Genetic Interactions Between Naturally Occurring Alleles at Quantitative Trait Loci and Mutant Alleles at Candidate Loci Affecting Bristle Number in *Drosophila melanogaster*

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ABSTRACT

Previously, we mapped quantitative trait loci (QTL) affecting response to short-term selection for abdominal bristle number to seven suggestive regions that contain loci involved in bristle development and/or that have adult bristle number mutant phenotypes, and are thus candidates for bristle number QTL in natural populations. To test the hypothesis that the factors contributing to selection response genetically interact with these candidate loci, high and low chromosomes from selection lines were crossed to chromosomes containing wild-type or mutant alleles at the candidate loci, and the numbers of bristles were recorded in *trans* heterozygotes. Quantitative failure to complement, detected as a significant selection line*cross effect by analysis of variance, can be interpreted as evidence for allelism or epistasis between the factors on selected chromosomes and the candidate loci. Mutations at some candidate loci (*bb, emc, h, Dl, Hairless*) showed strong interactions with selected chromosomes, whereas others interacted weakly (*ASC, abd, Scr*) or not at all (*N, mab, E(spl)*). These results support the hypothesis that some candidate loci, initially identified through mutations of large effect on bristle number, either harbor or are close members in the same genetic pathway as variants that contribute to standing variation in bristle number.

STANDING genetic variation for characters of evolutionary, medical, and agricultural importance is often quantitative in nature. It is generally believed that both randomly acting environmental forces and a number of genetic factors contribute to variation in these characters (FALCONER and MACKAY 1996). Identification of the loci contributing to continuous variation will likely have a great impact on future animal and plant breeding programs and on medical advances in identification and treatment of diseases with complex inheritance. The number and characteristics of the genes controlling standing variation in quantitative traits are also critical elements of models for the maintenance of quantitative variation within populations and for the evolutionary divergence of continuous characters. Despite years of speculation and considerable analysis, the nature of such variation remains an enigma (BARTON and TURELLI 1989; FALCONER and MACKAY 1996). In a previous study, we mapped factors of large effect that contributed to response to short-term selection for abdominal bristle number in *Drosophila melanogaster* (LONG *et al.* 1995). The short length of the selection experiment and the size of the population from which the experiment was initiated makes it likely that many of these factors were segregating in the wild population from which the selection experiment was initiated (HILL and KEIGHTLEY 1988; LONG *et al.* 1995).

For some purposes (*e.g.*, marker assisted selection) identification of the actual locus causing a quantitative effect is not necessary and accurately mapped factors will suffice. But many evolutionary inferences require determining the frequency spectrum of alleles at quantitative trait loci (QTL) in a randomly mating population. Despite recent progress in mapping QTL, it is still very difficult to move from a mapped factor to the actual genetic locus harboring a variant. The number of recombinants necessary to map a factor to a physical region small enough that it defines a single genetic locus is usually impractically large. A promising alternative in genetically developed organisms is to test if mapped factors are allelic to candidate genes, previously identified by mutant alleles of large effect that map to the same genetic location as QTL. Complementation testing of mutant alleles at candidate loci to chromosomes containing putative QTL is one such method. Complementation testing has been used in the past to infer allelism of mutations of large effect, which occurred during the course of long-term artificial selection experiments, to candidate loci (YOO 1980; FRANKHAM 1988; DOEBLEY *et al.* 1995).

Drosophila bristles are sensory organs of the peripheral nervous system. In recent years intense and successful investigation of the development of the peripheral ner-

This paper is dedicated to Richard A. Morton on the occasion of his promotion to Professor Emeritus.

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vous system of Drosophila has demonstrated the utility and power of this model in the elucidation of transcriptional regulation, lateral inhibition, and cell fate (reviewed by JAN and JAN 1994). Through these efforts a number of genes have been cloned and their roles extensively characterized with regards to the genetic pathways controlling bristle development and biochemical interactions among members of these pathways. The hypothesis that the alleles at QTL may be variants of small effect at the loci identified as being important in bristle development is supported by the observation of LONG *et al.* (1995) that the genetic intervals containing QTL of large effect also contain a number of these candidate loci, as well as studies that have found associations being molecular variants at candidate loci and variation in bristle number among sets of naturally occurring chromosomes (MACKAY and LANGLEY 1990; LAI *et al.* 1994). In the present paper, we test the hypotheses that the following candidate loci, listed by approximate map position (LINDSLEY and ZIMM 1992), genetically interact with *High* or *Low* chromosomes from LONG *et al.* (1995): *achaete-scute complex* (*ASC*, 1-0.0), *Notch* (*N*, 1-3.0), *bobbed* (*bb*, 1-66.0), *extramacrochaetae* (*emc*, 3-0.0), *hairy* (*h*, 3-26.5), *abdominal* (*abd*, 3-27.0), *Sex combs reduced* (*Scr*, 3-47.5), *malformed abdomen* (*mab*, 3-47.5), *Delta* (*Dl*, 3-66.2), *Hairless* (3-69.5), and *Enhancer of split* (*E(spl)*, 3-89.1). A number of the tested loci are well characterized loci involved in neurogenesis, such as *ASC*, *N*, *emc*, *h*, *Dl*, *Hairless*, and *E(spl)* (JAN and JAN 1994) (the gene symbol *H*, for *Hairless*, will not be used throughout this paper to avoid confusion with *H*, for *High* chromosome). In addition, other candidate genes that are not necessarily directly involved in neurogenesis were examined. These include two genes likely to have roles in cuticle formation [*abd* (LINDSLEY and ZIMM 1992) and *mab* (LEWIS *et al.* 1980)], *bb* because of its previous identification as a newly arising mutation capable of contributing to selection response (FRANKHAM 1988), and *Scr* largely due to its map position, reports of expression in abdominal regions (GINDHART *et al.* 1995; GORMAN and KAUFMAN 1995), and the effect of gain-of-function mutations at this locus on sternopleural bristle number (LINDSLEY and ZIMM 1992).

Here we investigate if complementation testing can be used to determine if naturally occurring alleles of quantitative effect genetically interact with mutant alleles at candidate loci. We used a modification of the commonly used complementation test to determine if *High* and *Low* extracted chromosomes "fail to complement" mutant alleles at candidate loci. The test involves a series of four crosses in which heterozygous combinations of either *High* or *Low* tester chromosomes affecting bristle number are made with a wild-type *Control* chromosome or a chromosome containing a mutation at the candidate gene (*Mutant*). If the effect of substituting a *Mutant* for a *Control* chromosome is the same in both *High* and *Low* chromosome backgrounds, then the *Mutant* chromosome is "complemented" by both the *High* and *Low* chromosomes. Alternately, if the effect of a *Control* to *Mutant* substitution are of a different magnitude in the two backgrounds, then a "failure to complement" is observed, the significance of which is indicated as a significant interaction term in a two-way ANOVA with main effect terms corresponding to the *High vs. Low* treatment, and the *Control vs. Mutant* treatment. Significant interactions imply that either the mapped factors are allelic to the candidate mutant alleles, mapped factors are dominant modifiers of the candidate mutant alleles, nonmapped factors are dominant modifiers of the candidate loci with no detectable effect of their own, or factors on the *High* or *Low* chromosomes are interacting with other QTL on the *Mutant* or *Control* chromosome.

MATERIALS AND METHODS

Drosophila stocks: The fly stocks used are described in Table 1. The *X* chromosome tester chromosomes were *High*, *Low*, or recombinant *X* chromosomes in an otherwise low autosomal background constructed for the earlier QTL mapping work (LONG *et al.* 1995). The use of recombinant chromosomes allows effects associated with failure to complement to be roughly mapped. Parentheses in the genotypes of the tester stock indicate the region to which a recombination (R) event or a small Low (L) segment have been localized.

FM7a, *In(1) dl-49*, *CyO*, and *TM8* are balancer chromosomes (LINDSLEY and ZIMM 1992). The *Control* strain used for the *X* chromosome complementation tests is the *X; bw; st* present in the Bloomington *bw; st* strain. An *FM7a; bw; st* strain was created by backcrossing the male progeny of a *FM7a* ♀ × *bw; st* ♂ cross to *bw; st*, then intercrossing *FM7a*/+; *bw; st* and +/*Y; bw; st* progeny and selecting for *FM7a* (+ throughout this paper indicates an uncontrolled, nonmutant chromosome). First chromosome candidate gene mutant alleles (*m*) were put into the *FM7a; bw; st* background by crossing the mutant to *FM7a; bw; st*, then backcrossing the *m*/*FM7a; bw*/+; *st*/+ F_1 progeny to *FM7a; bw; st* and intercrossing *m*/*FM7a; bw; st* F_2 progeny to create a stock, with the exceptions of Ybb^-, N^{55e11} and $(y\ sc)^-$.

The *bb* locus is located on both the *X* and *Y* chromosomes, thus complementation tests for this locus used a tester *X* chromosome crossed to either a *bb* or wild-type *Y* chromosome. The first generation of the cross used to introgress Ybb^- into the *bw; st* background was Ybb^- ♂ × *bw; st* ♀; otherwise the crosses were the same as above.

N^{55e11}/Y is lethal, so males in the second generation of the ^{N55e11} cross were N^{55e11}/Y; *bw*/w^+ *51b7*; *st*/+ [w^+ *51b7* covers the lethality of N^{55e11} in males (LINDSLEY and ZIMM 1992)]; otherwise the crosses were the same as above.

$(y\ sc)^-$ is lethal in hemizygotes and against the *scute* alleles common in the *FM* balancer series (LINDSLEY and ZIMM 1992), and therefore the crossing scheme for *ASC* mutant alleles was slightly modified. In the first generation *In(1) dl-49*/$(y\ sc)^-$; +; + was crossed to +/y^2 *Y60d*; *bw; st* y^2 *Y60d* covers $(y\ sc)^-$ (LINDSLEY and ZIMM 1992) and was substituted into *bw; st* in the same manner as Ybb^-). In generation two the resulting $(y\ sc)^-/y^2$ *Y60d*; *bw*/+; *st*/+ male progeny were backcrossed to *In(1) dl-49; bw; st* (*In(1) dl-49; bw; st* was created in a manner analogous to *FM7a; bw; st*). In generation three the resulting $(y\ sc)^-$/*In(1) dl-49*/y^2 *Y60d*; *bw; st* progeny were intercrossed to create a stock.

The third chromosome tester stocks, RLA 12.1 and RI3-79, originated from LONG *et al.* (1995) and were either *Low* or *High* for the third chromosome in an otherwise low first and second chromosome background. The chromosome *3* con-

TABLE 1

Drosophila stocks used in complementation tests

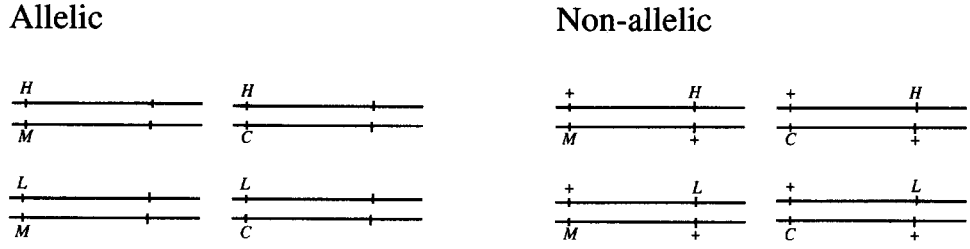
Abbreviation	Full genotype	Description of mutant	Source[a]
	X chromosome		
Tester			
Low X	RLA 25.2 = *L; L; L*		1
Low X Tip	RI1-25 = *L, R*{4C1-6A1}, *H; L; L*		1
High X	RI1-73 = *H; L; L*		1
Low X Centromere	RI1-71 = *H, L*{3C1-4A1}, *H, R*{11A7-17D1}, *L; L; L*		1
Control			
bw; st	*X; bw; st*		2
Mutant			
$(y\ ac)^-$	$(y\ ac)^-$ *88a15.1; bw; st*	Null	3
$(y\ sc)^-$	$(y\ sc)^-$ *DEB/In(1) dl-49; bw; st*	Null	3
N^{55e11}	N^{55e11}, *rb/FM7a; bw; st*	Weak LOF	4
bb^-	Ybb^1; *bw; st*	Weak LOF	5
	Chromosome *3*		
Tester			
Low	RLA 12.1 = *L; L; L*		1
High	RI3-79 = *L; L; H*		1
Control			
Sam ry^{506}	*Sam* ry^{506}		6
Mutant			
$emc^{P(3)-67}$	*S; S;* $emc^{P(3)-67}$	Weak LOF	7
emc^{H17}	+; +; emc^{H17}, *red/TM2,* p^p	Null	8
Df(3L) h-i22	+; +; *Df(3L) h-i22, Ki,* p^p*/TM3*	Null (*h* + *abd*)	4
abd^1	+; +; abd^1	? LOF	4
h^1	*S; S;* h^1	Weak LOF	9
h^{IL79K}	+; +; h^{IL79K}*/TM3*	Strong LOF	10
Df(3R) Scx2	+; +; *Df(3R) Scx2, red, e/TM3*	Null (*Scr* + *mab*)	4
mab^1	*L; L;* mab^1, *Ki,* p^p*/TM8*	? LOF	4
Scr^{17}	+; +; Scr^{17}*/TM6B*	Null	4
Dl^3	*S; S;* Dl^3	Weak LOF	9
$Hairless^1$	*L; L;* $Hairless^1$*/TM8*	Moderate LOF	4
$E(spl)^{Dr\nu bx22}$	*S; S;* $E(spl)^{Dr\nu bx22}$	Moderate LOF	11

H, L, S refer to *High, Low,* and *Samarkand* chromosomes, respectively. The origin of the *High* and *Low* chromosomes is described in LONG *et al.* (1995). The origin of the Samarkand strain used is described in LYMAN *et al.* (1996). LOF and null refer to a loss-of-function mutant and null (or deficiency) mutant, respectively. LOF mutant alleles have a modifier describing their magnitude or a ? when the magnitude of the mutant is unknown.

[a] 1, see LONG *et al.* (1995); 2, see MATERIALS AND METHODS for stock construction. *X, bw,* and *st* are chromosomes obtained from the Bloomington Stock Center; 3, from M. GREEN; 4, from Bloomington Stock Center; 5, from S. HAWLEY; 6, *Sam* ry^{506} is a highly inbred strain of Samarkand into which an isogenic third chromosome containing ry^{506} has been substituted (LYMAN *et al.* 1996); 7, newly induced *P*-element mutation in *Sam* ry^{506} background from T. F. C MACKAY; 8, from J. POSAKONY; 9, mutant backcrossed through females into *Sam* ry^{506} background for 20 generations; 10, from D. ISH-HOROWICZ; 11, from T. F. C. MACKAY.

trol stock was the highly inbred *Sam* ry^{506} (LYMAN *et al.* 1996). The h^1 and Dl^3 mutant stocks were backcrossed through females into the *Sam* background for ~20 generations, thus it is expected that the size of the introgressed fragment associated with the mutant alleles was ~10 cM (NAVEIRA and BARBADILLA 1992). The $emc^{P(3)-67}$ mutant stock was a newly induced *P*-element insertion at the *emc* locus (LYMAN *et al.* 1996). Two mutant alleles, $Hairless^1$ and mab^1, were put in a low first and second chromosome background by a two generation backcross through males to *L; CyO/L; TM8(Sb)/L* and recovering *L; L; TM8(Sb)/*$Hairless^1$ or mab^1 males and females to establish a stock. All the other mutant stocks used had uncontrolled, but nonmutant, first and second chromosome backgrounds (Table 1).

Complementation testing: For each candidate gene mutant tested for failure to complement, a set of four strains were used: a *Mutant* (*M*) strain containing a mutant allele at the candidate gene locus, a *Control* (*C*) strain presumed wild type at the candidate gene locus, a *High* (*H*) strain fixed for bristle number QTL increasing bristle number, and a *Low* (*L*) strain fixed for bristle number QTL decreasing bristle number (Figure 1 and Table 1). For each candidate gene mutant a set of four crosses were carried out: the *M* strain was crossed to both the *H* and the *L* strain, and the *C* strain was similarly crossed to the *H* and the *L* strains (Table 1). These four crosses resulted in a set of four *trans* heterozygote genotypic classes that could be compared to assess the relative degree of the failure of the *M* strain to complement the *H* or *L* strain (Fig-



Expected means by genotype

	M	C	row means
H	$\bar{X}_{HM}$	$\bar{X}_{HC}$	$\bar{X}_{H\cdot}$
L	$\bar{X}_{LM}$	$\bar{X}_{LC}$	$\bar{X}_{L\cdot}$
column means	$\bar{X}_{\cdot M}$	$\bar{X}_{\cdot C}$	

	M	C	row means
H	$\bar{X}_{+M;\,H+}$	$\bar{X}_{+C;\,H+}$	$\bar{X}_{+\cdot;\,H+}$
L	$\bar{X}_{+M;\,L+}$	$\bar{X}_{+C;\,L+}$	$\bar{X}_{+\cdot;\,L+}$
column means	$\bar{X}_{+M;\,\cdot+}$	$\bar{X}_{+C;\,\cdot+}$	

ANOVA tests

Source	Test	Test
H v. L	$\bar{X}_{H\cdot} - \bar{X}_{L\cdot} = 0$	$\bar{X}_{+\cdot;\,H+} - \bar{X}_{+\cdot;\,L+} = 0$
M v. C	$\bar{X}_{\cdot M} - \bar{X}_{\cdot C} = 0$	$\bar{X}_{+M;\,\cdot+} - \bar{X}_{+C;\,\cdot+} = 0$
complementation	$\bar{X}_{HM} + \bar{X}_{LC} - \bar{X}_{HC} - \bar{X}_{LM} = 0$	$\bar{X}_{+M;\,H+} + \bar{X}_{+C;\,L+} - \bar{X}_{+C;\,H+} - \bar{X}_{+M;\,L+} = 0$

Alternate interpretations of the test for complementation

$\bar{X}_{HM} - \bar{X}_{LM} = \bar{X}_{HC} - \bar{X}_{LC}$ and	$\bar{X}_{+M;\,H+} - \bar{X}_{+M;\,L+} = \bar{X}_{+C;\,H+} - \bar{X}_{+C;\,L+}$ and
$\bar{X}_{HM} - \bar{X}_{HC} = \bar{X}_{LM} - \bar{X}_{LC}$	$\bar{X}_{+M;\,H+} - \bar{X}_{+C;\,H+} = \bar{X}_{+M;\,L+} - \bar{X}_{+C;\,L+}$

FIGURE 1.—Statistical tests of failure to complement. (Top) The four genotypes compared in the quantitative complementation test in the case of high and low factors that are allelic to a mutant at a candidate gene (the left locus in each genotype), or are at a second modifier locus (the right locus in each genotype). (Bottom) The expected phenotypes of these four genotypes, and hypotheses tested in the ANOVA described in the text, for a model in which there is an allele on either the *High* or *Low* chromosome that is allelic to a candidate gene mutant, or a model in which there is an allele on either the *High* or *Low* chromosome that is a modifier of the candidate gene mutant. Abbreviations in the top part of the figure are as follows: high QTL allele (*H*), low QTL allele (*L*), mutant allele at a candidate locus (*M*), control allele at a candidate locus (*C*), and a wild-type allele (+) assumed to have the same effect on both the *Mutant* and *Control* chromosomes, or *High* and *Low* chromosomes depending on if the allele is at the candidate or modifier locus. In the bottom portion of the figure, nonsubscripted *H, L, M,* and *C* refer to *High, Low, Mutant,* and *Control* chromosomes, respectively, and $\bar{X}$ refers to expected phenotypic values with subscripts designating the genotype of individuals in that phenotypic class. Subscripted genotypes are of the form *ab;cd,* where *a* is the allelic state of the *High* or *Low* chromosome at the candidate locus, *b* is the allelic state of the *Mutant* or *Control* chromosome at the candidate locus, *c* is the allelic state of the *High* or *Low* chromosome at the modifier locus, *d* is the allelic state of the *Mutant* or *Control* chromosome at the modifier locus. · represents the average over alleles on the chromosomes and at the locus specified by its position in the subscript.

ure 1). The autosomal background of all flies examined for the first chromosome loci was *bw/L; st/L*. For the third chromosome loci, the first and second chromosomal background of the *Control* crosses was *Sam/L* or *Sam/Y; Sam/L,* whereas for the *M* crosses it was (*Sam/L* or *Sam/Y; Sam/L*) or (*L/L* or *L/Y; L/L*) or (+/*L* or +/*Y;* +/*L*), depending on the mutant tested (see Table 1). For each cross five replicate vials were initiated from a mass mating of two to 10 *M* or *C* females and a similar number of *H* or *L* males, varied to create a vial culture that was healthy, but not too densely crowded. The number of abdominal and sternopleural bristles was counted for 10 males and 10 females per replicate (only females were counted in the case of *X* chromosome loci and only males for *Ybb*$^-$). All crosses were carried out at 25° with a 12 hr day/night cycle, with parents being removed after several days, and the bristles of offspring counted after 12–20 days.

Statistical analysis: Evidence for a *M* chromosome failing to complement the *H* or *L* chromosome requires a quantitative comparison of the effect of a *C* (presumed wild type) to *M* chromosome substitution in a *L vs. H* chromosome background. The average effect of a *C* to *M* substitution in the *H* background is the difference between the means of flies of genotype *M/H* and *C/H* (*M/H* − *C/H*); similarly, the effect of a *C* to *M* substitution in the *L* background is *M/L* − *C/L*. If the *M* chromosome and QTL present on the *H* or *L* chromosome do not genetically interact, then we expect the effect of this substitution to be similar in the two backgrounds. If the *M* chromosome and QTL do genetically interact, then we expect failure of either the *H* or *L* chromosome to fully complement the *M* chromosome, which is detected as a difference in the average effect of a *C* to *M* substitution in the two backgrounds (*i.e.*, (*M/H* − *C/H*) − (*M/L* − *C/L*) ≠ 0). This is equivalent to the test for significance of the interaction term in the following linear model describing variation in bristle number:

$$Y_{ijkl} = \mu + T_i + A_j + TA_{ij} + rep_k(TA_{ij}) + \epsilon_{ijkl},$$

where T_i is the effect of *i*th Tester chromosome (*e.g., H vs. L*), A_j is the effect of the *j*th mutant allele (*e.g., M vs. C*), and $rep_k(TA_{ij})$ is the effect of replicate vials nested within the fully

TABLE 2

Means of *X* chromosome crosses and *P* values associated with complementation testing for full and specified contrast models

Mutant	Char[a]	Tester	Cross means[b]			Complementation[c]		
			M/T	C/T	Effect	Contrast	Effect[d]	*P*
(y ac)$^-$	AB	*Low*	15.14	15.60	−0.46	*H vs. L*	0.18	0.19
		Low Tip	16.66	16.06	0.60	*H vs. LT*	−0.88	0.29
		High	18.34	18.62	−0.28	*LT vs. L*	1.06	0.20
						Full		0.34
	SB	*Low*	16.34	17.04	−0.70	*H vs. L*	0.36	0.29
		Low Tip	17.12	16.72	0.40	*H vs. LT*	−0.74	0.04
		High	17.06	17.40	−0.34	*LT vs. L*	1.10	0.00
						Full		0.01
(y sc)$^-$	AB	*Low*	10.62	15.60	−4.98	*H vs. L*	−0.04	0.96
		Low Tip	10.88	16.06	−5.18	*H vs. LT*	0.16	0.83
		High	13.60	18.62	−5.02	*LT vs. L*	−0.20	0.79
						Full		0.96
	SB	*Low*	14.62	17.04	−2.42	*H vs. L*	0.26	0.41
		Low Tip	14.80	16.72	−1.92	*H vs. LT*	−0.24	0.31
		High	15.24	17.40	−2.16	*LT vs. L*	0.50	0.12
						Full		0.23
N^{55e11}	AB	*Low*	15.78	15.60	0.18	*H vs. L*	0.52	0.49
		Low Tip	15.64	16.06	−0.42	*H vs. LT*	1.12	0.20
		High	19.32	18.62	0.70	*LT vs. L*	−0.60	0.44
						Full		0.37
	SB	*Low*	20.26	17.04	3.22	*H vs. L*	0.10	0.81
		Low Tip	19.88	16.72	3.16	*H vs. LT*	0.16	0.72
		High	20.72	17.40	3.32	*LT vs. L*	−0.06	0.87
						Full		0.92
bb$^-$	AB	*Low*	11.16	12.82	−1.66	*Lc vs. L*	−0.76	0.20
		High	16.46	16.34	0.12	*H vs. Lc*	2.54	0.00
		Low Cent.	11.82	14.24	−2.42	Full		0.00
	SB	*Low*	15.64	15.38	0.26	*Lc vs. L*	0.06	0.90
		High	16.56	16.32	0.24	*H vs. Lc*	−0.08	0.84
		Low Cent.	16.32	16.00	0.32	Full		0.98

[a] Char is the character tested. AB, abdominal bristle number; SB, sternopleural bristle number.

[b] Cross means are the means of the specified genotypes. M, Mutant; T, Tester (*X; bw; st*), and Effect is the difference between these means.

[c] Complementation refers to the effects and *P* values associated with the test of failure to complement. Contrasts involve *Low (L), High (H), Low Tip (LT),* and *Low Centromere (Lc).* The effect associated with contrast *A vs. B* is $[(M/A - C/A) - (M/B - C/B)]$.

[d] Estimates of standard errors were comparable for *X* and third chromosome loci examined by complementation testing and averaged 0.24 (minimum = 0.16, maximum = 0.37) and 0.22 (minimum = 0.12, maximum = 0.30) bristles for male and female sternopleural bristle number, respectively, and averaged 0.25 (minimum = 0.19, maximum = 0.37) and 0.39 (minimum = 0.29, maximum = 0.53) bristles for male and female abdominal bristle number, respectively.

TA_{ij} crossed term. The significance of the interaction term was tested by an F ratio of

$$\frac{\textit{Mean Square}\ [TA]}{\textit{Mean Square}\ [rep(TA)]}.$$

For the *X* chromosome loci, *i* had three levels, *H, L,* and *Low Tip* (with *Low Centromere* being substituted for *Low Tip* for *Ybb*$^-$), with three subsequent contrasts: *H vs. L, H vs. Low Tip,* and *L vs. Low Tip.* For the third chromosome loci, *i* had two levels (*H* and *L*), and a cross-classified effect of sex.

Standard errors (SE) of estimates of effects associated with a *C* to *M* substitution in a given Tester "background" (*e.g.*, $M/L - C/L$), were estimated as

$$s.e. \cong \frac{2\sqrt{k \times MS_{rep}}}{\sqrt{N_i \times N_r}},$$

where $k = 1/6$ for the *X* chromosome crosses and 1/4 for the third chromosome crosses, MS_{rep} is the estimate of the Mean Square associated with the replicate within *TA* term of the ANOVA, N_i is the number of individuals per replicate measured, and N_r is the number of replicates carried out per TA_{ij}. The SE is approximate because it assumes the variances within each cross are equal (*e.g.*, for the third chromosome $\mathrm{Var}_{H/M} = \mathrm{Var}_{H/C} = \mathrm{Var}_{L/M} = \mathrm{Var}_{L/C} = 1/4\,\mathrm{Var}_{TA}$). The standard error of the effect associated with the actual effect of failure to complement [*e.g.*, for the third chromosome $(M/H - C/H) - (M/L - C/L)$], as opposed to the effect of a substitution in a given background, is estimated as twice the estimate of the above standard error. To address potential violations of the homoscedasticity assumption of ANOVA all analyses were also performed on square root of bristle number plus 10. This transformation was chosen because it appeared to remove a dependency of the variance in bristle number on the mean

TABLE 3

Means of third chromosome crosses and *p* values associated with complementation tests for full and sex-partitioned models

Mutant	Char[a]	Sex	Complementation[b] full model		Cross means[c]			Effect of mutant[d] in background		Complementation[e] by sex *P*
			P	*P**sex	*M/L*	*M/H*	*M/C*	*L*	*H*	
emc$^{P(3)-67}$	SB	M	0.234	0.592	17.5	19.2	19.4	0.34	0.58	0.465
		F			18.8	20.5	19.2	0.10	0.56	0.234
	AB	M	0.868	0.944	15.4	20.1	17.5	0.98	0.88	0.817
		F			16.5	21.3	20.7	0.74	0.70	0.956
emc^{H17} *red*	SB	M	0.000	0.023	18.7	21.3	18.8	1.58	2.72	0.062
		F			18.6	23.0	19.0	−0.10	3.02	0.000
	AB	M	0.309	0.548	13.7	18.1	16.0	−0.76	−1.16	0.594
		F			13.8	17.7	16.5	−1.94	−2.86	0.255
Df(3L)h-i22 Ki p^{p}	SB	M	0.027	0.457	17.0	19.2	Lethal	−0.12	0.58	0.126
		F			18.1	20.6	Lethal	−0.62	0.58	0.054
	AB	M	0.006	0.499	13.0	19.9	Lethal	−1.39	0.62	0.006
		F			14.2	20.3	Lethal	−1.50	−0.32	0.213
abd^{1}	SB	M	0.329	0.481	16.5	17.8	17.5	−0.58	−0.74	0.760
		F			18.5	19.3	18.3	−0.18	−0.70	0.099
	AB	M	0.111	0.732	13.9	19.9	17.7	−0.52	0.62	0.053
		F			16.3	21.9	21.5	0.54	1.32	0.418
h^{1}	SB	M	0.867	0.722	18.5	19.9	18.7	1.34	1.28	0.904
		F			19.5	21.0	18.8	0.84	1.02	0.711
	AB	M	0.938	0.958	15.4	20.2	16.2	0.96	0.94	0.969
		F			16.2	21.0	18.4	0.42	0.36	0.936
h^{1L79K}	SB	M	0.000	0.202	20.8	24.7	24.0	3.68	6.10	0.000
		F			21.8	26.2	23.5	3.12	6.26	0.000
	AB	M	0.268	0.486	14.8	20.5	18.4	0.36	1.20	0.084
		F			16.1	21.3	20.6	0.40	0.70	0.697
Df(3R)Scx2 red e	SB	M	0.000	0.819	19.3	22.7	21.3	2.14	4.12	0.000
		F			19.5	22.6	20.7	0.80	2.62	0.001
	AB	M	0.744	0.313	15.5	19.8	18.4	1.10	0.58	0.304
		F			14.5	19.6	20.2	−1.26	−1.00	0.665
mab^{1} *Ki p*^{p}	SB	M	0.737	0.360	14.8	16.5	17.6	−2.32	−2.10	0.641
		F			17.5	18.4	18.2	−1.16	−1.60	0.380
	AB	M	0.581	0.376	13.1	18.7	16.7	−1.36	−0.52	0.089
		F			10.4	15.1	17.4	−5.36	−5.52	0.882
Scr^{17}	SB	M	0.027	0.884	17.4	19.7	19.2	0.32	1.08	0.096
		F			19.6	21.7	20.5	0.90	1.74	0.062
	AB	M	0.264	0.416	15.2	19.2	16.9	0.80	−0.08	0.086
		F			15.5	20.1	20.3	−0.20	−0.48	0.706
Dl^{3}	SB	M	0.172	0.819	17.4	19.7	19.2	0.28	1.12	0.276
		F			20.6	22.9	21.1	1.92	2.88	0.135
	AB	M	0.000	0.779	18.2	26.5	22.2	3.76	7.24	0.000
		F			17.5	26.2	26.2	1.76	5.60	0.002
Hairless^{1}	SB	M	0.000	0.004	12.3	10.6	11.9	−4.86	−7.98	0.000
		F			13.6	13.5	14.5	−5.10	−6.52	0.019
	AB	M	0.605	0.066	11.1	15.0	12.8	−3.28	−4.30	0.039
		F			8.6	14.0	13.9	−7.10	−6.64	0.573
E(spl)^{Dmbx22}	SB	M	0.727	0.790	17.9	19.3	19.2	0.78	0.72	0.888
		F			20.1	21.2	20.3	1.36	1.18	0.650
	AB	M	0.386	0.866	15.6	19.9	17.6	1.16	0.60	0.174
		F			18.2	22.7	22.0	2.50	2.10	0.672

TABLE 3

Continued

Control	Char	Sex	*C/L*	*C/H*	*C/C*[f]
Sam ry[506]	SB	M	17.1	18.6	16.9
		F	18.7	20.0	17.6
	AB	M	14.4	19.3	15.2
		F	15.7	20.6	16.7

[a] Char is the character tested. AB, abdominal bristle number; SB, sternopleural bristle number.

[b] Complementation full model are the *P* values associated with the test for failure to complement for a full model including the effect of sex. *P* is the *P* value associated with the test for significance of the $T \times A$ term and *P** sex is the test for significance of the *sex* $\times$ $T \times A$ term (see MATERIALS AND METHODS).

[c] Cross means are the mean bristle numbers of genotypes of interest associated with all of the crosses performed. *M, Mutant; C, Control (Sam ry*[506]*); L, Low; H, High.*

[d] Effect of mutant in background are the estimated effect of a *Control* to *Mutant* substitution in a *Low (L) vs. High (H)* background. The effect is calculated as $(M/L - C/L)$ or $(M/H - C/H)$. The bottom portion of this table gives mean bristle numbers for genotypes associated with control crosses.

[e] Complementation by sex are the *P* values associated with the tests for failure to complement of a model where the two sexes are analyzed separately.

[f] As the *C/C* genotype is a completely homozygous fly, a column representing the effect of a *Control* to *Mutant* substitution in the *Control* background is not included.

bristle number more efficiently than a number of other transformations (*i.e.*, log and reciprocal) attempted.

RESULTS

General interpretation of results: The results of all complementation tests are presented in Tables 2 and 3, and the results for selected loci are plotted in Figures 2 and 3. Estimates of standard errors were comparable for *X* and third chromosome loci examined; their average, maximum, and minimum values are presented in the legend to Table 2.

Regressions of the standard deviation of bristle number on mean bristle number over all experimental treatments (*i.e., C/H, C/L, M/H,* and *M/L*) by sex and character explained a significant amount of the variation in bristle number in the entire experiment [*i.e.*, 1% for male sternopleural bristle number ($P < 0.26$ with 1, 33 degrees of freedom), 26% for female sternopleural bristle number ($P < 0.00$ with 1, 44 degrees of freedom), 11% for male abdominal bristle number ($P < 0.03$ with 1, 33 degrees of freedom), and 7% for female abdominal bristle number ($P < 0.04$ with 1, 44 degrees of freedom)]. This relationship between the mean and variance in bristle number violates the homoscedasticity assumption of ANOVA and may result in false positive significance tests. Therefore, analyses were also performed on transformed bristle scores that do not show this dependency of the variance on the mean. With the exception of female sternopleural bristle number, the square root of bristle number plus 10 transformation resulted in a data set in which the regression of standard deviations on means by experimental treatment did not explain a significant proportion of the total variation in mean bristle number [*i.e.*, 0% for male sternopleural bristle number ($P < 0.62$ with 1, 33 degrees of freedom), 8% for female sternopleural bristle number ($P < 0.03$ with 1, 44 degrees of freedom), 0% for male abdominal bristle number ($P < 0.53$ with 1, 33 degrees of freedom), and 0% for female abdominal bristle number ($P < 0.81$ with 1, 44 degrees of freedom)]. Analyses of the transformed data resulted in nearly identical *P* values associated with the tests of failure to complement as did the analyses of the untransformed data. Thus, all further reference to *P* values associated with failure to complement are from the analyses of raw bristle scores.

Results by candidate loci: In the sections that follow, the results of the complementation testing are described in detail for each of the candidate loci tested, and are ordered by cytogenetic divisions containing the mutant alleles. A brief description of the known function of the wild-type gene product is included so that subsequent discussion of results can remain general.

***achaete-scute* complex (1-0.0):** The *achaete-scute* complex (*ASC*) encodes multiple transcription factors capable of auto- and crossregulation, whose expression patterns define proneural regions (MARTINEZ and MODOLELL 1991). Loss-of-function mutations at the *ASC* complex reduce the size of the proneural region and subsequently reduce the number of sensory bristles on the fly (CAMPUZANO and MODOLELL 1992). Two deficiencies were used to test for complementation in the *ASC, (y ac)*$^-$ *88a15.1* and *(y sc)*$^-$ *DEB. (y ac)*$^-$ is a hemizygous and homozygous viable/fertile deficiency for the region from *yellow* to *achaete,* and *(y sc)*$^-$ *DEB* is a hemizygous and homozygous lethal deficiency from *yellow* to *scute* (OLSEN and GREEN 1982). The substitution of *(y ac)*$^-$ for the *Control* chromosome generally had effects close to zero. Although only statistically significant for sternopleural bristle number, the effect of a *Control* to *Mutant* substitution in the *Low X Tip* chromosome background was positive, whereas the effect of a *Control* to *Mutant* substitution for both the *High* and *Low* backgrounds was negative (Table 2 and Figure 2). Although we cannot show that *(y ac)*$^-$ fails to complement the *High*

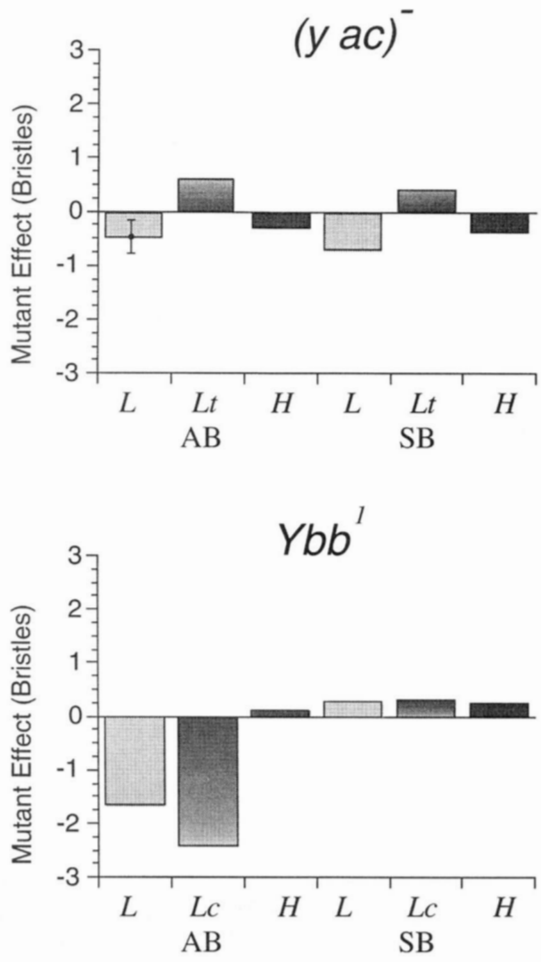


FIGURE 2.—The effect (in bristles) of a *Control* to *Mutant* chromosome substitution in different backgrounds (see text) for abdominal (AB) and sternopleural (SB) bristle number for the first chromosome complementation tests. The error bar depicted in the $(y\ ac)^-$ panel is 1 SE and is similar for all tests. The difference between bars within character classes is proportional to the failure to complement. First chromosome effects for $(y\ ac)^-$ and Ybb^- are for females and males, respectively. Effects of a *Control* to *Mutant* chromosome substitution were assayed in three different backgrounds (L, *Low X* chromosome; Lt, *Low X Tip* chromosome; H, *High X* chromosome).

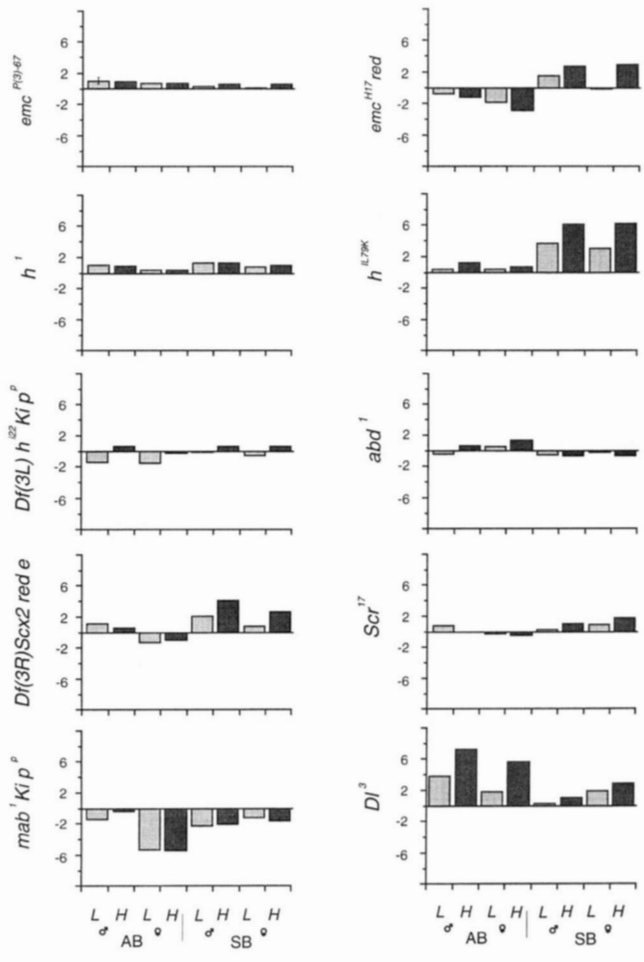


FIGURE 3.—The effect (in bristles) of a *Control* to *Mutant* chromosome substitution in different backgrounds (see text) and for both sexes for abdominal (AB) and sternopleural (SB) bristle number for the third chromosome complementation tests. The error bar depicted in the $emc^{P(3)-67}$ panel is 1 SE and is similar for all tests. Effects were estimated in a *Low* (L) and *High* (H) chromosomal background.

vs. Low chromosome for the *y ac* region, $(y\ ac)^-$ appears to be involved in nonadditive interactions with low factors located near the tip of the first chromosome and high factors located elsewhere on the first chromosome. Complementation tests involving the $(y\ sc)^-$ deficiency, although not significant, showed the same pattern of effects for sternopleural bristle number as the tests using the $(y\ ac)^-$ deficiency (Table 2). Abdominal bristle number effects associated with the $(y\ sc)^-$ complementation tests were similar in all three backgrounds, providing little support for failure to complement (Table 2).

***Notch* (1-3.0):** The gene product of the *Notch* (*N*) locus is a transmembrane protein that appears to be the receptor for the *Delta* gene product (ARTAVANIS-TSAKONAS *et al.* 1995). *Notch* and *Delta* are currently believed to be the primary molecules involved in the cell-cell communication that drives the process of lateral inhibition among sensory organ precursor cells (JAN and JAN 1994; ARTAVANIS-TSAKONAS *et al.* 1995). Loss-of-function mutations in *Notch* cause an overcommitment to a neurogenic fate and result in an increase in bristle number. We tested one allele of *Notch*, N^{55e11}, a weak loss-of-function mutant (KIDD *et al.* 1983; LINDSLEY and ZIMM 1992). N^{55e11} provided little evidence for failure to complement the tester chromosomes. Although showing the classic dominant nicking of the wing margins, N^{55e11} had only a small effect on abdominal and sternopleural bristle number, increasing them by only one and three bristles, respectively (Table 2).

***bobbed* (1-66.0):** The *bobbed* (*bb*) rRNA locus is organized as a tandem array and is located in the nucleolus organizing regions of the *X* and *Y* chromosomes (TARTOF 1975). The *bobbed* locus is known to have a very high mutation rate (10^{-4} gamete^{-1} generation^{-1}), with loss-of-function mutations decreasing bristle number (TARTOF 1975; FRANKHAM 1988). Both a *Low X* chromosome and an *Low X centromere* chromosome provided

strong evidence of failure to complement Ybb^- for abdominal bristle number (see Table 2 and Figure 2). The observation that only a very small centromeric region need be low to give failure to complement a *bobbed* mutant suggests that failure to complement is due to allelism of the mapped low QTL to the *bobbed* locus.

***extramachrochaetae* (3-0.0):** *extramachrochaetae* (*emc*) encodes a basic Helix-Loop-Helix protein that is a negative regulator of transcription in the *ASC* (VAN DOREN *et al.* 1991, 1992; LINDSLEY and ZIMM 1992). Loss-of-function mutations result in an increase in bristle number, presumably because mutant alleles are less capable of downregulating the genes of the *ASC*. Two mutant alleles at *emc* were used to test for failure to complement this locus, $emc^{P(3)-67}$ and emc^{H17}. $emc^{P(3)-67}$ appears to complement the *High vs. Low* backgrounds for the third chromosome (see Table 3 and Figure 3). In addition, $emc^{P(3)-67}$ had only a weak single dose effect on abdominal and sternopleural bristle number of approximately one bristle. emc^{H17}, a small deficiency that is a complete null for *emc*, showed strong evidence for failure to complement the *High* and *Low* tester chromosomes for sternopleural bristle number (see Table 3 and Figure 3). It is notable that the average effect of the emc^{H17} mutant was to decrease abdominal bristle number in both the *High* and *Low* backgrounds.

***hairy* and *abdominal* (3-26.5) region:** The genomic region at map position 3-26.5 contains two candidate genes, *hairy* (*h*) and *abdominal* (*abd*), in close physical proximity. *h* encodes a Helix-Loop-Helix protein that is a negative regulator of *ASC* (OHSAKO *et al.* 1994; VAN DOREN *et al.* 1994). Loss-of-function mutations at *hairy* result in an overcommitment to the neural fate, and thus an increase in bristle number. Two mutant alleles, h^1, a weak loss-of-function allele, and $h^{IL.79K}$, a strong loss-of-function allele (INGHAM *et al.* 1985), were used in the complementation testing of this locus. The h^1 allele had a small single dose effect on abdominal and sternopleural bristles of approximately one bristle, but showed no evidence for failure to complement (see Table 3 and Figure 3). $h^{IL.79K}$ showed a similar single dose effect of one abdominal bristle, but a much larger effect on sternopleural bristles of approximately four to six bristles. The effect of a *Control* to $h^{IL.79K}$ substitution on sternopleural bristle number was significantly different in the *High* and *Low* third chromosome backgrounds, indicating that the selected chromosomes failed to fully complement this allele of *hairy*.

abdominal is a less well characterized locus, with only a few, presumed weak, loss-of-function mutations known (LINDSLEY and ZIMM 1992). abd^1 appears to complement the *High* and *Low* chromosomes. One dose of abd^1 tended to add one abdominal bristle and decrease sternopleural bristle number by one, despite the observation that abd^1 homozygotes tend to have reduced bristle number. A deficiency that uncovers both *abd* and *h*, *Df(3L) h-i22* (INGHAM *et al.* 1985; LINDSLEY and ZIMM 1992), was also used to test for complementation in this region. The qualitative pattern of the effects associated with *Control* to *Mutant* substitutions for this deficiency do not resemble those of $h^{IL.79K}$ for the case of sternopleural bristle number, yet there was significance evidence of failure to complement the male abdominal bristle number phenotype (Table 3 and Figure 3). Patterns of complementation for *Df(3L) h-i22* did qualitatively resemble those for abd^1 (Figure 3). This indicates that both *hairy* and *abdominal* are likely to be genetically interacting with factors on the *High* or *Low* chromosomes, and that abd^1 may not be a strong enough allele to observe significance in the complementation test.

***Sex combs reduced* and *malformed abdomen* (3-47.5) region:** LONG *et al.* (1995) mapped a factor with an effect of over five abdominal bristles in females to the region containing the *Antennapedia complex*. *Sex combs reduced* (*Scr*) and *malformed abdomen* (*mab*) are located in this complex (LEWIS *et al.* 1980; KAUFMAN *et al.* 1990; LINDSLEY and ZIMM 1992). *mab* is a nonhomeotic cuticle gene at which only a few mutations uncovered by saturation mutagenesis of the *ANTC* have been identified (LEWIS *et al.* 1980). These presumed loss-of-function mutations at *mab* result in cuticle defects and reductions in abdominal bristle number (A. D. LONG, unpublished data). mab^1 has a single dose effect that causes the loss of one to five bristles, as with the homozygous mab^1 mutant, effects are the largest for female abdominal bristle number (see Table 3 and Figure 3). Complementation tests involving mab^1 provided no evidence that this locus fails to complement the *High* and *Low* chromosomes. Additional complementation tests were carried out with a deficiency, *Df(3R)Scx2*, that uncovers both *mab* and *Scr* (as well as *pb, zen, bcd, Dfd, ftz,* and *Antp*) (KAUFMAN *et al.* 1990; LINDSLEY and ZIMM 1992). *Df(3R)Scx2* provided strong evidence for failure to complement the *High* and *Low* chromosomes for sternopleural bristle number, but qualitative patterns of *Control* to *Mutant* substitutions did not resemble those of mab^1 (Table 3 and Figure 3). To assess if the significance of the *Df(3R)Scx2* complementation test was due to *Scr*, as opposed to *mab*, we carried out complementation tests using a reported null *Scr* allele, Scr^{17} (LINDSLEY and ZIMM 1992). Scr^{17} complementation tests provided marginally significant evidence that sternopleural bristle number failed to fully complement the *High* and *Low* chromosomes (see Table 3 and Figure 3). The similar patterns of failure to complement of *Df(3R)Scx2* and Scr^{17} implicate *Scr* in the difference between the *High* and *Low* chromosomes.

***Delta* (3-66.0):** *Delta* (*Dl*) encodes a protein that provides an inhibitory signal important in determining cell fate in regions with the potential to form bristles (VASSIN *et al.* 1987; KOPCZYNSKI *et al.* 1988; LINDSLEY and ZIMM 1992). Loss-of-function *Dl* mutations result in an increase in bristle number (PARKS and MUSKAVITCH 1993). The Dl^3 allele used in the complementation tests had a large single dose effect on both abdominal and sternopleural bristle number of one to seven bristles

and showed significant and strong evidence for failure to complement abdominal bristle factors, with effects of complementation as large as four bristles (see Table 3 and Figure 3). Unlike the results of other complementation tests, Dl^3/H offspring show close to a qualitative bristle phenotype.

***Hairless* (3-69.5):** Loss-of-function *Hairless* mutations show a loss of sensory bristles (BANG *et al.* 1991). *Hairless* mutant alleles are known to suppress the effects of loss-of-function mutant alleles at *N* and *Dl*, suggesting that *Hairless* acts in the same pathway as the other neurogenic loci (DE LA CONCHA *et al.* 1988). *Hairless* has been cloned, but it is not yet clear how it interacts at the molecular level with other neurogenic gene products (BANG and POSAKONY 1992). The H^1 mutant allele has a large single dose effect on bristle number of from two to eight bristles (see Table 3). H^1 also provided strong evidence for failure to complement the *High* and *Low* third chromosomes for sternopleural bristle number and male abdominal bristle number. Unlike other examples of significant failure to complement where the extreme class shows a greater than additive effect (*e.g.*, $H/M - L/C > (H/C - L/C) + (L/M - L/C)$ for emc^{H17}, h^{IL79K}, and Dl^3), for H^1 failure to complement appears to be due to a less than additive effect [*i.e.*, $-(L/M - H/C) < -((L/C - H/C) + (H/M - H/C))$]. It is important to note that the comparison is different for *Hairless* than for *emc*, *h*, and *Dl* because loss-of-function *Hairless* mutant alleles cause a reduction in bristle number.

***Enhancer of Split* (3-89.0) region:** *Enhancer of Split* (*E(spl)*) is a complex neurogenic locus encoding at least 11 distinct transcripts (LINDSLEY and ZIMM 1992). Loss-of-function mutations result in a neurogenic phenotype with increased bristle numbers, but interallelic complementation patterns are complex (KLAMBT *et al.* 1989; DELIDAKIS *et al.* 1991). A deficiency for the *E(spl)* region, *Df(3R)Espl1* ($=E(spl)^{8D06}$) (DELIDAKIS *et al.* 1991), was used in initial complementation tests of this region, but because of strong deleterious viability effects the crosses were not completed. A moderate loss-of-function allele, $E(spl)^{Drvbx22}$ (*i.e.*, a revertant of *E(spl)D* also called $E(spl)^{bx22}$), which complements at least some other *E(spl)* mutant alleles (SHEPARD *et al.* 1989), provided no evidence for failure to complement the *High* and *Low* third chromosomes. The single dose effect of $E(spl)^{bx22}$ is small, resulting in an increase of one to two bristles. Apparent complementation may have resulted from the decision to use a weak *E(spl)* allele in the complementation test, or a lack of factors on the *High* or *Low* chromosomes that interact with the *E(spl)* locus.

DISCUSSION

Patterns of interaction between candidate genes and selected chromosomes: Mutations at some candidate loci (*bb*, *emc*, *h*, *Dl*, *Hairless*) showed strong interactions with the selected chromosomes, whereas others interacted weakly (*ASC*, *abd*, *Scr*) or not at all (*N*, *mab*, *E(spl)*). The highly significant failure to complement a *bb* mutant of both a *Low X* chromosome and a first chromosome low for the centromeric region, but otherwise high, suggests that a factor mapped to the centromeric region of the *X* chromosome in LONG *et al.* (1995) is an allele of *bobbed*. The observed failure of the *High* and *Low* chromosomes to complement a null allele of *emc* for sternopleural bristle number may be due to the same factor affecting primarily sternopleural bristle number mapped to the left tip of the third chromosome in LONG *et al.* (1995). Mutant *hairy* alleles also show strong evidence of failure to complement the selected chromosomes, similarly suggesting the involvement of *hairy* or modifiers of *hairy* in selection response. Alleles of *Delta* and *Hairless* also showed strong failure to complement the *High* and *Low* chromosomes, implying the involvement of *Delta* and *Hairless* or modifiers of these two loci in selection response. The observation that failure to complement in the case of *Hairless* involved less than, as opposed to greater than, additive gene action makes it possible that failure to complement in the case of *Hairless* may be due to a low *Hairless* allele on the *High* chromosome. A biological lower limit on bristle number does not explain the observed less than additive complementation results, as additive effects in the test would only require that *M/L* individuals have 9.1 and 12.1 sternopleural bristles (in males and females, respectively). Bristle counts of this magnitude are well within the range of normal bristle variation.

Failure to complement was observed for the $(y\ ac)^-$ deficiency, but not the $(y\ sc)^-$ deficiency (which includes *y* to *ac*), suggesting that failure to complement is mediated through interactions between *ac* and *sc*. Failure to complement $(y\ ac)^-$ was also dependent on having a first chromosome with a low tip in an otherwise high first chromosome background, suggesting additional epistatic interactions important in *ASC* complementation. It might be expected that the *ASC* would be involved in epistatic interactions as genes of the *ASC* are known to be regulated by a number of other Helix-Loop-Helix proteins (CAMPUZANO and MODOLELL 1992). This observation appears inconsistent with the earlier failure to detect epistatic effects associated with the factor mapped to the tip of the *X* chromosome (LONG *et al.* 1995). Weak evidence for failure to complement associated with an allele of *abd* may reflect failure to test a strong amorphic allele of this locus, as the significant failure to complement observed for a deficiency uncovering *abd* gave results that qualitatively resembled those for *abd* (see Figure 3). In the case of Scr^{17}, a reported null *Scr* allele, the nonsignificant effects associated with the complementation testing resembled those of the deficiency including *Scr* that was statistically significant (see Figure 3). The observation of qualitatively, but not quantitatively, similar complementation results for *Df(3R)Scx2* and Scr^{17} suggest that complementation at *Scr* is complex, or that Scr^{17} is not

a complete null allele. The small magnitude of the failure to complement in the *Scr* region does not reflect the large and highly significant factor mapped to this region in LONG *et al.* (1995). This may be due to additional factors in this region, which is known to be physically large relative to its recombination distance (ASHBURNER 1989, pp. 452–457).

Neither *N, mab,* or *E(spl)* provided evidence for failure to complement the *High* and *Low* chromosomes. Apparent complementation for these loci may reflect a lack of the involvement of these loci in selection response, or alleles used for complementation testing that did not have strong enough loss-of-function phenotypes. Choosing mutant alleles that are inappropriate for complementation tests against alleles of subtle effect may be common for candidate genes that exhibit complex patterns of complementation from similar analyses of mutant alleles of large effect. Such pseudo-allelism has been observed in the past for some of the loci tested here (*e.g., ASC* (GARCIA-BELLIDO 1979), *N* (WELSHONS and VON HALLE 1962), *ANTC* (KAUFMAN *et al.* 1990), and *E(spl)* (DELIDAKIS *et al.* 1991)).

In a number of cases one mutant allele at a candidate locus showed significant failure to complement the *High* and *Low* chromosomes whereas another apparently complemented these same chromosomes. Although confounded with background, in all the cases in which this occurred, the mutant allele closer to a complete loss-of-function resulted in failure to complement (*i.e.,* $emc^{P(3)\text{-}67}$ *vs.* emc^{H17}, h^{1} *vs.* h^{IL79K}, abd^{1} *vs. Df(3L)h-i22,* Scr^{17} *vs. Df(3R)Scx2*). Weaker alleles often showed similar qualitative patterns of failure to complement, suggesting that the choice of mutant allele to use in complementation tests of alleles of subtle effect is an important consideration.

The cytological location of the candidate loci that showed evidence for failure to complement the *High* and *Low* chromosomes are in close agreement with the cytological locations of the factors mapped in LONG *et al.* (1995). *bb* and the *ASC* correspond in location to factors mapped to the proximal and distal section of the *X* chromosome, respectively. Although, in the case of the factor mapped to the distal portion of the *X* chromosome, the region to which the QTL was mapped includes a very large physical region. For the third chromosome loci the cytological locations of *emc* (61D12), *Scr* (84B1-2), and *Dl* (92A1-2) were all within cytological intervals defined by flanking *roo* elements that corresponded to suggestive or significant peaks in probability: 61A1-64C1, 75C1-85E1, and 88D1-92E1 for *emc, Scr,* and *Dl,* respectively. The other third chromosome candidate loci that showed evidence for failure to complement (*h/abd* at 66D10-15, and *H* at 92E12-F2) are estimated to be physically very close to intervals associated with suggestive peaks. Based on an estimated 270 kb of DNA per lettered subdivision in Drosophila (ASHBURNER 1989), *h/abd* is ~1 megabase from the interval defined by *roo* markers at 64D1 and 66A1, and *Hairless* is ~270 kb from the interval defined by *roo* markers at 88D1 and 92E1. Currently, we do not know how well QTL mapping alogorithms perform on real data, as their accuracy can only be tested on datasets that satisfy assumptions that may not be be representative of the real world. It is also known that mapping algorithms can misposition peaks when there are multiple QTL in fairly tight linkage with one another. Given the small total map length of Drosophila, and the number of mapped factors observed in LONG *et al.* (1995), we find the correspondence between the position of mapped factors and candidate genes to be excellent.

General interpretation of ANOVAs: Figure 1 depicts the four genotypes generally used in the complementation tests and expected mean values associated with each genotype, in the case of failure to complement being due to either allelic (at the candidate locus) or nonallelic (at a modifier locus) factors. It is assumed that, in the case of allelism, a second site does not contribute to the genotypic means; and in the case of a nonallelism, the *High* and *Low* chromosomes have the same allele at the candidate gene being tested and the *Mutant* chromosome has the same allele as the *Control* chromosome at the modifier locus. In both cases, the main effect test of *High vs. Low* chromosomes averaged over *Mutant* and *Control* backgrounds tests the average effect of a *Low* to *High* chromosome substitution. This contrast is almost always significant, which is to be expected given the origin of the *High* and *Low* chromosomes. As the significance of this term is not dependent on the high or low factors residing at the candidate locus it is not discussed further. Similarly, the main effect test of *Mutant vs. Control* chromosomes averaged over *High* and *Low* backgrounds tests the effect of a *Control* to *Mutant* chromosome substitution. This contrast is almost always significant, which shows that the mutant alleles used in this experiment, although often considered to be recessive on a qualitative scale, are rarely completely recessive on a quantitative scale. *P* values associated with main effect tests are not presented, but an examination of differences in the means are fairly indicative of the significance of these observations.

The interpretation of the test for complementation (*i.e.,* the interaction term associated with the ANOVA), depends on which of the two situations in Figure 1 applies. In the case of allelic factors it can be seen that if an allele on the *High* or *Low* chromosome has a nonadditive gene action on the *Mutant,* but has otherwise additive effects, the complementation test will indicate an interaction. This would occur, for example, if a weak loss-of-function allele on the *High* chromosome behaves as a partial recessive against the *Mutant* chromosome, but in all other allelic combinations (*H/C, L/M, L/C*) shows additive gene action. There are many examples of gene action being dependent on particular allelic combinations in crosses between allelic mutants (LINDSLEY and ZIMM 1992) and will also generally be the case

for genes for which activity is a concave down function of dose (that is all loci for which recessive mutants exist) (KACSER and BURNS 1981, for example). A number of different forms of gene action could result in failure to complement, although it seems likely the test has the most power to detect failure to complement when a null (or strong loss-of-function) mutant allele at the candidate locus results in a weak loss-of-function phenotype for either the *High* or *Low* chromosome. If the weak loss-of-function mutant allele is paired with a wild-type or other weak loss-of-function allele, little phenotypic effect may be apparent.

In the case of nonallelic factors, if the modifier locus has an effect on bristle number that is not dependent on the state of the *Control vs. Mutant* chromosome, then the test for failure to complement will be nonsignificant. For the interaction term to be significant in the case of nonallelism, the differential effects of the high and low alleles must be through a dominant modification of the phenotype associated with the *Control* to *Mutant* chromosome substitution (*i.e.*, either the high or low allele is a dominant modifier of the candidate locus). Genetic screens for dominant modifiers are often used to identify genes that biochemically interact with the candidate gene they modify (WOLFNER and GOLDBERG 1994), suggesting that if observed failure to complement is due to such modifiers they are likely to be positioned close in the same pathway as the candidate genes employed in this study. That is, not only must the QTL reside in the same pathway as the candidate gene it modifies, but it must be close enough in the pathway that different naturally occurring alleles interact in a nonadditive way with the mutant alleles, suggesting potential biochemical interactions. If observed failure to complement is due to dominant modifiers of the candidate loci of this study, then these modifiers must either have no main effect as a homozygote, but happened to be fixed in the selection experiment; or they are the mapped factors of LONG *et al.* (1995). It seems unlikely that many alleles of substantial effect went unmapped in LONG *et al.* (1995) as the sum of the effects of the mapped factors accounted for close to the total difference between the parental lines that were used to generate the recombinants. If failure to complement is due to dominant modifiers of the candidate loci with an additional mapped main effect, it is surprising they map to the positions of known candidate genes, as opposed to known modifiers of these candidate loci (LINDSLEY and ZIMM 1992; LONG *et al.* 1995). Although LONG *et al.* (1995) did show that some mapped factors affecting bristle number were epistatic to one another, the genotypes compared were all homozygotes, in contrast to this *trans*-heterozygote test. It is possible that these tests may be detecting biologically different types of epistasis. At present it cannot be determined if failure to complement is due to allelism of mapped factors to candidate genes or dominant modifiers of the candidate genes. Allelism seems more parsimonious.

To eventually determine if interactions between mutant alleles at candidate loci and high and low factors fixed in the artificial selection experiment are due to allelism or epistasis, it will be necessary to perform additional experiments. Backcrossing high and low segments into an otherwise standardized genetic background will potentially eliminate loosely linked dominant modifiers, but not closely linked modifiers. Genetic methods using mutant markers are limited by the availability of markers not affecting bristle number that are physically close to the candidate region, and thus also cannot exclude closely linked modifiers. Gene transformation methods, although commonly used in Drosophila, are likely to suffer from position effects of the same order of magnitude as the alleles of quantitative effect mapped in LONG *et al.* (1995) and are limited to fairly small physical fragments and may therefore be inappropriate for loci with large regulatory regions (ASHBURNER 1989). Similarly, gene replacement is limited in the size of the fragment that can be substituted, and by the availability of *P*-element insertions in the region that one wishes to replace (GLOOR *et al.* 1991; NASSIF *et al.* 1994), and is therefore only likely to be a viable approach when other evidence suggests a small region containing a variant that may be the cause of bristle differences between the *High* and *Low* chromosomes.

The nature of the loci contributing to variation in quantitative traits: Three independent lines of evidence now point to the importance of candidate neurogenic loci as harboring alleles of small effect that contribute to naturally occurring variation in bristle number. First, restriction map variation at *scabrous* (LAI *et al.* 1994) and insertions of transposable elements in the *ASC* (MACKAY and LANGLEY 1990) are correlated with variation in bristle number among a sample of randomly sampled wild chromosomes. Second, some genetic intervals containing naturally occurring factors that cause differences between lines alternatively selected for high or low abdominal bristle number also contain candidate neurogenic loci (LONG *et al.* 1995). Third, the *High* and *Low* chromosomes of the above selection experiment in many cases fail to fully complement mutant alleles at candidate loci. Previous complementation testing experiments have implicated alleles at *bb* (FRANKHAM 1988; MACKAY and FRY 1996), *Dl* (YOO 1980; MACKAY and FRY 1996), *h*, and *H* (MACKAY and FRY 1996) as contributing to long term selection response. These latter instances of allelism reflect mutations that arose during the course of selection, as opposed to variants segregating in the population from which selection was initiated. It is possible that loci like *bb*, which are known to have a high mutation rate (FRANKHAM 1988), represent alleles that arose during the short term selection experiment of LONG *et al.* (1995). It seems unlikely that many of the other loci identified in this study, which are believed to have more typical mutation rates, are new mutations that occurred during the selection experiment.

To understand the evolution of quantitative traits, it

is important to understand the nature of the alleles at the individual QTL controlling quantitative variation. To distinguish between hypotheses regarding the forces maintaining quantitative genetic variation within populations, estimates of the effects associated with, the frequencies of, mutation rates at, and pleiotropy between variants at QTL will be required (BARTON and TURELLI 1989). There is some evidence that alleles of large effect at intermediate frequency make a substantial contribution to standing variation (LAI *et al.* 1994; LONG *et al.* 1995), although it has been argued that these results could be explained by rare factors of large effect (KEIGHTLEY 1995). Identification of the actual variants causing phenotypic differences among lines will greatly strengthen the claim that these variants are at intermediate frequency in natural populations. Identifying the genetic pathway containing factors contributing to short term selection response is an important step in eventually answering these questions.

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