# Association of single-nucleotide polymorphisms at the *Delta* locus with genotype by environment interaction for sensory bristle number in *Drosophila melanogaster*

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**Summary**

The nature of forces maintaining variation for quantitative traits can only be assessed at the level of individual genes affecting variation in the traits. Identification of single-nucleotide polymorphisms (SNPs) associated with variation in *Drosophila* sensory bristle number at the *Delta* (*Dl*) locus provides us with the opportunity to test a model for the maintenance of variation in bristle number by genotype by environment interaction (GEI). Under this model, genetic variation is maintained at a locus under stabilizing selection if phenotypic values of heterozygotes are more stable than homozygotes across a range of environments, and the mean allelic effect is much smaller than the standard deviation of allelic effects across environments. Homozygotes and heterozygotes for two SNPs at *Dl*, one affecting sternopleural and the other abdominal bristle number, were reared in five different environments. There was significant GEI for both bristle traits. Neither condition of the model was satisfied for *Dl* SNPs exhibiting GEI for sternopleural bristle number. Heterozygotes for the abdominal bristle number SNPs were indeed the most stable genotype for two of the three environment pairs exhibiting GEI, but the mean genotypic effect was greater than the standard deviation of effects across environments. Therefore, this mechanism of GEI seems unlikely to be responsible for maintaining the common bristle number polymorphisms at *Dl*.

## 1. Introduction

The paradox of widespread segregating variation for quantitative traits in natural populations, despite strong stabilizing and directional selection that should eliminate variation, is unresolved (Barton & Turelli, 1989). The problem is not a dearth of potential mechanisms that, in theory, lead to the maintenance of genetic variation, but rather a lack of empirical data with which to evaluate the theory. For example, some fraction of the variation for all quantitative traits must be that expected at equilibrium between the input of new deleterious alleles by mutation and their elimination by natural selection (Barton, 1990), and some fraction might be selectively neutral, with variation maintained by a balance between mutation and drift (Lynch & Hill, 1986). Overdominance of alleles associated with intermediate trait values (Robertson, 1967; Barton, 1990) and epistasis (Gimelfarb, 1989) will maintain variation at loci affect-

ing traits under stabilizing selection. Variation can also be maintained when there is genotype by environment interaction (GEI) for fitness, if alleles have opposing (Levene, 1953) or variable (Fry *et al.*, 1996) effects on fitness in different environments, or if heterozygotes are less sensitive than homozygotes to environmental variation (Gillespie & Turelli, 1989).

These mechanisms are not mutually exclusive, and one might expect heterogeneity in mechanisms promoting genetic variation between different quantitative trait loci (QTLs) affecting variation in a single trait, and even among different alleles at a single locus (Long *et al.*, 2000). (Here, QTL refers to the actual genetic locus at which alleles affecting the trait segregate in nature.) The difficulty of addressing this problem empirically is apparent: not only do we need to know which QTLs affect variation in the trait of interest, in a range of ecologically relevant environments, but we also need to know what molecular polymorphisms at the QTLs actually cause the difference in trait phenotype (the 'quantitative trait nucleotides' or QTNs), their allele frequencies and the

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fitness effects of different QTL genotypes, again in a range of environments.

Currently, no quantitative trait in any organism is understood at this level of detail. However, progress towards this goal has been made for sensory bristle number in *Drosophila melanogaster*. Abdominal and sternopleural bristle numbers have abundant genetic variation in natural populations and respond rapidly to artificial selection (e.g. Long *et al.*, 1995; Gurganus *et al.*, 1999). Bristle numbers are thought to be under strong stabilizing selection because mean bristle numbers are relatively stable across natural populations despite the potential for divergence. However, efforts to deduce the relationship between bristle numbers and fitness in the laboratory have reached contradictory conclusions, with experiments supporting strong (Kearsey & Barnes, 1970; Linney *et al.*, 1971; Nuzhdin *et al.*, 1995), moderate (Clayton *et al.*, 1957; Latter & Robertson, 1962; García-Dorado & González, 1996) or very weak (Spiers, 1974; Mackay, 1985) stabilizing selection. Such inconsistencies could well reflect underlying heterogeneity in the relationships of genotypes at segregating bristle number QTLs to fitness between the different populations used.

Several loci affecting peripheral nervous system (PNS) development are bristle-number QTLs, and molecular polymorphisms associated with QTNs with large effects on bristle number have been identified at these loci (Long *et al.*, 1998, 2000; Lyman *et al.*, 1999). The fitness effects of the QTL genotypes are not known. However, molecular polymorphisms at intermediate frequency are likely to be associated with causal QTNs that are also at intermediate frequency and not with rare QTNs with very large effects (Long *et al.*, 1998). Models for the maintenance of variation by mutation–selection balance predict that equilibrium levels of genetic variance are reached when the mutant allele is at low frequency (Barton & Turelli, 1989; Barton, 1990); therefore, polymorphisms at intermediate frequency are not consistent with this mechanism. Intermediate frequency polymorphisms are consistent with maintenance by some form of balancing selection or with selective neutrality.

'Real' stabilizing selection is selection based on the value of the trait, as opposed to 'apparent' stabilizing selection owing to overdominance of alleles associated with intermediate trait values (Robertson, 1967; Barton, 1990) or deleterious pleiotropic fitness effects of alleles causing extreme phenotypes (Barton, 1990; Keightley & Hill, 1990; Kondrashov & Turelli, 1992). For loci under such 'real' selection, intermediate frequency polymorphisms can be maintained by GEI for the trait (Gillespie & Turelli, 1989). This model assumes stabilizing selection for a single phenotype that is optimal in all environments, constant fitnesses across environments, alleles with different additive effects on the trait in different environments and that

the mean and variance of allelic effects across environments are small. The last of these assumptions thus requires that the mean differences of average allelic effects must be very small relative to the standard deviation of allelic effects across environments. Under this model, heterozygotes will tend to have lower phenotypic variance than homozygotes and higher mean fitness. Testing this model requires that we (1) identify a common molecular polymorphism at a QTL associated with variation in a trait that is under stabilizing selection and (2) determine genotypic values of homozygous and heterozygous QTL genotypes across a range of environments. Here, we report the results of this test for single nucleotide polymorphisms (SNPs) at the *Delta* (*Dl*) locus, which have been associated with variation for sternopleural and abdominal bristle number (Long *et al.*, 1998).

## 2. Materials and methods

### (i) *Construction of Drosophila stocks*

Approximately 60 isogenic third chromosomes were derived from wild-caught flies and substituted into the inbred Samarkand *X* and second chromosome genetic background. Near-isoallelic lines containing the wild-derived *Dl* allele and an average of 10 cM flanking genomic fragment to either side, in an otherwise Samarkand chromosome 3 background, were constructed from each of the whole chromosome substitution lines by ten generations of back-crossing. For further details, see Lyman & Mackay (1998).

### (ii) *SNP associations with sensory bristle number*

Long *et al.* (1998) conducted a survey of restriction site variation in the *Dl* locus and examined the association of molecular variation with phenotypic variation in sternopleural and abdominal bristle number. Two SNPs in *Dl* were in significant linkage disequilibrium with bristle number QTN, as judged by a permutation test.

An *Hae*III restriction site polymorphism in the second intron (*Hae*III $+8.6$, represented here by H) was associated with a difference in sternopleural bristle number in both sexes. In the near-isoallelic lines, the mean sternopleural bristle numbers ($\pm$ standard error) of flies homozygous for the presence (H11) of this restriction site were $19.12 \pm 0.14$ in males and $20.16 \pm 0.15$ in females. The mean sternopleural bristle numbers in flies homozygous for the absence (H00) of this restriction site were $18.53 \pm 0.10$ in males and $19.36 \pm 0.11$ in females. Averaged over both sexes, the H11 genotype produces $\sim 0.7$ more sternopleural bristles than does the H00 genotype.

An *Scr*FI restriction site polymorphism in the fifth intron (*Scr*FI $+18.6$, represented here by S) was associated with a difference in abdominal bristle

number in females only. In the near-isoallelic lines, the mean abdominal bristle numbers ($\pm$ standard error) of flies homozygous for the presence (S11) of this restriction site were $19 \cdot 22 \pm 0 \cdot 19$ in males and $22 \cdot 03 \pm 0 \cdot 27$ in females. The mean abdominal bristle numbers in flies homozygous for the absence (S00) of this restriction site were $19 \cdot 43 \pm 0 \cdot 17$ in males and $23 \cdot 21 \pm 0 \cdot 21$ in females. In females, the S00 genotype produces $\sim 1 \cdot 2$ more abdominal bristles than does the S11 genotype.

### (iii) Crosses between Dl near-isoallelic lines

Homozygous near-isoallelic *Delta* lines were grouped into four haplotypes according to the presence (1) or absence (0) of the H and S sites, respectively (i.e. 00, 01, 10, 11). Three lines of each haplotype were chosen at random for each of the bristle traits (the lines representing each haplotype are given in parentheses; see Long *et al.*, 1998, appendix): low sternopleural bristle number haplotypes 00 (33, 41, 51) and 01 (13, 22, 31); high sternopleural bristle number haplotypes 11 (15, 17, 84) and 10 (10, 50, 116); low abdominal bristle number haplotypes 11 (58, 111, 119) and 01 (19, 46, 95); high abdominal bristle number haplotypes 10 (10, 86, 116) and 00 (41, 53, 107).

The *Dl* near-isoallelic lines were crossed to generate $F_1$ genotypes that were homozygous or heterozygous for the focal SNPs but randomized for heterozygosity at all other polymorphic sites at *Dl* and for the introgressed regions surrounding *Dl*. Thus, six low sternopleural bristle number genotypes (H00) were constructed by a round-robin crossing scheme (with the subscripts denoting the three lines representing the designated haplotype): $00_1 \times 00_2$; $00_2 \times 00_3$; $00_3 \times 01_1$; $01_1 \times 01_2$; $01_2 \times 01_3$; $01_3 \times 00_1$. Progeny of all of these crosses are homozygous H00. Progeny of the first two crosses are homozygous S00, progeny of the third and sixth cross are heterozygous S01, and progeny of the fourth and fifth cross are homozygous S11. Six high sternopleural bristle number genotypes (H11) were constructed similarly: $11_1 \times 11_2$; $11_2 \times 11_3$; $11_3 \times 10_1$; $10_1 \times 10_2$; $10_2 \times 10_3$; $10_3 \times 11_1$. Crosses to create heterozygous genotypes at the SNP associated with sternopleural bristle number (H10) were: $11_1 \times 01_1$; $11_2 \times 01_2$; $11_3 \times 00_1$; $10_1 \times 01_3$; $10_2 \times 00_2$; $10_3 \times 00_3$. The same logic was used to construct six lines representing each of the three abdominal bristle number SNP genotypes (S00, S01, S11). Within each S genotype class, two genotypes were H11, two were H01, and two were H00.

### (iv) Culture conditions and bristle number phenotypes

Two replicate vials for each of the 36 $F_1$ genotypes produced by the above crosses of *Dl* near-isoallelic lines were reared in each of five environments:

standard cornmeal–agar–molasses medium at 18 °C, 25 °C and 28 °C, cornmeal–agar–molasses medium brought to a final concentration of 9% ethanol at 25 °C, and tomato paste medium (Fry *et al.*, 1996) at 25 °C. These environments had previously been shown to affect a measure of competitive fitness (Fry *et al.*, 1996). Ten males and ten females from each replicate vial were scored for either sternopleural (total number of bristles on the left and right sternopleural plates) or abdominal (the total number of bristles on the sixth abdominal sternite in females and the fifth sternite in males) bristle number, depending on whether the focal SNP was H or S, respectively. A total of 3,600 flies were scored for each bristle trait. The design was completely balanced.

### (v) Statistical analyses

Distribution statistics, analyses of variance of bristle number and tests of significance of *F*-ratios were estimated using SAS procedures MEANS and GLM (SAS Institute, 1988). Variance in bristle number was partitioned by three-way factorial analyses of variance (ANOVA) according to the full model,

$$Y = \mu + S + G + E + G \times S + G \times E + E \times S + G \times E \times S$$
$$+ L(G) + E \times L(G) + S \times L(G) + E \times S \times L(G)$$
$$+ R(E \times G \times L) + S \times R(E \times G \times L) + \text{Error}$$

where $S$, $G$ and $E$ are the fixed cross-classified effects of sex, SNP genotype and culture environment (respectively), $L$ and $R$ are random effects of six different $F_1$ lines within each SNP genotype and replicate vial (respectively), and parentheses indicate nested effects. Reduced analyses by sex and/or by genotype, and for all possible pairs of environments, were also conducted as appropriate.

## 3. Results

### (i) Sternopleural bristle number

Table 1 shows the ANOVA of sternopleural bristle number for the three H SNP genotypes across both sexes and all five environments, and Fig. 1 shows the mean bristle numbers of each genotype in each environment. The main effect of genotype in the ANOVA was highly significant, confirming that this site is associated with variation in sternopleural bristle number. Rearing environment also had a highly significant effect on mean bristle number, with the highest mean bristle number at the lowest developmental temperature and the lowest mean bristle number in the ethanol-supplemented medium (Fig. 1). The SNP genotype by rearing environment interaction term was also highly significant, fulfilling the first criterion of Gillespie & Turelli's model (Gillespie & Turelli, 1989).

Table 1. *Analyses of variance of bristle number, pooled across sexes and all five environments. Sources of variation are sex (S), SNP genotype (G), environment (E), F₁ line cross (L) and vial replicate (R)*

| Source | d.f. | Sternopleural bristles | | | Abdominal bristles | | |
|---|---|---|---|---|---|---|---|
| | | MS | F | p | MS | F | p |
| S | 1 | 390·1 | 147 | 0·0001 | 7906 | 233 | 0·0001 |
| G | 2 | 1095 | 29·1 | 0·0001 | 1303 | 16·1 | 0·0002 |
| E | 4 | 226·2 | 55·7 | 0·0001 | 367·1 | 31·7 | 0·0001 |
| $G \times S$ | 2 | 6·370 | 2·41 | 0·1 | 227·0 | 6·70 | 0·008 |
| $G \times E$ | 8 | 17·50 | 4·31 | 0·0004 | 22·30 | 1·93 | 0·07 |
| $E \times S$ | 4 | 4·902 | 1·55 | 0·2 | 10·38 | 1·80 | 0·1 |
| $G \times E \times S$ | 8 | 1·220 | 0·39 | 0·9 | 9·023 | 1·56 | 0·2 |
| $L(G)$ | 15 | 37·65 | 10·7 | 0·0001 | 81·08 | 2·04 | 0·07 |
| $E \times L(G)$ | 60 | 4·058 | 1·08 | 0·4 | 11·57 | 1·80 | 0·03 |
| $S \times L(G)$ | 15 | 2·646 | 0·834 | 0·6 | 33·87 | 5·86 | 0·0001 |
| $E \times S \times L(G)$ | 60 | 3·171 | 1·32 | 0·1 | 5·776 | 1·21 | 0·2 |
| $R(G \times E \times L)$ | 90 | 2·994 | 1·25 | 0·2 | 5·400 | 1·13 | 0·3 |
| $S \times R(G \times E \times L)$ | 90 | 2·400 | 1·16 | 0·2 | 4·760 | 1·13 | 0·2 |
| Error | 3240 | 2·073 | | | 4·205 | | |
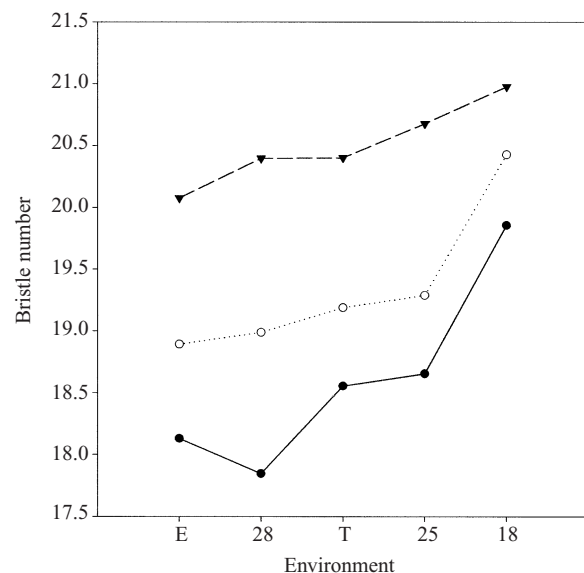


Fig. 1. Mean sternopleural bristle number of H00 (filled circles), H10 (open circles) and H11 (triangles) single-nucleotide-polymorphism genotypes for the *Dl* locus in five environments: standard culture medium at 18 °C, 25 °C and 28 °C, standard culture medium supplemented with 9 % ethanol at 25 °C (E) and tomato paste medium at 25 °C (T).

However, inspection of Fig. 1 suggests that the pattern of GEI is not consistent with one of the requirements of the model: that the heterozygous genotype is more stable than the homozygous genotypes across environments. Rather, it appears that H11 homozygotes are the most stable genotype, and statistical analyses bear this out. ANOVAs were computed separately for each genotype, pooled across sexes and environments (data not shown). Both sex and environment were fixed effects. The effect of

environment was significant for all three genotypes: H11, $F_{4,20} = 5.08$, $p = 0.005$; H10, $F_{4,20} = 25.6$, $p < 0.0001$; H00, $F_{4,20} = 44.5$, $p < 0.0001$. The proportion of the total sums of squares explained by the effect of environment was, however, much smaller for the H11 genotype (3·2 %) than for the H10 (11·1 %) or H11 (19·3 %) genotypes.

It is possible that GEI is not significant for all pairs of environments and that closer examination of the pairs that contribute to the overall significance of this term could reveal different patterns. Therefore, we computed separate ANOVAs for each of the ten pairwise environmental comparisons. There was significant GEI for sternopleural bristle number for five of these contrasts: 18 °C and 25 °C ($p < 0.0001$); 18 °C and 28 °C ($p = 0.0004$); 18 °C and ethanol medium ($p = 0.05$); 18 °C and tomato medium ($p = 0.03$); and 28 °C and tomato medium ($p = 0.002$). However, examination of the difference in mean bristle number in each of these environment pairs for each genotype (a measure of the environmental sensitivity) shows that, in all cases, the H11 genotype was the least sensitive and the H00 genotype the most sensitive, with the heterozygotes being intermediate (Table 2).

Furthermore, the assumption that the mean genotypic effect across environments is much smaller than the standard deviation of effects across environments is not true for these data. Genotypic values (*a*) were estimated as one-half of the difference between H11 and H00 homozygotes (Falconer & Mackay, 1996), averaged over sexes, within each environment. The mean value of *a* was 0·85 and the standard deviation across environments was 0·316. Thus, neither the mean nor the variance of allelic effects across environments was small and the mean effect was 2·7

Table 2. *Differences between line means (*environmental sensitivities*) for pairs of environments contributing to genotype by environment interaction for genotypes at the* Delta (Dl) *locus. The standard errors are given in parentheses*

| SNP genotype | Environment pair | Environmental sensitivities | | |
|---|---|---|---|---|
| *Sternopleural bristles* | | *H00* | *H10* | *H11* |
| | 18 °C–28 °C | 2·008 (0·127) | 1·438 (0·142) | 0·579 (0·148) |
| | 18 °C–25 °C | 1·200 (0·123) | 1·138 (0·139) | 0·300 (0·143) |
| | 18 °C–Ethanol | 1·725 (0·120) | 1·533 (0·133) | 0·900 (0·128) |
| | 18 °C–Tomato | 1·300 (0·124) | 1·238 (0·121) | 0·575 (0·132) |
| | 28 °C–Tomato | −0·708 (0·114) | −0·200 (0·131) | −0·004 (0·152) |
| *Abdominal bristles* | | *S00* | *S10* | *S11* |
| | 25 °C–28 °C | 1·300 (0·276) | −0·233 (0·285) | −0·792 (0·284) |
| | 25 °C–Tomato | 1·050 (0·308) | −0·008 (0·251) | −0·800 (0·274) |
| | 25 °C–Ethanol | 0·117 (0·293) | −0·167 (0·299) | −1·058 (0·284) |

times greater than the standard deviation across environments, in violation of the model assumption.

### (ii) *Abdominal bristle number*

The ANOVA of abdominal bristle number (Table 1) shows that there were highly significant differences in bristle number between the three SNP genotypes and between the five environments, but that the SNP GEI term was not significant, although it approached nominal significance ($p = 0·07$). However, the SNP genotype by sex interaction term was highly significant, which was not surprising given the previous observation that this SNP had a female-specific effect on abdominal bristle number (Long *et al.*, 1998). We ran reduced ANOVAs for each sex separately (data not shown). In males, the effects of SNP genotype ($p = 0·06$) and SNP GEI ($p = 0·4$) were not significant. However, in females, the effect of SNP genotype was highly significant ($p < 0·0001$) and the SNP GEI term reached nominal significance ($p = 0·04$). Therefore, we restricted further analyses to females only.

Mean abdominal bristle numbers in females are shown for each genotype in each environment in Fig. 2. The highest mean bristle number was at 18 °C and the lowest at 28 °C. Analyses of environmental sensitivities of each genotype over all five environments (data not shown) do not indicate that the heterozygote is the most stable genotype. The effect of environment was significant for all three genotypes: S11, $F_{4,20} = 16·104$, $p < 0·0001$; S10, $F_{4,20} = 4·31$, $p < 0·01$; S00, $F_{4,20} = 6·35$, $p < 0·002$. The proportion of the total sums of squares explained by the effect of environment was, however, much larger for the S11 genotype (15·8 %) than the S10 (7·5 %) or S00 (7·6 %) genotypes.

Inspection of Fig. 2 indicates that there might be some pairs of environments contributing to GEI for which the heterozygote is the most stable genotype.
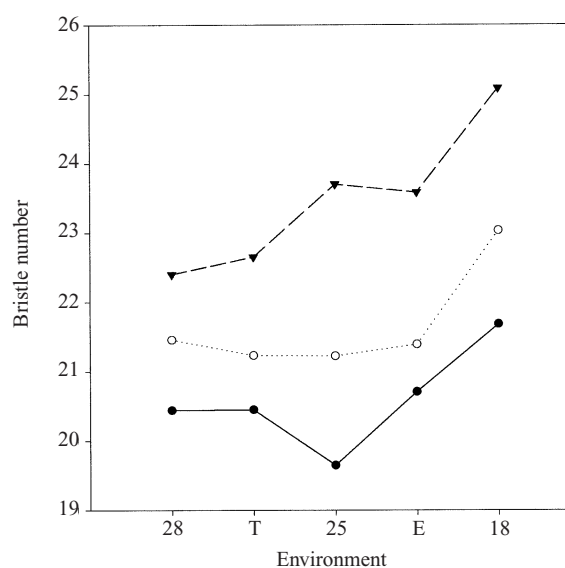


Fig. 2. Mean female abdominal bristle number of S11 (filled circles), S10 (open circles) and S00 (triangles) single-nucleotide-polymorphism genotypes for the *Dl* locus in five environments: standard culture medium at 18 °C, 25 °C and 28 °C, standard culture medium supplemented with 9 % ethanol at 25 °C (E) and tomato paste medium at 25 °C (T).

We again computed separate ANOVAs for each of the ten pairwise environmental comparisons. There was significant GEI for abdominal bristle number in females for three of these contrasts: 25 °C and 28 °C ($p = 0·02$); 25 °C and tomato medium ($p = 0·02$); and 25 °C and ethanol medium ($p = 0·04$). Furthermore, examination of the environmental sensitivity for each genotype revealed that, for two of these pairs (25 °C and 28 °C, and 25 °C and tomato medium), the S10 genotype was indeed less sensitive than either homozygote (Table 2).

However, the assumption that the mean genotypic effect is smaller than the standard deviation of effects across environments is not met for the abdominal

bristle number polymorphism. The mean genotypic value was 1·45 over all five environments and 1·39 over the four environments that contribute to significant GEI. The standard deviation of allelic effects was 0·429 over all five environments and 0·468 over the four environments that contribute to GEI. Thus, the mean allelic effect is approximately three times as large as the standard deviation of effects across environments.

## 4. Discussion

Many fundamental questions in evolutionary quantitative genetics have remained unanswered because the theoretical underpinnings are in terms of the fitness effects of individual genes, whereas observations have been at the level of collective properties of all genes affecting variation in traits of interest. As QTLs for many traits begin to be resolved into discrete genetic loci and indeed into QTNs at each of these loci, the prospects are bright for empirically addressing such long-standing issues as the nature of the forces that maintain variation for quantitative traits and the genetic basis of adaptation.

*Drosophila* sensory bristle numbers are classic examples of traits that are under presumed stabilizing selection but for which large amounts of additive genetic variation segregates in natural populations (e.g. Long *et al.*, 1995; Gurganus *et al.*, 1999). Identification of SNPs in linkage disequilibrium with bristle number at several genetic loci corresponding to bristle number QTL (Long *et al.*, 1998; 2000; Lyman *et al.*, 1999) define, for the first time, genotypes of functional alleles affecting variation in bristle number. This in turn provides us with the opportunity to test, one locus at a time and in combination, which evolutionary forces act to maintain variation for bristle number. Here, we have begun this process by evaluating the prediction of one particular model of maintenance of genetic variation by GEI (Gillespie & Turelli, 1989): that the phenotypic values of heterozygous genotypes are more stable across a range of environments than are phenotypic values of homozygous genotypes.

Homozygous and heterozygous genotypes for two SNPs at the *Dl* locus, one of which affects sternopleural and the other abdominal bristle number, were constructed by crossing six different near-isoallelic lines for the *Dl* gene region so as to randomize heterozygosity for other segregating sites within each SNP genotype class. When reared in five different environments, there was strong SNP GEI for sternopleural bristle number for males and females, and SNP GEI for abdominal bristle number in females only. Although the rank order of the different genotypes was, in both cases, the same in the different environments,

the additive effects of the bristle number SNPs varied more than twofold. For example, genotypic values ranged from 0·56 sternopleural bristle at 18 °C and 1·28 sternopleural bristles at 28 °C; and, in females, from 0·98 abdominal bristle at 28 °C to 2·03 abdominal bristles at 25 °C. Furthermore, the degree of dominance ($d/a$, where $d$ is the difference between the mean bristle number of the heterozygous SNP genotype and the mean bristle number of the two homozygous genotypes (Falconer & Mackay, 1996)) can also vary between environments. At 28 °C, the S polymorphism affecting female abdominal bristle number is additive ($d/a = -0·04$) but, in the ethanol-supplemented culture medium, the low allele is partially dominant ($d/a = -0·52$). Thus, the amount of additive and dominance variance attributable to the QTNs at *Dl* will be different depending on the environment in which the flies were reared.

These observations are germane to the proposal to use SNP associations to determine the genetic basis of complex human diseases (Lander, 1996; Collins *et al.*, 1997). It is assumed that causal associations will replicate in different populations, whereas spurious associations produced by population admixture will not. However, GEI and sex-specific SNP effects reduce the power of association studies within populations and could lead to non-reproducibility of the effects of causal SNPs across populations. Explicit inclusion of sex and common demographic factors in the experimental design of SNP association studies, with concomitant increases in sample sizes to give sufficient power to test all combinations of parameters, will be necessary to determine the full range of SNP effects, and to evaluate environmental risk factors.

Our data do not provide support for the maintenance of genetic variation for bristle number by the mechanism proposed by Gillespie & Turelli (1989). Although heterozygotes for the abdominal bristle number polymorphism were more stable than homozygotes across two of the three environmental contrasts showing GEI for this trait, this was not true for the sternopleural bristle number polymorphism, for which one of the homozygotes was the least environmentally sensitive. In neither case was the model assumption of small means and variances of allelic effects across environments met.

This conclusion must be tempered by several caveats. First and foremost, a fair test of this model, or of any other model invoking GEI, requires that the means and variances of effects be measured in whatever range of environments is relevant to maintaining the observed variation in nature. As these conditions are not known, it is quite possible that the relevant factors were not included among the lab environments tested. Second, *Dl* is just one of many loci affecting variation in bristle number and the mechanisms maintaining variation might vary from

locus to locus. Third, the model assumes real stabilizing selection acting on *Dl* through its effect on bristle number, for which we have no direct evidence. Fourth, we do not know that the SNPs associated with sternopleural and abdominal bristle number are themselves the causal QTNs; all we know is that they are in linkage disequilibrium with the causal QTNs. Thus, crosses to generate homozygote and heterozygote genotypes at the focal SNPs might not have produced homozygous and heterozygous genotypes at the causal QTNs. This does not appear to be a problem, however, because the SNP genotypes recapitulate the expected differences in bristle number phenotypes. Despite these caveats, it is plausible that the assumptions of Gillespie & Turelli's model (Gillespie & Turelli, 1989) regarding small means and variances of allelic effects across environments are overly restrictive and compromise the generality of the model (Gimelfarb, 1990). A more realistic model of maintenance of variation by GEI would incorporate larger differences in mean allelic effects than the variance of effects across environments, as observed for the *Dl* polymorphisms.

Evaluation of other models for the maintenance of variation of the bristle number polymorphisms at *Dl* requires that we estimate the fitnesses of the three genotypes at each polymorphic site. Knowledge of markers in strong linkage disequilibrium with the causal QTNs (and, ultimately, of the causal QTNs themselves) opens up the possibility of applying the whole gamut of population genetic approaches that have been used to infer selection on allozyme and other polymorphisms, both in the wild (Endler, 1986) and in the laboratory, to the problem of selection on loci affecting variation for quantitative traits. Given the somewhat chequered history of such attempts, however, it might be unrealistic to assume that we could directly measure the fitness effects of all loci affecting variation in any trait. Selection acting on any one locus affecting a quantitative trait in any one environment at any point in time is likely to be quite weak, particularly if selection at the level of the trait is weak (Kingsolver *et al.*, 2001) or when there are many variable QTLs to consider (Kimura, 1983). In addition, as noted above, one needs to consider the whole range of environments that are ecologically relevant. However, there is a rich body of population genetics theory for inferring the action of historical selection from data on DNA sequence variation (Hartl & Clark, 1997; Wayne & Simonsen, 1998). When applied to sequences of cloned QTLs, it will be possible to detect the signatures of purifying selection, selective sweeps, balancing selection and neutrally evolving polymorphisms, as exemplified by the demonstration that domestication of maize was accompanied by selection in the 5′ regulatory region of *Teosinte-branched1* (Wang *et al.*, 1999).

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