with large effects. *Genet. Res.* **35**, 19–31.