The Genetic Architecture of Selection Response: Inferences From Fine-Scale Mapping of Bristle Number Quantitative Trait Loci in *Drosophila melanogaster*

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Manuscript received June 30, 1998
Accepted for publication July 21, 1999

ABSTRACT

Quantitative trait loci (QTL) affecting responses and correlated responses to selection for abdominal and sternopleural bristle number have been mapped with high resolution to the *X* and third chromosomes. Advanced intercross recombinant isogenic chromosomes were constructed from high and low selection lines in an unselected inbred background, and QTL were detected using composite interval mapping and high density transposable element marker maps. We mapped a total of 26 bristle number QTL with large effects, which were in or immediately adjacent to intervals previously inferred to contain bristle number QTL on these chromosomes. The QTL contributing to response to selection for high bristle number were not the same as those contributing to response to selection for low bristle number, suggesting that distributions of allelic effects per locus may be asymmetrical. Correlated responses were more often attributable to loose linkage than pleiotropy or close linkage. Bristle number QTL mapping to the same locations have been inferred in studies with different parental strains. Of the 26 QTL, 20 mapped to locations consistent with candidate genes affecting peripheral nervous system development and/or bristle number. This facilitates determining the molecular basis of quantitative variation and allele frequencies by associating molecular variation at the candidate genes with phenotypic variation in bristle number in samples of alleles from nature.

THE bulk of phenotypic variation in populations is quantitative as opposed to qualitative; of degree rather than kind. Continuous variation for quantitative traits is caused by genetic complexity—segregating alleles at multiple loci—as well as sensitivity of the phenotypic expression of these alleles to environmental variation (Falconer and Mackay 1996). Many diseases, growth and production characters, aspects of morphology, physiology, and behavior are quantitative characters. Therefore, understanding the genetic and environmental factors affecting variation in quantitative trait phenotypes is of interest in medicine, agriculture, and evolution. Specifically, knowledge of the following properties of quantitative traits is required: the loci (quantitative trait loci, or QTL) at which mutational and segregating variation occurs; the distribution of homozygous, heterozygous, epistatic, and pleiotropic allelic effects at each locus; environmental sensitivities of QTL alleles; allele frequencies; and mutation rates.

The first stage of the genetic dissection of a quantitative trait, therefore, is to map the QTL causing genetic variation in the trait phenotype by linkage to marker loci, the segregation of which can be scored unambiguously. With the growing availability of dense polymorphic marker linkage maps, initial coarse mapping of QTL is feasible in a wide range of organisms. However, the ultimate goal of understanding the genetic architecture of quantitative traits at the level of genetic loci is most likely to be achieved using a genetically tractable organism and a model trait for which candidate genes involved in the biochemical or developmental pathways leading to the trait phenotype have been identified. One such model system is the number of sensory bristles in *Drosophila melanogaster*. The numbers of abdominal and sternopleural bristles have been used for over 50 years to estimate fundamental quantitative genetic parameters and to check quantitative genetic theory and were the first quantitative traits for which a comprehensive effort was made to estimate map positions of QTL (Breese and Mather 1957; Thoday 1979; Shrimpton and Robertson 1988). Further, bristles are external sensory organs of the peripheral nervous system (PNS), and the many loci controlling PNS development that have been characterized genetically and molecularly (Campos-Ortega 1993; Jan and Jan 1993) are candidate genes at which alleles affecting variation in bristle number in nature could segregate.

Previously, we mapped QTL causing response to divergent artificial selection for abdominal (Long *et al.* 1995) and sternopleural (Gurganus *et al.* 1999) bristle number from the Raleigh natural population to the *X* and third chromosomes. In each case, populations of recombinant isogenic (RI) chromosomes were constructed

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from a single generation of recombination between isogenic high and low parental line chromosomes and substituted into the low isogenic selection line backgrounds. Recombination breakpoints were determined using highly polymorphic cytological insertion sites of *roo* transposable elements, which provide a dense (4 cM) informative marker map. The power to detect QTL was increased by reducing the within-marker class phenotypic variation in three ways: (1) by measuring multiple individuals per RI line; (2) by mapping one chromosome at a time in a common isogenic background; and (3) by using composite interval mapping (Zeng 1994) to account for the segregation of chromosomally linked QTL when computing the probability that no QTL exists in each test interval. We detected two *X* chromosome and five chromosome *3* QTL affecting the direct response to selection for abdominal bristle number, and two *X* chromosome and six chromosome *3* QTL affecting the direct response to selection for sternopleural bristle number.

The results of these experiments generate questions that can only be addressed by further fine-scale mapping efforts.

1. Evidence that the estimated QTL map positions and effects might be accurate comes from overlapping map positions of QTL affecting correlated response to sternopleural (abdominal) bristle number with those determined from direct response to sternopleural (abdominal) bristle number (Gurganus *et al.* 1999). It is nevertheless important to confirm the QTL map positions and effects by independent replication.
2. The *roo* transposable element insertion sites provided an even denser marker map than could be utilized, given the limited number of recombination events in a single generation. Consequently, many intervals within which the QTL mapped were quite large. To realize the ultimate goal of determining QTL locations at the level of genetic locus, much finer-scale mapping involving multiple rounds of recombination must be undertaken.
3. Many of the mapped QTL had large effects and were inferred to be at intermediate frequency in the base population. However, with large intervals, one cannot distinguish multiple linked QTL with small effects from a single QTL with a large effect, and again, further recombination is necessary to discriminate between the former, infinitesimal model of allelic effects from the Robertsonian (Robertson 1967) exponential model.
4. Many intervals contained QTL affecting both bristle characters, and finer-scale mapping is required to distinguish close linkage from pleiotropy as the cause of these associations.
5. The initial experiments mapped the divergence between high and low QTL alleles. While this design maximizes the power to detect QTL, a consequence is that there is no information on whether the response to selection was due to both high and low alleles at each QTL or whether response at each locus was in one or the other direction. It is necessary, therefore, to map the high and low selection lines relative to an unselected background to make inferences about the distribution of allelic effects.
6. A potentially powerful technique for refining QTL map positions is by quantitative complementation tests to deficiencies and candidate loci (Long *et al.* 1996; Mackay and Fry 1996; Gurganus *et al.* 1999). However, failure to complement in these tests can be attributable to allelism or epistasis. The latter interpretation is less likely if the alleles at other QTL on the test chromosome are not extreme, which again requires introgression of each QTL into a wild-type genetic background.

Here, we report results of fine-scale mapping of QTL affecting the response to selection for increased and decreased bristle number relative to an unselected line. This experiment utilized the same selected chromosomes as Long *et al.* (1995) and Gurganus *et al.* (1999), and the map positions and effects of the QTL detected in the three studies are compared.

MATERIALS AND METHODS

Drosophila stocks: The balancer chromosomes and marker genes used are described in Lindsley and Zimm (1992). All cultures were reared in shell vials with 10 ml cornmeal-agar-molasses medium at 25°. The high and low bristle number parental lines used to establish the advanced intercross mapping populations were the same lines used by Long *et al.* (1995) and Gurganus *et al.* (1999) to map QTL affecting response to selection for abdominal and sternopleural bristle number, respectively, from the Raleigh base population.

LAB: An isogenic strain derived from a line selected for low abdominal bristle number (Long *et al.* 1995).

H3AB: An isogenic strain in which the third chromosome derives from a line selected for high abdominal bristle number, and the first and second chromosomes are co-isogenic with *LAB* (Long *et al.* 1995).

LST1 and LST2: Two independent isogenic strains derived from a line selected for low sternopleural bristle number. *LST1* was used to map chromosome *3*, and *LST2* to map *X* chromosome bristle number QTL (Gurganus *et al.* 1999).

H3ST: An isogenic strain in which the third chromosome derives from a line selected for high sternopleural bristle number, and the first and second chromosomes are co-isogenic with *LST1* (Gurganus *et al.* 1999).

H1ST: An isogenic strain in which the *X* chromosome derives from a line selected for high sternopleural bristle number, and the second and third chromosomes are co-isogenic with *LST2* (Gurganus *et al.* 1999).

Inbred Samarkand (Sam): This wild-type strain, derived from a standard laboratory stock by >230 generations of continuous full-sib inbreeding (Lyman *et al.* 1996), was used as the recurrent parent into which high and low selection line alleles were introgressed.

Sam balancer stocks: Balancer stocks of genotype *Sam C(1)DX,*

y w f (abbreviated *Sam XX* below), *Sam FM4*, and *Sam TM6B, Tb/Sb* (abbreviated *Sam Tb/Sb* below) were constructed by substituting the balancer chromosomes into the inbred *Sam* background (Lyman *et al.* 1996).

Mapping populations: Six mapping populations—C*1*, HST; C*1*, LST; C*3*, HST; C*3*, LST; C*3*, HAB; and C*3*, LAB—were constructed by initially crossing *H1ST*, *LST2*, *H3ST*, *LST1*, *H3AB*, and *LAB* males, respectively, to inbred *Sam* females. Females of each population were then backcrossed to *Sam* males for three generations with 100–200 females mated to ~50 males per population per generation. The populations were then maintained by mass transfer with 5–10 replicate vials per population. Recombinant isogenic chromosomes were extracted during random mating generations 5–10.

To construct isogenic *X* chromosome lines (populations C*1*, HST and C*1*, LST), single males were crossed to *Sam XX* females (G1). Male progeny were crossed to (1) *Sam FM4* and to (2) *Sam XX* females (G2), and in the following generation (G3) female *FM4* heterozygote progeny of cross (1) were mated to male progeny of cross (2). The *FM4* balancer chromosome was eliminated at G4 to produce an isogenic *X* chromosome line. To construct isogenic chromosome *3* lines (populations C*3*, HST; C*3*, LST; C*3*, HAB; and C*3*, LAB), single males were crossed to *Sam Tb/Sb* females (G1). Individual *Tb* males were backcrossed to *Sam Tb/Sb* females (G2), and at G3, *Tb* males and females were mated *inter se*. The *Tb* balancer chromosome was eliminated at G4 to produce the isogenic C*3* lines.

Each of the isogenic lines was genotyped for insertion sites of *roo* elements (see below), and those that were recombinants between the parental selection lines and *Sam* were retained for further analysis (the majority of the extracted chromosomes were nonrecombinant *Sam*). Although these lines had been backcrossed for a total of five generations to *Sam*, the background genotype was further purified by backcrossing each RI chromosome *1* (*3*) line to *Sam XX* (*Sam Tb/Sb*) for three generations and reextracting the chromosomes as described above.

RI line genotypes: *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 line patterns.

RI line phenotypes: Abdominal and sternopleural bristle number was scored on 10 males and 10 females in each of two replicate vials of all RI lines, for a total of 40 individuals scored per line. The number of bristles on the fifth abdominal sternite of males and sixth of females, and the number of bristles on the left (L) and right (R) sternopleural plates was recorded. Two sternopleural bristle traits were analyzed: the total number of bristles ($L + R$), and bristle number asymmetry ($|L - R|/(L + R)$).

Analyses of variance: Analysis of variance (ANOVA) was used to partition the variance in bristle number and in bristle number asymmetry in each population into sources attributable to sex (S, fixed), line (L, random), $S \times L$ interaction, replicate, $R(L)$, $S \times R(L)$, and error, E. Analyses of variance of each bristle trait were also computed for males and females separately. F-ratio tests of significance and estimates of variance components were computed using SAS GLM and VARCOMP procedures (SAS Institute 1988).

QTL mapping analysis: The multiple opportunities for recombination afforded during the construction of the advanced intercross lines result in expanded genetic maps for each population of RI chromosomes. 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 stepwise regression. A conditioning window of 15 cM was used, such that only markers 15 cM away from the markers flanking the test interval were included in the model. The likelihood-ratio (LR) test statistic 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. Two significance levels were used to infer the presence of QTL. First, 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 were exceeded by the permutation maximum LR statistics <50 times are significant at $\alpha = 0.05$ under the null hypothesis. Second, the Bonferroni correction for multiple tests, in which critical values of the LR test statistic exceeding χ^2 [2, 0.05/n], where n is the number of independent tests per mapping population, was used as a less stringent significance threshold. The number of independent tests per chromosome was estimated as ($C/50 + 1$), where C is the total estimated map length of the "expanded" genetic map.

RESULTS

Mapping populations and bristle number phenotypes: Six advanced intercross populations of RI lines were established by repeated crossing of *X* chromosomes selected for high and low sternopleural bristle number and third chromosomes divergently selected for abdominal and sternopleural bristle number to an unselected inbred strain, *Sam*. A total of 266 RI lines were scored for abdominal and sternopleural bristle number phenotypes. The numbers of RI lines, mean bristle numbers, and estimated magnitudes of genetic (V_L) and environmental (V_R) variance, as well as the genetic correlations of bristle number between the sexes, are given for each mapping population in Table 1. There was highly significant genetic variance between each of the selected chromosomes and *Sam* in both sexes and for the two

TABLE 1

Mapping population statistics

Mapping population	Number of RI lines	Trait	Sex	Mean	V_L[a]	V_R[b]	r_G[c]
C*1*, HST	47	AB	♂	17.28	1.208****	4.006	0.858**
			♀	19.38	2.950****	4.737	
		ST	♂	18.90	1.341****	3.305	0.837***
			♀	18.94	1.600****	3.183	
C*1*, LST	44	AB	♂	15.98	4.314****	3.503	0.953****
			♀	16.79	12.072****	4.190	
		ST	♂	16.67	4.784****	2.707	0.990 (NS)
			♀	16.81	5.743****	2.745	
C*3*, HST	45	AB	♂	17.78	1.597****	4.298	0.832*
			♀	18.80	1.124****	5.618	
		ST	♂	21.68	5.995****	4.434	0.985 (NS)
			♀	21.64	5.966****	4.509	
C*3*, LST	28	AB	♂	16.91	0.932****	2.921	0.801*
			♀	18.58	1.015****	3.402	
		ST	♂	17.65	3.215****	2.552	0.980*
			♀	17.84	2.645****	2.145	
C*3*, HAB	46	AB	♂	18.92	3.327****	4.721	0.953 (NS)
			♀	21.14	3.541****	6.203	
		ST	♂	20.16	2.808****	3.339	0.966 (NS)
			♀	19.85	2.813****	3.265	
C*3*, LAB	56	AB	♂	15.05	1.820****	4.176	0.893****
			♀	16.47	4.621****	6.377	
		ST	♂	19.29	1.186****	2.637	0.943*
			♀	19.55	0.974****	2.636	

C*1* and C*3* denote chromosomes *1* and *3*, respectively; H refers to selection for high and L to selection for low sternopleural (ST) or abdominal (AB) bristle number. NS, $P > 0.05$; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $0.0001 < P < 0.001$; **** $P < 0.0001$.

[a] Among-line variance component from single-sex analyses of variance of bristle number. *P* values are from *F*-ratio tests of significance of the line term in these analyses.

[b] The sum of the between-replicate and within-replicate (error) variance components from single-sex analyses of variance of bristle number.

[c] The genetic correlation between the sexes computed from variance components as described in the text. *P* values are from *F*-ratio tests of significance of the sex × line interaction term from combined-sex analyses of variance and indicate whether the genetic correlations are significantly different from unity.

bristle traits in all populations. The genetic correlation of abdominal (sternopleural) bristle number between males and females was significantly different from unity in 5 (3) of the six mapping populations, suggesting some QTL affecting bristle number have sex-specific effects. There was significant among-line variation for asymmetry of sternopleural bristle number in only one analysis: females of the C*3*, HAB population (data not shown).

Marker genotypes: The cytogenetic insertion sites of *roo* transposable elements were determined for each of the RI lines. Markers were informative provided at least one recombination event was observed between them. The informative markers and their map positions in centimorgans, estimated from observed recombination frequencies using the Kosambi map function, are given for each population in Table 2. All genetic maps were expanded relative to the standard map (Lindsley and Zimm 1992) as expected given multiple opportunities for recombination. There were 26 (22) informative markers for the C*1*, HST (LST) population, for an average interval size of 14.3 (11.4) cM on the expanded map. The corresponding interval sizes on the standard map are 2.56 cM for C*1*, HST and 3.08 cM for C*1*, LST; *i.e.*, the *X* chromosome maps were expanded by factors of 5.6 and 3.7, respectively. There were 39, 30, 36, and 30 informative markers for, respectively, C*3* populations HST, LST, HAB, and LAB. The average interval size for the C*3* populations was 9.67 cM on the expanded map, which corresponds to 3.12 cM on the standard map, and an average map expansion by a factor of 3.1.

QTL mapping: QTL affecting abdominal and sternopleural bristle number in each sex were mapped by linkage to molecular markers in each population using a composite interval mapping method (Zeng 1993, 1994). Briefly, the likelihood that a QTL is present in a test interval is evaluated relative to the null hypothesis (no QTL in the interval), while simultaneously accounting for the segregation of chromosomally linked QTL by fitting as cofactors additional markers that explain varia-

TABLE 2

Polymorphic markers and estimated map positions (centimorgans)

No.[a]	Cyt.[b]	cM[c]	No.	Cyt.	cM	No.	Cyt.	cM	No.	Cyt.	cM	No.	Cyt.	cM	No.	Cyt.	cM	No.	Cyt.	cM
C*1*, HST																				
1	1A	0.00	2	1C	18.15	3	1E	26.94	4	2A	42.65	5	4A	60.80	6	4B	65.16	7	4D	69.52
8	5D	87.67	9	6AB	92.03	10	7A	105.38	11	7B	107.55	12	7D	111.92	13	8E	132.58	14	9A	145.93
15	9B	159.28	16	9E	168.07	17	10B	186.22	18	11E	206.89	19	12C	225.04	20	12E	240.76	21	13A	249.54
22	14B	262.89	23	15C	271.68	24	16A	289.83	25	17EF	318.77	26	19BD	357.72						
C*1*, LST																				
1	2B	0.00	2	2C	2.27	3	3C	18.75	4	4B	30.32	5	4D	34.88	6	4EF	39.44	7	6AB	51.00
8	7A	65.00	9	7B	69.56	10	7D	74.12	11	9B	98.65	12	9F	107.84	13	10D	124.32	14	12B	154.91
15	13A	161.77	16	13D	166.34	17	14B	180.33	18	16F	191.89	19	17B	194.16	20	18A	203.36	21	18C	222.41
22	19E	238.89																		
C*3*, HST																				
1	61B	0.00	2	62B	12.49	3	62D	14.53	4	62E	24.88	5	64D	33.11	6	65D	47.81	7	65F	51.90
8	66C	68.85	9	67A	81.35	10	67E	87.50	11	69F	93.65	12	72F	95.69	13	73C	97.73	14	75C	103.88
15	77E	107.97	16	78E	110.01	17	81F	116.16	18	82F	118.20	19	83A	120.24	20	84A	122.28	21	85A	124.33
22	85D	128.42	23	86D	136.65	24	87A	138.69	25	88E	153.39	26	89A	163.73	27	89F	187.91	28	90B	192.00
29	91B	200.23	30	91F	210.57	31	92C	220.92	32	92E	229.15	33	93D	233.24	34	96F	272.27	35	97A	274.31
36	98C	282.54	37	98E	290.78	38	99B	301.12	39	100C	311.47									
C*3*, LST																				
1	61A	0.00	2	61E	2.86	3	62B	11.52	4	62E	20.17	5	63F	44.86	6	64B	50.60	7	64E	53.46
8	66C	78.15	9	67A	92.85	10	67E	114.03	11	69D	122.68	12	70C	131.34	13	70E	140.00	14	75B	142.86
15	85D	145.72	16	85F	148.58	17	86B	154.32	18	87A	160.06	19	87D	165.79	20	88E	171.52	21	89A	186.12
22	89E	194.77	23	90B	197.63	24	91F	209.27	25	94E	251.27	26	95A	259.92	27	97C	281.11	28	98C	292.74
29	98F	295.61	30	99B	298.47															
C*3*, HAB																				
1	61A	0.00	2	61E	3.78	3	62B	9.46	4	62D	13.24	5	66C	48.19	6	67A	61.72	7	67C	71.27
8	67E	88.95	9	68A	94.64	10	69D	112.32	11	72F	127.89	12	77E	145.57	13	79A	147.47	14	81F	149.36
15	83A	151.25	16	83D	153.14	17	86D	155.03	18	87D	162.64	19	89A	178.21	20	89F	181.99	21	90B	183.88
22	91B	195.40	23	91F	203.01	24	92E	214.53	25	93F	228.06	26	94B	229.95	27	94DE	235.63	28	95A	245.18
29	96B	290.25	30	96F	312.33	31	97C	314.22	32	98B	316.11	33	98E	321.80	34	99B	331.34	35	99F	333.23
36	100C	335.12																		
C*3*, LAB																				
1	61E	0.00	2	62B	4.94	3	62D	6.58	4	64B	23.59	5	64E	28.53	6	66A	74.06	7	66C	75.70
8	67A	85.67	9	67E	97.36	10	68C	107.33	11	69D	113.92	12	70C	140.85	13	72E	149.12	14	75C	154.05
15	77B	162.33	16	83F	163.97	17	84B	165.61	18	88B	172.21	19	88E	175.50	20	89A	178.78	21	89D	182.07
22	89F	185.35	23	90B	188.64	24	91B	200.32	25	91F	206.92	26	92C	210.21	27	94EF	235.01	28	97C	271.50
29	98E	292.29	30	99B	307.50															

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

TABLE 3

Bristle number QTL

			Interval range		Peak LR				
Population	Trait	Sex	Cyt.[a]	M[b]	Cyt.	M	LR[c]	Effect[d]	σ_A[e]
C*1*, HST	ST	♂	1A–1C	0.0101–0.1301	1A	0.0701	17.365	4.249	3.669
			1E–2A	0.2695–0.4195	2A	0.3795	16.015	−2.811	−2.427
			9E–11E	1.8108–1.9023	10B	1.8623	11.166	1.558	1.345
		♀	*1A–1C*	*0.0201–0.1301*	*1A*	*0.0701*	*20.220*	*4.825*	*3.815*
			1E–4A	0.3295–0.4966	2A	0.3795	14.144	−4.631	−3.661
	AB	♂	1A–1E	0.1001–0.2216	1C	0.1816	14.209	−1.655	−1.506
			1E–2A	0.3395–0.4195	2A	0.4195	14.281	−1.624	−1.477
			9E–11E	1.8508–1.8723	10B	1.8623	10.661	1.604	1.459
		♀	*1A–1C*	*0.0101–0.1201*	*1A*	*0.0701*	*51.561*	*7.117*	*4.144*
			1E–2A	*0.3195–0.3895*	*2A*	*0.3795*	*59.348*	*−8.295*	*−4.830*
			5D–7A	0.8868–1.0304	6AB	0.9604	16.820	2.841	1.654
			7D–8E	1.1792–1.3192	8E	1.2492	17.338	−3.499	−2.037
C*1*, LST	ST	♂	*2B–3C*	*0.0001–0.1728*	*2C*	*0.0628*	*67.090*	*−4.196*	*−1.918*
		♀	*2B–3C*	*0.0001–0.1728*	*2B*	*0.0001*	*75.653*	*−4.404*	*−1.838*
	AB	♂	*2B–3C*	*0.0001–0.1628*	*2B*	*0.0101*	*62.568*	*−4.032*	*−1.941*
		♀	*2B–3C*	*0.0001–0.1628*	*2B*	*0.0001*	*79.337*	*−6.722*	*−1.935*
			9F–10D	1.1985–1.2385	10D	1.2385	11.310	1.836	0.528
			12B–13D	1.5892–1.6478	13A	1.6178	11.890	−1.931	−0.556
C*3*, HST	ST	♂	*61B–62B*	*0.0001–0.1101*	*61B*	*0.0001*	*16.454*	*2.348*	*0.959*
			64D–65F	0.3812–0.4612	65D	0.4212	10.282	1.840	0.751
		♀	*61B–62E*	*0.0001–0.2454*	*61B*	*0.0001*	*33.902*	*2.879*	*1.179*
			67A–69F	*0.8536–0.9251*	*67E*	*0.8951*	*15.433*	*1.850*	*0.757*
			89F–91F	*1.9192–2.0424*	*91B*	*1.9601*	*16.542*	*−2.123*	*−0.869*
			93D–98C	*2.6125–2.7532*	*96F*	*2.6825*	*12.397*	*1.703*	*0.697*
	AB	♂	*92E–96F*	*2.3116–2.5525*	*93D*	*2.4325*	*14.799*	*1.553*	*1.229*
C*3*, LST	ST	♂	*66C–67E*	*0.8416–0.9786*	*67A*	*0.9286*	*13.551*	*−1.742*	*−0.972*
			70C–85D	*1.3735–1.4287*	*70E*	*1.4001*	*13.241*	*−1.838*	*−1.025*
			85D–87A	*1.4773–1.5933*	*86B*	*1.5433*	*15.231*	*−1.978*	*−1.103*
		♀	*66C–67E*	*0.8516–0.9586*	*67A*	*0.9286*	*14.091*	*−1.425*	*−0.876*
			70C–85D	*1.3435–1.4487*	*70E*	*1.4001*	*19.814*	*−1.906*	*−1.172*
			85D–87D	*1.4673–1.6207*	*86B*	*1.5433*	*21.509*	*−1.983*	*−1.219*
			90B–94E	*2.0364–2.2028*	*91F*	*2.0928*	*16.177*	*−1.457*	*−0.896*
	AB	♂	*70C–75B*	*1.3335–1.4101*	*70E*	*1.3735*	*15.709*	*1.325*	*1.372*
			85F–87A	*1.5160–1.5633*	*86B*	*1.5433*	*14.326*	*1.312*	*1.359*
			89A–90B	*1.8913–1.9678*	*89E*	*1.9478*	*16.001*	*−1.267*	*−1.312*
			95A–97C	2.7393–2.7693	97C	2.7493	10.028	0.890	0.922
		♀	*88E–89A*	*1.7354–1.8054*	*88E*	*1.7654*	*14.295*	*−1.754*	*−1.741*
			89A–91F	*1.9313–2.0064*	*89E*	*1.9478*	*13.873*	*−1.475*	*−1.464*

(*Continued*)

tion in the trait, situated 15 cM away from the test interval. The conditioning markers were chosen by forward step-wise regression. The markers used as cofactors were not necessarily the same in the different analyses and varied in number from 2 to 10. The results of these analyses are summarized in Table 3 and Figures 1–6. QTL are reported in Table 3 at two significance levels: those for which the LR test statistic exceeds the permutation threshold, which is the more stringent criterion, and those for which the LR test statistics exceed the χ^2 [2, 0.05/n] critical value (n is the number of independent tests). Less stringent thresholds are considered because we wish to pool information across sexes, traits, and populations. The permutation thresholds varied according to the trait and sex analyzed, and are shown in the figures. The thresholds based on the number of independent tests were 10.15 for the C*1*, HST and C*3*, HAB populations; 9.88 for C*3*, HST, C*3*, LST, and C*3*, LAB; and 9.57 for C*1*, LST.

Within each population, we have estimates of map positions and effects of QTL affecting two bristle traits in both sexes. As the QTL map positions from the different analyses often coincide, we can use these analyses to deduce a minimum number of bristle number QTL for each population. We inferred that there was statistical support for the presence of a QTL within a population if (1) the LR test statistic exceeded the permutation threshold in any one of the four analyses or (2) the LR

TABLE 3

(Continued)

Population	Trait	Sex	Interval range		Peak LR			Effect[d]	σ_A[e]
			Cyt.[a]	M[b]	Cyt.	M	LR[c]		
C*3*, HAB	ST	♂	*61A–62D*	*0.0001–0.0301*	*61A*	*0.0001*	*14.886*	*2.073*	*1.237*
			61E–62D	*0.0579–0.1247*	*62B*	*0.0947*	*15.354*	*2.224*	*1.327*
			62D–66C	*0.1425–0.2225*	*62D*	*0.1825*	*13.365*	*2.939*	*1.754*
			87D–89A	*1.7465–1.7765*	*89A*	*1.7765*	*12.690*	*−1.713*	*−1.022*
	ST	♀	*87D–91B*	*1.6765–1.9389*	*89F*	*1.8200*	*18.575*	*−2.240*	*−1.335*
	AB	♂	*72F–87D*	*1.3190–1.6204*	*86D*	*1.5504*	*45.360*	*3.154*	*1.729*
			92E–94DE	*2.1454–2.3496*	*94B*	*2.2996*	*21.323*	*1.417*	*0.777*
			97C–100C	*3.1423–3.3424*	*98E*	*3.2181*	*24.764*	*1.825*	*1.000*
		♀	*72F–89A*	*1.3690–1.7365*	*86D*	*1.5504*	*58.754*	*4.691*	*2.493*
			92E–94DE	2.2254–2.2996	94B	2.2907	10.539	1.386	0.736
C*3*, LAB	ST	♂	*61E–64B*	*0.0001–0.2359*	*62D*	*0.0959*	*17.810*	*−1.058*	*−0.972*
			68C–70C	*1.0734–1.2194*	*68C*	*1.0934*	*23.003*	*1.645*	*1.511*
			70C–89A	*1.4086–1.7851*	*84B*	*1.6562*	*26.453*	*−1.391*	*−1.277*
		♀	64E–66A	0.3854–0.5554	64E	0.4654	10.694	−1.093	−1.108
			67E–69D	*1.0437–1.1334*	*68C*	*1.0934*	*14.461*	*1.316*	*1.333*
			75C–83F	1.5907–1.6334	77B	1.6234	11.465	−1.221	−1.237
	AB	♂	*70C–75C*	*1.4786–1.5013*	*72E*	*1.4913*	*11.512*	*−1.068*	*−0.792*
			92C–97C	*2.1022–2.5902*	*92C*	*2.2222*	*41.078*	*−2.291*	*−1.698*
		♀	61E–62B	0.0001	61E	0.0001	10.878	1.444	0.672
			64B–64E	*0.2360–0.2660*	*64B*	*0.2360*	*18.604*	*−2.128*	*−0.990*
			66A–67A	*0.7407–0.8471*	*66C*	*0.7871*	*20.567*	*1.595*	*0.742*
			67A–67E	*0.9168–0.9368*	*67E*	*0.9268*	*12.649*	*1.511*	*0.703*
			70C–75C	*1.4586–1.5313*	*72E*	*1.4913*	*17.636*	*−1.753*	*−0.816*
			90B–94EF	*1.8865–2.3422*	*92C*	*2.1222*	*48.844*	*−4.019*	*−1.870*
			94EF–97C	2.4002–2.5302	94EF	2.4602	11.205	−1.487	−0.692

Entries in italic font refer to QTL for which the LR test statistic exceeds the permutation threshold. Entries in regular type refer to QTL for which the LR test statistic exceeds χ^2 [2, 0.05/*n*], where *n* is the number of independent markers.

[a] Cytogenetic interval.
[b] Estimated map position in Morgans.
[c] Likelihood-ratio test statistic.
[d] QTL effect in bristles relative to *Sam*.
[e] QTL effect in genetic standard deviation units.

test statistic exceeded the χ^2 threshold corrected for multiple independent tests in at least two separate analyses. Multiple QTL in a region were inferred if two or more significant LR peaks were separated by LR scores below the threshold value; otherwise a single QTL was inferred at the location of the largest significant LR statistic. [This procedure is somewhat *ad hoc*. Multiple trait composite interval mapping (Jiang and Zeng 1995) combines information from two or more traits (or the same trait in both sexes) to estimate QTL map positions and main and interaction QTL effects, and is expected to be more precise than single trait analysis. However, software applications for this method are not yet publicly available.]

For ease of reference, we designate the bristle number QTL below as "Bn," followed in parentheses by the cytological location of the marker nearest the peak LR statistic. The terminology is for convenience only and is not meant to convey the impression that the QTL have been mapped to the level of genetic locus; hence, the QTL names are not italicized as is conventional for Drosophila gene names. All effects are relative to *Sam*.

C1, HST: At least three QTL were detected. There are two LR peaks at the tip of the *X* chromosome in all analyses. The first maps to the 1A–1C interval and is associated with increased sternopleural bristle number in males and females, increased abdominal bristle number in females, and decreased abdominal bristle number in males. Bn(2A) decreases both bristle traits in males and females, and Bn(10B) is associated with increased sternopleural and abdominal bristle number in males only. There was suggestive evidence for two additional QTL with female-specific effects on abdominal bristle number in this population.

C1, LST: There is at least one QTL at the tip of the *X* chromosome associated with decreased sternopleural and abdominal bristle number in both sexes. In all analyses the LR test statistics displayed two peaks, one at 0.01 or 1.01 cM (2B), and one at 5.28 or 6.28 cM (between 2B and 2C). Although this region may be genetically

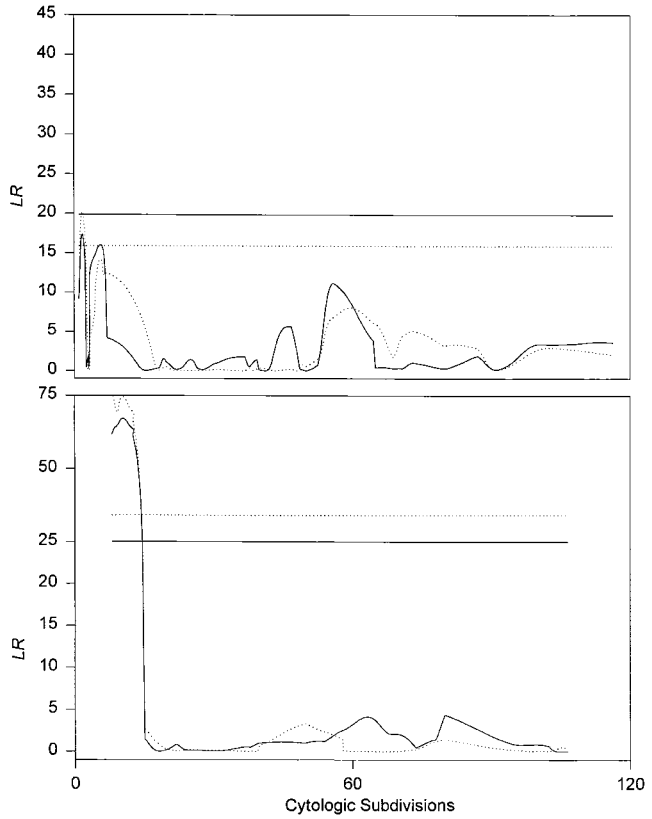

Figure 1.—*X* chromosome QTL affecting response to selection for high (top) and low (bottom) sternopleural bristle number. The likelihood ratio (LR) statistic is plotted against cytogenetic map location at the level of polytene band subdivision. Each of the numbered *X* chromosome polytene bands (1–20) consists of 6 subdivisions (A–F), for a total of 120 subdivisions. The solid lines are for QTL affecting male bristle number and the dotted lines are for QTL affecting female bristle number. The horizontal lines represent the critical value of the LR statistic for an experimentwise type I error rate determined by permuting the trait and marker data 1000 times.

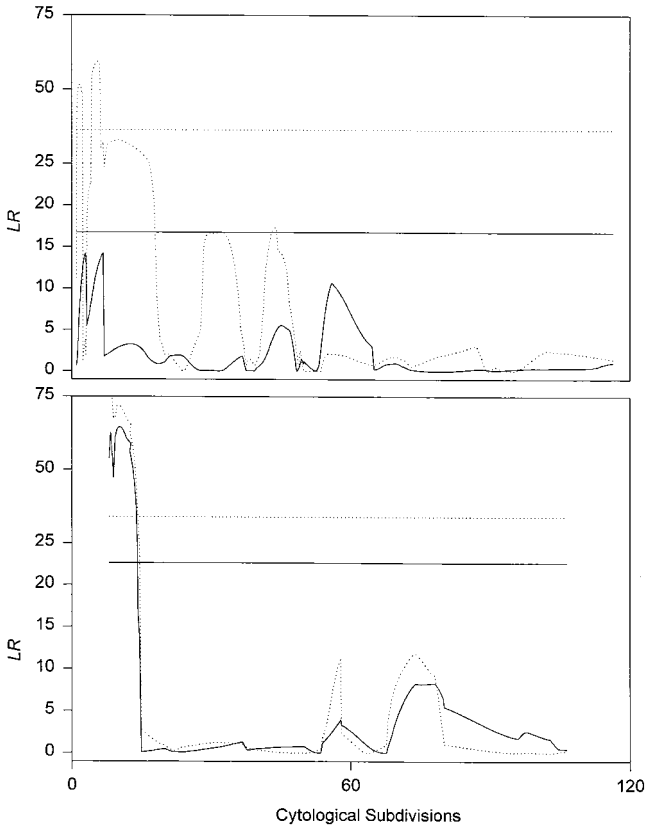

Figure 2.—*X* chromosome QTL affecting correlated response to selection in abdominal bristle number in the high (top) and low (bottom) sternopleural bristle number selection lines. The axes and notation are as in Figure 1.

complex, we refer to a single QTL as Bn(2B, 2C). Two QTL in this population reached the suggestive significance threshold in females.

C3, HST: There were four QTL affecting sternopleural bristle number in this population. Bn(61B) increased sternopleural bristle number in both sexes. The remaining three sternopleural bristle QTL were significant in females only; Bn(67E) and Bn(96F) were associated with increased, and Bn(91B) was associated with decreased bristle number. Only one QTL affecting abdominal bristle number was detected, Bn(93D); this QTL was associated with increased bristle number in males. There was suggestive evidence for the presence of an additional QTL affecting increased sternopleural bristle number in males [Bn(65D)].

C3, LST: Six QTL were detected in this population. Bn(67A), Bn(70E), and Bn(86B) were associated with decreased sternopleural bristle number in both sexes; the latter two QTL were also associated with a significant increase in male abdominal bristle number. Bn(91F) was significantly associated with decreased sternopleural bristle number in females. Bn(89E) and Bn(88E) were associated with decreased abdominal bristle number, the former in both sexes and the latter in females. There was suggestive evidence for one further QTL, Bn(97C), affecting abdominal bristle number in males.

C3, HAB: At least three QTL were detected with effects on abdominal bristle number. Bn(86D) and Bn(94B) increased abdominal bristle number in both sexes, and Bn(98E) increased abdominal bristle number in females. According to the decision rule that multiple QTL are inferred in a region if the LR test statistic drops below the significance threshold and then rises above it again, there appear to be three closely linked QTL affecting male sternopleural bristle number at the tip of chromosome *3*. However, the magnitude of the fluctuation in LR scores is trivial. To be conservative, we postulate a single QTL, Bn(61A, 62D), in this region. There were formally two other sternopleural bristle number QTL in this population, Bn(89A), affecting male bristle number, and Bn(89F), affecting female bristle number. However, their proximity and similarity of effects in both sexes, as well as the form of the likelihood profiles, suggest that there is only one QTL in this region, Bn(89A, 89F).

C3, LAB: Two QTL decreased abdominal bristle num-

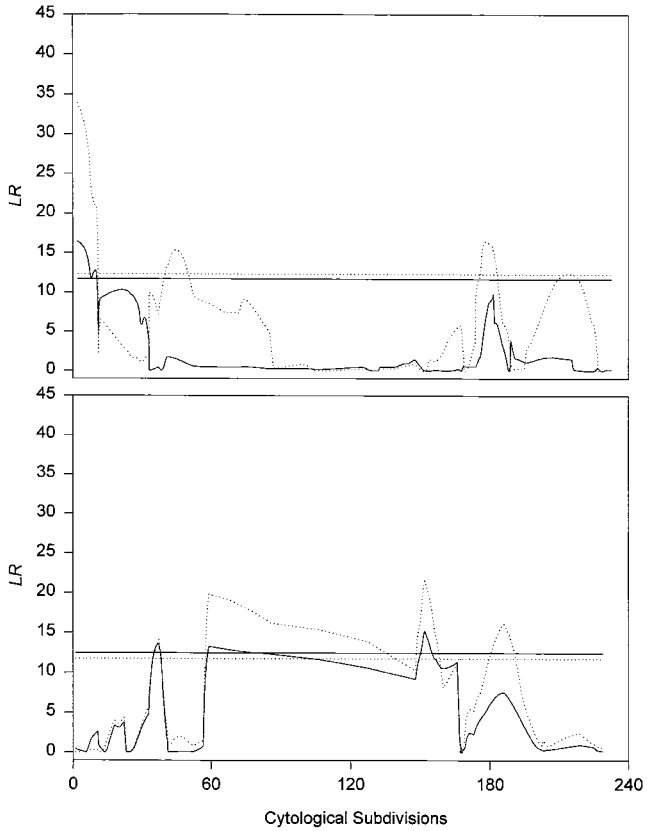

Figure 3.—Chromosome *3* QTL affecting response to selection for high (top) and low (bottom) sternopleural bristle number. Each of the chromosome *3* polytene bands (61–100) consists of 6 subdivisions (A–F) for a total of 240 subdivisions. Otherwise, the axes and notation are as in Figure 1.

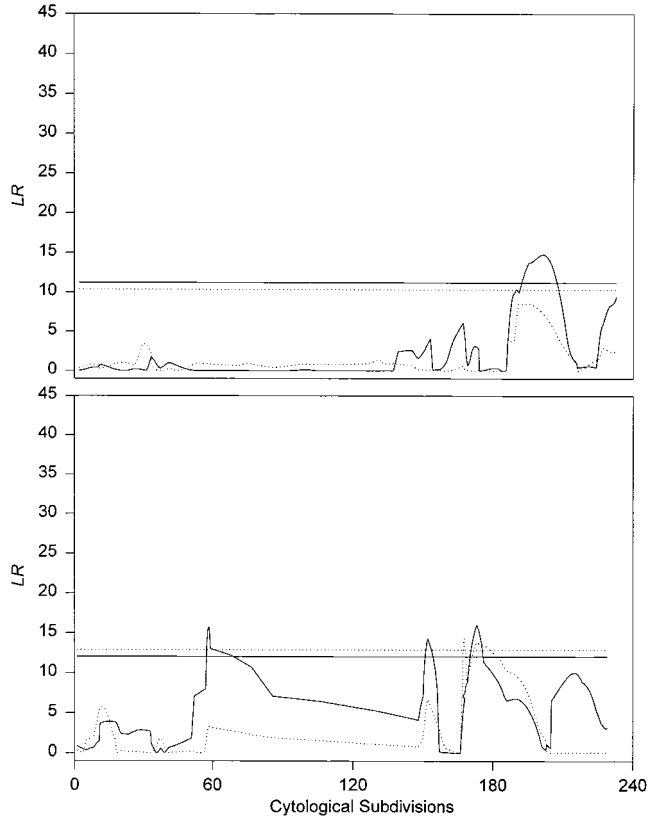

Figure 4.—Chromosome *3* QTL affecting correlated response to selection in abdominal bristle number in the high (top) and low (bottom) sternopleural bristle number selection lines. The axes and notation are as in Figure 3.

ber significantly in both sexes, Bn(72E) and Bn(92C). In addition, three abdominal bristle QTL exceeded the permutation threshold in females only, one of which decreased [Bn(64B)], and two of which increased [Bn(66C) and Bn(67E)] abdominal bristle number. There was suggestive evidence for two more female abdominal bristle QTL, at 61E and 94EF. At least three QTL affecting sternopleural bristle number were detected. Bn(62D) affected this trait in males, and Bn(68C) in both sexes. The third QTL covered a large pericentromeric region and appeared to have two peaks in the likelihood profile in both males and females (although the evidence for a female QTL in this region is suggestive only). One peak is at 77B and the other at 84B. Although this is a large physical distance, the restriction of recombination near the centromere results in a shrinkage of the genetic distance on the expanded map to only 3.3 cM. As the estimated effects are similar at both likelihood peaks, we adopt the conservative interpretation that there is only one QTL affecting sternopleural bristle number in this region, Bn(77B, 84B).

To tally the total number of QTL detected and to infer whether response to divergent selection is caused by alleles at the same or different loci, it is necessary to merge the QTL maps for the different populations. A QTL was judged to be the same in two or more populations if the map positions coincided with the same marker in those instances where the marker was present in both populations or if the map positions relative to flanking markers matched. All QTL exceeding the lower significance threshold were included in this consideration. Those that were detected at the lower threshold in only a single analysis were considered to be spurious. However, a suggestive QTL in one population that coincided with at least one suggestive QTL in another population, or with QTL that were formally significant as judged by the permutation tests, were included in the final total. The results are given in Table 4.

In total, we have mapped 26 bristle number QTL, 4 on the *X* and 22 on chromosome *3*. Four QTL were inferred to be present in two populations, and 2 were detected in three populations. The QTL alleles fall into five categories, based on the sign of the effect and trait affected:

1. QTL alleles that affect the selected trait in the direction of selection and are thus associated with response to selection.
2. QTL alleles that affect the selected trait in the opposite direction to selection. Such QTL may occur on the selected chromosome if they are closely linked

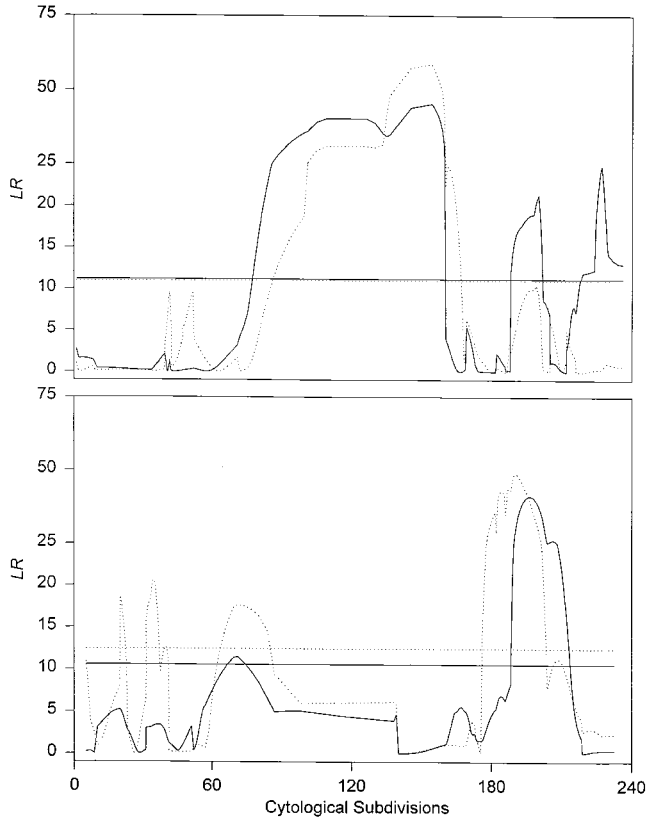

Figure 5.—Chromosome *3* QTL affecting response to selection for high (top) and low (bottom) abdominal bristle number. The axes and notation are as in Figure 3.

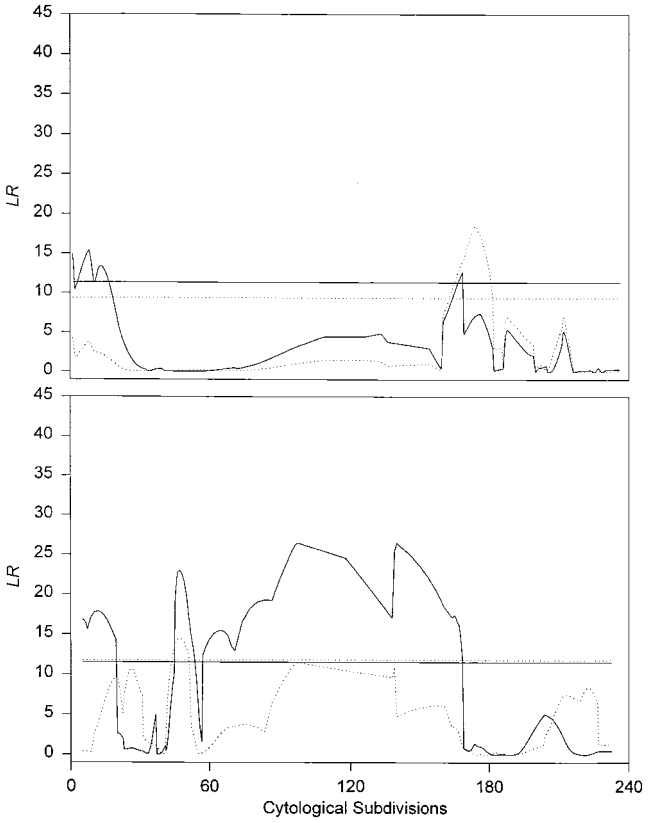

Figure 6.—Chromosome *3* QTL affecting correlated response to selection in sternopleural bristle number in the high (top) and low (bottom) abdominal bristle number selection lines. The axes and notation are as in Figure 3.

to a selected QTL; they also contribute to selection response, albeit in an undesired manner. The two sternopleural bristle QTL with large and opposite effects at the tip of the *X* chromosome in the HST population may well be an example of alleles in close repulsion linkage in the base population—the markers corresponding to the peak LR statistics, 1A and 1E, are in a region of very restricted recombination and are only 0.3 map units apart on the standard map (Lindsley and Zimm 1992). QTL alleles were included in this category if they were <15 cM from the putative selected QTL on the standard map.

3. Correlated response to selection can be attributed to QTL alleles that contribute to direct response to selection but that also have pleiotropic effects on the correlated character.
4. Correlated selection response can also occur if QTL alleles affecting the correlated trait are linked to QTL affecting the directly selected trait. We inferred correlated responses were attributable to pleiotropy or very close linkage if the QTL affecting the directly selected and correlated trait mapped to identical locations, and to loose linkage if the QTL affecting the correlated trait mapped within 15 cM of the directly selected QTL.
5. The final category includes alleles with effects that do not fall into any of the previous categories. This includes QTL alleles in a selection line with effects on the selected trait in the opposite direction to selection but that are not obviously linked to selected QTL. This could occur if the allele has beneficial pleiotropic fitness effects, if it has an epistatic effect in the opposite direction to its main effect, if the effect is small, or indeed if the QTL is a false positive. QTL alleles with "wrong" signs may also have originated from *Sam*. Each of the 34 QTL alleles is assigned to one or more of these categories in Table 4.

Three of the four *X* chromosome QTL affected the selected trait, sternopleural bristle number, in the direction of selection. Two of these QTL mapped to the high chromosome and one on the low selected chromosome. Fifteen QTL alleles were associated with response to direct selection on chromosome *3*: four each for the high and low sternopleural chromosomes, and three for the high and four for the low abdominal chromosomes. Perhaps surprisingly, the QTL alleles associated with direct response to selection for high bristle number were totally different from those associated with response to selection for low bristle number. Several QTL alleles affected the selected trait in the opposite direction to selection. In addition to the QTL at the tip of

TABLE 4

QTL locations and effects

QTL	Population	Effect[a]	Interpretation[b]	Candidate genes[c]
1 Bn(1A–C)	C*1*, HST	+AB♀, −AB♂, +ST♀, +ST♀	R, CRP	*Achaete-scute* complex (1B)
2 Bn(2A)	C*1*, HST	−AB♂, −AB♀, −ST♂, −ST♀	RL Bn(2A), CRL-P	
3 Bn(2B, 2C)	C*1*, LST	−AB♂, −AB♀, −ST♂, −ST♀	R, CRP	*shaggy* (3B), *Notch* (3C)
4 Bn(10B–D)	C*1*, HST	+AB♂, +ST♂	R, CRP	
	C*1*, LST	+AB♀	US	
5 Bn(61A, 62D)	C*3*, HAB	+ST♂	US	*extramacrochaetae* (61C), *rhomboid* (62A)
	C*3*, HST	+ST♂, +ST♀	R	
	C*3*, LAB	+AB♀, −ST♂	RL Bn(64B), CRL Bn(64B)	
6 Bn(64B)	C*3*, LAB	−AB♀,	R	*pavarotti* (64B)
7 Bn(64E, 65D)	C*3*, LAB	−ST♀	CRL Bn(64B)	*scratch* (64D), *melted* (65E), *que mao* (65F)
	C*3*, HST	+ST♂	R	
8 Bn(66C)	C*3*, LAB	+AB♀	RL Bn(64B)	*pebble* (66B), *hairy* (66D)
9 Bn(67A)	C*3*, LST	−ST♂, −ST♀	R	*abdominal* (66C–67A)
10 Bn(67A-E)	C*3*, LAB	+AB♀	RL Bn(72E)	*astray* (67B)
11 Bn(67E, 69F)	C*3*, HST	+ST♀	R	
12 Bn(68C)	C*3*, LAB	+ST♂, +ST♀	CRL Bn(72E)	*cyclin A* (68E), *polychaetoid* (69C–70A)
13 Bn(70E)	C*3*, LST	+AB♂, −ST♂, −ST♀	R, CRP	*tartan* (70A), *Bearded* (71A)
14 Bn(72E)	C*3*, LAB	−AB♂, −AB♀	R	
15 Bn(77B, 84B)	C*3*, LAB	−ST♂, −ST♀	CRL Bn(72E)	*P(3)*78A, *P(3)*79D, *Malformed abdomen* (84B), *Sex-combs-reduced* (84B); *missensed* (84D), *atonal, hearty* (84F)
16 Bn(86B)	C*3*, LST	+AB♂, −ST♂, −ST♀	R, CRP	*dorsotonals* (86C)
17 Bn(86D)	C*3*, HAB	+AB♂	R	*prospero* (86E)
18 Bn(88E, 89A)	C*3*, LST	−AB♀	CRL Bn(86B), Bn(91F)	
	C*3*, HAB	−ST♂	CRL Bn(86D)	
19 Bn(89E-F)	C*3*, LST	−AB♂, −AB♀	CRL Bn(86B), Bn(91F)	*abdominal A, Abdominal B* (89E)
	C*3*, HAB	−ST♀	CRL Bn(86D)	*couch potato* (90DE)
20 Bn(91B)	C*3*, HST	−ST♀	RL Bn(94EF, 97C)	*P(3)*91A
21 Bn(91F)	C*3*, LST	−ST♀	R	*P(3)*91F
22 Bn(92C)	C*3*, LAB	−AB♂, −AB♀	R	*Delta* (92A), *bonus* (92E), *Hairless* (92F)
23 Bn(93D)	C*3*, HST	+AB♂	CRL Bn(94EF, 97C)	*P(3)*93B
24 Bn(94B)	C*3*, HAB	+AB♂, +AB♀	R	
25 Bn(94EF, 97C)	C*3*, HST	+ST♀	R	*pointed* (94E), *Enhancer-of-split* (96F), *P(3)*97C
	C*3*, LST	+AB♂	CRL Bn(91F)	
	C*3*, LAB	−AB♀	R	
26 Bn(98E)	C*3*, HAB	+AB♂	R	*P(3)*98DF, *string* (99A)

[a] QTL are listed as having significant effects in one sex only if the peak LR statistic reached formal significance in that sex but not the other. From Figures 1–6, it can be seen that there was often, but not always, an accompanying nonsignificant peak in the likelihood profile of the other sex. The significance of sex-specific effects cannot at present be tested using composite interval mapping.

[b] R, direct response to selection; RL, selection response due to linkage; CRP, pleiotropic correlated response; CRL, correlated response due to linkage; US, unselected.

[c] Sources for candidate genes are Lindsley and Zimm (1992), Jan and Jan (1993), and Salzberg *et al.* (1997). *P(3)* insertional mutations with adult bristle number phenotypes are from Lyman *et al.* (1996).

the *X* chromosome mentioned above, one QTL in the high sternopleural bristle line and three QTL in the low sternopleural bristle line fell into this category. They are all closely linked to selected QTL but could also be *Sam* alleles.

X chromosome QTL affecting correlated responses in abdominal bristle number mapped to the same location as those affecting direct selection response and can be inferred to be attributable to pleiotropy or close linkage. Six chromosome *3* QTL were deduced to be associated with correlated responses in abdominal bristle number, one on the high and five on the low sternopleural chromosome. Of these QTL, two mapped to the same location as QTL affecting direct selection response, and the others were within 15 cM of a directly selected QTL. Similarly, six chromosome *3* QTL were hypothesized to be associated with correlated responses in sternopleural bristle number, one on the high and five on the low selected chromosome. None mapped to the same location as QTL with effects in the direction of selection, but were within 15 map units of selected QTL. Thus, correlated responses from pleiotropic effects of directly selected QTL (or close linkage to these QTL) are less common (5) than correlated responses attributable to linkage of QTL affecting the correlated trait with QTL affecting the directly selected trait (10). The effects of two QTL alleles could not be interpreted as arising from direct or correlated selection response.

Composite interval mapping analyses were also performed for asymmetry in sternopleural bristle number. Two QTL for asymmetry were detected in females of the C*3*, HAB population, for which there was significant among-line variation in asymmetry. The QTL mapped to 61E (LR = 9.70) and 89F (LR = 9.53); both are nearly significant at the permutation threshold of 9.78. Although there was no among-line variation in asymmetry in any of the other populations, a QTL affecting asymmetry was mapped between 11E and 12C in the C*1*, HST population. The LR statistic for the QTL was 13.59, which exceeds the permutation threshold of 12.67.

DISCUSSION

Consistency: Because QTL mapping requires very large sample sizes and there are serious problems with multiple testing in the context of genome scans, it is always necessary to confirm the map positions of QTL by independent replication. Previously, QTL affecting response to selection for abdominal bristle number and correlated response in sternopleural bristle number were mapped on the C*3*, HAB and C*3*, LAB chromosomes, relative to each other, by Long *et al.* (1995). Similarly, QTL affecting divergent response to selection for sternopleural bristle number and correlated response in abdominal bristle number were mapped on the C*1*, HST, C*1*, LST, C*3*, HST, and C*3*, LST chromosomes by Gurganus *et al.* (1999). To what extent do the map positions of QTL detected in this study coincide with those identified previously?

The abdominal bristle QTL of Long *et al.* (1995) were located in intervals 61A–64C, 66A–67C, 75C–85E, 89D–92E, and 96B–96F. In this study, QTL affecting response to selection for abdominal bristle number Bn(61A, 62D), Bn(64B), Bn(66C), Bn(67A-E), Bn(72E), Bn(86D), Bn(92C), and Bn(94EF, 97C) map at, or adjacent to, the first four of these intervals. Further, QTL Bn(94B) and Bn(98E) flank the final interval. Long *et al.* (1995) mapped QTL affecting correlated response in sternopleural bristle number to the 61A–64C and 89D–92E intervals. We mapped at least two QTL in and near the first region affecting sternopleural bristle number [Bn(61A, 62D) and Bn(64E)] and two in the latter [Bn(88E, 89A) and Bn(89E-F)]. Given that a slightly different composite interval mapping analysis was used by Long *et al.* (1995)—all markers >10 cM away from the test interval were included in the multiple regression model, rather than those significant by forward stepwise regression, and map distances were taken from the standard map, rather than estimated from the data—the agreement between the two studies is excellent.

The composite interval mapping analysis of Gurganus *et al.* (1999) detected a major factor affecting response to selection for sternopleural bristle number and correlated response in abdominal bristle number at the tip of the *X* chromosome (1A–3E), corresponding to our QTL Bn(1A–C), Bn(2A), and Bn(2B, 2C). A QTL affecting sternopleural and abdominal bristle number was detected at 9A–12C; this corresponds to Bn(10B–D). Gurganus *et al.* (1999) mapped a QTL affecting female abdominal bristle number to the 5D–8E interval. In this analysis, a QTL affecting female abdominal bristle number at 8E was significant on the basis of the χ^2 threshold but did not exceed the permutation threshold and was not included in the final tabulation. The correspondence between the two data sets is thus excellent.

In the analyses of Gurganus *et al.* (1999), there were six chromosome *3* QTL affecting direct response to selection for sternopleural bristle number and three chromosome *3* QTL affecting correlated response in abdominal bristle number. The most likely positions of the sternopleural bristle QTL were between 61D–62D, 65F–69F, 70E–75B, 87F–89A, 89A–89E, and 98F–100C, and of the abdominal bristle QTL, between 65F–70E, 70E–75B, and 89E–90F. Several of our factors affecting response to selection for sternopleural bristle number [Bn(61A, 62D), Bn(64E, 65D), Bn(67A), Bn(67E, 69F), and Bn(70E)] and correlated response in abdominal bristle number [Bn(70E), Bn(88E, 89A), and Bn(89E–F)] map within or adjacent to these intervals. Although additional QTL affecting direct and correlated selection response not detected by Gurganus *et al.* (1999) were

mapped in this study, overall there is good agreement between the two experiments.

Magnitude of effects: Some of the QTL detected in the studies of Long *et al.* (1995) and Gurganus *et al.* (1999) had large effects. The mean effect of the five chromosome *3* abdominal bristle QTL was 2.4 bristles in males and 3.5 bristles in females, with ranges from 1.4–3.6 and 2.4–5.6 in males and females, respectively (Long *et al.* 1995). Similarly, the mean effect of *X* and third chromosome sternopleural bristle QTL was 1.1 in males and females, with a range of 0.18–1.6 in males and 0.29–1.5 in females (Gurganus *et al.* 1999). However, the intervals in which the QTL with large effects resided were rather wide, leaving unresolved the issue of whether a few factors with large effects or multiple linked factors with smaller effects accounted for the selection response.

We have clarified here that the minimum number of factors accounting for selection response in abdominal bristle number on chromosome *3* has grown from 5 to 10. The mean absolute value of abdominal bristle QTL effects has concomitantly dropped to 2.0 in males and 2.2 in females, with ranges of 1.1–3.2 in males and 1.4–4.7 in females. The minimum number of factors on chromosomes *1* and *3* accounting for selection response in sternopleural bristle number has risen from 2 and 6 to, respectively, 4 and 9. However, the mean absolute value of sternopleural bristle QTL effects has increased to 2.5 in males and 2.7 in females, with ranges from 1.6–4.2 and 1.4–4.8 in males and females, respectively. The effects of the QTL detected here were thus of the same magnitude, if not larger, as those estimated previously. The solution to the apparent paradox is that many of our mapped factors had effects opposite to the direction of selection; presumably these factors were on the selected chromosome as a consequence of hitchhiking along with the selected loci. Finer-scale mapping of bristle number QTL does not result in the detection of more QTL with smaller and smaller effects: the distribution of QTL effects is more close to exponential than infinitesimal.

There was good agreement between the total divergence in bristle number of parental selected chromosomes and the sum of effects of mapped QTL in previous analyses. The divergence in abdominal bristle number between the *H3AB* and *LAB* and strains was 13.7 bristles, and the sum of the abdominal bristle QTL effects mapped by Long *et al.* (1995) was 14.8 bristles. Similarly, the divergence in sternopleural bristle number between the *H1ST* and *LST2* strains was 2.3 bristles and between the *H3ST* and *LST1* strains was 7.7 bristles; the sum of sternopleural bristle QTL effects detected by Gurganus *et al.* (1999) was 1.2 bristles for *X* chromosome QTL and 6.5 bristles for chromosome *3* QTL. The summed effects of bristle number QTL mapped in this study agree less well with the divergences between the original parental lines. The total divergence between high and low chromosome *3* abdominal bristle QTL is only 10 bristles, nearly 4 bristles less than that between the parental lines. On the other hand, the total divergence between high and low *X* and chromosome *3* sternopleural bristle QTL—5.9 and 10.4 bristles—exceeds that between the parental lines by 3.6 and 2.7 bristles, respectively. A plausible explanation for these observations is epistasis: the previous estimates of parental line divergence and QTL effects were made in the low selection line background, whereas effects were estimated here in a wild-type background. If this explanation is true, the epistatic effects are in opposite directions for the two bristle traits, with high abdominal bristle alleles enhanced and high sternopleural bristle alleles diminished in the background of the low selection line.

Correlated effects: Previously, it was found that QTL affecting correlated response to selection mapped to a subset of the same intervals in which factors affecting direct response to selection were mapped. However, the intervals were often large, and consequently it was not possible to interpret the cause of correlated responses as due to pleiotropy or linkage. Here we have shown that two-thirds of the QTL associated with correlated selection responses were linked to the QTL associated with direct selection response, and one-third were associated with the same markers as those affecting direct responses. Pleiotropy can be presumed to be the cause of the latter correlated responses pending further finer scale mapping.

Distribution of allelic effects per locus: In no case did we observe that the QTL associated with response to selection for high sternopleural or abdominal bristle number were the same as those associated with response to selection for low bristle number. Therefore, we can infer that the distribution of allelic effects at loci affecting selection response is not such that a high- and low-effect allele segregates at each locus at intermediate frequency. If the distribution of allelic effects is symmetrical, then we must infer that high and low alleles of large effect are rare, so the probability of sampling the high allele in the high line and the low allele in the low line is very small. However, the results of experiments in which selection limits for bristle number were compared between single-pair bottleneck and larger base populations suggest that many of the alleles affecting selection response are at intermediate frequency in nature (Robertson 1968; Frankham 1980). It is therefore possible that the distribution of allelic effects is asymmetrical at the majority of loci contributing to selection response, such that there exists a common allele with either a high or low effect on bristle number relative to other alleles segregating at the locus.

Six of the bristle number QTL were detected in more than one population. None of the QTL alleles detected in more than one population had the same effects in each. QTL alleles in different populations affected the same trait in opposite directions or had effects on a

different trait and/or sex. Either there are multiple alleles with differing effects at some of the loci affecting selection response or our description of these QTL as allelic based on their map positions and marker associations is incorrect.

If our contention is that at least some of the QTL affecting variation in bristle number in nature are alleles of loci affecting PNS development (see below), then one might expect asymmetrical distributions of effects of naturally occurring alleles and multiple alleles with varying pleiotropic effects, based on distributions of mutational allelic effects at these loci. For example, loss-of-function mutations at the *achaete-scute* complex, which is required to initiate sensory organ development, generally lead to loss of bristles and hairs; and loss-of-function mutations at *Delta*, which acts to suppress sensory organ development, generally lead to increases in bristle number (Lindsley and Zimm 1992).

Other populations: Are the same loci responsible for genetic variation in bristle number in different populations? This question can only be addressed by repeating the experiments described here for the Raleigh population on a number of different populations. This is a rather daunting task, but comparison of these results with earlier and more recent studies suggests that some loci may be polymorphic for alleles affecting variation in bristle number in many populations. Wolstenholme and Thoday (1963) mapped two-third chromosome sternopleural bristle number QTL to the 85A–87C interval. Spickett and Thoday (1966) mapped four QTL affecting response to selection for high sternopleural bristle number, two at 3C and 13F on the *X* chromosome and two in the 67C–67D region. More recently, Gurganus *et al.* (1998) mapped QTL affecting sternopleural and abdominal bristle number in a panel of recombinant inbred lines derived from two unselected inbred strains. Two *X* chromosome QTL associated with markers at 1B and 4C, and seven chromosome *3* QTL associated with markers at 61D, 67D, 79E, 88E, 92A, 94D, and 96F. Finally, Nuzhdin *et al.* (1998) mapped sternopleural bristle number QTL segregating between two wild-type inbred strains by assessing changes in neutral transposable element marker frequencies during response to divergent selection. Three QTL were detected, one on the *X* chromosome in the 4B–6A interval and two on chromosome *3* between 92C–94E and 97B–98E. The agreement in map positions of QTL in these studies with those reported here is striking. Many bristle number QTL mapped over a period of 35 years, using different base populations, mapping methods, and statistical analyses, coincide with positions of QTL we have mapped in the Raleigh population. Further study of loci that contribute to genetic variation in bristle number within populations and that are also polymorphic in different populations promises to be rewarding in terms of understanding the evolutionary forces maintaining quantitative genetic variation (Barton and Turelli 1989).

Candidate genes: As noted above, one class of loci that are candidate genes at which naturally occurring variation for bristle number might segregate are the many loci that act in the progressive determination of the PNS (Jan and Jan 1993). Candidate genes with map positions consistent with those of the bristle number QTL are listed in Table 4. Of the 26 bristle number QTL, the map positions of 16 coincided with at least one gene known to be involved with PNS development (Jan and Jan 1993; Salzberg *et al.* 1997); one coincided with a known mutation with an adult bristle number phenotype, but whose role in bristle development is unknown (Lindsley and Zimm 1992); and three coincided with *P*-element insertional mutations with adult bristle number phenotypes, but which are otherwise uncharacterized (Lyman *et al.* 1996). If QTL map to the same location as candidate genes that have been cloned and sequenced, the actual molecular basis of the mutation associated with the QTL allele and its frequency can be inferred by associating molecular polymorphisms at the candidate gene with phenotypic variation in bristle number in a large sample of alleles from nature (Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998).

However, some bristle number QTL with large effects, for example, Bn(10B–D), Bn(72E), and Bn(94B), do not map near known genes with bristle number phenotypes. More precise and finer-scale mapping of these loci may be feasible using overlapping deficiencies and the recombinant isogenic lines generated in the course of this study. Such studies might reveal that these loci do correspond to known loci with bristle number phenotypes; alternatively, they may not so correspond and further study may reveal novel loci discovered using quantitative genetic analysis of naturally occurring variation.

We thank Jeff Leips and two anonymous reviewers for comments on the manuscript. This work was supported by National Institutes of Health grants GM-45146 and GM-45344 to T.F.C.M., and by the W. M. Keck Foundation.

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Communicating editor: C. Haley