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High-Resolution Mapping of Quantitative Trait Loci for Sternopleural Bristle Number in *Drosophila melanogaster*

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Manuscript received April 15, 1998
Accepted for publication March 31, 1999

ABSTRACT

We have mapped quantitative trait loci (QTL) harboring naturally occurring allelic variation for Drosophila bristle number. Lines with high (H) and low (L) sternopleural bristle number were derived by artificial selection from a large base population. Isogenic H and L sublines were extracted from the selection lines, and populations of *X* and third chromosome H/L recombinant isogenic lines were constructed in the homozygous low line background. The polymorphic cytological locations of *roo* transposable elements provided a dense molecular marker map with an average intermarker distance of 4.5 cM. Two *X* chromosome and six chromosome *3* QTL affecting response to selection for sternopleural bristle number and three *X* chromosome and three chromosome *3* QTL affecting correlated response in abdominal bristle number were detected using a composite interval mapping method. The average effects of bristle number QTL were moderately large, and some had sex-specific effects. Epistasis between QTL affecting sternopleural bristle number was common, and interaction effects were large. Many of the intervals containing bristle number QTL coincided with those mapped in previous studies. However, resolution of bristle number QTL to the level of genetic loci is not trivial, because the genomic regions containing bristle number QTL often did not contain obvious candidate loci, and results of quantitative complementation tests to mutations at candidate loci affecting adult bristle number were ambiguous.

CONTINUOUS phenotypic variation among individuals in natural populations is ubiquitous for most morphological, behavioral, physiological, and life history traits. Continuous variation for such "quantitative" traits results jointly from the simultaneous segregation of multiple genes [or quantitative trait loci (QTL)] affecting the trait, each of which has effects too small to be perceived over the noise of segregation of the remaining QTL, and effects of environmental variation, which further blur the distinction between genotypes (Falconer and Mackay 1996). Knowledge of the genetic properties of QTL (the genetic loci at which mutational and segregating variation occurs, mutation rates and mutational effects at these loci, effects and frequencies of segregating alleles) and their effects in different environments is critical in several biological contexts. Quantitative genetic variation for complex human diseases is more common than major gene mutations; hence, the proportion of people affected by genes of small effect is greater than for genes with large effects. Quantitative characters are generally considered to be the substrate of phenotypic evolution and are the traits selected by animal and plant breeders to improve productivity of agriculturally important species. Finally, as there is naturally occurring variation for (almost) all phenotypes, it is possible that quantitative genetic analysis can be used as a gene discovery tool by screening for segregating alleles in nature rather than for mutations.

The lack of a 1:1 correspondence between genotype and phenotype of quantitative traits has stimulated the development of biometric analyses (Mather and Jinks 1971; Bulmer 1985; Falconer and Mackay 1996) and stymied the genetic dissection of quantitative traits. Although the principles of QTL analysis have been realized for >70 yr (Sax 1923), their application had been limited largely to the model system of Drosophila bristle number (reviewed by Thoday 1979) until the recent development of high-density linkage maps of polymorphic marker loci in many species (reviewed by Tanksley 1993). In species amenable to inbreeding, the most efficient way to map QTL is to cross two lines that are fixed for alternate alleles at QTL and marker loci and to score individuals from backcross, F_2, or other segregating generations for their quantitative trait phenotype and marker genotype. The positions and effects of QTL can be inferred from differences in mean phenotype between marker genotype classes.

Despite the apparent simplicity of this approach, there are many statistical and genetical issues to consider when designing and interpreting such experiments (Falconer and Mackay 1996; Lynch and Walsh 1998). Large experiments are needed to detect QTL of

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even moderate effect. The effect of a QTL depends on the mean difference between homozygous genotypes and the phenotypic standard deviation. Therefore, designs that minimize the latter, such as measuring multiple individuals of the same genotype from recombinant inbred (RI) lines and reducing genetic variation from segregating QTL on chromosomes other than the one of interest, will considerably increase the power to detect QTL with small effects. QTL mapping using line cross analysis will usually underestimate the true number of loci causing variation in a trait, not just for reasons of limited power, but because only the QTL that are genetically variable between the two parental lines can be detected. If inferences are to be made about the loci and allelic effects in nature, a good genetic sample of naturally occurring variation is necessary. Statistical issues include the problem of testing multiple markers for linkage to QTL and controlling for the cosegregation of multiple QTL affecting the trait.

Even the most rigorous effort to map QTL by linkage to markers in a line-cross analysis will succeed only in delineating genomic regions of interest. Further progress in resolving the QTL to the level of genetic locus is likely to depend on whether there are candidate genes affecting the trait in the region to which the QTL maps. This in turn depends on a sound understanding of what genes affect the metabolic and developmental pathways leading to the trait phenotype. It also depends on whether naturally occurring variation for quantitative trait phenotypes often occurs at the same loci at which mutations with large effects on the phenotype arise, a not unreasonable hypothesis, but one that needs to be tested. Postulating candidate genetic loci corresponding to QTL hardly constitutes proof that the candidate gene and the QTL are one and the same entity. Complementation tests of QTL alleles with mutations or deficiencies at the candidate locus can establish whether they interact genetically, and population level tests of association of molecular variation at the candidate locus and phenotypic variation in the quantitative trait are necessary to convincingly resolve QTL to the level of genetic locus and to identify molecular polymorphisms responsible for variation in quantitative trait phenotypes (Mackay 1996).

Currently, the best case scenario for the description of a quantitative trait in terms of complex genetics rather than statistics is for a phenotype that is the end product of a well-understood metabolic or developmental pathway in a genetically tractable model organism. The numbers of sensory sternopleural and abdominal bristles of *Drosophila melanogaster* are one such model system (Mackay 1995, 1996). Used as model quantitative traits for >40 yr, bristle numbers are typical morphological quantitative traits. They have high heritabilities (Clayton *et al.* 1957) and stable mean phenotypes in nature, possibly as a consequence of selection for intermediate bristle number (Linney *et al.* 1971). Bristles are external sensory organs of the peripheral nervous system. Many loci affecting peripheral nervous system development have been characterized genetically and molecularly, and placed in a developmental pathway (reviewed by Campos-Ortega 1993; Jan and Jan 1993). These genes are candidate bristle number QTL, at which naturally occurring variation could segregate.

Here we report the results of a study to map QTL on the *X* and third chromosomes affecting naturally occurring variation for sternopleural bristle number. To maximize the opportunity to map alleles affecting bristle number that segregate at intermediate frequency in nature, the parental lines were derived from lines selected for 25 generations for increased and decreased bristle number from a single natural population. Restriction of selection to 25 generations reduces the chance that new mutations affecting bristle number contribute to selection response (Hill 1982), while bringing to high frequency alleles of moderately small effect on the trait. Insertion sites of neutral, highly polymorphic, *roo* transposable elements were used to provide a dense (4-cM) cytogenetic molecular map. The power to detect QTL of small effect was increased genetically by replacing the background genotype with that of the homozygous low parental line and by using the RI design, both of which reduce variation within marker classes, and statistically by using composite interval mapping (Zeng 1994) to control for segregation of chromosomally linked QTL. Finally, quantitative complementation tests (Long *et al.* 1996; Mackay and Fry 1996) to mutations at candidate bristle number loci were performed to test the hypothesis that naturally occurring alleles at bristle number QTL interact with the mutant alleles. Previously, we used the same design to map QTL affecting response to selection for abdominal bristle number in this population (Long *et al.* 1995, 1996), and here we compare the results of the two studies.

MATERIALS AND METHODS

Drosophila Stock Construction

All cultures were reared in shell vials with 10 ml cornmeal-agar-molasses medium at 25°, unless otherwise noted.

Selection lines: The base population consisted of 62 isofemale lines collected from the Raleigh Farmer's Market in May 1988. To equalize the contribution of each isofemale line, females and males from each line were crossed in series ($1 \times 2, 2 \times 3, \ldots, 62 \times 1$) in separate vials. After 48 hr, one male and one fertilized female from each cross were placed in a communal bottle and allowed to lay eggs. The parents (G_0) were discarded after 3 days. One hundred virgin females and males were collected from the offspring (G_1) and scored for sternopleural bristle number (the sum of the numbers of bristles on the right and left sternopleural plates). The 25 highest and lowest scoring individuals from each sex were selected as parents of the high and low lines, respectively. The lines were maintained each generation by selecting the 25 most extreme individuals of 100 scored of each sex, for a total of 25 generations of selection.

Isogenic sublines: At generation 26, the 20 most extreme males from the high and low lines were used to construct isogenic sublines for mapping. All chromosome balancer stocks referred to below are described in Lindsley and Zimm (1992). The multiple balancer stocks are of *P* cytotype for the P–M system of hybrid dysgenesis (Kidwell *et al.* 1977). Single males from the high and low selection lines (genotype *S1/Y*; $S2_i/S2_j$; $S3_i/S3_j$, where *S* indicates a high or low selected chromosome, *1*, *2*, and *3* refer to the *X*, second and third chromosomes, respectively, and *i* and *j* indicate nonisogenic homologues) were crossed to females of a stock with C(*1*)*DX* and $T(2,3)ap^{Xa}$ (abbreviated *Xa* below) in an inbred Harwich background (G_1). At G_2, a single male progeny of genotype *S1/Y*; $Xa/S2_i$; $S3_i$ was crossed to females of the balancer stock *Basc*; *Xa/SM5,Cy; TM6B, Tb* (abbreviated *B*; *Xa/Cy*; *Tb* below). Male ($B/Y;Cy/S2_i;Tb/S3_i$) and female ($B/S1;Cy/S2_i;\ Tb/S3_i$) progeny carrying isogenic copies of single *X*, second, and third chromosomes of the selection line were intercrossed (G_3), and at G_4 $S1/Y$; $Cy/S2_i$; $Tb/S3_i$ males were mated to their $B/S1;\ Cy/S2_i;\ Tb/S3_i$ sibs. At G_5 five males and five females of each of the resultant eight genotypic classes (less if $S2_i$ or $S3_i$ harbored recessive lethals) were scored for abdominal (the number of bristles on the most posterior abdominal sternite; segment 5 of males, 6 of females) and sternopleural bristle number. One isogenic high (IH) and two low (IL1, IL2) lines with bristle numbers near those of the mass-mated selection lines at the end of the selection experiment were retained for mapping the genetic factors contributing to the high-low divergence in bristle number.

Recombinant isogenic chromosome *3* lines: To construct high–low sternopleural bristle number RI lines in a low isogenic selection background, we first crossed the isogenic high (IH) and isogenic low (IL1) selection lines (G_1). At G_2, the heterozygous female progeny (genotype *L1/H1; L2/H2; L3/H3*) were crossed to males from a stock in which the *TM6B, Tb* balancer chromosome and *Sb* marker had been placed in the low isogenic *X* and chromosome *2* (C*2*) background (*L1; L2; Tb/Sb*). Single *Tb/L3H3* (where *L3H3* represents a potential recombination between the third chromosomes from the high and low selection lines) males were collected from this cross and backcrossed for five generations to females of the *L1;L2;Tb/Sb* marker stock (G_3–G_7). At G_8, *Tb/L3H3* males and females were crossed to one another to create a single homozygous recombinant C*3* line, *L1; L2; Tb/L3H3*. At this point, larvae were checked by analysis of insertion sites of *roo* element markers (see below) to verify that C*2* was isogenic *L2* for each RI line. Males from any lines found with heterozygosity on C*2* were backcrossed for four more generations (G_9–G_{12}) to females of the *L1; L2; Tb/Sb* marker stock. At G_{13}, *Tb/L3H3* males and females were intercrossed and homozygosity of the second chromosome confirmed by analysis of *roo* insertion sites.

Recombinant isogenic C*1* lines: First, a chromosome substitution line of genotype *H1; L2; L3* was constructed using the IH and IL2 isogenic selection lines. Females from the *B*; *Xa/Cy*; *Tb* balancer stock were crossed in small mass matings to males from either the high or low isogenic lines (G_1). From the high line cross, G_2 females of genotype *B/H1; Cy/H2; Tb/H3* were crossed to *B/Y; Cy/H2; Tb/H3* males. From the low line cross, G_2 *B/L1; Cy/L2; Tb/L3* females were backcrossed to males of the low isogenic selection line. G_3 females from the low line cross of genotype *B/L1; L2; L3* were mated to G_3 *H1/Y; Cy/H2; Tb/H3* males from the high line cross. At G_4, *B/H1; Cy/L2; Tb/L3* females and *B/Y; Cy/L2; Tb/L3* males were crossed *inter se*, and at G_5, females of genotype *B/H1; L2; L3* were crossed to *H1/Y; L2; L3* males. At G_6, *H1; L2; L3* females and males were selected. To create the C*1* RI lines, *L1; L2; L3* females were crossed to *H1; L2; L3* males (G_7). At G_8, *H1/L1; L2; L3* females were backcrossed to *L1; L2; L3* males. At G_9, single *H1L1/Y; L2; L3* males were crossed to *FM4/L1; L2; L3* females. The single males from G_9 were saved and mated to their *FM4/H1L1; L2; L3* daughters to obtain *H1L1; L2; L3* homozygous lines.

QTL Mapping

Quantitative traits: Ten males and 10 females from each of two replicate vials, a total of 40 flies per RI line, were scored for sternopleural and abdominal bristle number.

***In situ* hybridization:** *roo* insertion sites were determined by *in situ* hybridization of biotin-labeled *roo* DNA to polytene salivary chromosomes of third instar larvae, according to the procedure of Shrimpton *et al.* (1986). Phage probes containing a complete copy of *roo* (Scherer *et al.* 1982) 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 horseradish peroxidase/3,3′-diaminobenzidine. Two high quality preparations from each RI line were scored for sites of insertion of *roo* elements at the level of cytological band subdivision on the standard Bridge's map (LeFevre 1976). This provides a map where the order of markers is known and recombination breakpoints in the RI lines are easily determined by the change of *roo* insertion sites from the parental high and low line patterns. Only lines with marker genotypes consistent with a small number of recombination events were retained for analysis. Markers in the same cytological positions in the parental lines are uninformative and were not used to construct the marker genotype.

Mapping analysis: The map positions of the *roo* markers were estimated from the observed recombination frequencies between pairs of markers, *r*, using the Kosambi mapping function, $d = \frac{1}{4} \ln[(1 + 2r)/(1 - 2r)]$, where *d* is the distance between adjacent markers in morgans. Composite interval mapping (Zeng 1993, 1994), as implemented by the QTL Cartographer software (Basten *et al.* 1994, 1997), was used to test the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait, while simultaneously controlling for the effects of chromosomally linked QTL by multiple regression on markers outside the test interval. These analyses were conducted on least-squares line means separately for each trait and sex. The conditioning markers were chosen for each analysis by forward selection–backward elimination stepwise regression. The analysis uses a "conditioning window," such that only markers *x* cM away from the markers flanking the test interval are included in the model. The size of this conditioning window is arbitrary and can affect the outcome. We used a range of conditioning windows to check the stability of the models. 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). The test statistic at a genomic location is distributed as χ^2 with 2 d.f. under the null hypothesis, and was evaluated every centimorgan.

The experiment-wise significance level was determined by permutation. Empirical distributions of LR test statistics under the null hypothesis of no association between any of the intervals and trait values were obtained for each analysis by randomly permuting the trait data 1000 times and calculating the maximum LR statistic across all intervals for each permutation. LR statistics from the original data that exceed the 50th highest permutation LR statistic are significant at $\alpha = 0.05$ under the null hypothesis (Churchill and Doerge 1994; Doerge and Churchill 1996).

Epistatic effects: The significance of pairwise epistatic interactions between nonadjacent bristle number QTL detected in the composite interval mapping analyses was evaluated by multiple regression on least-squares line means. For each interaction tested, a model was fitted that included the marker closest to each significant QTL peak and the interaction effect of interest. The significance of the interaction was tested by an *F* ratio statistic, with the mean square of the interaction as the numerator and the error mean square as the denominator.

The effects of significant two-locus interactions were estimated from the least-squares means of the four marker locus classes as $[(\bar{X}_{LL} + \bar{X}_{HH}) - (\bar{X}_{LH} + \bar{X}_{HL})]$, where the first subscript denotes the genotype (high, H, or low, L) at the first marker locus, and the second subscript denotes the genotype of the second marker locus. Standard errors of the interaction effects were estimated as

$$\sqrt{\frac{\text{MSI}}{n_{LL} + n_{HH} + n_{LH} + n_{HL} - 4}},$$

where MSI is the interaction mean square from the multiple regression model, and *n* is the number of lines in each of the four marker classes described above.

Quantitative Complementation Tests

Candidate loci: Candidate genetic loci corresponding to bristle number QTL are genes with mutant effects on sensory bristle number that map approximately to the same genetic locations as bristle number QTL. The candidate loci (allele, map position) used in complementation tests to naturally occurring bristle number QTL were as follows: *scute* (*sc*, 1-0.0); *Notch* (*spl*, 1-3.0); *cut* (ct^6, 1-20.0); *extra-macrochaetae* ($emc^{P(3)-67}$, $emc^{P(3)-5}$, 3-0.0); *que mao* (*qm*, 3-23.0) (Lai *et al.* 1998); *hairy* (*h*, 3-26.5); *abdominal* (*abd*, 3-27); *polychaetoid* (*pyd*, 3-39); *Sex-combs-reduced* (*Scr*, 3-47.5); *Delta* (Dl^3, 3-66.2); *Hairless* (*H*; 3-69.5), and *Enhancer-of-split* ($E(spl)^D$, 3-89.1). Mutant alleles of the candidate loci were introgressed into the isogenic strain $Sam\ ry^{506}$ (Lyman *et al.* 1996) by 10 generations of repeated backcrossing. Two independent *P[lArB]* element insertion alleles of *emc* in the $Sam\ ry^{506}$ background have been generated in this laboratory (Lyman *et al.* 1996) and were used for complementation crosses. The $E(spl)^D$ allele was not introgressed into $Sam\ ry^{506}$, but the *X* and second chromosome background was coisogenic with the control strain.

Chromosome substitution lines: *X* chromosome substitution lines of genotype *H1; L2; L3* in the IL2 genetic background and chromosome *3* substitution lines of genotype *L1; L2; H3* in the IL1 genetic background were constructed as described above.

Quantitative complementation test: The test for quantitative failure to complement is a test for a difference in heterozygous effect of high and low selected chromosomes in a control and mutant background (Long *et al.* 1996; Mackay and Fry 1996). For the *X* chromosome loci, the *H1; L2; L3* and IL2 strains were each crossed to the control $Sam\ ry^{506}$ stock (the control cross) and to the derivative of $Sam\ ry^{506}$ into which *X* chromosome mutations at candidate bristle loci had been introgressed (the tester cross). Similarly, for the chromosome *3* loci, the *L1; L2; H3* and IL1 strains were each crossed to the control stock and the chromosome *3* tester stocks. In each case, there are four F_1 genotypes: *H*/*Sam*, *L*/*Sam*, *H*/*Sam m*, and *L*/*Sam m*. A difference in mean bristle number phenotype between *H*/*Sam* and *L*/*Sam* and between *H*/*Sam m* and *L*/*Sam m* is expected because the bristle number QTL on the *H* and *L* selected chromosomes act additively. This difference is reflected in a significant effect of line in a two-way analysis of variance (ANOVA) of bristle number, where line and cross are fixed main effects. Whether or not there is a difference in bristle number between *H*/*Sam* − *H*/*Sam m* and *L*/*Sam* − *L*/*Sam m* genotypes depends on the heterozygous effect of the mutant, and any linked non-*Sam* genomic fragments, on bristle number. Such a difference would be detected as a significant effect of cross in the ANOVA. Whether the contrast (*H*/*Sam* − *L*/*Sam*) − (*H*/*Sam m* − *L*/*Sam m*) = 0 is tested by the significance of the cross × line interaction term of the ANOVA. A significant interaction is interpreted as failure to complement. That is, alleles at bristle number QTL in one or both of the selection lines interact with the mutation at the candidate bristle locus.

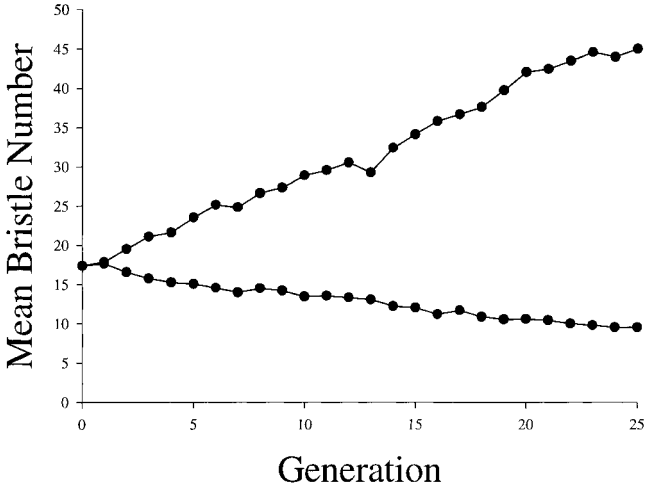

Figure 1.—Response to 25 generations of divergent artificial selection for sternopleural bristle number.

There were a total of four crosses (10 *Sam* or *Sam m* males × 10 H or L substitution line females) for each candidate locus. For each cross, four replicates of 10 males and 10 females each were scored for sternopleural and abdominal bristle number, except for the C*1* candidates in which only females were scored. As the total number of flies scored (13 candidate locus mutations × 4 crosses/locus × 4 replicates/cross × 10 individuals/sex × 2 sexes = 4160 individuals) was too large for the entire experiment to be executed at one time, the replicates were set up sequentially, but all 13 mutations were assessed simultaneously within each replicate.

Complementation test analysis: The data were analyzed by four-way ANOVA for sternopleural and abdominal bristle number separately, using the model

$$Y = \mu + C + S + L + R + C \times S + C \times L + C \times R + L \times S + L \times R + S \times R + C \times L \times S + C \times L \times R + C \times S \times R + S \times L \times R + C \times S \times L \times R + \text{error},$$

where *C* is the cross (mutant or control), *S* is sex, *L* is line (high or low), and *R* is replicate (1–4). The main effects of *C*, *S*, and *L* were considered fixed, with *R* random. Any test in which the cross × line or cross × sex × line terms had *P* values of 0.05 or less was interpreted as a failure to complement.

RESULTS

Selection response: The response to divergent selection for sternopleural bristle number from the Raleigh population is shown in Figure 1. Response to 25 genera-

tions of selection should be primarily from alleles segregating in the base population and not from new mutations (Hill 1982). The average sternopleural bristle number from generations 23–25 was 44.5 in the high line and 9.6 in the low line. Responses were asymmetrical: 27.2 bristles in the high line and 7.8 bristles in the low line. There was a correlated response in abdominal bristle number, with mean abdominal bristle numbers in the high and low lines at G_{25} of 22.2 and 11.1, respectively. The correlated responses were also asymmetrical: 3.8 in the high line and 7.3 in the low line. The realized heritability (h^2) and additive genetic variance (V_A) of sternopleural bristle number were estimated by restricted maximum likelihood from the first 10 generations of divergence. These estimates were $h^2 = 0.34$ and $V_A = 1.43$, in good agreement with previous estimates from other wild populations for this trait (*e.g.*, López-Fanjul and Hill 1973a,b).

Isogenic sublines were constructed from the high and low selection lines using balancer chromosomes. One IH and two IL (IL1, IL2) sublines with sternopleural bristles closest to those of the selection lines were saved for mapping. The mean sternopleural bristle numbers in the isogenic sublines were 40.53 for IH, 9.54 for IL1, and 9.63 for IL2. Mean abdominal bristle numbers recorded were 16.18 for IH, 7.7 for IL1, and 12.38 for IL2.

RI lines: 129 RI C*1* (*X*) lines and 74 RI C*3* lines were established and made homozygous in a low selection line background. Mean sternopleural and abdominal bristle numbers were determined for each RI line from 20 animals of each sex. The data set was completely balanced, with 5160 flies scored for the *X* chromosome and 3040 flies scored for the third chromosome RI lines. The average sternopleural bristle score among the RI lines ranged from 9.45 to 12.63 in the *X* lines and from 9.8 to 19.6 in the C*3* lines. The average abdominal bristle number ranged from 9.55 to 19.63 in the *X* lines and 5.83 to 12.9 in the C*3* lines.

Cytological sites of *roo* insertion were scored for each RI line. There were 15 informative *roo* markers on the *X* chromosome and 29 on C*3*. Two markers on the *X* and six on C*3* were not informative because there were no crossovers between them. The informative markers and their estimated map positions based on observed recombination fractions are given in Table 1. The average spacing of informative *X* chromosome markers was 4.1 cM and of the third chromosome markers was 4.7 cM, for a mean intermarker distance throughout the portion of the genome tested of 4.5 cM. Most recombinant chromosomes had a single crossover event. The largest interval without an informative marker was 11.3 cM on the *X* chromosome (between markers 5D and 8E) and 27 cM on chromosome *3* (between markers 65F and 69F). Some of the RI lines had no detectable recombination based on the *roo* markers: 39 (7) of the *X* (C*3*) chromosome lines had the low and 25 (8) had

TABLE 1

Polymorphic markers and estimated map positions

No.[a]	Cyt[b]	cM[c]	No.	Cyt	cM	No.	Cyt	cM	No.	Cyt	cM	No.	Cyt	cM
								Chromosome *1*						
1	1A	0.0	2	3E	0.8	3	3F	2.4	4	4F	6.4	5	5D	13.0	6	8E	24.3
7	9A	32.7	8	11E	40.2	9	12C	41.8	10	13D	45.8	11	15D	49.8	12	16A	51.4
13	16F	53.8	14	18A	56.2	15	18D	57.0									
								Chromosome *3*						
1	61D	0.0	2	62D	4.4	3	64B	7.2	4	64C	8.6	5	65D	13.0	6	65F	15.8
7	69F	42.8	8	70E	45.6	9	75B	50.0	10	84F	54.3	11	86B	57.2	12	87F	58.6
13	89A	66.1	14	89B	70.4	15	89E	73.3	16	90F	77.6	17	91B	80.5	18	92C	83.4
19	92E	84.8	20	93D	87.6	21	94E	96.7	22	95A	98.1	23	96F	107.3	24	97A	108.7
25	97B	111.5	26	97C	112.9	27	98E	122.1	28	98F	126.4	29	100C	132.3			

[a] Marker number.
[b] Cytogenetic insertion site.
[c] Estimated map position in centimorgans.

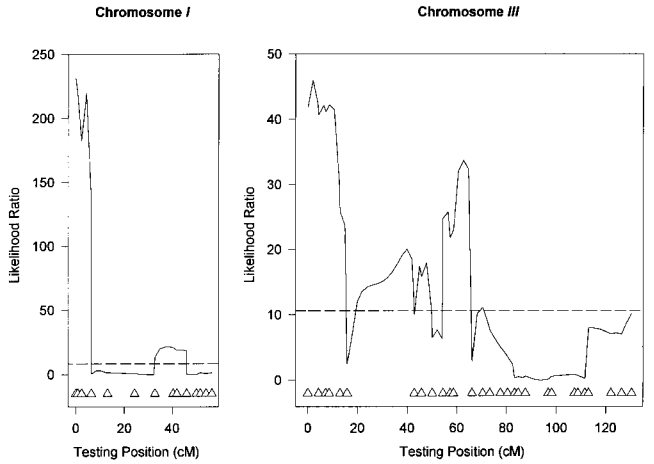

Figure 2.—Composite interval mapping results for male sternopleural bristle number. The triangles on the *x*-axis correspond to the cytogenetic markers, and the interval sizes correspond to map distances between markers, in centimorgans, estimated from the observed recombination between markers. The *y*-axis is the value of the likelihood-ratio (LR) test statistic. The horizontal dashed line indicates the threshold for significance of LR test statistics obtained by permutation. The permutation thresholds were 8.4 for chromosome *1* and 10.6 for chromosome *3*.

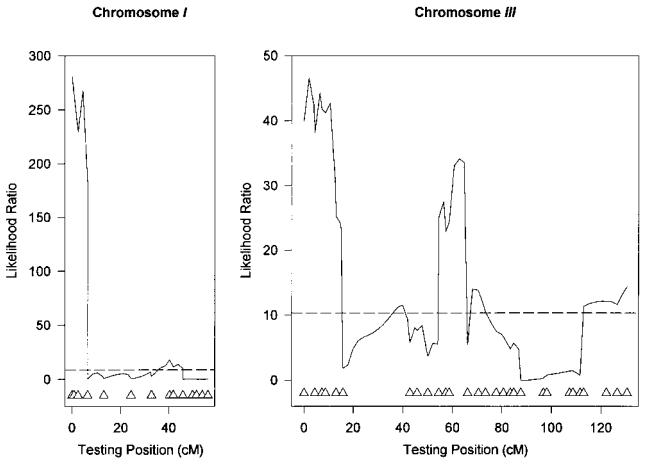

Figure 3.—Composite interval mapping results for female sternopleural bristle number. The *x*- and *y*-axes are as in Figure 2. Significance thresholds, obtained by permutation, were 8.6 and 10.3 for the first and third chromosomes, respectively.

the high parental *roo* marker patterns. Mean bristle numbers and *roo* insertion sites are given for all *X* and C*3* RI lines in the appendices 1 and 2.

QTL mapping: Factors affecting divergence in bristle number between high and low selected chromosomes were mapped using a composite interval mapping method (Zeng 1994). Single markers significantly associated with variation in the trait phenotype were chosen using a forward selection–backward elimination multiple regression procedure. The test for the presence of a QTL at a location within an interval was then conditioned on the markers *x* cM from the markers flanking the test interval. To investigate the effect of varying *x*, the window size, on estimates of QTL positions, analyses were done for four different window sizes: 2, 5, 10, and 15 cM. The significance thresholds for the LR test statistic were determined by permutation separately for each analysis.

Varying the size of the conditioning window had little effect on the location of the significant QTL peaks, although the breadth of the significant regions increased with increasing window size. The QTL likelihood profiles for sternopleural and abdominal bristle number in each sex are depicted in Figures 2–5 for models using a window size of 15 cM (chromosome *3* sternopleural bristle number) and 5 cM (chromosome *1* sternopleural and abdominal bristle number, chromosome *3* abdominal bristle number). These particular models are presented as the best compromise between breadth and significance of QTL peaks and to minimize differences between QTL detected in males and females. The permutation-derived significance thresholds are shown on the graphs—they range from LR statistics of 7.5 to 8.6 for the *X* chromosome analyses and 10.2 to 10.6 for the chromosome *3* analyses. The map positions, LR values, and estimated effects of significant QTL are given for these models in Tables 2 (chromosome *1*) and 3 (chromosome *3*).

The picture for the *X* chromosome is simple: there are two QTL associated with response to selection for sternopleural bristle number and correlated response in abdominal bristle number in both sexes. The QTL located at the tip of the *X* chromosome had very strong statistical support, with LR test statistics >200. This region may be complex genetically, as there were two consistent peaks in each of the analyses. A third *X* chromosome QTL was associated with female abdominal

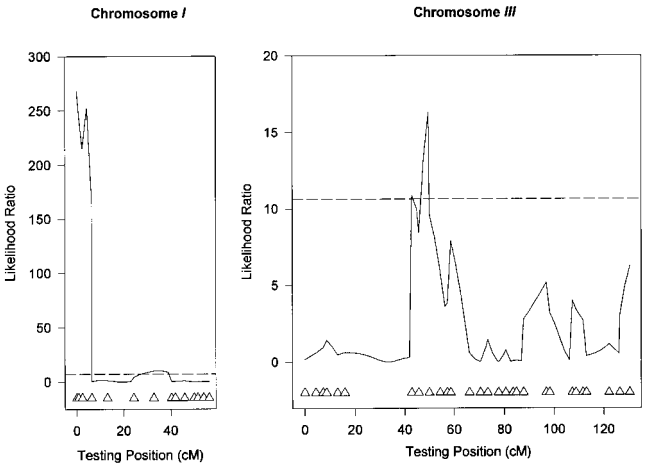

Figure 4.—Composite interval mapping results for male abdominal bristle number. The *x*- and *y*-axes are as in Figure 2. Significance thresholds, obtained by permutation, were 7.1 and 10.6 for the first and third chromosomes, respectively.

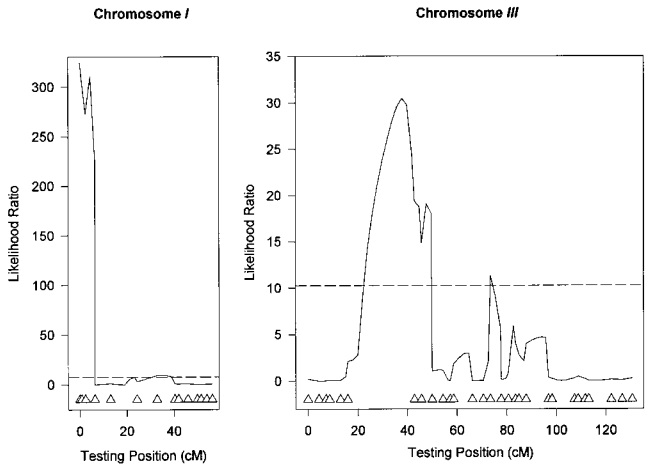

Figure 5.—Composite interval mapping results for female abdominal bristle number. The *x*- and *y*-axes are as in Figure 2. Significance thresholds, obtained by permutation, were 7.5 and 10.2 for the first and third chromosomes, respectively.

bristle number. The sum of the *X* chromosome QTL effects, averaged over sexes, was 1.22 sternopleural bristles and 3.04 abdominal bristles. The total divergence between the high and low *X* chromosomes was 2.30 sternopleural bristles and 5.24 abdominal bristles. Thus, the mapped QTL accounted for 53% of the total divergence in sternopleural bristle number and 58% of the divergence in abdominal bristle number. However, the fit of the QTL models was excellent, with 90% of the variance explained. There are two possible, and nonmutually exclusive, explanations for this discrepancy. One is that we have underestimated the number of QTL. If there is truly a second QTL at the tip of the *X* chromosome, as indicated by the second LR peak, the summed sternopleural and abdominal QTL effects would be 2.21 and 5.96, respectively; and 96% (114%) of the divergence of the parental lines in sternopleural (abdominal) bristle number would be accounted for. The second possibility is that the QTL interact synergistically.

There were multiple QTL affecting sternopleural bristle number on the third chromosome. Some of the QTL peaks were in adjacent intervals. We have adopted the convention that distinct QTL are reported if significant LR peaks are separated by LR scores that fall below the significance threshold. According to this criterion, there were five QTL affecting male and five QTL affecting female sternopleural bristle number. Three QTL mapped to the same location in both sexes—the QTL at the tip of the left arm of the chromosome (61D–62D), which showed signs of genetic complexity, and the QTL in the 65F–69F and 87F–89A intervals. The fourth QTL, which mapped to position *3*-70.46 (89B–89E) in males and *3*-68.11 (89A–89B) in females, is most parsimoniously viewed as being the same QTL in both sexes. The remaining two QTL were sex-specific: the one in interval 70E–75B was expressed in males, and the one near the telomere of *3*R, between 98F–100C, was expressed in females.

The effects of the sternopleural bristle number QTL were moderately large, averaging 1.3 bristles (or 1.1 σ_A, 0.63 σ_P; where σ_A and σ_P, are, respectively, the additive genetic and phenotypic standard deviation units of the base population). The range of QTL effects was from 0.70 to 1.62 bristles (0.59–1.35 σ_A; 0.34–0.79 σ_P). The cumulative effect of the QTL was 6.49 bristles averaged over males and females. The mapped QTL accounted for 85% of the 7.68 bristle divergence between the high and low selected third chromosomes.

Two QTL affecting abdominal bristle number were detected in males and in females. The male abdominal bristle QTL are in adjacent intervals, 69F–70E and 70E–75B. The first female abdominal bristle QTL covers a broad range, from 65F to 75B, with the peak LR in the 65F–69F interval. However, there is a second peak from

TABLE 2

Chromosome *1* bristle number QTL

		Map position (range)		Map position (LR peak)			
Trait	Sex	cM[a]	Cyto[b]	cM	Cyto	LR[c]	Effect[d]
ST	Male	0.01–6.40	1A–4F	0.01	1A–3E	231.4	0.890
		32.78–45.86	9A–15D	38.78	9A–11E	21.54	0.183
ST	Female	0.01–6.40	1A–4F	0.01	1A–3E	281.0	1.074
		36.78–43.86	9A–13D	40.29	11E–12C	18.08	0.291
AB	Male	0.01–6.40	1A–4F	0.01	1A–3E	268.1	2.577
		28.29–38.78	8E–11E	34.78	9A–11E	10.15	−0.304
AB	Female	0.01–6.40	1A–4F	0.01	1A–3E	324.1	3.278
		23.05	5D–8E	3.05	5D–8E	7.71	0.874
		30.29–38.78	8E–11E	34.78	9A–11E	9.42	−0.352

[a] Map position in centimorgans.
[b] Cytogenetic interval.
[c] Likelihood-ratio test statistic.
[d] QTL effect in bristles.

TABLE 3

Chromosome *3* bristle number QTL

		Map position (range)		Map position (LR peak)			
Trait	Sex	cM[a]	Cyto[b]	cM	Cyto	LR[c]	Effect[d]
ST	Male	0.01–14.99	61D–65F	2.01	61D–62D	46.00	1.542
		19.85–41.85	65F–69F	39.85	65F–69F	20.09	1.534
		44.80–49.66	69F–75B	47.66	70E–75B	17.97	1.420
		54.36–64.63	84F–89A	62.63	87F–89A	33.74	1.623
		70.11–70.46	89A–89E	70.46	89B–89E	11.11	0.815
ST	Female	0.01–14.99	61D–65F	2.01	61D–62D	46.52	1.534
		37.85–39.85	65F–69F	39.85	65F–69F	11.52	1.262
		54.36–64.63	84F–89A	62.63	87F–89A	34.14	1.507
		68.11–72.46	89A–89E	68.11	89A–89B	14.00	1.041
		113.01–130.48	97C–100C	130.48	98F–100C	14.34	0.704
AB	Male	42.80–44.80	69F–70E	42.80	69F–70E	10.89	0.797
		47.66–49.66	70E–75B	49.66	70E–75B	16.30	1.039
AB	Female	23.85–49.66	65F–75B	37.85	65F–69F	30.47	1.497
		73.32	89E–90F	73.32	89E–90F	11.31	1.176

[a] Map position in centimorgans.
[b] Cytogenetic interval.
[c] Likelihood-ratio test statistic.
[d] QTL effect in bristles.

70E to 75E. It is likely that there are two abdominal bristle QTL with effects in both sexes in this region. The abdominal bristle QTL in the 89E–90F interval is female-specific.

Epistatic effects: Interaction effects between pairs of nonadjacent QTL were estimated using multiple regression models that included all QTL with significant main effects and the interaction of interest. No significant interactions were found between QTL affecting abdominal bristle number. However, of the 15 tests for epistasis between QTL affecting sternopleural bristle number, six (40%) were significant. Estimates of the epistatic effects are given in Table 4 and are depicted graphically in Figure 6. The interactions involve the two *X* chromosome QTL and the QTL associated with the third chromosome markers 61D (three interactions), 69F (two interactions), 89B (two interactions), and 100C (two interactions). Five of the six epistatic interactions were positive. The epistatic effect between the *X* chromosome

TABLE 4

Epistatic interactions for sternopleural bristle number

Chromosome	Interaction	Effect ± SE
X	1A × 11E	0.33 ± 0.08**
3	61D × 69F	1.38 ± 0.31*
	61D × 89B	0.94 ± 0.22***
	61D × 100C	0.92 ± 0.22***
	61D × 69F	0.33 ± 0.08**
	69F × 75B	−6.07 ± 0.57***
	89B × 100C	0.69 ± 0.16*

* $P \leq 0.05$; ** $P < 0.001$; *** $P < 0.0001$.

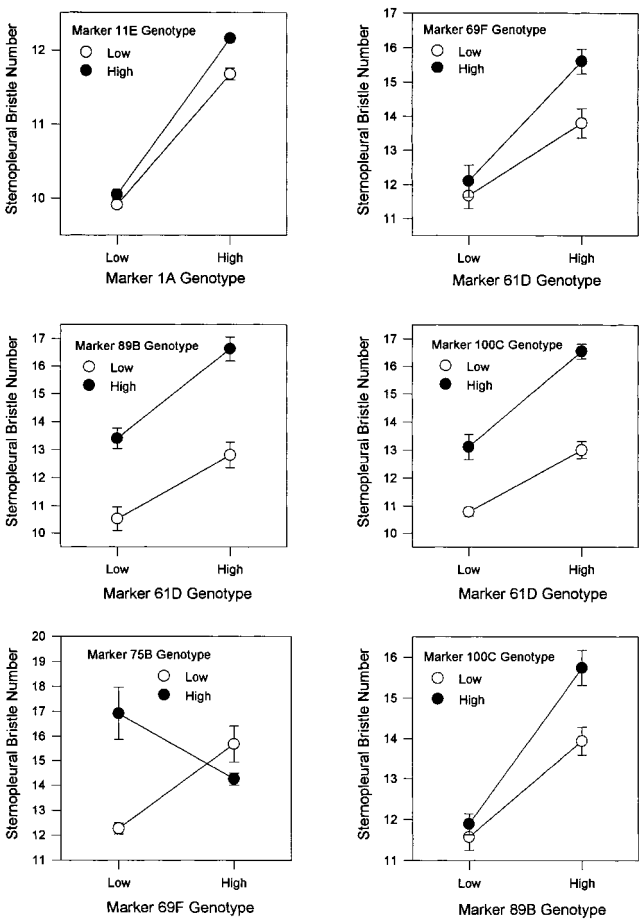

Figure 6.—Epistatic interactions between sternopleural bristle number QTL.

QTL was not large (0.33 bristle), whereas the average of the four positive chromosome *3* epistatic effects was 0.98 bristle—the same magnitude as the main effects.

The epistatic interaction between the sternopleural bristle QTL associated with markers 69F and 75B was larger than any of the main QTL effects detected—six bristles—and was, surprisingly, negative. Positive, not negative, interactions are expected between alleles at loci associated with response to directional artificial selection. However, negative interactions between loci might be expected for a trait that is under stabilizing natural selection (Robertson 1967; Kondrashov and Turelli 1992).

The observations of extensive epistatic interactions between bristle number QTL are interesting with respect to the genetic architecture of the trait. However, they complicate the interpretation of the main QTL effects, because the estimates presented are not strictly valid in the presence of epistasis. There were not sufficient degrees of freedom in our data set to evaluate a model that included all significant QTL and the significant pairwise interactions.

Quantitative complementation tests: The resolution of QTL mapping by linkage to marker loci from analysis of F_2 segregating genotypes from a cross of divergent parental lines is limited by the low levels of recombination between closely linked markers. Often, candidate loci with functional effects on the quantitative trait map to the same intervals at the QTL. It is therefore possible to test whether QTL alleles interact genetically with mutant and wild-type candidate locus alleles. If so, we can conclude that either naturally occurring variation for the quantitative trait is attributable in part to allelic variation at the candidate locus or that alleles at another QTL interact epistatically with the candidate locus alleles. In the former case, the QTL has been mapped to the level of genetic locus, bypassing the need for fine-scale recombination mapping. However, the ambiguous interpretation of a genetic interaction between candidate locus and QTL alleles (allelism or epistasis) motivates the need to evaluate interactions between QTL alleles and candidate loci with map positions that do *not* correspond to those of QTL as well as those with positions that concur.

Of the 10 intervals with significant effects on bristle number, 5 included candidate loci with effects on adult bristle number for which we had available suitable strains for complementation tests. The positional candidate loci tested (and the QTL interval to which they correspond) were as follows: the *achaete-scute complex* (*ASC*, 1B) and *Notch* (*N*, 3C) [1A–4F]; *cut* (*ct*, 7B) [5D–8E]; *extramacrochaetae* (*emc*, 61D) [61D–62D]; *que mao* (*qm*, 65F), *hairy* (*h*, 66D), and *abdominal* (*abd*, 66D–67A) [65F–69F]; and *polychaetoid* (*pyd*, 69C–70A) [69F–70E]. The map positions of four candidate loci, *Sex combs-reduced* (*Scr*, 84B), *Delta* (*Dl*, 92A), *Hairless* (*H*, 92E–92F), and *Enhancer-of-split* [*E(spl)*, 96F] do not coincide with those of significant QTL. All 12 candidate loci were tested for quantitative complementation with QTL alleles by crossing mutant and wild-type candidate locus alleles to the high and low selected chromosomes and assessing the effect of the cross (mutant or wild type) × line (high or low selected chromosome) interaction by ANOVA of bristle number of the four heterozygous genotypes. If this interaction term is significant, the effect of substituting a mutant for a wild-type allele at the candidate locus is different in the high and low selected backgrounds, and there is quantitative failure to complement (Long *et al.* 1996; Mackay and Fry 1996). A nonsignificant cross × line interaction term indicates that the difference in bristle number between mutant and wild-type heterozygotes is independent of genetic background, *i.e.*, quantitative complementation.

The results of the quantitative complementation tests are given in Table 5. There was no evidence for failure of the QTL alleles on the high and low *X* chromosomes to complement the sternopleural bristle number effects of the *X* chromosome candidate locus mutations (*sc*, *spl*, and ct^6). Likewise, high and low chromosome *3* QTL alleles complemented the sternopleural bristle effects of mutations at *H* and one of the two *emc* mutant alleles tested. There was, however, significant failure of high and low chromosome *3* QTL alleles to complement the other *emc* mutant allele and alleles of the remaining C*3* candidate loci for sternopleural number. None of the abdominal bristle number complementation effects were significantly different from zero, although the cross × line interaction was close to formal significance for both *sc* and *spl* ($P = 0.06$).

DISCUSSION

Numbers of QTL: A total of eight QTL affecting sternopleural bristle number were detected, two of which mapped to the *X* and six to the third chromosome. It is interesting that the estimate of the minimum number of loci contributing to selection response from mapping QTL agrees with an estimate from a biometrical analysis. The IH isogenic strain derived from the high sternopleural bristle selection line was crossed reciprocally to both the IL1 and IL2 strains derived from the low selection line. Each of the four F_2 populations was selected for high and low sternopleural bristle number for four generations, with an intensity of 10 selected males and females out of 20 of each sex scored per generation. The realized heritability was estimated by restricted maximum-likelihood analysis of the divergent selection response by the same method as Mackay *et al.* (1994), and the additive genetic variance of sternopleural bristle number, V_A, was estimated from the product of the heritability and the phenotypic variance. The average V_A of the four replicate lines (± empirical standard error) was 9.294 ± 0.378, and the average divergence, *D*, between the parental lines was 30.94 bris-

TABLE 5

Quantitative complementation tests

	Sternopleural bristles			Abdominal bristles		
Mutation	$H(M-C)$[a]	$L(M-C)$[b]	$C \times L$[c]	$H(M-C)$	$L(M-C)$	$C \times L$
sc	−0.68	−0.65	NS	−2.93	−1.78	*
spl	−0.68	−1.00	NS	−0.30	0.90	*
ct^6	−0.35	−0.30	NS	−0.68	−0.33	NS
$emc^{P(3)5}$	0.74	−0.28	**	−0.13	−0.79	NS
$emc^{P(3)67}$	0.88	0.30	NS	0.60	0.24	NS
qm	1.30	−0.09	***	0.02	0.22	NS
h	1.21	−0.05	***	1.08	0.41	NS
abd	0.06	−0.83	**	−0.85	−0.98	NS
pyd	0.79	−0.75	***	0.57	0.99	NS
Scr	2.70	1.83	*	−1.73	−1.44	NS
Dl	1.63	−0.76	***	1.65	2.62	NS
H	−3.76	−3.20	NS	−3.17	−3.06	NS
E(spl)	0.21	−0.38	*	−0.51	−0.25	NS

NS, $P > 0.05$; *, $0.10 > P > 0.05$; **, $0.05 > P > 0.01$; ***, $0.01 > P > 0.001$; ****, $P < 0.001$.

[a] The difference in bristle number between heterozygotes for mutant and wild-type (control) alleles at the candidate loci in the high-selection line background, averaged over sexes and replicates.

[b] The difference in bristle number between heterozygotes for mutant and wild-type (control) alleles at the candidate loci in the low-selection line background, averaged over sexes and replicates.

[c] The results of tests of significance of the cross × line interaction term from four-way factorial analyses of variance of bristle number, in which variance in bristle number was partitioned into sources attributable to the main effects of cross, *C* (mutant or control allele at the candidate locus); line, *L* (high or low selection line); sex, *S*; and replicate, *R*; and all their interactions. The $C \times L$ term tests whether the complementation effect $[H(M-C) - L(M-C)]$ is significantly different from zero.

tles. Thus, the effective number of loci, n_e, contributing to divergence between the parental lines is $n_e = D^2/8\,V_A$ (Wright 1968; Lande 1981; Zeng *et al.* 1990; Falconer and Mackay 1996), or ~13. This estimate is biased downward by unequal allelic effects, but the other assumptions of the method, *i.e.*, allele frequencies equal to 0.5 and all increasing (decreasing) alleles fixed in the high (low) lines, are likely to hold well in this case. Linkage also induces a large bias toward underestimating the number of loci, particularly in Drosophila, with only three major chromosomes and no male recombination, but this is mitigated by estimating V_A from four generations of selection response, during which loosely linked QTL will have been separated by recombination. We mapped eight QTL on the *X* and third chromosomes, which is ~60% of the Drosophila genome. Thus, we estimate ~13 QTL affecting sternopleural bristle number in the whole genome, assuming a random genomic distribution of bristle number QTL, which exactly corresponds to the estimate of 13 effective factors.

QTL effects: The homozygous effects of bristle number QTL were moderately large, but variable. For example, the QTL at the tip of the *X* chromosome had a threefold greater effect on sternopleural bristle number than the other *X* chromosome QTL. These data are consistent with the distribution of QTL effects first proposed by Robertson (1967), whereby a few leading QTL account for the majority of the selection response. This model is also supported by Shrimpton and Robertson's (1988b) analysis of factors affecting selection response for sternopleural bristle number and by distributions of effects of *P*-element insertional mutations affecting bristle number (Mackay *et al.* 1992; Lyman *et al.* 1996). However, it is not possible to say from these data whether the effects of QTL are due to allelic variation at a single locus or to variation at multiple linked loci in the significant intervals.

A second caveat regarding the estimates of homozygous QTL effects is that these effects were estimated using a model that did not take epistatic interactions into account, and interaction effects of sternopleural bristle number QTL were substantial and common. These observations are consistent with those of Long *et al.* (1995), who found significant pairwise interactions between chromosome *3* QTL for both abdominal and sternopleural bristle number, and with those of Shrimpton and Robertson (1988a,b), who found epistasis between QTL in section 2 (~66D–73A) and section 5 (~93D–99B) of their high sternopleural bristle number chromosome *3*. These observations seem contradictory to the widely held dictum that genetic variance for bristle number is predominantly additive, with little contribution from dominance and interaction variance. However, the theoretical genetic covariances among relatives include fractions of the additive genetic variance plus additive × additive and higher-order interaction

variances (Falconer and Mackay 1996). The contribution of interaction variance can only be partitioned from the total additive variance in experiments of heroic proportions. In practice, additive × additive and the higher-order interaction variance components are confounded with the additive variance, and the conclusion that epistatic effects are unimportant based on variance component analyses is unwarranted.

Two of the eight QTL affecting sternopleural bristle number had sex-specific effects. Variation in the sex dimorphism of QTL affecting bristle number is emerging as a common feature of the genetic architecture of bristle traits. Sex-specific effects have been observed for abdominal bristle number QTL (Clayton and Robertson 1957; Frankham 1968; Long *et al.* 1995; Gurganus *et al.* 1998; Lyman and Mackay 1998), *P*-element-induced (Mackay *et al.* 1992; Lyman *et al.* 1996) and spontaneous (Mackay *et al.* 1994, 1995) mutations affecting bristle number, and molecular polymorphisms in the candidate loci *ASC* (Mackay and Langley 1990) and *Dl* (Long *et al.* 1998) associated with naturally occurring variation in bristle number. This property of QTL should be taken into account when evaluating evolutionary models for the maintenance of variation of these traits (*e.g.*, Lande 1975; Turelli 1984; Barton 1990; Keightley and Hill 1990; Kondrashov and Turelli 1992).

Gene frequency: Indirect evidence that genes affecting variation in bristle number might be at intermediate frequency in natural populations comes from experiments in which selection limits from large and from single-pair bottleneck base populations are compared. If rare alleles are largely responsible for selection response, the limit will be severely reduced in the bottlenecked lines. This is not the case (Robertson 1968; Frankham 1980), suggesting that the majority of alleles contributing to selection response are at intermediate frequencies. However, the large variance in response among long-term selection lines from the same population indicates that rare genes with large effects are also present in natural populations (Robertson 1968; Yoo 1980).

Direct estimates of gene frequency at loci causing variation for bristle number in nature will only be possible when bristle number QTL have been resolved to the level of genetic locus; and, further, the molecular polymorphisms at the loci responsible for the phenotypic variation have been identified. If alleles responsible for quantitative genetic variation are at intermediate frequencies in nature, then the same alleles should be responsible for response to selection in replicate lines derived from the same base population, provided the selection lines are not too small. In this regard, it is interesting that several of the intervals containing QTL affecting direct response to sternopleural bristle number from the Raleigh population were also found to be responsible for correlated response in sternopleural bristle number by Long *et al.* (1995); and, conversely, intervals containing QTL associated with direct response to selection for abdominal bristle number by Long *et al.* (1995) were found here to overlap those associated with correlated response in abdominal bristle number. The region at the tip of the *X* chromosome affected direct and correlated responses of both bristle traits in both studies. Two third-chromosome intervals associated with direct response to selection for sternopleural bristle number, 61D–62D and 98F–100C, overlapped those associated with correlated response in sternopleural bristle number, 61A–64C and 97F–100C. Similarly, two third-chromosome intervals containing QTL affecting direct response in abdominal bristle number, 66A–67C and 89D–92E, overlapped those affecting correlated response in abdominal bristle number, 65F–69F and 89A–90F.

Other populations: The issue of whether the same loci cause segregating variation for quantitative traits in different populations is central to our eventual understanding of the relative strengths of the evolutionary forces maintaining this variation. Natural populations of *D. melanogaster* have similar mean numbers of sternopleural ($\bar{x}$ = 18.6; López-Fanjul and Hill 1973b) and abdominal ($\bar{x}$ = 21.4; Reeve and Robertson 1954) bristles. Indirect evidence that genetic variation for bristle number within populations is largely attributable to segregation at the same loci comes from experiments in which response to long-term selection for bristle number is compared for lines derived from independent populations and from crosses between them. With no epistasis, the response from the synthetic population will exceed those of the single populations if different loci affect variation in the trait, but not if the same loci segregate in the parental populations. López-Fanjul and Hill (1973a,b) used this technique to infer that variation for sternopleural bristle number occurred at the same loci in three geographically distinct populations (Kaduna, Nigeria; Pacific, United States; and Canberra, Australia) that had been maintained in the laboratory for several years, but that a fourth population (Stellenbosch, South Africa) contained genetic variation that was not present in the laboratory populations.

More direct evidence that alleles at the same loci are at least partly responsible for variation in bristle number in different populations comes from comparing the map positions of bristle number QTL from different base populations. Gurganus *et al.* (1998) mapped QTL affecting abdominal and sternopleural bristle number by linkage to *roo* markers in a panel of recombinant inbred lines derived from two independent laboratory strains, Oregon and 2b. One *X* chromosome QTL affecting abdominal bristle number (1B–5D) and one affecting sternopleural bristle number (3E–5D) coincided with the large QTL at the tip of the *X* chromosome affecting both bristle traits in the Raleigh population (this article; Long *et al.* 1995). The positions of the four

third-chromosome QTL affecting sternopleural bristle number (61A–63A, 69D–87B, 82D–91D, 94D–98A) overlapped those of the QTL affecting sternopleural bristle number in this study and that of Long *et al.* (1995). Similarly, the positions of the three third-chromosome QTL affecting abdominal bristle number (65D–68B, 91A–93B, 91D–96A) detected by Gurganus *et al.* (1998) overlapped the two intervals containing abdominal bristle number QTL that were mapped both in this study and that of Long *et al.* (1995).

Nuzhdin *et al.* (1998) mapped QTL affecting sternopleural bristle number by monitoring the change in gene frequency of neutral transposable element marker alleles during response to divergent selection from an Oregon × Canton synthetic base. One chromosome *1* (4B–6A) and two chromosome *3* (92C–94E, 97B–98E) QTL were detected. Their positions correspond to those of sternopleural bristle number QTL detected by Gurganus *et al.* (1998; which is not surprising, given that these studies used the Oregon strain as a common parent), Long *et al.* (1995), and this article.

Several independent studies on high sternopleural bristle number third chromosomes derived from the Kaduna population in Edinburgh, using linkage to visible morphological markers (Piper 1972), backcrossing with selection (Mostafa 1963), and interval mapping combined with progeny testing (Shrimpton and Robertson 1988a,b), all showed that the greatest effect was concentrated between 66C–73A, corresponding to our chromosome *3* QTL in intervals 65F–69F and 70E–75B. Finally, Thoday and his colleagues pioneered the use of interval mapping and progeny testing using visible recessive markers to map sternopleural bristle number QTL. Factors affecting response to selection for high sternopleural bristle number from replicate synthetic populations derived from *dumpy* and *vestigial* mutant stocks of unknown background and an Oregon strain were mapped to the *X* (1B–5B, 10A–15F; Spickett and Thoday 1966) and third (two factors between 66D–69C; Thoday *et al.* 1964) chromosomes. The chromosome *1* regions overlap the large factor at the tip found in the Raleigh population and Oregon-derived lines, and the QTL at 9A–11E detected in this study. The chromosome *3* region corresponds to our QTL in the 65F–69F interval and the Kaduna region with the largest total sternopleural bristle effect. Wolstenholme and Thoday (1963) mapped two factors affecting response to disruptive selection for sternopleural bristle number from a wild-caught isofemale line to the 85A–87C interval, which corresponds to the 84F–89A interval containing sternopleural bristle QTL reported here, and the 82D–91D interval associated with divergence in sternopleural bristle number between the Oregon and 2b strains (Gurganus *et al.* 1998).

The actual number of loci affecting variation in bristle number is likely to be much larger than that inferred from initial coarse-mapping analyses (Shrimpton and Robertson 1988b). Nevertheless, it is encouraging that the same relevant genomic regions are recurring in studies that span a period of 35 yr, for populations of diverse origin, and using different mapping methodologies. This strongly suggests that considerable progress in understanding naturally occurring variation for bristle number can be made by studying the loci that contribute the most variation within populations and that are also polymorphic in multiple populations.

Candidate loci: The data we require to understand the genetic architecture of bristle number, or any quantitative trait, are the numbers and additive allelic effects of segregating loci, epistatic and pleiotropic effects, allele frequencies, and mutation rates and mutational effects at these loci. Coarse localization of QTL is only the first step of a multistage process toward identifying the actual genetic loci causing quantitative variation and serves to narrow the genomic regions on which to focus further effort. Unfortunately, different mapping methods tend to pinpoint different, though usually adjacent, intervals associated with variation in the trait. The practice of conditioning on other significant intervals when estimating the position and effect of the test interval is advantageous in that it reduces the within-line variance by taking account of segregating QTL. However, the choice of window size to use in the conditioning is arbitrary, and the width and significance of intervals can be altered by varying the window size.

The second stage is to refine the map positions of the QTL within intervals by further recombination. There is a limit to the resolution that can be achieved, particularly in regions of low recombination, and methods for further fine-scale mapping need to be investigated. In Drosophila, deficiency mapping across intervals and complementation tests to candidate loci within the intervals may be used to fine-map QTL, in the latter case to the level of genetic locus. It is important to note that several of the intervals affecting high–low divergence in sternopleural bristle number did *not* contain obvious candidate genes. Not all the loci in the bristle development pathway have been identified by mutagenesis; it is possible that novel loci may be found by analyzing naturally occurring variants. It is also possible that naturally occurring variants at essential genes have unexpected and undocumented effects on adult bristle number. For example, the *smooth* and *que mao* loci encode, respectively, a homologue of human heterogeneous nuclear ribonucleoprotein L (zur Lage *et al.* 1997) and geranylgeranyl pyrophosphate synthase (Lai *et al.* 1998) and were identified by *P*-element insertional mutations that severely decreased bristle number. Meiotic and deficiency mapping are the only options for localizing QTL more precisely in these regions.

Here, we tested whether mutations at 12 candidate loci with large effects on adult sternopleural bristle number interacted with high and low QTL alleles on the selected chromosomes. Eight of the candidate loci were

in intervals to which QTL mapped, and four were in intervals that did not contain QTL. All possible results were obtained: complementation (*sc*, *spl*, *ct*, *emc*) and failure to complement (*emc*, *qm*, *h*, *abd*, *pyd*) where the candidate is in a significant interval; and complementation (*H*) and failure to complement [*Dl*, *Scr*, *E(spl)*] where the candidate is not in a significant interval. The interpretation in each case is ambiguous.

1. Complementation of a candidate mutation in a significant interval can occur if the QTL are not allelic to the candidate gene, but an important caveat here is that complementation or failure to complement can be allele-specific (Long *et al.* 1996; Mackay and Fry 1996; this article). It is important in the future to use null alleles of the candidates in these tests to avoid this potential ambiguity. Although the *sc* allele used complemented the bristle number QTL at the tip of the *X*, naturally occurring insertional variation at the *ASC* has been shown to be associated with variation in sternopleural and abdominal bristle number (Mackay and Langley 1990).
2. Failure of QTL to complement a candidate mutation in a significant interval can occur if the QTL are allelic to the candidate gene or if there is epistasis between the QTL alleles and candidate gene mutation. Because epistatic interactions between chromosome *3* QTL were common and substantial, both interpretations are possible.
3. Complementation of a candidate mutation in a nonsignificant interval is to be expected, provided the mapping results are accurate and epistasis is not important. However, one should apply the same standards here and ensure that null mutations at the candidate genes are used in these tests in the future.
4. Failure to complement of a candidate locus that maps to a nonsignificant interval could be attributable to epistasis or to allelism if the effect of the interval was too small to be detected in the mapping experiment. This result was obtained for *Dl*, and independent evidence favors the latter interpretation. Naturally occurring alleles in 20-cM regions containing the *Dl* locus failed to complement the same *Dl* mutation used in this study for sternopleural bristle number (Lyman and Mackay 1998), and a polymorphism in the second intron of *Dl* was associated with an effect of 0.65 sternopleural bristles (Long *et al.* 1998). An effect this small could not be significant in our sample of chromosome *3* RI lines, but the complementation tests were larger and thus had more power to detect small effects.

These results do not negate the utility of the complementation approach to refine QTL map locations; however, they do suggest that the outcome of these tests will be less ambiguous if applied to introgressed genomic regions containing the candidate QTL in a standard, nonselected background, and if a null allele of the candidate gene is used. The challenge of the future is to resolve QTL into constituent genetic loci.

This work was supported by National Institutes of Health grants GM-45344 and GM-45146 to T.F.C.M.

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Communicating editor: A. G. Clark

APPENDIX 1

Phenotypic and marker data for the *X* chromosome

	Sternopleural bristles				Abdominal bristles																		
	Males		Females		Males		Females		Markers														
Line	Mean	SE	Mean	SE	Mean	SE	Mean	SE	1A	3E	3F	4F	5D	8E	9A	11E	12C	13D	15D	16A	16F	18A	18D
HLL	12.05	0.16	12.83	0.17	17.65	0.26	17.80	0.31	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
LLL	9.93	0.15	9.93	0.14	12.13	0.25	10.93	0.26	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
1	9.65	0.22	9.90	0.14	13.05	0.30	11.10	0.24	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
2	9.85	0.22	9.55	0.31	13.45	0.28	11.10	0.38	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
3	9.40	0.20	9.75	0.22	13.30	0.34	11.90	0.31	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
10	11.10	0.22	11.85	0.19	18.35	0.36	17.90	0.40	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L
15	9.60	0.24	10.20	0.17	11.30	0.29	9.90	0.39	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H
17	9.50	0.21	10.10	0.17	12.55	0.43	9.50	0.38	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
19	9.25	0.25	10.20	0.19	13.40	0.28	11.45	0.34	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
26	9.35	0.22	9.85	0.16	13.50	0.24	10.95	0.36	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H
27	11.10	0.19	11.80	0.24	17.75	0.30	17.00	0.48	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L
28	9.50	0.19	10.00	0.17	13.50	0.39	11.25	0.33	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
32	9.45	0.26	9.70	0.16	13.75	0.40	11.25	0.28	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
33	9.60	0.15	10.35	0.19	13.20	0.40	11.55	0.25	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H
36	9.50	0.23	10.00	0.20	12.80	0.28	11.50	0.34	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
41	11.30	0.25	12.85	0.19	18.55	0.32	18.25	0.37	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L
44	9.90	0.22	9.80	0.21	13.20	0.38	11.95	0.30	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H
46	9.50	0.23	9.90	0.19	13.25	0.25	10.85	0.42	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
48	11.75	0.21	11.85	0.16	18.60	0.37	17.85	0.29	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
54	10.00	0.19	10.10	0.19	13.90	0.26	11.60	0.52	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
55	10.25	0.49	10.20	0.27	12.95	0.45	10.95	0.32	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
56	10.20	0.25	10.35	0.20	12.45	0.32	10.50	0.34	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
57	11.55	0.25	12.05	0.22	17.35	0.29	17.40	0.40	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L
61	9.85	0.19	9.65	0.25	12.85	0.33	10.65	0.38	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
65	9.95	0.23	9.90	0.20	13.10	0.26	10.65	0.36	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
66	11.45	0.23	12.30	0.19	18.15	0.30	17.90	0.31	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L
71	9.85	0.25	10.55	0.23	12.80	0.40	11.75	0.50	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H
73	9.90	0.26	9.95	0.22	12.65	0.25	9.75	0.39	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
76	11.90	0.14	12.40	0.23	18.65	0.40	16.35	0.46	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
78	9.95	0.18	10.00	0.14	14.40	0.44	11.30	0.25	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
79	11.75	0.17	12.50	0.13	17.70	0.35	17.25	0.32	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
81	10.05	0.25	9.90	0.19	13.65	0.43	10.55	0.35	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
83	11.90	0.21	12.55	0.23	18.95	0.29	17.55	0.43	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L
93	9.95	0.18	10.40	0.19	12.45	0.30	10.10	0.24	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
94	11.15	0.26	11.60	0.22	17.35	0.75	16.20	0.59	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
96	10.00	0.21	10.35	0.19	13.90	0.31	11.10	0.36	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H
97	9.65	0.25	10.25	0.19	14.20	0.35	12.10	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
98	9.60	0.24	9.85	0.16	13.60	0.26	11.15	0.29	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H

(*continued*)

APPENDIX 1

(Continued)

	Sternopleural bristles				Abdominal bristles																	
	Males		Females		Males		Females		Markers													
Line	Mean	SE	Mean	SE	Mean	SE	Mean	SE	1A	3E	3F	4F	5D	8E	9A	11E	12C	13D	15D	16A	16F	18A	18D
100	11.55	0.24	12.35	0.18	18.50	0.40	18.25	0.37	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
102	10.10	0.12	10.30	0.19	13.45	0.37	11.85	0.25	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
104	9.60	0.24	9.95	0.13	12.85	0.22	11.10	0.45	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H
106	9.95	0.21	10.25	0.20	14.10	0.36	12.25	0.41	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H
108	9.75	0.20	10.20	0.17	13.10	0.27	11.30	0.20	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H
113	11.45	0.21	12.60	0.23	17.50	0.36	18.15	0.34	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
114	10.30	0.25	9.95	0.19	13.50	0.42	11.55	0.37	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H
115	11.70	0.29	12.45	0.18	19.25	0.42	17.65	0.55	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
116	11.35	0.24	12.60	0.16	18.40	0.31	17.95	0.47	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L
117	12.20	0.23	13.00	0.25	18.25	0.30	17.05	0.30	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
118	9.65	0.20	10.30	0.20	13.45	0.28	11.85	0.45	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
119	12.15	0.25	11.95	0.19	17.95	0.42	16.65	0.42	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
122	12.50	0.24	12.55	0.24	18.10	0.45	17.50	0.35	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
123	9.95	0.18	10.10	0.17	13.10	0.25	11.35	0.33	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
126	9.95	0.21	10.00	0.19	13.50	0.23	11.85	0.36	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
127	12.25	0.16	13.00	0.23	18.65	0.47	18.05	0.42	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
128	12.20	0.24	12.75	0.30	17.95	0.45	17.80	0.45	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
132	9.65	0.23	9.95	0.21	13.00	0.36	11.30	0.29	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H
133	11.60	0.23	12.30	0.21	17.55	0.48	17.85	0.38	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L
136	11.90	0.23	12.45	0.26	17.25	0.32	18.15	0.36	H	H	H	H	H	H	H	H	H	L	L	L	L	L	H
138	11.90	0.25	13.00	0.30	18.35	0.39	18.05	0.29	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
139	9.65	0.22	10.55	0.22	12.85	0.33	11.40	0.30	L	L	H	H	H	L	L	L	L	L	L	L	L	L	L
144	11.45	0.27	11.90	0.21	17.65	0.42	16.10	0.54	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L
145	11.75	0.22	12.60	0.22	17.75	0.47	16.90	0.45	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
146	9.85	0.20	10.45	0.21	13.20	0.40	12.10	0.44	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H
147	9.45	0.23	10.15	0.29	12.55	0.30	10.55	0.39	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
148	10.15	0.33	10.10	0.14	11.80	0.30	9.95	0.34	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H
152	11.65	0.34	12.75	0.26	16.95	0.51	17.25	0.39	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
154	12.25	0.27	12.70	0.24	17.55	0.40	16.50	0.49	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
155	9.90	0.22	10.00	0.19	13.05	0.28	10.55	0.30	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
158	11.45	0.17	12.70	0.27	18.05	0.30	16.05	0.57	H	H	H	L	L	L	L	H	H	H	H	H	H	H	H
160	11.35	0.24	12.40	0.23	17.65	0.49	17.35	0.49	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L
163	9.90	0.26	10.35	0.19	13.90	0.27	12.90	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
166	11.90	0.23	11.95	0.19	18.20	0.38	18.10	0.35	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L
167	9.70	0.20	9.95	0.19	13.45	0.33	10.90	0.39	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
168	11.80	0.26	12.55	0.23	17.40	0.45	16.45	0.62	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
169	9.95	0.23	10.00	0.24	12.45	0.34	10.35	0.29	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H

(*continued*)

APPENDIX 1

(Continued)

	Sternopleural bristles				Abdominal bristles																		
	Males		Females		Males		Females		Markers														
Line	Mean	SE	Mean	SE	Mean	SE	Mean	SE	1A	3E	3F	4F	5D	8E	9A	11E	12C	13D	15D	16A	16F	18A	18D
170	12.35	0.18	12.45	0.17	17.00	0.43	17.15	0.36	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
171	11.70	0.25	12.30	0.20	18.00	0.43	18.60	0.35	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
173	9.30	0.20	9.65	0.18	13.10	0.42	11.10	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
175	11.05	0.31	11.80	0.21	18.95	0.30	18.35	0.35	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L
177	11.55	0.13	12.05	0.17	17.55	0.43	18.65	0.40	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
178	11.20	0.44	11.35	0.41	14.20	0.76	12.20	0.60	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H
179	11.65	0.20	12.50	0.26	17.75	0.41	17.65	0.34	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
180	11.25	0.21	12.10	0.17	16.80	0.34	17.35	0.49	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
183	9.10	0.21	10.15	0.18	11.80	0.29	11.55	0.35	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
191	9.90	0.21	10.00	0.17	12.00	0.33	9.65	0.36	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
192	9.80	0.19	10.20	0.23	12.70	0.34	10.40	0.23	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
193	11.15	0.29	12.10	0.22	20.05	0.39	19.20	0.42	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L
194	12.20	0.21	12.35	0.19	18.00	0.37	18.00	0.27	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
197	9.15	0.20	9.75	0.23	13.30	0.28	11.40	0.27	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
199	9.50	0.18	9.70	0.27	13.55	0.28	12.05	0.43	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
201	11.85	0.22	12.20	0.24	17.45	0.63	17.10	0.42	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
206	9.90	0.17	10.25	0.23	12.20	0.32	12.05	0.36	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
208	11.85	0.19	12.20	0.21	17.90	0.36	18.45	0.43	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
209	9.35	0.27	9.95	0.15	12.10	0.27	11.40	0.28	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
210	11.85	0.15	12.30	0.21	17.55	0.32	17.00	0.52	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
211	11.80	0.26	12.25	0.20	18.40	0.38	17.95	0.51	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L
213	9.45	0.13	9.85	0.19	12.35	0.40	11.20	0.36	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
219	9.65	0.24	9.90	0.17	12.85	0.32	11.70	0.36	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H
228	9.45	0.23	9.95	0.21	10.40	0.55	8.70	0.45	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H
229	11.50	0.23	11.65	0.20	18.00	0.39	18.35	0.32	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L
231	11.45	0.18	12.10	0.21	17.35	0.38	18.85	0.36	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L
234	9.65	0.22	10.05	0.15	12.85	0.30	11.25	0.40	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
336	9.60	0.24	9.95	0.18	9.35	0.77	10.50	0.30	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
337	9.60	0.22	9.65	0.22	12.40	0.43	11.70	0.31	L	L	L	L	L	H	H	L	L	L	L	L	L	L	L
338	11.40	0.19	11.90	0.22	17.50	0.48	18.30	0.45	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L
340	9.50	0.15	9.90	0.19	13.25	0.27	12.20	0.40	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
342	11.75	0.31	12.25	0.21	17.50	0.45	17.50	0.42	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
347	9.90	0.20	10.70	0.27	11.75	0.66	11.00	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
349	9.75	0.16	9.45	0.21	13.25	0.40	13.05	0.33	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H
350	9.45	0.23	10.35	0.22	12.35	0.28	10.50	0.38	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
351	9.95	0.25	9.80	0.17	11.40	0.66	10.95	0.48	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
354	11.20	0.29	12.05	0.30	16.95	0.53	17.70	0.57	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L
355	9.50	0.27	10.40	0.20	12.80	0.28	10.70	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

(*continued*)

APPENDIX 1

(Continued)

	Sternopleural bristles				Abdominal bristles																			
	Males		Females		Males		Females		Markers															
Line	Mean	SE	Mean	SE	Mean	SE	Mean	SE	1A	3E	3F	4F	5D	8E	9A	11E	12C	13D	15D	16A	16F	18A	18D
357	10.45	0.23	10.60	0.23	12.70	0.24	11.55	0.31	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H
358	9.90	0.21	10.15	0.15	11.90	0.47	10.70	0.49	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H
259	9.85	0.18	10.10	0.17	12.35	0.28	10.55	0.31	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H
360	11.80	0.17	12.20	0.26	17.80	0.34	15.90	0.44	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L
362	11.85	0.25	12.30	0.19	17.80	0.44	16.30	0.57	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L
363	9.80	0.21	10.00	0.16	13.25	0.24	11.10	0.53	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
365	11.35	0.24	12.25	0.20	16.90	0.32	18.40	0.39	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
368	11.85	0.23	12.20	0.15	17.35	0.29	16.75	0.61	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
370	11.80	0.22	12.80	0.27	18.15	0.60	17.80	0.55	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
371	10.05	0.22	10.00	0.25	13.90	0.39	12.30	0.33	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
372	9.60	0.23	10.35	0.16	12.75	0.35	11.10	0.49	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
373	11.25	0.24	12.15	0.18	17.20	0.54	16.75	0.52	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L
374	9.75	0.28	10.30	0.22	12.45	0.30	10.90	0.24	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H
376	10.85	0.19	11.75	0.30	16.35	0.41	16.40	0.48	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L
377	9.75	0.20	10.00	0.25	11.90	0.52	10.65	0.53	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H
378	10.30	0.24	10.20	0.18	12.35	0.22	10.05	0.46	L	L	L	H	H	H	H	H	H	H	H	H	L	L	L
379	12.20	0.22	12.45	0.19	17.15	0.38	16.75	0.26	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
380	11.35	0.20	12.10	0.22	18.45	0.46	17.50	0.38	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L

APPENDIX 2

Phenotypic and marker data for the third chromosome

	Sternopleural bristles				Abdominal bristles				Markers																												
	Males		Females		Males		Females																														
Line	Mean	SE	Mean	SE	Mean	SE	Mean	SE	61D	62D	64B	64C	65D	65F	69F	70E	75B	84F	86B	87F	89A	89B	89E	90F	91B	92C	92E	93D	94E	95A	96F	97A	97B	97C	98E	98F	100C
LLH	15.25	0.38	15.00	0.25	11.03	0.33	6.05	0.40	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
LLL	9.65	0.24	10.23	0.16	9.48	0.30	5.85	0.31	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
11	Absent		13.20	0.26	Absent		9.25	0.37	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
15	12.10	0.61	12.45	0.30	12.00	0.49	7.50	0.51	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
21	15.30	0.77	15.85	0.31	9.75	0.29	5.40	0.53	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L
23	15.45	0.77	14.50	0.25	11.95	0.43	6.85	0.50	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L
24	17.50	0.88	19.20	0.41	12.80	0.42	5.95	0.49	H	H	H	H	H	H	H	H	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
31	11.19	0.56	11.50	0.23	12.69	0.34	9.65	0.38	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
33	10.35	0.52	10.55	0.12	11.85	0.27	9.75	0.29	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
41	16.05	0.80	16.55	0.32	14.70	0.32	9.60	0.51	H	H	H	H	H	H	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
46	10.55	0.53	10.70	0.18	9.20	0.38	6.50	0.40	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
48	9.95	0.50	9.65	0.10	10.65	0.36	9.15	0.29	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
52	10.25	0.51	10.40	0.24	11.75	0.43	7.65	0.37	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L
53	10.60	0.53	10.70	0.16	11.15	0.33	9.30	0.30	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	L	H
54	14.55	0.73	14.25	0.37	9.30	0.49	4.00	0.47	H	H	H	H	H	H	H	L	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
62	16.45	0.82	16.17	0.42	10.00	0.94	6.00	1.23	H	H	H	H	H	H	H	H	H	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
63	14.25	0.71	14.40	0.35	13.15	0.40	10.1	0.34	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H
64	Absent		10.80	0.25	Absent		9.55	0.47	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
65	16.55	0.83	14.95	0.39	10.70	0.54	6.25	0.61	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
72	17.60	0.88	18.10	0.53	11.60	0.51	5.10	0.41	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	H
81	13.95	0.70	14.45	0.39	12.55	0.54	8.60	0.61	H	H	H	H	H	H	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L
84	10.55	0.53	10.85	0.22	12.10	0.23	10.0	0.29	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H
85	10.30	0.52	10.85	0.28	13.70	0.34	12.0	0.27	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H
91	15.80	0.79	16.60	0.44	11.05	0.55	4.25	0.50	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
96	15.25	0.76	15.90	0.33	10.50	0.48	4.35	0.45	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L
101	16.30	0.82	16.20	0.33	11.55	0.35	7.15	0.35	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L
102	11.15	0.56	12.00	0.28	8.30	0.39	5.10	0.42	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L
106	12.10	0.61	12.55	0.19	10.60	0.44	5.45	0.40	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L
111	12.20	0.61	11.90	0.25	10.95	0.31	6.20	0.48	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	H
122	13.70	0.69	14.10	0.33	12.75	0.25	9.75	0.32	L	L	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H
123	10.40	0.52	10.40	0.25	11.15	0.31	9.70	0.29	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H
124	10.05	0.50	10.73	0.22	11.10	0.36	8.73	0.39	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H
126	17.20	0.86	17.45	0.49	13.55	0.33	6.65	0.64	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
134	11.35	0.57	11.15	0.27	11.30	0.36	8.10	0.46	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
146	11.20	0.56	11.50	0.23	14.15	0.38	9.85	0.36	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
153	13.95	0.70	14.20	0.43	10.90	0.36	6.40	0.43	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H
154	13.90	0.70	14.30	0.26	9.80	0.29	7.45	0.39	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L
156	10.60	0.28	10.10	0.24	10.85	0.24	8.35	0.46	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	L	L	L	L	L	L	L	L	L
161	17.20	0.53	17.65	0.41	10.05	0.53	3.55	0.32	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
162	13.05	0.36	13.05	0.40	11.85	0.51	8.40	0.42	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H
163	10.85	0.29	11.45	0.24	10.75	0.40	5.60	0.58	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
164	18.10	0.42	18.00	0.32	11.35	0.38	6.10	0.46	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	H	H	H
171	16.00	0.39	15.35	0.33	12.00	0.35	5.45	0.53	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
172	12.15	0.25	12.40	0.27	10.95	0.60	8.10	0.37	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

(*continued*)

APPENDIX 2
(Continued)

	Sternopleural bristles				Abdominal bristles				Markers																												
	Males		Females		Males		Females																															
Line	Mean	SE	Mean	SE	Mean	SE	Mean	SE	61D	62D	64B	64C	65D	65F	69F	70E	75B	84F	86B	87F	89A	89B	89E	90F	91B	92C	92E	93D	94E	95A	96F	97A	97B	97C	98E	98F	100C
173	13.95	0.40	14.40	0.40	10.10	0.61	4.15	0.48	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L
175	12.40	0.33	12.05	0.27	11.30	0.58	4.35	0.42	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L
177	10.11	0.22	9.83	0.45	11.21	0.42	8.50	0.84	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
213	9.75	0.23	9.95	0.19	9.10	0.47	4.30	0.37	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
214	10.00	0.28	10.75	0.26	10.15	0.44	4.75	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H
215	13.65	0.42	14.20	0.28	11.45	0.58	8.00	0.48	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H
217	18.85	0.36	19.45	0.38	11.55	0.46	6.05	0.46	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
218	12.45	0.27	12.80	0.30	12.30	0.45	7.65	0.62	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H
219	14.50	0.28	13.95	0.25	11.25	0.35	7.20	0.41	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L
202	18.25	0.37	19.00	0.40	10.80	0.62	5.20	0.46	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
221	12.40	0.36	13.15	0.29	8.65	0.42	5.90	0.41	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
223	17.61	0.41	17.25	0.46	11.89	0.49	7.65	0.40	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
224	18.90	0.41	20.35	0.58	10.90	0.59	4.55	0.56	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
225	15.65	0.52	15.75	0.39	7.70	0.54	3.95	0.40	H	H	H	H	H	H	H	H	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L
226	10.20	0.28	10.00	0.30	8.55	0.27	5.15	0.43	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
227	10.70	0.16	11.05	0.22	11.10	0.38	8.00	0.32	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
228	11.45	0.30	11.20	0.23	12.75	0.35	9.20	0.40	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
231	10.40	0.27	10.70	0.26	11.10	0.27	6.90	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
232	10.60	0.24	11.00	0.21	10.90	0.37	9.00	0.58	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H
233	13.25	0.37	13.25	0.20	12.00	0.37	9.70	0.36	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
234	14.05	0.29	14.80	0.34	11.45	0.50	6.65	0.60	H	H	H	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
236	9.75	0.27	10.10	0.19	11.60	0.35	9.15	0.32	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
237	14.35	0.39	15.00	0.45	8.10	0.54	5.55	0.65	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H
204	17.25	0.24	16.35	0.38	11.65	0.43	6.00	0.67	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
240	11.70	0.27	11.80	0.28	12.75	0.48	9.00	0.35	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H
205	11.65	0.25	12.55	0.29	12.70	0.45	9.40	0.47	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H
252	12.45	1.56	13.80	0.25	10.70	0.46	4.35	0.43	H	H	H	H	H	H	L	L	L	L	L	L	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
254	9.90	1.18	10.65	0.29	10.05	0.41	6.50	0.46	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H
257	9.85	0.96	10.55	0.21	11.10	0.34	7.60	0.55	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	H
208	18.15	1.49	17.35	0.38	12.35	0.55	4.85	0.56	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H