Genotype-Environment Interaction at Quantitative Trait Loci Affecting Sensory Bristle Number in *Drosophila melanogaster*

Marjorie C. Gurganus,*,[1] James D. Fry,*,[2] Sergey V. Nuzhdin,*,[3] Elena G. Pasyukova,*,† Richard F. Lyman* and Trudy F. C. Mackay*

**Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695 and †Institute of Molecular Genetics of Russian Academy of Sciences, Moscow 123182, Russia*

Manuscript received November 17, 1997
Accepted for publication May 11, 1998

ABSTRACT

The magnitude of segregating variation for bristle number in *Drosophila melanogaster* exceeds that predicted from models of mutation-selection balance. To evaluate the hypothesis that genotype-environment interaction (GEI) maintains variation for bristle number in nature, we quantified the extent of GEI for abdominal and sternopleural bristles among 98 recombinant inbred lines, derived from two homozygous laboratory strains, in three temperature environments. There was considerable GEI for both bristle traits, which was mainly attributable to changes in rank order of line means. We conducted a genome-wide screen for quantitative trait loci (QTLs) affecting bristle number in each sex and temperature environment, using a dense (3.2-cM) marker map of polymorphic insertion sites of *roo* transposable elements. Nine sternopleural and 11 abdominal bristle number QTLs were detected. Significant GEI was exhibited by 14 QTLs, but there was heterogeneity among QTLs in their sensitivity to thermal and sexual environments. To further evaluate the hypothesis that GEI maintains variation for bristle number, we require estimates of allelic effects across environments at genetic loci affecting the traits. This level of resolution may be achievable for Drosophila bristle number because candidate loci affecting bristle development often map to the same location as bristle number QTLs.

A major focus of evolutionary quantitative genetics in recent years has been to evaluate the hypothesis that ubiquitous naturally occurring variation for quantitative traits (Mousseau and Roff 1987; Roff and Mousseau 1987; Houle 1992) can be maintained in the face of continual erosion of genetic variability by strong directional and stabilizing natural selection (Endler 1986), by reintroduction of variation each generation by mutation (Barton and Turelli 1989). The exact quantitative theoretical relationships between the rate of production of mutational variance affecting a quantitative trait and the intensity of selection vary depending on the model assumptions, but in all cases, there is a positive association between the amount of genetic variation maintained in an equilibrium population (V_G) and the input of mutational variation per generation (V_M; Clayton and Robertson 1955). For example, if mutations affecting quantitative traits have deleterious pleiotropic fitness effects, V_G is related to V_M as $V_G = V_M/s$, where s is the average selection coefficient against a heterozygous mutation, and is sufficiently large that mutant homozygotes are rare (Barton 1990). However, when this hypothesis is evaluated for Drosophila abdominal and sternopleural bristle number, for which many independent estimates of V_M (reviewed by Keightley *et al.* 1993; Houle *et al.* 1996) and the fitness effects of spontaneous mutations affecting bristle number (Mackay *et al.* 1995; Nuzhdin *et al.* 1995) have been made, it is inadequate to explain the large amounts of segregating variation observed in natural populations. Therefore, alternative mechanisms must be sought to explain the maintenance of variation in these traits in the face of weak mutation and strong selection.

The mean phenotypic value for a quantitative trait of a defined genotype is not a constant but can vary according to the environment. A plot of the mean phenotypic value of a genotype across a range of environments is its norm of reaction, or environmental sensitivity. Variation among genotypes in environmental sensitivity contributes to genotype × environment interaction (GEI) variance, which could lead to levels of maintained variation in excess of those expected from an equilibrium between mutation and selection in a single environment. For example, Via and Lande (1987) describe a model in which GEI for a character between two environments is quantified by the cross-environment genetic correlation, r_{GE}, which reflects the extent to which the same genes pleiotropically affect

Corresponding author: Trudy F. C. Mackay, Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695. E-mail: trudy_mackay@ncsu.edu

[1] *Present address:* Max Planck Institute of Chemical Ecology, Tatzendpromenade 1A, D-07745 Jena, Germany.

[2] *Present address:* Department of Biology, Utah State University, Logan, UT 84322-5305.

[3] *Present address:* Department of Evolution and Ecology, University of California, Davis, CA 95616.

the trait in the different conditions (Falconer 1960). If there is stabilizing selection within environments but disruptive selection between environments, GEI will increase the equilibrium genetic variance above the level expected from mutation-selection balance alone if $r_{GE} = -1$. Alternatively, if a trait exhibits GEI such that additive allelic effects fluctuate randomly across environments, and there is stabilizing selection for a single phenotype in all environments (*i.e.*, no disruptive selection between environments), variation will be maintained by overdominance because the across-environment variance decreases as the number of heterozygous loci increases (Gillespie and Turelli 1989).

Empirical evaluation of the extent to which GEI affects quantitative genetic variation for traits under stabilizing selection promises to be difficult, requiring knowledge of the magnitude of GEI under the exact environmental circumstances experienced by the population, the nature of selection acting on the trait within and between environments, the types and frequencies of relevant environments, and the genetic basis of GEI (Via *et al.* 1995). In this article, we have begun to determine the genetic basis of GEI using the *Drosophila melanogaster* bristle number model system. There is substantial GEI for sternopleural bristle number segregating between inbred strains of *D. melanogaster* (Caligari and Mather 1975), for *Drosophila pseudoobscura* abdominal bristle number among isogenic second-chromosome lines sampled from nature (Gupta and Lewontin 1982), and for new mutations affecting both abdominal and sternopleural bristle number (Mackay and Lyman 1998). Furthermore, Drosophila bristle number is one of the few quantitative traits for which quantitative trait loci (QTLs) at which mutational and segregating variation occur have been mapped with high resolution, in some cases to the level of genetic locus (reviewed by Mackay 1995, 1996). Here, we determine the extent to which QTLs affecting bristle number exhibit GEI by mapping bristle number QTLs that segregate in a panel of recombinant inbred (RI) lines derived from two unselected inbred strains by linkage to highly polymorphic transposable element markers, in each of three temperature environments. There is considerable GEI for bristle number and variation among loci in the association between mean effects and sensitivity.

MATERIALS AND METHODS

Drosophila stocks: Two unrelated lines, Oregon R (Lindsley and Zimm 1992) and 2b (Pasyukova and Nuzhdin 1993), were made isogenic using balancer chromosomes to suppress recombination (Pasyukova and Nuzhdin 1993). The parental lines were crossed, and the F_1 progeny were backcrossed to 2b. An advanced intercross design, in which the backcross progeny were randomly mated for four generations, was used to increase recombination between markers and QTLs and hence the precision of mapping. In the fifth generation, 200 individual pairs were selected, and the progeny of each pair maintained by full-sib mating for 25 generations. The 98 RI lines that survived inbreeding were maintained subsequently by mass matings of 20 pairs per generation.

Bristle number phenotype: Two replicate vials of each RI line, and the two parental lines, were reared at 18°, 25°, and 29°. A total of 10 males and 10 females from each vial were scored for abdominal (the total number of hairs on the sixth abdominal sternite in females and on the fifth abdominal sternite in males) and sternopleural (the total number of macrochaetes and microchaetes on the left and right sternopleural plates) bristle number. The total sample size was thus 3 temperatures × 98 lines/temperature × 2 replicates/line/temperature × 2 sexes/replicate × 10 individuals/sex = 11,760 flies scored for each bristle trait. The design was completely balanced.

Marker genotype: Insertion sites of high-copy-number transposable elements provide convenient polymorphic molecular markers for mapping (Long *et al.* 1995; Nuzhdin *et al.* 1997; M. C. Gurganus, S. V. Nuzhdin and T. F. C. Mackay, unpublished results). *roo* transposable element insertion sites were determined by *in situ* hybridization of a biotin-labeled plasmid containing the full-length *roo* transposable element (Long *et al.* 1995) to polytene salivary gland chromosomes of third instar larvae. Probes were labeled with biotinylated dATP (bio-7-dATP; Bethesda Research Laboratories, Gaithersburg, MD) by nick translation. Hybridization was detected using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and visualized with diaminobenzidine (Long *et al.* 1995; Nuzhdin *et al.* 1997). Five slides were scored per line, and markers were classified as homozygous Oregon, homozygous 2b, or heterozygous. There were 92 *roo* markers in total, with 22 on the *X* chromosome, 27 on chromosome *2*, and 43 on chromosome *3*. The *roo* elements provide a dense marker map with an average intermarker distance of 3.2 cM (Nuzhdin *et al.* 1997) on the standard genetic map (Lindsley and Zimm 1992). The fourth chromosome of Oregon was marked by the recessive morphological marker spa^{pol}, which also segregated among the RI lines.

The additional generations of recombination during the construction of the RI lines resulted in an effective map expansion; although the standard map length of the Drosophila genome is 294 cM (Lindsley and Zimm 1992), the effective map length in the RI line population was 690 cM, as estimated by summing the recombination rates using the observed recombination between markers (r) and the Kosambi mapping function relating observed recombination to distance (d) between them in centimorgans ($100d = 0.25 \ln[(1 + 2r)/(1 - 2r)]$). There was no recombination between 16 markers and the next adjacent marker, leaving 76 informative markers and an average interval size on the expanded map of 9.2 cM.

Analysis of variance of bristle number: Analysis of variance (ANOVA) was used to partition variation among the recombinant inbred lines for each bristle character into sources attributable to line (L, random), sex (S, fixed), and temperature (T, fixed) according to the model

$$y = \mu + T + S + L + T \times S + T \times L + S \times L + T \times S \times L + R(T \times L) + S \times R(T \times L) + E,$$

where μ is the overall mean, R refers to replicate vial, E is the within vial error variance, and parentheses represent the nesting of an effect. Similar analyses were done considering the three possible pairs of temperatures and for each temperature environment. In addition, all analyses were run separately for males and females. Tests of significance of F ratios and estimates of variance components were obtained using SAS procedures GLM and VARCOMP (SAS Institute, Inc. 1988), respectively.

Genetic correlations of bristle number in the two sexes and

in pairs of temperature environments (r_{GE}) were computed from the variance components as $\sigma^2_{L12}/(\sigma^2_{L1} \times \sigma^2_{L2})$, where σ^2_{L12} is the variance among lines from the joint analysis across temperature or sex environments, and σ^2_{L1} and σ^2_{L2} are the variances among lines from the analyses in environments 1 and 2, respectively (Robertson 1959).

Significant GEI of loci affecting bristle number with the sex and/or temperature environment can arise from two sources: from the departure of the genetic correlation (r_{GE}) of bristle-number effects across environments from unity, and from changes in among-line variance of bristle number in the different environments. Their relative contributions are given by the relationship

$$\sigma^2_{GE} = \sum_{i<j}[2\,\sigma_{Li}\sigma_{Lj}(1 - r_{ij}) + (\sigma_{Li} - \sigma_{Lj})^2]/t(t-1)$$

(Cockerham 1963), where σ^2_{GE} is the $G \times E$ interaction variance component; t is the number of environments; σ_{Li} and σ_{Lj} are the square roots of the among-line variance components in environments i and j, respectively; and r_{ij} is the genetic correlation between environments. The first term is the contribution from lack of perfect correlation between environments, and the second is from differences in variance.

The variance of means across pairs of environments was estimated as $\sigma^2_M = 0.25(\sigma^2_{L1} + \sigma^2_{L2}) + 0.5\sigma^2_{L12}$. The variance of sensitivity was estimated as $\sigma^2_S = (\sigma^2_{L1} + \sigma^2_{L2} - 2\,\sigma^2_{L12})/D^2$. ($D$ is the difference in mean bristle number between the environments 1 and 2, where environment 1 has a higher overall mean value of the trait than environment 2.) The covariance between the mean and sensitivity was estimated as $\text{cov}_{MS} = (\sigma^2_{L1} - \sigma^2_{L2})/2D$ (Falconer 1990). The genetic correlation between the mean and sensitivity, r_{MS}, was thus estimated as $r_{MS} = \text{cov}_{MS}/(\sigma^2_M \times \sigma^2_S)^{1/2}$.

QTL mapping: We used two different procedures to map QTLs affecting bristle number in each sex and environment. Single-marker analysis is arguably more appropriate than interval mapping for advanced generation crosses because the probability of multiple recombination events between marker intervals is much higher than in F_2 and backcross generations. Therefore, we first used a sequential search procedure combined with permutation testing to evaluate associations between the molecular markers and trait phenotypes. Single-marker F statistics for the regressions of mean trait values on marker genotypes were computed for all markers for each sex, temperature environment, and bristle trait separately. To overcome the problems of multiple tests and correlated markers in setting the correct experiment-wise Type I error rate at $\alpha = 0.05$, we determined the empirical F distribution under the null hypothesis of no association between any of the markers and trait values by randomly permuting the trait data among marker classes 2000 times and by calculating the maximum F statistic (F_{MAX}) across all markers for each permutation. The number of times that F_{MAX} exceeded the F statistic from the original data was recorded. The 100th highest F_{MAX} is the empirical critical F corresponding to $\alpha = 0.05$ under the null hypothesis (Churchill and Doerge 1994; Doerge and Churchill 1996). To address the issue of segregating unlinked QTLs inflating the within-marker-class variance and hence reducing the power to detect additional QTLs (Zeng 1993, 1994; Jansen and Stam 1994), we used a sequential search procedure for significant marker-QTL associations (Doerge and Churchill 1996). For each category, the effects of the most significant marker (having the highest F statistic from the original data) were removed using the SAS procedure GLM (SAS Institute, Inc. 1988) with the model y = marker and calculating the residuals. The permutation procedure was then repeated on the residuals until no further markers were significant. F statistics (Type III SS) for regressions of line mean trait values on multiple markers were calculated using the SAS procedure GLM (SAS Institute, Inc. 1988). Permutation tests for major QTLs and a sequential search for additional QTLs (Churchill and Doerge 1994; Doerge and Churchill 1996) were performed in Mathematica Version 2.2 (Wolfram 1991).

We also analyzed these data using a composite interval mapping procedure (Zeng 1993, 1994), which tests the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait, while statistically accounting for the effects of additional segregating QTLs by multiple regression on markers outside the tested interval. The interval sizes in cM were estimated from the observed recombination between markers and the Kosambi mapping function, as described above. The likelihood ratio test statistic LR is $-2\ln(L_0/L_1)$, where L_0/L_1 is the ratio of the likelihood under the null hypothesis (there is no QTL in the interval) to the alternative hypothesis (there is a QTL in the interval). This test statistic at a genomic location is distributed as chi-squared with 1 d.f. under the null hypothesis. The genome-wide significance level for a Type I error rate of 0.05, taking multiple tests into account, is 11.6. These analyses were performed using the QTL Cartographer software Version 1.09a (J. C. Basten, B. S. Weir and Z.-B. Zeng; fttp://esssjp.stat.ncsu.edu/pub/qtlcart/), 10 background markers, and a window size of 5 cM.

RESULTS

Three temperatures: Mean sternopleural and abdominal bristle numbers of the two parental strains, Oregon and 2b, and of the 98 RI lines derived from them, are in the wild-type range for these characters (Table 1). There is genetic variation between the parental strains for both bristle traits: Averaged over temperature and sex, 2b has more sternopleural and abdominal bristles than Oregon. Temperature and bristle number are negatively correlated, averaged over both parental lines and sexes. However, the two parental lines respond differently to changes in temperature, for both bristle traits; *i.e.*, there is genetic variation in the temperature reaction norms, or there is GEI variance. Both bristle traits are sexually dimorphic, with females having on average more bristles than males, and the magnitude of the sex dimorphism of abdominal and sternopleural bristle number varies between the parental lines (sex $\times$ line interaction). For abdominal bristle number, the magnitude of the difference between lines in sex dimorphism clearly varies with temperature (temperature $\times$ sex $\times$ line interaction). These lines are thus suitable material to use to investigate the genetic basis of variation in reaction norms.

Mean bristle numbers of the Oregon/2b RI lines in the three temperature environments are given in Figures 1 (sternopleural bristles) and 2 (abdominal bristles). Although the mean bristle numbers of all RI lines are bracketed by the two parental line means for each sex and bristle trait (Table 1), the range of means of the RI lines exceeds those of the parental lines for each trait and temperature. Such transgressive segregation is a common phenomenon for progeny generations derived from parental lines that are not selected for the

TABLE 1

Mean sternopleural and abdominal bristle numbers of inbred Oregon and 2b, and RI lines derived from them, in three temperature environments

		Oregon		2b		RI	
Trait	Temperature	Male	Female	Male	Female	Male	Female
ST	18°	19.50	21.05	23.40	23.35	20.69	21.61
		(0.34)	(0.45)	(0.51)	(0.51)	(0.13)	(0.15)
	25°	18.10	19.40	20.00	20.80	19.04	20.02
		(0.62)	(0.74)	(0.59)	(0.53)	(0.14)	(0.16)
	29°	16.50	17.50	19.35	22.15	18.67	19.41
		(0.44)	(0.32)	(0.51)	(0.43)	(0.18)	(0.22)
AB	18°	17.00	18.55	16.80	18.15	16.38	17.91
		(0.49)	(0.45)	(0.51)	(0.67)	(0.12)	(0.14)
	25°	15.25	16.35	16.75	20.90	15.79	17.18
		(0.70)	(0.55)	(0.92)	(0.55)	(0.14)	(0.15)
	29°	14.45	15.70	16.55	20.20	14.95	17.08
		(0.40)	(0.43)	(0.36)	(0.44)	(0.14)	(0.12)

ST, sternopleural; AB, abdominal; mean, ± SE.

measured trait. Increasing and decreasing alleles at loci affecting the trait are in dispersion in the parental lines and recombine to produce more extreme phenotypes in the progeny.

The variation among the RI lines in the three temperatures is quantified in Table 2, which gives the full ANOVAs for the two bristle traits. The main effects of temperature (T), sex (S) and line (L) are all highly significant, for both bristle traits. The $T \times L$ and $S \times L$ GEI terms are also highly significant for both bristle traits, indicating that the crossing of temperature reaction norms of the RI lines demonstrated in Figures

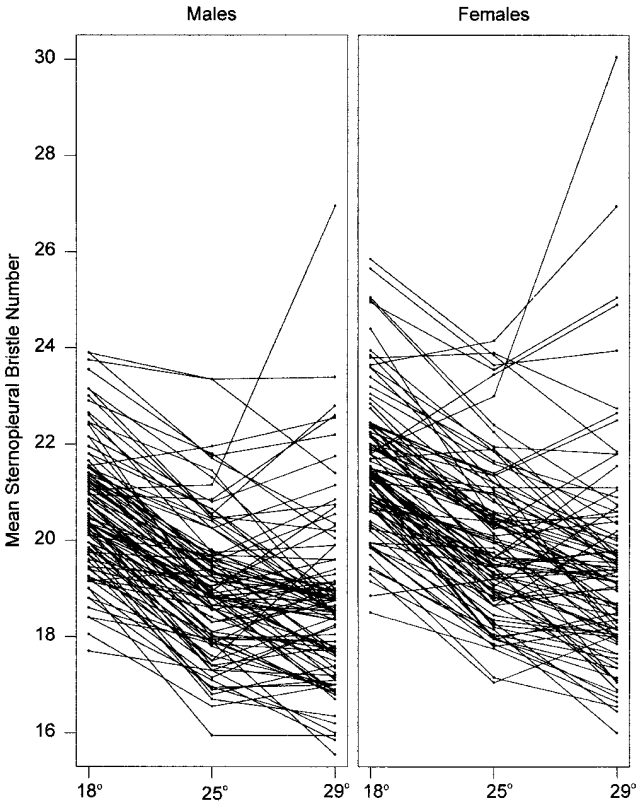

Figure 1.—Norms of reaction of sternopleural bristle number for 98 Oregon/2b recombinant inbred lines in three temperature environments.

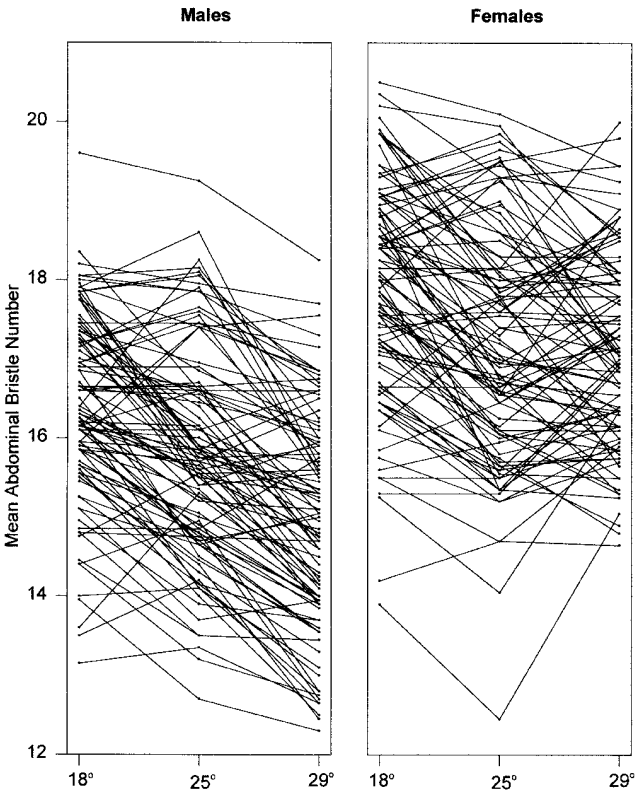

Figure 2.—Norms of reaction of abdominal bristle number for 98 Oregon/2b recombinant inbred lines in three temperature environments.

TABLE 2

ANOVA of bristle numbers for the recombinant inbred lines, pooled over three temperature environments and sexes

		Sternopleural bristles			Abdominal bristles		
Source	d.f.	Mean square	*P*	Variance component	Mean square	*P*	Variance component
T	2	4783.59	0.0001	—	1269.32	0.0001	—
S	1	2298.44	0.0001	—	8369.58	0.0001	—
L	97	244.04	0.0001	1.691	157.81	0.0001	1.116
$T \times S$	2	14.92	0.0198	—	151.50	0.0001	—
$T \times L$	194	31.91	0.0001	0.536	14.50	0.0001	0.162
$S \times L$	97	13.00	0.0001	0.155	15.25	0.0001	0.157
$T \times S \times L$	194	3.73	0.2309	0.017	5.82	0.0495	0.056
$R(T \times L)$	294	10.12	0.0001	0.337	6.89	0.0005	0.110
$S \times R(T \times L)$	294	3.39	0.0655	0.039	4.70	0.0001	0.147
E	10,584	3.00		3.001	3.24		3.237

T, temperature; *S*, sex; *L*, line; *R*, replicate; *E*, environment.

1 and 2 are statistically significant, and that, furthermore, the magnitude of the sex dimorphism for bristle number is not constant across lines. The three-way $T \times S \times L$ GEI term is marginally significant for abdominal bristle number. The remaining terms in the ANOVA are due to uncontrolled environment.

The variance components for the random effects are also given in Table 2. The among-line variance component is an estimate of the genetic variance (V_G) segregating between the two homozygous parental lines (Falconer and Mackay 1996). One can gauge the relative importance of the GEI variance, or the environment-specific among-line variance in bristle number, to the genetic variation common to all environments by the ratio of the sum of all significant interaction variance components ($V_I = V(T \times L) + V(S \times L) + V(T \times S \times L)$) to V_G. Estimates of V_I/V_G from the analyses over three temperatures are quite high: 0.41 for sternopleural bristle number and 0.34 for abdominal bristle number.

Two temperatures: Among-line and GEI variance components from ANOVAs of bristle number in pairs of temperatures are given in Table 3. In the analyses pooled across sexes, the among line variance of sternopleural bristle number is highly significant at all temperatures but is greatest for the 25–29° contrast. The $S \times L$ interaction is also significant for all temperature pairs and is of the same magnitude for each contrast. The $T \times L$ interaction is small and only marginally significant between 18–25° but highly significant for the other temperature pairs. Estimates of V_I/V_G for sternopleural bristle number consequently vary among temperature pairs, from 0.14 (18–25°), to 0.33 (25–29°), to 0.88 (18–29°). The among-line variance of sternopleural bristle number from the separate sex analyses is 32% greater in females than males, but the ratio of $T \times L$ interaction to V_G for the different temperature pairs is constant across sexes (Table 3).

The among-line variance of abdominal bristle number is also highly significant for each pair of temperatures considered, but its magnitude is least for the 18–29° contrast. The $S \times L$ and $T \times L$ interactions for abdominal bristle number are all formally significant, but their magnitude varies. The $S \times L$ interaction variance at 18–29° is about one-half its value in the other contrasts, and the $T \times L$ interaction variance at 18–29° is over twice the $T \times L$ interactions estimated for 18–25° and 25–29°. The $T \times S \times L$ variance is highly significant for the 18–29° contrast. The ratio V_I/V_G is consequently higher (0.52) for 18–29° than for 18–25° (0.27) or 25–29° (0.22). Although the average among-line variance of abdominal bristle number is similar for males and females, the relative ratio of the $T \times L$ interaction to V_G is on average greater for females (0.28) than males (0.07). Female abdominal bristle number is far more variable in response to temperature than is male abdominal bristle number, for the same genotypes. Furthermore, as expected from the significant $T \times S \times L$ variance component, the ratios of the $T \times L$ interactions to V_G vary between the sexes among the different temperature pairs. For males, $V_I/V_G = 0.09$ (18°–25°), 0.03 (25–29°), and 0.11 (18–29°); whereas for females, $V_I/V_G = 0.09$ (18–25°), 0.20 (25–29°), and 0.77 (18–29°).

One temperature: Among-line and $S \times L$ variance components from ANOVAs of bristle number within each temperature are given in Table 4. The among-line variance of sternopleural bristle number increases with temperature by 20% between 18° and 25° and by 89% between 25° and 29° (see Figure 1). The $S \times L$ interaction also increases as temperature increases (by 22% between 18° and 25° and 31% between 25° and 29°). Consequently, the among-line variance of female stern-

TABLE 3

Summary of among-line (L), $T \times L$, and $S \times L$ interaction variance components from ANOVAs of ST and AB bristle number of recombinant inbred lines across three temperature environments

		Sexes pooled				Males		Females	
Trait	Temperature	L	$T \times L$	$S \times L$	$T \times S \times L$	L	$T \times L$	L	$T \times L$
ST	18°, 25°, 29°	1.691 ***	0.536 ***	0.155 ***	0.017 NS	1.591 ***	0.457 ***	2.099 ***	0.650 ***
	18°, 25°	1.559 ***	0.087 *	0.144 ***	0.005 NS	1.561 ***	0.072 NS	1.845 ***	0.112 *
	25°, 29°	2.067 ***	0.524 ***	0.166 ***	0.024 NS	1.844 ***	0.482 ***	2.622 ***	0.612 ***
	18°, 29°	1.446 ***	0.998 ***	0.153 ***	0.022 NS	1.368 ***	0.816 ***	1.830 ***	1.225 ***
AB	18°, 25°, 29°	1.116 ***	0.162 ***	0.157 ***	0.056 *	1.311 ***	0.092 *	1.235 ***	0.344 ***
	18°, 25°	1.192 ***	0.115 *	0.212 ***	0.014 NS	1.214 ***	0.109 *	1.593 ***	0.150 **
	25°, 29°	1.245 ***	0.114 **	0.165 ***	0.035 NS	1.517 ***	0.040 NS	1.304 ***	0.259 ***
	18°, 29°	0.910 ***	0.257 ***	0.094 *	0.119 **	1.201 ***	0.127 *	0.807 ***	0.625 ***

P values are from F ratio tests of significance of mean squares. NS, not significant; $P > 0.05$; *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***$P < 0.001$. See legends to Tables 1 and 2 for definitions of abbreviations.

opleural bristle number is 51% greater than that of males at 29° but only 20% greater than the male variance at 18° and 25°. These changes of variance with temperature are not proportional to mean sternopleural bristle numbers because the changes in mean are in the opposite direction.

The pattern of among-line variance of abdominal bristle number across temperatures is more complicated.

TABLE 4

Summary of among-line (L) and $S \times L$ interaction variance components from ANOVAs of ST and AB bristle number of recombinant inbred lines across three temperature environments

		Sexes pooled		Males	Females
Trait	Temperature	L	$S \times L$	L	L
ST	18°	1.499 ***	0.135 **	1.490 ***	1.778 ***
	25°	1.792 ***	0.164 ***	1.776 ***	2.136 ***
	29°	3.390 ***	0.216 ***	2.877 ***	4.333 ***
AB	18°	1.114 ***	0.239 ***	1.094 ***	1.612 ***
	25°	1.500 ***	0.213 **	1.552 ***	1.874 ***
	29°	1.219 ***	0.187 ***	1.562 ***	1.252 ***

P values are from F ratio tests of significance of mean squares. **, $0.001 < P < 0.01$; ***, $P < 0.001$. See legends to Tables 1 and 2 for definitions of abbreviations.

Averaged over sexes, expressed genetic variance is greatest in the "home" environment of 25°; among-line variance is reduced by 23% of this value at 29° and by 34% at 18°. However, the $S \times L$ interaction variance is greatest at 18° and least at 29°, reflecting the different patterns of response of among-line variance to temperature in males and females. For males, variance among lines is nearly equivalent at 25° and 29° and is reduced by 42% at 18°; whereas for females, among-line variance is greatest at 25° and is reduced from its maximum by 16% at 18° and by 50% at 29°. Therefore, the among-line variance for female abdominal bristle number is 47% greater than males at 18°, 21% greater than males at 25°, and 25% less than males at 29°.

Genotype environment correlations: The variance component genetic correlations of bristle number between males and females of the RI lines are given in Table 5. Although the correlations are higher for sternopleural bristle number (on average, 0.92) than abdominal bristle number (on average, 0.88), they are all significantly different from 1. Averaged over the three temperature treatments, 89% of the $S \times L$ interaction for sternopleural bristle number was attributable to changes of rank order of bristle number effects in males and females. For abdominal bristle number, over 99% of the three temperature $S \times L$ interaction variance was caused by changes of rank. Thus, for both bristle traits, the significant departure of the genetic correlations between the sexes from 1 is primarily caused by crossing of reaction norms between the sexes, rather than by differences in among line variance between males and females.

TABLE 5

Variance component genetic correlations of bristle number between males and females for recombinant inbred lines in three temperature environments

Temperature	Sternopleural bristles	Abdominal bristles
18°	0.921**	0.839***
25°	0.920***	0.879**
29°	0.960***	0.872***

P values are for significance of departure of the genetic correlation from unit from the $S \times L$ interaction term of the appropriate analysis of variance. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$. See legend to Table 2 for definitions of abbreviations.

Variance component genetic correlations of bristle number between pairs of temperature environments are given in Table 6. The picture for sternopleural bristles is straightforward. The cross-environment genetic correlation of sternopleural bristle number is high between 18° and 25° (0.95) and only marginally significantly different from 1. The genetic correlations of sternopleural bristle number are highly significantly different from unity between 25° and 29° and between 18° and 29°, but the correlation is lowest (0.64) between the two extreme temperatures. Cross-environment correlations for this trait are very similar for males and females. 80% of the total $T \times L$ interaction variance is attributable to changes of rank order of line means across temperatures. The pattern of genetic correlations across environments for abdominal bristle number is more complex, as the sexes respond differently to changes in environment. Recall that the $T \times L$ interaction variance for male abdominal bristle number is in general lower than for female abdominal bristle number. Thus, the genetic correlation for males is high, averaged over all three environments (0.93), and only marginally significantly different from 1; in fact, r_{GE} is not significantly different from 1 between 25° and 29°. However, r_{GE} for female abdominal bristle number is highly significantly different from 1 in all temperature pairs, and the pattern of magnitudes of the correlation follows that for sternopleural bristle number: highest between 18° and 25° (0.92) and lowest between 18° and 29° (0.57). In males, 85% of the total three temperature-by-line interaction variance is caused by changes in rank; in females, rank order change accounts for 95% of the $T \times L$ interaction.

TABLE 6

Variance component genotype × environment correlations of bristle number for recombinant inbred lines across three temperature environments

Temperature	Sex	Sternopleural bristles	Abdominal bristles
18–25°	Both	0.951*	0.922*
	Males	0.960^{NS}	0.932*
	Females	0.947*	0.917**
25–29°	Both	0.839***	0.921*
	Males	0.816***	0.975^{NS}
	Females	0.862***	0.851***
18–29°	Both	0.641***	0.781***
	Males	0.661***	0.919*
	Females	0.660***	0.568***

P values are for significance of departure of the genetic correlation from unity from the $T \times L$ interaction term of the appropriate analysis of variance. NS, not significant; $P > 0.05$; *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$. See legend to Table 2 for definitions of abbreviations.

Correlations between mean and sensitivity: The variance among lines in each environment and covariance among lines in pairs of environments can be expressed in terms of variance among lines in mean performance in the two environments (σ^2_M), variance among lines in environmental sensitivity (σ^2_S), and the covariance (cov_{MS}) and correlation (r_{MS}) of mean and sensitivity (Falconer 1990). Estimates of these parameters are given in Table 7. For sternopleural bristle number, both σ^2_M and σ^2_S, averaged over sexes, are least between 18° and 25°, and maximal between 25° and 29°. Between 18° and 25°, σ^2_S is over 20 times less than σ^2_M, whereas between 25° and 29°, σ^2_S is 1.9 times greater than σ^2_M. Between 18° and 25°, the correlation between the mean and sensitivity is approximately −0.3, whereas between 25° and 29° and 18° and 29°, r_{MS} increases to −0.5. The above contrasts follow the same pattern for males and females considered separately.

The variance of mean abdominal bristle number is very similar across all temperature pairs, both averaged over sexes and for males and females separately. As for sternopleural bristle number, the maximum σ^2_S is between 25° and 29° (where it is 25% less than the variance in mean, averaged over sexes), and the minimum is between 18° and 29°. (At this temperature pair, σ^2_S is 2.6 times less than σ^2_M.) However, σ^2_S for abdominal bristle number varies across the sexes between 25° and 29° and 18° and 29°, with the variance of sensitivity of males 400 and 14 times less, respectively, than the variance of sensitivity of females. Overall, correlations between the mean and sensitivity are lower for abdominal ($r_{MS} = -0.03$, averaged over sexes and all three temperature pairs) than for sternopleural (average $r_{MS} = -0.42$) bristle number. Furthermore, r_{MS} for abdominal bristle number appears to differ in both sign and magnitude between males and females and across temperature pairs. For example, between 18° and 25°, the correlation between means and sensitivities is −0.4 both averaged over sexes and in males; whereas between 25° and 29°, $r_{MS} = 0.3$, both averaged over sexes and in females.

QTL-marker associations: Insertion sites of the *roo* transposable elements were analyzed for five larvae of each of the 98 RI lines. Because transposable element insertion sites are dominant markers, it was necessary to

TABLE 7

Estimates of the variance of means (σ^2_M) and sensitivities (σ^2_S), and the covariance (cov_{MS}) and correlation (r_{MS}) between mean and sensitivity for ST and AB bristle number in pairs of temperature environments

Trait	Temperature	Sex	σ^2_M	σ^2_S	cov_{MS}	r_{MS}
ST	18–25°	Males	1.597	0.053	−0.087	−0.300
		Females	1.901	0.088	−0.113	−0.275
		Both	1.602	0.066	−0.090	−0.278
	25–29°	Males	2.086	6.992	−1.482	−0.388
		Females	2.928	3.306	−1.805	−0.580
		Both	2.329	4.360	−1.630	−0.512
	18–29°	Males	1.775	0.399	−0.343	−0.408
		Females	2.443	0.506	−0.581	−0.522
		Both	1.945	0.448	−0.448	−0.480
AB	18–25°	Males	1.269	0.608	−0.383	−0.436
		Females	1.638	0.561	−0.180	−0.187
		Both	1.249	0.523	−0.290	−0.359
	25–29°	Males	1.537	0.113	−0.006	−0.014
		Females	1.434	52.979	3.149	0.361
		Both	1.302	1.043	0.300	0.257
	18–29°	Males	1.265	0.123	−0.163	−0.413
		Females	1.120	1.813	0.217	0.152
		Both	1.038	0.401	−0.046	−0.072

See legend to Table 1 for definitions of abbreviations.

genotype multiple individuals from each line to identify heterozygous sites. Each site was scored as homozygous Oregon, homozygous 2b, or heterozygous. The mean homozygosity averaged over all markers and lines was 0.956, but 60 of the 98 RI lines were heterozygous for at least one marker, and only eight markers were fixed in all lines. For segregating markers, we do not know the genotype of the individuals for which bristle number was measured, but we assumed all were heterozygous in the analyses below.

We used a sequential search procedure for QTLs and a permutation test to determine the experimentwise empirical *F* statistic corresponding to $\alpha = 0.05$ (Churchill and Doerge 1994; Doerge and Churchill 1996). The first step in the analysis was a single-marker analysis, in which the bristle number phenotypes were regressed on each marker genotype, separately for each bristle trait, temperature, and sex combination. The bristle number phenotypes were randomly permuted across the marker genotypes 2000 times, and the empirical critical *F* corresponding to $\alpha = 0.05$ under the null hypothesis was determined to be 11.5. Several markers exceeded this critical value for all the analyses. Therefore, a second iteration was performed, in which the effects of the most significant marker from the first step were removed by calculating the residuals from the model $y =$ marker (with the highest *F* statistic) + error. We continued to add markers that exceeded the empirical threshold *F* statistic to the model in this stepwise fashion until no further significant markers were detected. The 11 markers showing significant association with sternopleural bristle number and 13 markers associated with abdominal bristle with their *F* statistics from the sequential search are shown in Table 8.

In addition, we compared the significant markers from the sequential search procedure to those detected by a composite interval mapping procedure (Zeng 1993, 1994). The results from the composite interval mapping analyses were nearly identical to those of the sequential search, with only minor variations from the inclusion or exclusion of markers at the border of significant intervals. Since the exact results of composite interval mapping can depend on the number of background markers and the window size specified in the model, the choice of both of which is somewhat arbitrary, we have presented for simplicity the results from the sequential search procedure.

All significant markers from the sequential search were fitted as cofactors in a multiple regression analysis of the entire data set (separately for the two bristle traits), according to the model

$$y = \mu + T + S + S \times T + \sum M_j + \sum (T \times M_j) + \sum (S \times M_j) + \sum (S \times T \times M_j) + L(M_j) + S \times L(M_j) + T \times L(M_j) + E,$$

where μ, T, S, L, and E are as defined above, and M_j is the set of j markers found during permutation analysis to be associated with bristle number. The reduced model

$$y = \mu + \sum M_j + L(M_j) + E$$

TABLE 8

Sequential search for markers associated with bristle number

		Sternopleural bristles						Abdominal bristles					
		Males			Females			Males			Females		
Iteration		18°	25°	29°	18°	25°	29°	18°	25°	29°	18°	25°	29°
1	Marker	96F	96F	35BC	96F	96F	43E	92A	38A	94D	35BC	35BC	57C
	F	31.63	30.89	21.8	42.59	32.42	31.79	25.03	34.72	26.97	23.42	35.23	21.28
2	Marker	88E	79E	61D	63A	43E	96F	43A	57D	38A	29F	57C	50D
	F	30.79	22.44	11.77	20.10	20.44	22.49	22.92	26.58	25.23	18.01	25.06	15.79
3	Marker	57C	63A	End	4F	57F	End	57D	92A	57C	67D	21E	35BC
	F	11.86	21.34		20.47	23.56		30.23	27.32	24.35	13.39	15.15	11.72
4	Marker	End	48D		End	61D		21E	21E	50D	57D	End	1B
	F		12.21			14.63		18.54	15.49	13.16	12.89		17.20
5	Marker		57F			End		End	End	21E	44F		End
	F		18.01							17.05	13.90		

Markers are identified by their cytological location. The *F* statistics are from regressions of bristle number on marker genotype.

was run on the six temperature-sex categories separately. Sternopleural bristle markers 57C and 63A and abdominal bristle markers 38A and 57C were not significant in any of these models, most probably because they were linked to the same QTL as an adjacent marker in the model. These markers were removed from the analyses, and marker effects were reestimated for the remaining significant 9 sternopleural and 11 abdominal bristle number markers. The markers associated with sternopleural bristle number are mostly different from those associated with abdominal bristle number, as expected from the low-correlation among-line means for these characters ($r = 0.27$, averaged over temperatures and sexes).

The associations between marker genotypes and bristle number phenotypes were determined by treating the two homozygous marker classes and the heterozygous marker class separately. We tested whether the mean bristle numbers of the two homozygous marker classes were significantly different from each other. Furthermore, we tested whether the mean bristle number of lines containing heterozygotes differed from the average of the two homozygotes for each marker. The results of the tests of significance for each marker associated with bristle number in the full model and in the reduced models are given in Table 9. The significant effects are mostly for differences between homozygous marker classes, which is not unexpected since the number of heterozygous lines for most markers is very small, giving little power to detect heterozygous effects. However, there are two significant deviations from additivity: marker 35BC for sternopleural bristle number and marker 94D for abdominal bristle number. Although some markers are associated with bristle number in all sexes and environments (sternopleural bristle markers 57F, 61D, and 96F and abdominal bristle markers 21E and 57D), most have effects that are restricted to one sex (*e.g.*, abdominal bristle marker 92A), one or two temperatures (*e.g.*, sternopleural bristle marker 88E and abdominal bristle marker 67D), or are both temperature- and sex-specific (*e.g.*, sternopleural bristle marker 43E and abdominal bristle marker 1B). Of the 19 markers associated with variation in bristle number among the RI lines, 14 exhibit GEI with sex and/or temperature.

Homozygous effects: Estimates of homozygous effects of markers associated with bristle number are given in Tables 10 (sternopleural bristles) and 11 (abdominal bristles). Significant homozygous effects range from 0.52 to 1.82 sternopleural bristles (0.39 and 1.24 genetic standard deviation units, respectively) and 0.32 to 2.13 abdominal bristles (0.31 and 1.70 genetic standard deviation units, respectively). The proportion of V_G contributed by each QTL was estimated as $p(1 - p)a^2$, where p is the marker frequency and a is the difference in bristle number between homozygous marker classes. This estimate assumes additivity, which is true for most markers, and that the markers and QTLs are tightly linked, so the marker frequency is equal to the QTL frequency. Significant single-marker contributions to genetic variance range from 2.9 to 35% V_G for sternopleural bristles (Table 10) and from 2.5 to 27% V_G for abdominal bristles. Collectively, the significant sternopleural bristle markers explain 66, 70, and 57% of the variance among RI line means at 18°, 25°, and 29°, respectively. The significant abdominal bristle markers explain 70, 73, and 68% of the variance among RI lines at 18°, 25°, and 29°, respectively. The temperature × marker effects are as large as main effects, ranging from 0.33 to 1.84 bristles (data not shown).

Overdominance: Two significant deviations from additivity were found: sternopleural bristle marker 35BC and abdominal bristle marker 94D. The homozygous effect of 35BC on sternopleural bristle number is not

TABLE 9

Summary of significant marker effects from multiple regression of bristle number phenotypes on marker genotypes

		Sternopleural bristles								Abdominal bristles					
		18°		25°		29°				18°		25°		29°	
Marker	Three temperatures[a]	Male	Female	Male	Female	Male	Female	Marker	Three temperatures	Male	Female	Male	Female	Male	Female
4F	*M**, *S***	**	***	NS	NS	NS	*	1B	*T****, *ST**	NS	NS	NS	NS	NS	***
35BC	*T**	NS	NS	NS	NS	*†	NS	21E	*M****	***	*	**	**	**	*
43E	*ST**	NS	NS	NS	*	NS	*	29F	*M**, *S*	NS	***	NS	*	NS	NS
48D	*M**, *T**	NS	NS	**	NS	**	**	35BC	*M****	NS	**	**	***	**	**
57F	*M****, *ST**	**	*	***	***	**	***	43A	*ST***	NS	NS	NS	NS	NS	NS
61D	*M****	***	***	***	***	**	**	44F	*ST***	NS	*	**	NS	NS	NS
79E	*S****	NS	NS	***	NS	NS	NS	50D	*T***	NS	NS	NS	*	*	**
88E[b]		**	*					57D	*M****	***	***	***	***	***	***
96F	*M****	***	***	***	***	**	***	67D	*T****, *ST**	*	***	NS	NS	NS	NS
								92A	*M**, *S***	***	NS	**	NS	*	NS
								94D	*M***	**†	NS	*	NS	***	*

P values in the body of the table are for homozygous effect from the reduced model for each temperature and sex separately. NS, not significant; †, heterozygous effects; $P > 0.05$; *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

[a] *M*, main effect; *T*, marker × temperature effect; *S*, marker × sex effect; *ST*, marker × sex × temperature effect from full model including all markers, temperatures, and both sexes.

[b] Note that this marker is significant only at 18° but nevertheless does not contribute to an overall marker × temperature effect.

TABLE 10

Effects of sternopleural bristle number QTLs in three temperature environments

		Males				Females			
Marker	Temperature	*a*	SE	σ_G	$\%V_G$	*a*	SE	σ_G	$\%V_G$
4F	18°	**−0.91**	**(0.28)**	**−0.74**	**6.83**	**−1.37**	**(0.30)**	**−1.03**	**13.01**
	25°	−0.01	(0.28)	−0.01	0.00	−0.59	(0.32)	−0.40	1.99
	29°	−0.50	(0.44)	−0.29	1.08	**−1.13**	**(0.49)**	**−0.54**	**3.64**
35BC	18°	0.21	(0.33)	0.17	0.45	0.13	(0.35)	0.10	0.14
	25°	0.32	(0.32)	0.24	0.91	0.53	(0.37)	0.36	2.09
	29°	0.86	(0.51)	0.50	4.03	0.64	(0.57)	0.31	1.50
43E	18°	−0.06	(0.45)	−0.05	0.02	−0.11	(0.48)	−0.08	0.06
	25°	0.49	(0.44)	0.37	1.24	**1.05**	**(0.51)**	**0.72**	**4.67**
	29°	0.37	(0.71)	0.22	0.43	**1.71**	**(0.78)**	**0.82**	**6.10**
48D	18°	0.35	(0.43)	0.28	0.83	0.57	(0.46)	0.42	1.85
	25°	**1.17**	**(0.42)**	**0.88**	**7.89**	0.83	(0.49)	0.57	3.31
	29°	**2.25**	**(0.68)**	**1.33**	**18.23**	**2.34**	**(0.75)**	**1.13**	**13.11**
57F	18°	**0.78**	**(0.23)**	**0.64**	**7.71**	**0.52**	**(0.25)**	**0.39**	**2.86**
	25°	**1.14**	**(0.22)**	**0.86**	**13.83**	**1.28**	**(0.26)**	**0.88**	**14.55**
	29°	**1.02**	**(0.36)**	**0.60**	**6.85**	**1.33**	**(0.40)**	**0.64**	**7.73**
61D	18°	**0.77**	**(0.20)**	**0.63**	**9.96**	**1.10**	**(0.22)**	**0.83**	**16.82**
	25°	**0.83**	**(0.20)**	**0.63**	**9.66**	**0.98**	**(0.23)**	**0.67**	**11.06**
	29°	**0.97**	**(0.32)**	**0.57**	**8.15**	**1.16**	**(0.35)**	**0.56**	**7.67**
79E	18°	0.13	(0.25)	0.11	0.28	−0.50	(0.27)	−0.38	3.28
	25°	**0.97**	**(0.24)**	**0.73**	**12.35**	0.11	(0.28)	0.07	0.13
	29°	−0.01	(0.39)	−0.01	0.00	−0.53	(0.43)	−0.25	1.51
88E	18°	**0.73**	**(0.26)**	**0.60**	**7.87**	**0.61**	**(0.28)**	**0.46**	**4.56**
	25°	0.09	(0.26)	0.07	0.11	0.14	(0.30)	0.09	0.19
	29°	0.13	(0.41)	0.08	0.14	−0.25	(0.45)	−0.12	0.33
96F	18°	**−1.53**	**(0.23)**	**−1.26**	**31.26**	**−1.77**	**(0.24)**	**−1.33**	**35.00**
	25°	**−1.70**	**(0.22)**	**−1.28**	**32.24**	**−1.82**	**(0.26)**	**−1.24**	**30.49**
	29°	**−1.10**	**(0.36)**	**−0.65**	**8.37**	**−1.54**	**(0.39)**	**−0.74**	**10.84**

Significant effects indicated in boldface type; *a*, homozygous effects for the 2b marker relative to Oregon; σ_G, genetic standard deviation units; $\%V_G$, percent genetic variation.

significant overall, or for any sex and temperature combination (Tables 9, 10). However, at 29°, this marker has a significant ($P = 0.01$) effect in heterozygotes of 1.87 sternopleural bristles (relative to the mean of 2b and Oregon homozyogtes for this marker) in males. Abdominal bristle marker 94D has significant effects of −0.61 bristle in males at 25° and of −1.05 and −0.59 bristle in males and females, respectively, at 29°. This marker also has a significant ($P = 0.003$) heterozygous effect of 1.38 abdominal bristle in males at 18°. Therefore, heterozygous effects are temperature- and sex-specific (*i.e.*, exhibit GEI). Since the frequency of heterozygotes is very low for most markers in these RI lines (0.07 for marker 35BC and 0.05 for marker 94D, for example), our power to detect heterozygous effects in general and overdominant effects in particular is limited.

DISCUSSION

Levels of naturally occurring variation for Drosophila abdominal and sternopleural bristle number far exceed those predicted from a balance between mutational input and selective elimination (Barton 1990; Caballero and Keightley 1994; Houle *et al.* 1996). It is quite likely, therefore, that an additional mechanism or mechanisms act(s) to preserve genetic variation for these traits. One possibility is that variation is maintained in the face of environmental heterogeneity by GEI (Felsenstein 1976; Via and Lande 1987; Gillespie and Turelli 1989).

Here, we have confirmed that the first criterion for the maintenance of variation by GEI is met: There is substantial GEI variance for sensory bristle number among a panel of RI lines established from two homozygous laboratory strains. The total genotype × temperature, genotype × sex, and genotype × temperature × sex interaction variance was over 40% (30%) the genetic variance of sternopleural (abdominal) bristle number when all three temperatures were considered. The relative magnitude of the GEI variance was, however, greatest between the most extreme temperature pair (88% and 52% of the total genetic variance for sternopleural and abdominal bristles, respectively, averaged over sexes). There is genetic variation for sex dimorphism of both bristle traits (genotype × sex interaction). For

TABLE 11

Effects of abdominal bristle number QTLs in three temperature environments

		Males				Females			
Marker	Temperature	*a*	SE	σ_G	$\%V_G$	*a*	SE	σ_G	$\%V_G$
1B	18°	−0.20	(0.17)	−0.20	0.89	−0.09	(0.22)	−0.07	0.12
	25°	0.04	(0.19)	0.03	0.02	0.23	(0.21)	0.17	0.68
	29°	0.32	(0.20)	0.26	1.51	**1.06**	**(0.20)**	**0.94**	**20.58**
21E	18°	**−0.56**	**(0.16)**	**−0.55**	**7.38**	**−0.47**	**(0.21)**	**−0.37**	**3.27**
	25°	**−0.60**	**(0.18)**	**−0.48**	**5.56**	**−0.56**	**(0.20)**	**−0.41**	**4.02**
	29°	**−0.67**	**(0.20)**	**−0.53**	**6.89**	**−0.41**	**(0.19)**	**−0.36**	**3.21**
29F	18°	−0.33	(0.18)	−0.32	2.31	**−0.88**	**(0.23)**	**−0.70**	**10.78**
	25°	−0.16	(0.20)	−0.13	0.37	**−0.47**	**(0.23)**	**−0.35**	**2.68**
	29°	−0.22	(0.22)	−0.18	0.70	−0.17	(0.21)	−0.15	0.53
35BC	18°	0.40	(0.26)	0.40	2.52	**1.11**	**(0.34)**	**0.87**	**12.12**
	25°	**0.80**	**(0.30)**	**0.64**	**6.59**	**1.34**	**(0.33)**	**0.98**	**15.21**
	29°	**1.01**	**(0.32)**	**0.81**	**10.32**	**0.83**	**(0.31)**	**0.74**	**8.79**
43A	18°	0.74	(0.54)	0.74	7.39	−0.39	(0.69)	−0.31	1.31
	25°	−0.47	(0.60)	−0.38	1.95	0.88	(0.68)	0.64	5.59
	29°	−0.15	(0.65)	−0.12	0.19	−0.29	(0.63)	−0.26	0.92
44F	18°	0.30	(0.61)	0.30	0.82	**1.69**	**(0.79)**	**1.33**	**16.20**
	25°	**2.13**	**(0.68)**	**1.70**	**26.57**	0.85	(0.77)	0.62	3.52
	29°	1.05	(0.74)	0.84	6.38	1.00	(0.72)	0.89	7.21
50D	18°	−0.09	(0.27)	−0.09	0.07	0.04	(0.36)	0.03	0.01
	25°	0.32	(0.31)	0.26	0.61	**0.88**	**(0.35)**	**0.64**	**3.81**
	29°	**0.74**	**(0.34)**	**0.60**	**3.28**	**0.92**	**(0.32)**	**0.82**	**6.31**
57D	18°	**0.99**	**(0.16)**	**0.98**	**21.51**	**0.95**	**(0.21)**	**0.75**	**12.48**
	25°	**1.17**	**(0.18)**	**0.93**	**19.65**	**1.13**	**(0.21)**	**0.82**	**15.21**
	29°	**0.97**	**(0.20)**	**0.78**	**13.50**	**1.05**	**(0.19)**	**0.94**	**19.84**
67D	18°	**−0.32**	**(0.16)**	**−0.31**	**2.47**	**−0.84**	**(0.20)**	**−0.66**	**10.83**
	25°	0.15	(0.18)	0.12	0.38	−0.26	(0.20)	−0.19	0.89
	29°	0.07	(0.19)	0.05	0.07	0.24	(0.18)	0.22	1.19
92A	18°	**−1.04**	**(0.20)**	**−1.03**	**23.32**	0.09	(0.26)	0.07	0.11
	25°	**−0.75**	**(0.23)**	**−0.60**	**8.04**	−0.36	(0.26)	−0.27	1.56
	29°	**−0.57**	**(0.25)**	**−0.45**	**4.57**	−0.11	(0.24)	−0.10	0.21
94D	18°	−0.40	(0.21)	−0.40	3.09	−0.44	(0.27)	−0.35	2.35
	25°	**−0.61**	**(0.24)**	**−0.49**	**4.79**	−0.40	(0.27)	−0.29	1.70
	29°	**−1.05**	**(0.26)**	**−0.84**	**13.95**	**−0.59**	**(0.25)**	**−0.53**	**5.52**

Significant effects indicated in boldface type; *a*, homozygous effects for the 2b marker relative to Oregon; σ_G, genetic standard deviation units; $\%V_G$, percent genetic variation.

sternopleural bristle number, the magnitude of this interaction variance is similar across all pairs of environments; it is the genotype × temperature interaction variance that increases between 18° and 29°, in both sexes. The relative magnitude of the genotype × temperature interaction variance for abdominal bristle number not only changes with the pair of temperatures considered but with sex; GEI for this trait is greater for females than males at all temperatures, especially between 25° and 29° and 18° and 29°.

Similar patterns of GEI variance of sternopleural and abdominal bristle number in response to thermal environments greater in females than in males, but much more pronounced for abdominal bristle number, and of increased genotype × temperature interaction variance between 18° and 29°, have been observed for new spontaneous mutations affecting these traits (Mackay and Lyman 1998). Caligari and Mather (1975) also reported significant genotype × temperature interaction (but not genotype × sex interaction) for sternopleural bristle number in their diallel cross-analysis of chromosome substitution lines between two laboratory strains; and Gupta and Lewontin (1982) found substantial variation in temperature reaction norms of abdominal bristle number among homozygous and heterozygous second chromosomes of *D. pseudoobscura*, both within and between sexes. Since only a limited number of genotypes and environments have been sampled in these studies, and harsher environmental contrasts like those prevalent in nature are likely to invoke greater GEI than the relatively mild conditions tested in the laboratory (Kondrashov and Houle 1994), the conclusion that sensory bristle numbers exhibit large amounts of GEI is inescapable. Estimates of genetic and mutational variance obtained under standard laboratory conditions may thus have little relevance to the true parameters

required in models of maintenance of genetic variance and response to natural and artificial selection (Gillespie and Turelli 1989). Future quantitative genetic analyses using model organisms should incorporate ecologically reasonable environmental treatments whenever possible to partly ameliorate this problem. Furthermore, our observation that GEI may be sex-specific (Mackay and Lyman 1998; this article) adds an unexpected complexity to the issue of maintenance of quantitative variation by GEI. However, genotype by sex variation is often observed for autosomal genes affecting bristle number (Long *et al.* 1995; Mackay *et al.* 1995; Lyman *et al.* 1996) and other quantitative traits (Anholt *et al.* 1996; Mackay *et al.* 1996; Nuzhdin *et al.* 1997).

Predictions of whether genetic variance can be maintained by GEI depend on the genetic basis of GEI and the nature and intensity of selection acting on the trait within and between environments, as well as on the nature and frequencies of relevant environments. Here, we have begun to examine the genetic basis of GEI by mapping QTLs affecting sternopleural and abdominal bristle number in each of the temperature environments and by linkage to polymorphic insertion sites of *roo* transposable element markers. Of the 19 markers associated with variation in bristle number in at least one temperature and sex, 6 had significant main effects only; the remaining 14 markers had significant interactions with temperature (5 markers), sex (4 markers) and/or sex × temperature (6 markers). Clearly, there is heterogeneity among QTLs affecting bristle number in their sensitivity to thermal and sexual environments.

At the trait level, we observed positive genetic correlations of bristle number across environments. In the model of Via and Lande (1987), maintenance of a level of segregating genetic variation beyond that expected from the equilibrium between mutation and selection by GEI is not assured unless the cross-environment genetic correlation is −1. Therefore, we can ask to what extent the sign of effects changes across environments, and to what extent the magnitude, but not direction of significant effects changes among those QTLs exhibiting GEI. There is one example of a change of sign: abdominal bristle marker 43A. The 2b allele is associated with increased bristle number in males and decreased bristle number in females at 18°, relative to the Oregon allele, but decreased bristle number in males and increased bristle number in females at 25°. At this marker, there is a negative association between the phenotypes across both temperature and sex environments, and a significant genotype × temperature × sex interaction. This pattern of GEI is one that could potentially result in the maintenance of polymorphism for abdominal bristle number, depending on the form of selection. For all but one other marker for which GEI was observed, interactions were attributable to detection of the QTL in one sex and/or in one or two environments; the exception was a change in relative magnitude of sternopleural bristle marker 57F in males and females across temperatures.

Genetic correlations between the mean and sensitivity, averaged over all loci, were generally negative. Values of r_{MS} for sternopleural bristle number in contrasts involving 29° were approximately −0.5. Averaged over all pairs of environments, the correlations between means and sensitivities of abdominal bristle number were close to zero, suggesting that the mean and sensitivity are largely under the control of different genes. However, between each pair of environments, r_{MS} is moderately high in one sex, although the sign of the correlation and the sex with the highest absolute value follows no discernable pattern. At the level of individual QTLs, the correlation between mean performance across pairs of environments and sensitivity is poor: Some markers are associated with a large effect on the mean but are insensitive to the environment, whereas others with small effects on the mean are relatively more sensitive. For each of the markers associated with a significant effect on bristle number in at least one of a pair of environments, we computed the mean effect as $M = 0.5(X_H + X_L)$, and the sensitivity as $S = (X_H - X_L)/D$, where X_H and X_L are the effects in the environment that confer high and low bristle number, respectively, averaged over all loci, and D is the mean difference between environments (Falconer 1990). The correlations between the mean and sensitivity, averaged over all markers and pairs of environments in which at least one significant effect was detected, were −0.25 and −0.44 for male and female sternopleural bristle number, respectively, and −0.20 and 0.18 for male and female abdominal bristle number, respectively, in good agreement with the more precise estimates from among-line variance components. There are no apparent constraints to the independent evolution of the mean and sensitivity of bristle number.

We cannot use our mapping results to discriminate between pleiotropy and epistasis (Scheiner 1993; Via *et al.* 1995) as the root cause of GEI because a QTL that is mapped in a restricted set of environments could logically be either a conditionally expressed bristle gene or a conditionally expressed gene that does not itself directly affect bristle number but interacts with one or more bristle number loci. In the latter, epistatic case, the interacting locus will be mapped by virtue of its interaction effect. With only 98 RI lines, we do not have enough degrees of freedom to test statistically for all pairwise interactions of our mapped factors. Neither do these data directly address the issue of the relative magnitudes of GEI for heterozygotes and homozygotes for QTLs affecting the trait, as required to evaluate the model of Gillespie and Turelli (1989) since most loci are homozygous in the RI lines. However, for one marker, significant marker genotype by temperature and sex environment effects were found for heterozygotes but not homozygotes.

TABLE 12

Cytogenetic markers (map position) and candidate bristle number loci

Marker	Interval[a]	Candidate loci[b]	Reference[c]
1B (1-0)	1B-5D (1.0–1.15)	*ASC* (1-0.0); *N* (1-3.0)	6, 7, 11, 13, 16
4F (1-10)	3E-5D (1.4–1.15)		7, 13, 16, 18
21E (2-1)	21E-22F (2.1–2.4)		18
29F (2-32)	22F-33E (2.4–2.45)	*Sp* (2-22.0); *numb* (2-35.0); *bib* (2-34.7)	2, 6, 7
35BC (2-50)	34EF-38E (2.48–2.54)		
43A (2-55)	39A-44F (2.54–2.58)		
43E (2-57)	33E-49D (2.45–2.67)		
44F (2-58)	44C-46C (2.58–2.59)		
48D (2-64)	46C-49D (2.64–2.67)	*sca* (2-66.7)	3, 8, 12, 14
50D (2-70)	50B-50F (2.69–2.70)		
57D (2-97)	50F-57F (2.70–2.97)	*sm* (91.5)	9, 14
57F (2-97)	50F-60E (2.70–2.107)	*sm* (91.5)	9, 14
61D (3-0)	61A-63A (3.0–3.3)	*emc* (3-0.0)	13, 14, 15, 16
67D (3-33)	65D-68B (3.22–3.35)	*qm* (3-23); *abd* (3-26.5); *h* (3-27); *Hsp22-Hsp-G3* (3-28); *Sod* (3-34.6)	5, 10, 13, 14, 15
79E (3-47)	69D-87B (3.39–3.51)	*mab* (3.47); *dsx* (3-48); *atonal* (3-48); *neu* (3-50)	1, 4, 10, 13, 16
88E (3-57)	82D-91D (3.47–3.64)	*Tft* (3-53.6); *abdA*, *abdB* (3-58)	10, 13, 16
92A (3-65)	91A-93B (3.63–3.70)	*Dl* (3-66.2); *H* (3-69.5)	13, 14, 15, 17, 18
94D (3-76)	91D-96A (3.64–3.84)		10, 13, 16, 18
96F (3-89)	94D-98A (3.76–3.100)	*E(spl)* (3-89.1); *Pr* (3-90)	10, 13, 14, 16, 18

[a] Approximate bounds on intervals were determined by the position of the first nonsignificant marker flanking the significant marker.

[b] Gene names are given in Lindsley and Zimm (1992). Candidate genes affect bristle number development or have bristle number mutant phenotypes, with the exception of the small heat shock proteins (*Hsp22-Hsp-G3*) and *Superoxide dismutase* (*Sod*), which are listed because they are sensitive to environmental conditions.

[c] References are to studies mapping bristle number QTLs in which the same interval was significant: (1) Breese and Mather 1957; (2) Gibson and Thoday 1962; (3) McBride and Robertson 1963; (4) Wolstenholme and Thoday 1963; (5) Thoday *et al.* 1964; (6) Spickett and Thoday 1966; (7) Davies 1971; (8) Frankham 1980; (9) Frankham and Nurthen 1981; (10) Shrimpton and Robertson 1988; (11) Mackay and Langley 1990; (12) Lai *et al.* 1994; (13) Long *et al.* 1995; (14) Mackay and Fry 1996; (15) Long *et al.* 1996; (16) M. C. Gurganus, S. V. Nuzhdin and T. F. C. Mackay, unpublished results; (17) Long *et al.* 1998; and (18) Nuzhdin *et al.* 1988.

To further resolve outstanding questions regarding the feasibility that quantitative genetic variation is maintained by GEI, we need to go beyond QTL mapping to the actual loci causing variation in the trait. For a given locus, we require data on homozygous and heterozygous effects of alleles affecting the trait in different environments to determine empirically whether there are negative correlations in effects across environments, negative correlations between mean performance and sensitivity, and/or differences between homozygotes and heterozygotes in sensitivity, all for a realistic sample of alleles and environments. Discrimination of epistasis or pleiotropy as the cause of GEI requires similar analyses for all possible two-locus genotypes.

Initial QTL analysis falls far short of this goal. One can only detect QTLs if there is a difference between the parental strains in alleles affecting the trait. Thus QTLs detected between two lines will be a sample of the total number of loci causing variation in the trait, in the environments chosen for analysis. If many QTL alleles are conditionally expressed in nonstandard laboratory environments, QTLs segregating even between one pair of lines can be missed if the analysis is done in a single environment. Here, sternopleural bristle markers 4F, 35BC, and 88E and abdominal bristle markers 1B and 67D were detected only in 18° and/or 29° environments. It is not clear how many additional QTLs would be found if other environments were considered. The issue of genetic sampling is also complex. The expected difference in QTL maps between a random pair of strains depends on the distribution of allelic effects at the loci affecting the trait in nature. If there are a large number of alleles segregating at each locus, any pair of strains is likely to contain different alleles, and all QTLs could in principle be mapped using any two homozygous lines, provided the experimental design had sufficient power to detect small allelic differences. On the other hand, if there are only two alleles at each locus, the probability of capturing alternate alleles in two random strains depends on the gene frequency. Loci at which allele frequencies are intermediate will be sampled more frequently than loci with one common and one rare allele; intermediate gene frequencies are more likely to be the result of balancing selection than rare alleles, which might be maintained by mutation-selection balance. We can extract no information about gene frequency, and hence what QTLs are *a priori* candidates for GEI analysis, from a single mapping study.

Neither can we ascertain whether the QTL alleles were initially segregating in the natural populations from which the parental lines were derived, or whether they were new mutations that occurred during and after the parental lines were made homozygous. Finally, QTLs are mapped to a large genetic region, even in fine-scale mapping studies such as the one described here. QTLs are not genetic loci, and progress toward defining a QTL at this level can proceed either by fine-scale recombination mapping or by searching for candidate genes with developmental or biochemical effects on the trait that have been identified by mutational analysis and that map to the QTL region. The former approach is limited by the size of the allelic effect and is likely to succeed only when effects are large, and the latter is limited by our understanding of the trait.

Drosophila bristle number has been used as a model quantitative trait for over half a century. There have been many efforts to map the loci affecting variation in bristle number, using different base populations and different methods (reviewed by Mackay 1996). If some QTLs are maintained by balancing selection, one predicts that they would be at intermediate frequency in many populations and thus would be mapped in multiple studies. (Concordance of QTL map position across studies is not proof of balancing selection because the mapped QTLs could correspond to different bristle number loci, and a high mutation rate would give the same result.) There are also a large number of candidate genetic loci that might segregate for alleles affecting variation in bristle number in nature (Mackay 1995, 1996)—many of these are loci controlling sensory bristle development (Campos-Ortega 1993; Jan and Jan 1993), others are not developmental loci but have strong mutant bristle number phenotypes (Lindsley and Zimm 1992). Failure of QTL alleles to complement candidate gene mutations (Mackay and Fry 1996; Long *et al.* 1996) and association of molecular polymorphism in naturally segregating alleles of candidate genes with quantitative trait phenotypes (Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998) can implicate the candidate gene as the genetic locus corresponding to the QTL. Therefore, the most promising QTL regions for future GEI analysis are those that have been mapped previously and in which there are good candidate genes affecting bristle number.

The markers associated with bristle number QTLs in this study, candidate genes in the region, and previous studies detecting bristle number QTLs in these regions are summarized in Table 12. Several gene regions are detected repeatedly across studies, and for some of these regions, quantitative complementation and/or allelic association tests implicate candidate loci controlling bristle development as the genetic locus at which QTL alleles segregate (*achaete-scute*, *scabrous*, *hairy*, and *Delta*). Studying the interactions between environment and genome in creating a phenotype is a particularly difficult problem for continuously varying traits, but in the future, analysis of allelic effects across environments at loci responsible for quantitative variation in Drosophila bristle number may provide a solid foundation for understanding the genetic basis of environmental sensitivity in general.

We thank Jeff Leips and Marta Wayne for comments on the manuscript. This work was supported by National Institutes of Health grants GM 45344 and GM 45146 to T.F.C.M., National Science Foundation Grant DEB 9317754 to J.D.F. and T.F.C.M., and grant 97-04-48101 from the Russian Foundation of Basic Research to E.G.P.

LITERATURE CITED

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

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

Barton, N. H., and M. Turelli, 1989 Evolutionary quantitative genetics: How little do we know? Annu. Rev. Genet. **23:** 337–370.

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

Caballero, A., and P. D. Keightley, 1994 A pleiotropic nonadditive model of variation in quantitative traits. Genetics **138:** 883–900.

Caligari, P. D. S., and K. Mather, 1975 Genotype-environment interaction. III. Interactions in *Drosophila melanogaster*. Proc. R. Soc. Lond. B. Biol. Sci. **191:** 387–411.

Campos-Ortega, J. A., 1993 Early neurogenesis in *Drosophila melanogaster*, pp. 1091–1129 in *The Development of Drosophila melanogaster*, Vol. 2, edited by M. Bate and A. Martinez Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Clayton, G., and A. Robertson, 1955 Mutation and quantitative variation. Am. Nat. **89:** 151–158.

Cockerham, C. C., 1963 Estimation of genetic variances, pp. 53–94 in *Statistical Genetics and Plant Breeding*, edited by W. D. Hanson and H. F. Robertson. National Academy of Sciences–National Research Council, Washington, DC.

Davies, R. W., 1971 The genetic relationship of two quantitative characters in *Drosophila melanogaster*. II. Location of the effects. Genetics **69:** 363–375.

Doerge, R. W., and G. A. Churchill, 1996 Permutation tests for multiple loci affecting a quantitative character. Genetics **142:** 285–294.

Endler, J. A., 1986 *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.

Falconer, D. S., 1960 Selection of mice for growth on high and low planes of nutrition. Genet. Res. **1:** 91–113.

Falconer, D. S., 1990 Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. Genet. Res. **56:** 57–70.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics*, Ed. 4. Addison Wesley Longman, Harlow, Essex, UK.

Felsenstein, J., 1976 The theoretical population genetics of variable selection and migration. Annu. Rev. Genet. **10:** 253–280.

Frankham, R., 1980 The founder effect and response to artificial selection in Drosophila, pp. 87–90 in *Selection Experiments in Laboratory and Domestic Animals*, edited by A. Robertson. Commonwealth Agricultural Bureaux, Slough, UK.

Frankham, R., and R. K. Nurthen, 1981 Forging links between population and quantitative genetics. Theor. Appl. Genet. **59:** 251–263.

Gibson, J. B., and J. M. Thoday, 1962 Effects of disruptive selection. VI. Analysis of a second chromosome polymorphism. Heredity **17:** 1–26.

Gillespie, J. H., and M. Turelli, 1989 Genotype-environment inter-

actions and the maintenance of polygenic variation. Genetics **121:** 129–138.

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

Houle, D., 1992 Comparing evolvability and variability of quantitative traits. Genetics **130:** 195–204.

Houle, D., B. Morikawa and M. Lynch, 1996 Comparing mutational variabilities. Genetics **143:** 1467–1483.

Jan, Y. N., and L. Y. Jan, 1993 The peripheral nervous system, pp. 1207–1244 in *The Development of Drosophila melanogaster*, Vol. 2, edited by M. Bate and A. Martinez Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Jansen, R. C., and P. Stam, 1994 High resolution of quantitative traits into multiple loci via interval mapping. Genetics **136:** 1447–1455.

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

Kondrashov, A. S., and D. Houle, 1994 Genotype-environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. Proc. R. Soc. Lond. Ser. B Biol. Sci. **258:** 221–227.

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

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

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

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

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

Mackay, T. F. C., 1995 The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system. Trends Genet. **11:** 464–470.

Mackay, T. F. C., 1996 The nature of quantitative genetic variation revisited: lessons from *Drosophila* bristles. BioEssays **18:** 113–121.

Mackay, T. F. C., and J. D. Fry, 1996 Polygenic mutation in *Drosophila melanogaster*: Genetic interactions between selection lines and candidate quantitative trait loci. Genetics **144:** 671–688.

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

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

Mackay, T. F. C., J. B. Hackett, R. F. Lyman, M. L. Wayne and R. R. H. Anholt, 1996 Quantitative genetic variation of odor-guided behavior in a natural population of *Drosophila melanogaster*. Genetics **144:** 727–735.

Mackay, T. F. C., and R. F. Lyman, 1998 Polygenic mutation in *Drosophila melanogaster*: Genotype × environment interaction for spontaneous mutations affecting bristle number. Genetica (in press).

McBride, G., and A. Robertson, 1963 Selection using assortative mating in *Drosophila melanogaster*. Genet. Res. **4:** 356–369.

Mousseau, T. A., and D. A. Roff, 1987 Natural selection and the heritability of fitness components. Heredity **59:** 181–197.

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

Nuzhdin, S. V. , E. G. Pasyukova, C. L. Dilda and T. F. C. Mackay, 1997 Sex-specific quantitative trait loci affecting longevity. Proc. Natl. Acad. Sci. USA **94:** 9734–9739.

Nuzhdin, S. V., P. D. Keightley, E. G. Pasyukova and E. A. Morozova, 1998 Mapping quantitative trait loci affecting *Drosophila melanogaster* sternopleural bristle number using changes of marker frequencies in divergently selected lines. Genet. Res. (in press).

Pasyukova, E. G., and S. V. Nuzhdin, 1993 *Doc* and *copia* instability in an isogenic *Drosophila melanogaster* stock. Mol. Gen. Genet. **240:** 302–306.

Roff, D. A., and T. A. Mousseau, 1987 Quantitative genetics and fitness: lessons from *Drosophila*. Heredity **58:** 103–118.

Robertson, A., 1959 The sampling variance of the genetic correlation coefficient. Biometrics **15:** 469–485.

SAS Institute, Inc., 1988 *SAS/STAT User's Guide, Release 6.03 Edition*. SAS Institute Inc., Cary, NC.

Scheiner, S. M., 1993 Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst. **24:** 35–68.

Shrimpton, A. E., and A. Robertson, 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.

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

Thoday, J. M., J. B. Gibson and S. G. Spickett, 1964 Regular responses to selection. 2. Recombinations and accelerated response. Genet. Res. **5:** 1–19.

Via, S., R. Gomulkiewicz, G. de Jong, S. M. Scheiner, C. D. Schlichting *et al.*, 1995 Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol. Evol. **10:** 212–217.

Via, S., and R. Lande, 1987 Evolution of genetic variation in a spatially heterogeneous environment. Genet. Res. **49:** 147–156.

Wolfram, S., 1991 *Mathematica*, 2/e. Addison-Wesley Publishing Company, Inc., New York.

Wolstenholme, D. R., and J. M. Thoday, 1963 Effects of disruptive selection. VII. A third chromosome polymorphism. Heredity **10:** 413–431.

Zeng, Z.-B., 1993 Theoretical basis of precision mapping of quantitative trait loci. Proc. Natl. Acad. Sci. USA **90:** 10972–10976.

Zeng, Z.-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

Communicating editor: C. Haley