Both Naturally Occurring Insertions of Transposable Elements and Intermediate Frequency Polymorphisms at the *achaete-scute* Complex Are Associated With Variation in Bristle Number in *Drosophila melanogaster*

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ABSTRACT

A restriction enzyme survey of a 110-kb region including the *achaete scute* complex (*ASC*) examined 14 polymorphic molecular markers in a sample of 56 naturally occurring chromosomes. Large insertions as a class were associated with a reduction in both sternopleural and abdominal bristle number, supporting deleterious mutation-selection equilibrium models for the maintenance of quantitative genetic variation. Two polymorphic sites were independently associated with variation in bristle number measured in two genetic backgrounds as assessed by a permutation test. A 6-bp deletion near *sc* α is associated with sternopleural bristle number variation in both sexes and a 3.4-kb insertion between *sc* β and *sc* γ is associated with abdominal bristle number variation in females. Under an additive genetic model, the small deletion polymorphism near *sc* α accounts for 25% of the total *X* chromosome genetic variation in sternopleural bristle number, and the 3.4 kb insertion accounts for 22% of the total *X* chromosome variation in female abdominal bristle number. The observation of common polymorphisms associated with variation in bristle number is more parsimoniously explained by models that incorporate balancing selection or assume variants affecting bristle number are neutral, than mutation-selection equilibrium models.

MANY characters of evolutionary, medical, and agricultural importance are quantitative in nature. Both environmental and genetic factors contribute to variation in these traits, with the contribution from genetic factors often being modeled as the sum of a number of independent and additively acting factors (Falconer and Mackay 1996). Identifying the actual genetic loci underlying variation in quantitative traits will result in advances in both medical and agricultural genetics. Estimates of the number of loci contributing to continuous variation, the effects and number of alleles at each locus, epistatic effects between loci, and genotype-by-environment interactions (*e.g.*, classical GEI, allele-by-sex interactions) have important implications for evolutionary models that attempt to explain both the maintenance of quantitative variation and divergence in quantitative traits between species (Barton and Turelli 1989; Orr and Coyne 1992). Identification of the actual DNA polymorphisms that contribute to continuous variation will allow connections to be forged between molecular evolutionary theory, molecular population genetics, and quantitative genetics. These fields have strong theoretical connections, but there are few data directly linking the evolution of molecules to the evolution of morphological characters. Despite the importance of describing the nature of the genes contributing to continuous variation, knowledge is still largely limited to statistical descriptions of observed phenotypic variation, under assumed genetic models that may be unrealistically simple at the molecular level (Barton and Turelli 1989; Falconer and Mackay 1996).

Quantitative traits have proven to be resistant to genetic dissection, because large numbers of individuals and/or carefully controlled genetic backgrounds must be employed to identify factors contributing to a small fraction of the variation in a character. In this respect, the number of abdominal and sternopleural bristles in *Drosophila melanogaster* is a tractable model system for initially dissecting the genetic basis of continuous variation. Bristle number variation has been well characterized using classical quantitative genetic approaches (Falconer and Mackay 1996), and a number of studies have documented stabilizing selection on bristle number (Linney *et al.* 1971; Nuzhdin *et al.* 1995; Garcia-Dorado and Gonzalez 1996). Abdominal and sternopleural bristles are external sensory organs of the peripheral nervous system. A number of mutants have been identified that affect bristle spacing and patterning, and the genetic loci that harbor these mutants have been cloned and characterized biochemically (Jan

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and Jan 1994). Quantitative trait locus (QTL) mapping has shown that a small number of factors may account for much of the short-term response to artificial selection on bristle number, and QTL (not all statistically significant) map to genetic locations approximately coincident with candidate bristle loci (Long *et al.* 1995). Further experiments have shown that mutants at candidate genes important in bristle development fail to fully complement chromosomes that are the result of short-term selection on bristle number (Long *et al.* 1996) and that molecular variants at these candidate loci are associated with a significant fraction of the total phenotypic variation in bristle number among a set of wild-derived chromosomes reared in a laboratory environment (Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998). Cumulatively these experiments suggest that a small number of loci may contribute to the majority of standing genetic variation in bristle number and that some of these loci may be candidate genes that have been identified through mutants of large effect and are known to be important in bristle development. The generality of these observations, and possible extensions that will allow mechanisms such as epistasis to be incorporated in models for the maintenance of quantitative variation and phenotypic evolution, will rely on the results of a thorough survey of a number of candidate loci.

The QTL mapping study of Long *et al.* (1995) mapped a factor distal to 4C1-2 on the *X* chromosome that had an effect of approximately three abdominal bristles in males and five abdominal bristles in females. The difference in bristle number associated with this factor between males and females was significant. A second QTL mapping study of Gurganus *et al.* (1999) found strong support for a QTL contributing to both sternopleural and abdominal bristle number at the tip of the *X* chromosome. This region contains two candidate genes, which are hypothesized to harbor alleles of subtle effect contributing to standing variation in bristle number: the *achaete-scute* complex *ASC* at 1B1-4 and the *Notch* locus at 3C7 (see Figure 2 and last paragraph of p. 1279 of Long *et al.* 1995; Gurganus *et al.* 1999). The *ASC* encodes multiple transcription factors capable of auto- and cross-regulation, whose expression patterns define proneural regions (Martinez and Modolell 1991), and loss-of-function mutations at the *ASC* reduce the size of the proneural region and subsequently the number of sensory bristles on the fly (Campuzano and Modolell 1992). Conflicting support that the *ASC* may harbor alleles contributing to standing variation came from quantitative complementation tests of both *achaete* and *achaete-scute* deletions to high or low chromosomes that were the result of short-term selection for abdominal bristle number (Long *et al.* 1996). The *achaete* deletion complemented the abdominal bristle number phenotype but failed to complement the sternopleural bristle number phenotype of the lines, while the *achaete-scute* deletion complemented both abdominal and sternopleural bristle number phenotypes.

We report here the results of a study to test for associations between DNA polymorphisms in the *ASC* gene region and variation in abdominal and sternopleural bristle number among a sample of chromosomes extracted from a natural population. This association study approach has been used in previous studies of bristle number variation (Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998) and is likely to play a role in the future study of complex human diseases (Risch and Merikangas 1996; Long *et al.* 1997). This survey replicates the earlier observation that individually rare large insertions are associated as a class with reduced numbers of sternopleural and abdominal bristles (Mackay and Langley 1990). Earlier work is also extended through the inclusion of a greater number of polymorphic markers, recording bristle phenotypes in both homozygous (or hemizygous) *ASC* introgression lines in addition to *X* chromosome substitution lines, and the examination of a new set of wild-extracted chromosomes. The inclusion of additional polymorphic markers throughout the *ASC* gene region allows tests for associations between polymorphic DNA markers and variation in bristle number to be examined. Such associations may represent different mechanisms for the maintenance of quantitive genetic variation than associations between large insertions and variation in bristle number (Lai *et al.* 1994; Long *et al.* 1998).

MATERIALS AND METHODS

Drosophila stocks: All lines used in this study were derived from a collection of isofemale lines from the Raleigh (North Carolina) Farmers Market in 1988. Two sets of *X* chromosome lines were constructed: *X* chromosome substitution lines, in which entire wild-derived *X* chromosomes were substituted into the standard, highly inbred Samarkand ry^{506} (*Sam*) background (Long *et al.* 1998; Lyman and Mackay 1998); and introgression lines derived from the substitution lines, in which the tips of wild *X* chromosomes were introgressed onto the *Sam X* chromosome.

To construct the substitution lines, females from the isogenic stocks were crossed to *Sam X; SM5 Cy/Sp; TM3 Sb* ry^{RK}/H males. Single $+_{1i}$; *SM5 Cy*/$+_{2i}$; *TM3 Sb* $ry^{RK}/+_{3i}$ ($+_i$ denotes a wild-derived chromosome from isofemale strain *i*) sons were crossed to *C(1)DX y w f Sam* ry^{506} females, and $+_{1i}$; *SM5 Cy/Sam2; TM3 Sb* ry^{RK}/ry^{506} male progeny were crossed to both *FM4 B Sam* ry^{506} and *C(1)DX y w f Sam* ry^{506} females. *FM4 B*/$+_{1i}$; *Sam2;* ry^{506} daughters of the first cross were mated to $+_{1i}$; *Sam2;* ry^{506} sons of the second, and the *FM4* balancer chromosome was eliminated in the following generation to produce an isochromosomal substitution line, denoted *W* below.

In addition to a set of whole chromosome substitution lines a set of introgression lines containing a wild-derived distal region of the *X* chromosome in an otherwise *SAM* background were generated. We first derived a strain in which a viable *Notch* allele, *split* (*spl*) had been backcrossed through females into the *Sam* ry^{506} background for 20 generations. Each of the wild *X* chromosome substitution lines was backcrossed through females to the *spl Sam* ry^{506} stock for 10 generations,

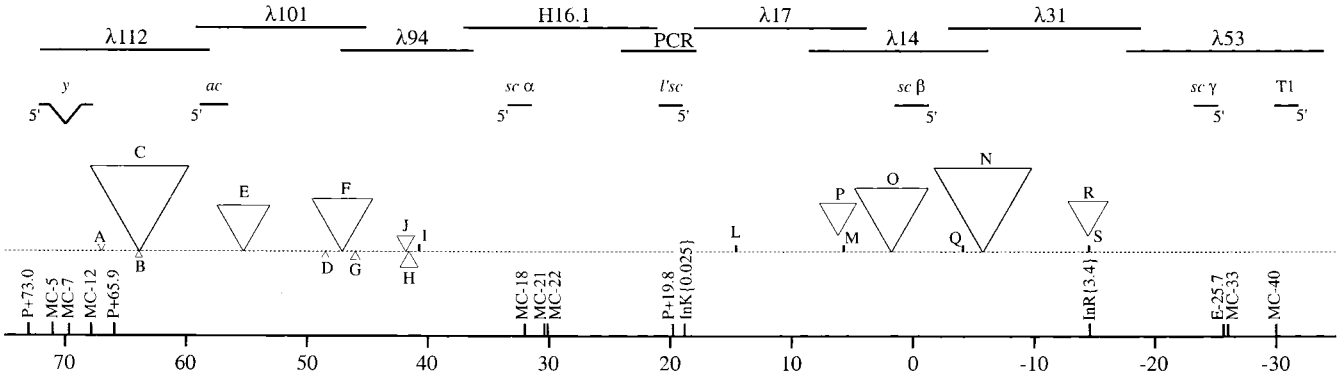

Figure 1.—Summary of restriction map variation in the *ASC* region. (A) Seven phage clones (prefixed with λ) covering much of the *ASC* used in the Southern blotting portion of the survey (Campuzano *et al.* 1985). Phage clones were unavailable for the region covering from 37 to 18, so a 16.1-kb *Hin*dIII fragment (H16.1) isolated from DS06327 (BDGP Project Members 1994) and a Long PCR fragment were used in this portion of the survey. (B) Transcripts encoded by the *ASC* gene region. (C) Inserts (▽) and deletions (△) observed in the Southern blotting portion of the survey. The size of the triangle is proportional to the size of the insert, with inserts <100 bp in size being represented by a line. (D) The relative positions of "common" polymorphisms employed in the association study. Polymorphisms prefixed with a "P" or "E" are *Pst*I or *Eco*RI polymorphisms observed the Southern portion of the survey. Polymorphisms prefixed with an "MC" are PCR-RFLPs designed to amplify polymorphisms identified in Martin-Campos *et al.* (1992). The number following the MC corresponds to the polymorphism number in Table 3 of the same article (some of these variants are restriction site variants and some are common insertion/deletion variants). In K and In R are common insertion deletion variants observed in the Southern portion of the survey.

with two independent backcross populations per chromosome line. After 10 backcross generations, the wild *X* chromosomes were reextracted by crossing single males to *FM4 B Sam ry*^{506} and *C(1)DX y w f Sam ry*^{506} females, as described above. After 10 backcross generations the expected length of the introgressed fragment is 10 cM to either side of *Notch* (Crow and Kimura 1970, pp. 94–95). However *Notch* is at 1–3.0 near the tip of the *X* chromosome, and the total length of the introgressed fragments is thus expected to be slightly smaller than 13 cM. These lines are referred to as B, for backcross, background below. Some of the backcross lines are likely to have experienced a recombination event, such that the introgression line is *ASC*^{SAM}, as opposed to *ASC*^{wild}. Although the probability of this event is approximately one-quarter (*i.e.*, $1 - 0.97^{10}$), it is unlikely to have happened in both replicate introgression lines. As scoring of the molecular markers was carried out on the nonintrogressed (W) lines, recombination events resulting in a *ASC*^{SAM} allele will weaken associations between DNA polymorphisms and bristle number variation. In addition, these recombination events will only have an effect on analyses carried out on the introgression (B) lines.

Restriction map analysis: The *ASC* region was examined at two levels of resolution to assess associations between DNA polymorphisms detectable with restriction endonucleases and variation in bristle number. The first level of resolution was a six-cutter survey of the entire *ASC* region using Southern blots of genomic DNA. Each line was cut with four different restriction endonucleases (*Eco*RI, *Hin*dIII, *Bam*HI, and *Pst*I), separated by gel electrophoresis, blotted onto nylon membranes, and probed with each of the DNA fragments covering the ~110-kb *ASC* gene region as shown in Figure 1. The methods employed in the restriction map survey are described in greater detail in Long *et al.* (1998). This six-cutter survey resulted in the identification of a number of insertion/deletion events throughout the *ASC* gene region and four polymorphic restriction sites.

As the six-cutter survey detected very few polymorphic sites with which to examine DNA polymorphism/phenotypic associations, a PCR-restriction fragment length polymorphism (RFLP) strategy was employed to survey additional polymorphisms. PCR primers were designed to amplify small genomic DNA fragments, which included polymorphisms that had been previously identified by Martin-Campos *et al.* (1992). In some cases, this required sequencing cloned fragments from the *ASC* region, but in most cases primers were designed on the basis of published sequence. The primers used to amplify these fragments, the PCR cycling parameters employed, and the restriction endonucleases used to identify a given site are described in Table 1. PCR products were resolved on Synergel (Research Products International, Mt. Prospect, IL) as described in Long *et al.* (1998) for all PCR-RFLPs, with the exception of polymorphism MC-18. This variant is a small deletion that is difficult to score on Synergel, so a small amount of αP^{32} dCTP was added to the PCR reaction and the resulting product was resolved on a 6% denaturing polyacrylamide (sequencing) gel and visualized using autoradiography. Eight additional common variants were detected using this method; five were size variants and three were single nucleotide polymorphisms.

Bristle number phenotypes: Abdominal and sternopleural bristle numbers were scored on 10 individuals of each sex in each of two replicate vials for the *X* chromosome substitution lines and 10 individuals of each sex in each independent introgression line for the *ASC* region introgressions. Abdominal bristle number was scored as the number of bristles on the fifth abdominal sternite of males and the sixth of females. Sternopleural bristle number was scored as the sum of the total number of macrochaetae and microchaetae on the left and right sternopleural plates. All cultures were reared on 10 ml of cornmeal-agar-molasses medium in shell vials at 25°.

Statistical analysis of molecular marker/phenotype associations: The marker data consist of a combination of sites recognized in the six-cutter portion of the survey, additional sites recognized in the PCR-RFLP portion of the survey, and small insertion/deletion variants surveyed in the PCR-RFLP portion of the survey. For each line bristle number was measured in each of two genetic backgrounds, *W* and *B.* The analysis of molecular marker/bristle number associations was carried out on arithmetic mean bristle number for each background/line/sex combination. As in a previous study, mean bristle

TABLE 1

Primers used in the survey of four-cutter variation in the *ASC*

Name	Primer sequence	Anneal	Size (bp)	Post-PCR
MC5-F	CCAATTGTTAAGATTATATGATGC	56	233	
MC5-R	TTAAGATATCCTTGAAGAGCGTG			
MC7-F	TAGGACTGAAAGAGCACATGTC	56	346	*Sau*3A
MC7-R	ATTTCGACCGATTTCCGCTCCAG			
MC12-F	GGAGTGGAGGTGCCAAAGGCC	56	382	*Hae*III
MC12-R	TTGCGTAAACTCTTAACCTTC			
MC18-F	TTCCTTACCTGTGCAGGCAGC	58	225	*Dde*I[a]
MC18-R	TTTCCGGATAACGATCAACAG			
MC21/22-F	AGGGGATCCATCTATTGCATCAGG	56	480	*Dde*I[b]
MC21/22-R	GATCGGGTTAAAACTACCTCAC			
MC33-F	TGGTGGGAGGCACATGCACC	61	241	
MC33-R	GCGAAGTTATCCTCCTTTCTCGGC			
MC40-F	GATGAATTCATGGTGTGTTTTCGGTGTCGC	56[c]	4 kb	*Hae*III
MC40-R	CCGCGAAAACCCGCGATAAACTGCTACCG			

Primer names are designated MC followed by a number: the MC refers to Martin-Campos *et al.* (1992) and the number corresponds to the polymorphism numbers of Table 3 of that article. PCR reactions were as follows: ~20 ng of genomic DNA, 50 mm KCl, 10 mm Tris (pH 8.8), 1.5 mm $MgCl_2$, 0.1% Triton X-100, 50 μm each dNTP, 0.5 μm each primer, 1 unit of *Taq* polymerase in a 25-μl reaction volume. PCR reactions were initially denatured for 1 min at 92° and then cycled 35 times (45 sec at 92°, 45 sec at correct anneal temperature, and 1 min at 72°). When specified, restriction digests were carried out without any purification of the PCR products and polymorphisms were scored on ethidium bromide-stained "Synergels" as described in the text, with the exceptions listed below.

[a] A 4-bp deletion scored in a 79-bp *Dde*I fragment on a denaturing polyacrylamide gel.

[b] MC22 is a 6-bp insertion in the 232-bp *Dde*I fragment and MC21 is a 12-bp insertion in the 248-bp *Dde*I fragment.

[c] This PCR fragment was amplified using Long PCR and the conditions described in Long *et al.* (1998).

scores for each sex and genetic background were transformed to have zero mean and unit variance and principal component scores were derived for each sex over the two genetic backgrounds (Long *et al.* 1998). These manipulations resulted in a set of four principal component scores: male abdominal, female abdominal, male sternopleural, and female sternopleural bristle number. Principal component scores were analyzed separately for each of the two bristle characters: $Y_{ijk} = \mu + s_i + m_j + (s*m)_{ij} + c(m)_{k(j)} + s*c(m)_{ik(j)}$, where Y_{ijk} is the principal component score of the *i*th sex with the *j*th allele for the *k*th wild chromosome at the molecular marker under consideration, *s* is the effect of sex, *m* the effect of marker, and *c* the effect of wild-derived chromosome. The *F* statistics, ϕ_m and ϕ_{s*m}, associated with the model terms of marker and sex-by-marker, respectively (tested with the chromosome in marker and sex-by-chromosome in marker mean squares, respectively) are assessed for statistical significance.

A previously described permutation testing approach was used to determine if any of the calculated ϕ_m and ϕ_{s*m}'s for each bristle character and the *m* molecular markers in the study are associated with bristle number variation (Churchill and Doerge 1994; Long *et al.* 1998). This approach is appropriate as the *m* molecular markers in the study are often highly correlated, that is, they show significant linkage disequilibrium. If a marker is found to be significantly associated with bristle number variation in the first round of the permutation test, its effect can be statistically removed from the data and additional permutation testing rounds can be sequentially carried out to identify additional significantly associated markers (Doerge and Churchill 1996). Effects associated with a given marker were statistically removed by obtaining residuals by separately fitting a model for each bristle trait and sex combination in which that marker was the sole predictor variable. Effects of, and standard errors associated with, significant markers were also calculated according to published methods (Long *et al.* 1998).

RESULTS

Patterns of bristle variation among Raleigh *X* chromosomes: For each of the 56 lines studied the mean bristle number by sex and genetic background is listed in the appendix. In Figure 2 histograms are plotted for each of the bristle characters, sexes, and genetic backgrounds employed in the study. The average bristle number over lines appears normally distributed, although there is one line (line 2) that is an obvious outlier for abdominal bristle number. Line 2 has a typical average sternopleural bristle number.

Table 2 gives estimates of the correlations of line means between different measures of bristle number in the two sexes and genetic backgrounds. The line mean correlations are upwardly biased estimates of the true genetic correlations by an amount that is inversely proportional to the number of individuals scored per line. As the number of individuals scored per line was large, the magnitude of the bias is not great. For abdominal bristle number bristle counts are highly correlated over genetic backgrounds and sexes. This trend is similarly observed for sternopleural bristle number, with the exception that sternopleural bristle number in the back-

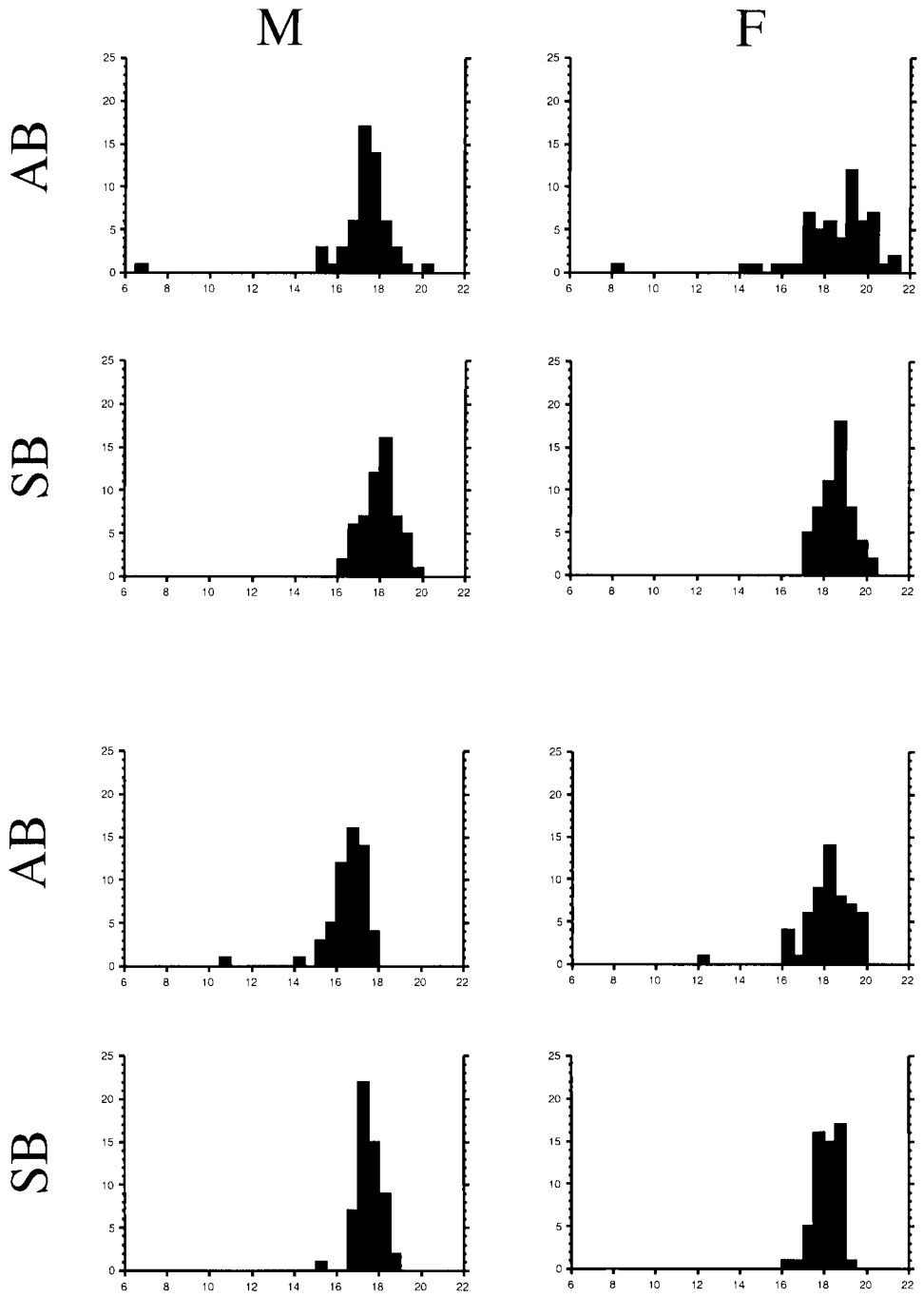

Figure 2.—Histograms showing the distribution of line means for abdominal and sternopleural bristle number for males and females, in both the whole chromosome (top) and backcross chromosome (bottom) genetic backgrounds. The outlier for abdominal bristle number in both genetic backgrounds and sexes is line 2. Line 2 harbors insertion N, the largest insertion observed in the survey.

cross background, although approaching statistical significance, is not correlated with sternopleural bristle number in the whole chromosome background. Bristle counts for abdominal and sternopleural bristle number are not correlated with one another within the whole chromosome background nor between the whole chromosome background and backcross background. Nonetheless, the two different bristle characters are correlated with one another within the backcross background. Correlations are only expected to be the same (statistical fluctuations aside) if the loci affecting bristle number only reside at the introgressed tip of the *X* chromosome or the pleiotropic effects of genes on abdominal and sternopleural bristle number are the same for all loci. The variance in bristle number among lines is higher for abdominal bristle number than sternopleural bristle number, and within a given bristle character the variance is higher in the whole chromosome than the backcross chromosome genetic background. These patterns of variation are in accord with previous studies of bristle variation (Lyman and Mackay 1998).

Molecular population genetics of the *ASC* region: For each line the molecular marker genotypes are listed in the appendix. Figure 1 summarizes the region surveyed

TABLE 2

Correlations and variances over lines between the different sexes, bristle traits, and genetic backgrounds employed

	WABM	WABF	BABM	BABF	WSBM	WSBF	BSBM	BSBF
WABM	*1.69*	0.83***	0.74***	0.70***	0.26	0.06	0.26	0.21
WABF		*2.00*	0.70***	0.81***	0.20	0.13	0.24	0.20
BABM			*1.09*	0.85	0.06	−0.05	0.32*	0.27*
BABF				*1.23*	0.04	0.02	0.29*	0.33*
WSBM					*0.80*	0.83***	0.47***	0.23
WSBF						*0.72*	0.37**	0.21
BSBM							*0.57*	0.61***
BSBF								*0.54*

Pearson product-moment correlations with asterisks indicating a difference from zero: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. These significance levels are not corrected for multiple tests. Along the diagonal (in italics) is the variance in bristle number associated with each genetic background/trait/sex combination. The first letter of each label corresponds to genetic background (W, whole *X* chromosome; B, fragments of the *X* chromosome including the *ASC* introgressed into a *SAM* background), the next two letters indicate the bristle trait measured (AB, abdominal bristle number; SB, sternopleural bristle number), and the last letter corresponds to the sex (M, male; F, female). The variance and covariance estimates among line means in this table include V_e and are thus not estimates of corresponding genetic parameters.

in this study. The four six-cutters of this survey uncovered nine polymorphic sites, whereas the earlier study of Aguadé *et al.* (1989) uncovered seven polymorphic sites using the same set of restriction enzymes and a different sample of wild chromosomes from Raleigh. Five of the nine polymorphic sites observed in this study were not observed in the previous study (P + 73.0, H + 5.7, E − 16.9, E − 26.5, and P − 27.9); all of these sites were unique in the current survey with the exception of P + 73.0, which was seen twice. Aguadé *et al.* (1989) observed three unique sites that were not seen in the present study (P + 65.1, B − 0.4, and E − 4.4). Rare sites will often be absent from replicate samples drawn from the same population, thus the observation of a different set of rare sites seen in this study and the earlier study of Aguadé *et al.* (1989) is consistent with little difference between the two samples. The wild chromosomes used in these two studies were collected approximately a decade apart from the same geographic location; the combined data from the two studies with respect to common restriction sites suggest that patterns of restriction map variation in the *ASC* are fairly stable over short-term evolutionary periods.

The six-cutter survey of the *ASC* region uncovered 19 insertion/deletion (in/del) polymorphisms. Of the 19 insertion/deletion polymorphisms, 7 were 3.0 kb or larger, 15 were 100 bp or larger, and 4 were smaller than 100 bp. There were 15 unique in/del polymorphisms in the sample, 2 in/del polymorphisms were observed twice (In A and Del Q), and 2 in/del polymorphisms were at high frequency (In K and In R). Although common in the present sample, In K was not observed in the previous study of Aguadé *et al.* (1989), whereas In R was observed in the earlier study.

Previous studies that have surveyed restriction map variation in the *ASC* region have concluded that there is less variation in this region than at other loci in Drosophila (Aguadé *et al.* 1989; Martin-Campos *et al.* 1992). The results of the six-cutter portion of this survey support these earlier observations, as the estimate of θ (Hudson 1982) for the *ASC* is 0.0017. For neutral sex-linked loci the expectation of θ is equal to $3N\mu$, where N is the effective population size and μ the neutral mutation rate. $3N\mu$ can also be estimated from the average pairwise heterozygosity, π, which is estimated as 0.0011 (Engels 1981). Under a neutral model of molecular evolution, estimates of π and θ should be equal to one another. Departures from equality, which can be measured by Tajima's *D* statistic, are often assumed to be due to nonneutral evolution (Kaplan *et al.* 1989; Tajima 1989). Tajima's *D* for the six-cutter data is −0.937, providing little evidence for a departure from neutrality (Braverman *et al.* 1996). We did not estimate levels of nucleotide variation from the PCR-RFLP portion of the survey as these markers were chosen because they were polymorphic in a previous survey.

Figure 3 is a plot of the pairwise disequilibrium between each pair of polymorphic sites in this study. It is evident that there is a great deal of linkage disequilibrium in the *ASC* region, apparently much more than other loci that have been examined for a similar sample of wild chromosomes extracted from Raleigh (Lai *et al.* 1994; Long *et al.* 1998). High levels of linkage disequilibrium are expected in the *ASC*, as this locus is located in a region with relatively low levels of recombination per physical length. $3Nc$ (an estimate of 3 times the effective population size times the probability of a recombinational event per gamete per generation between the end points of the region under consideration) is estimated to be 51.8 on the basis of the six-cutter data

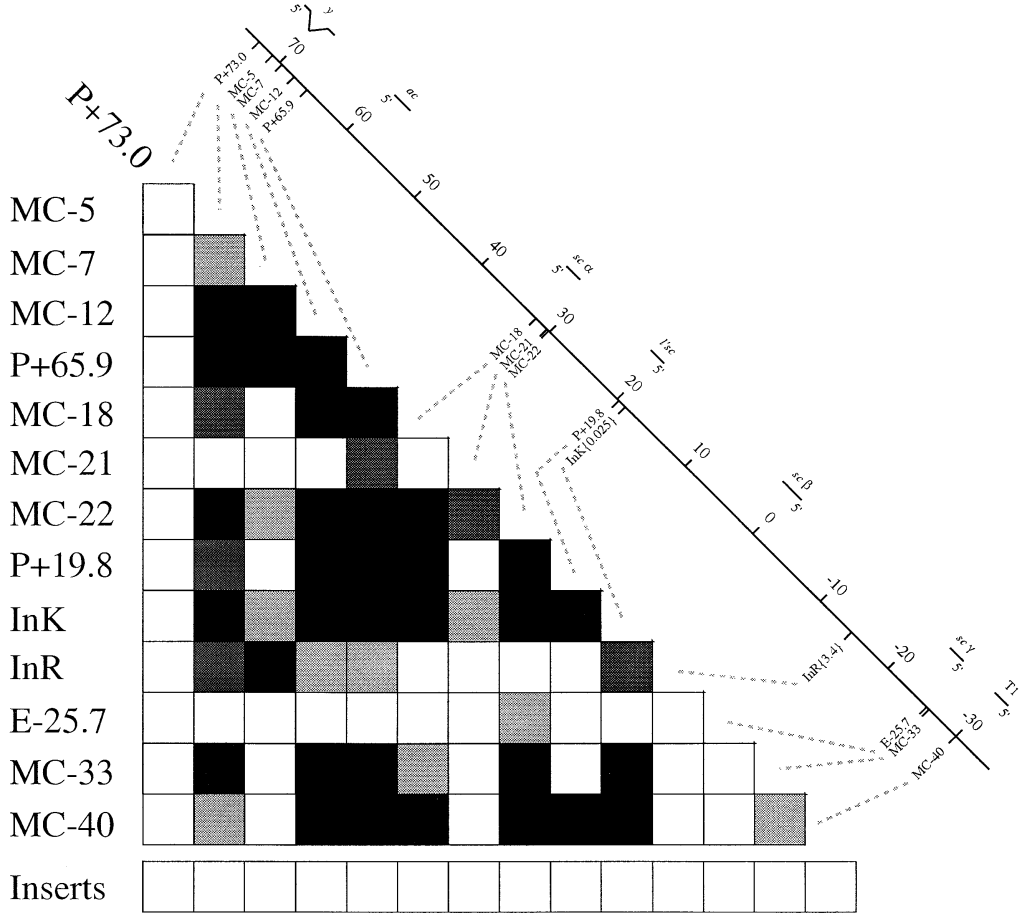

Figure 3.—Patterns of pairwise disequilibrium between "common" polymorphisms employed in the association study and large insertions as a class. Squares within the disequilibrium grid are shaded according to the significance level of disequilibrium between the sites (not corrected for multiple tests) as assessed by a Fisher's exact test; solid squares, $P < 0.005$; shaded squares, $P < 0.01$; lightly shaded squares, $P < 0.05$; and open squares, $P > 0.05$.

(Hudson 1987). The region surveyed was ~104 kb, thus for the *ASC* region one 3*Nc* is equal to ~2 kb, a ratio of disequilibrium map distance to physical distance ~10 times smaller than the more typical *Delta* gene region (Long *et al.* 1998). The estimate of 3*Nc* for the *ASC* region based on a combination of six-cutter data and PCR-RFLP data was somewhat smaller; 3*Nc* for this set of markers was estimated to be 20.0. It is not clear if the estimate of 3*Nc* based on the inclusion of the PCR-RFLP data is a more or less accurate reflection of the estimate of 3*Nc* for the region based solely on the six-cutter data. The estimate based on the inclusion of the PCR-RFLP data includes more polymorphic markers, but the PCR-RFLP markers are not randomly ascertained. Regardless, given the rather large sampling variance of estimates of 3*Nc* it is unlikely that these two estimates are significantly different from one another (Hudson 1987).

Associations between molecular variants and bristle number: We tested whether the 14 polymorphic markers with a frequency of three or greater in the survey plus a dummy variable corresponding to the presence or absence of large insertions are associated with variation in the principal component scores associated with bristle number variation as assayed in two genetic backgrounds (W and B). Table 3 lists the results of tests for an association assessed using a sequential permutation testing procedure (Churchill and Doerge 1994; Doerge and Churchill 1996). The first principal component accounts for 87, 90, 73, and 60% of the total variation in male abdominal, female abdominal, male sternopleural, and female sternopleural bristle number, respectively. For the first principal component the permutation test resulted in In R being associated with a significant sex-by-abdominal bristle number interaction ($P < 0.010$). The effect of In R was statistically removed from the first principal component scores and the analysis rerun. This resulted in MC-22 being significantly associated with variation in bristle number ($P < 0.029$). When the effects of both In R and MC-22 were removed by regression no other markers remained statistically significant. These analyses demonstrate that In R and MC-22 are independently associated with variation in bristle number. No significant associations between polymorphic sites in the *ASC* and variation in the second principal component scores were observed.

An earlier study showed that large insertions as a class in the *ASC* region are associated with variation in abdominal and sternopleural bristle number (Mackay and Langley 1990). In this study, tests for associations between insertions as a class and variation in bristle number were assessed using a permutation-based one-

TABLE 3

Results of permutation testing procedure used to evaluate the significance of marker/bristle trait associations

Response variable[a]	Set of predictors[b]	Permutation test round 1			Permutation test round 2		
		Character	Marker	$P <$	Character	Marker	$P <$
PC1	15 markers	AB*sex	In R	0.010	SB	MC-22	0.029
	Inserts[c, d]	SB		0.023			
W	15 markers[e]						
	Inserts[c]	SB		0.016			
B	15 markers[f]	AB*sex	In R	0.003	AB*sex	MC-21	0.044
	Inserts[c]	AB		0.025			

[a] Variable tested for significance using the permutation testing procedure. PC1, principal component one; W, whole *X* chromosome substitution lines; B, introgression lines obtained by backcrossing. No markers were significant for principal component two.

[b] Set of predictors used in the permutation testing model. 15 markers, the set of all polymorphic markers plus a dummy variable corresponding to the presence or absence of large inserts (see appendix); Inserts, a dummy variable corresponding to the presence or absence of large inserts (excluding In R).

[c] All insert main effect tests are one-tailed.

[d] AB is $P < 0.074$.

[e] MC-22 for SB is $P < 0.058$; In R for AB*sex is $P < 0.072$.

[f] E-25.7 for SB is $P < 0.073$ in permutation test round 3; MC-22 for SB gave no indication of significance.

tailed test of significance. The rationale for a one-tailed test is that insertions in the *ASC* region are expected to reduce bristle number, consistent with loss-of-function mutations in *ASC* (Lindsley and Zimm 1992) and the earlier association observed by Mackay and Langley (1990). A one-tailed permutation test is carried out by premultiplying each of the ϕ statistics by $a + 1$ if the mean bristle number of the noninsert-containing lines is greater than the insert-containing lines and $a - 1$ otherwise. Associations between large insertions as a class and a sex-mediated effect on bristle number were assessed using a two-tailed test, as there is no *a priori* expectation that effects associated with insertions would be larger in one sex than the other. When all large insertions were considered as a class, variation in abdominal bristle number was associated with the presence/absence of large inserts ($P < 0.026$) and variation in sternopleural bristle number approached significance ($P < 0.093$; results not shown). Neither abdominal nor sternopleural bristle number was associated with a large insert-by-sex interaction, in contrast to a previous study (Mackay and Langley 1990). Given that In R was associated with bristle number variation on its own, we also tested for associations with large insertions as a group excluding In R. A further justification for considering In R separately from the other insertion/deletion polymorphisms is the atypically high frequency of In R in this survey and earlier surveys of variation in the *ASC* region. Table 3 presents the results of permutation tests designed to detect association between the presence of "large insertions" (those >50 bp) in the *ASC* and bristle number variation. When In R is not grouped with the other large insertions, the presence of large insertions is associated with variation in sternopleural bristle number ($P < 0.023$) and weakly associated with variation in abdominal bristle number ($P < 0.074$). Similar to the case of all large insertions, sex-by-large insertions excluding In R are not significant for either bristle trait. In no instance is the presence of large insertions associated with variation in the second principal component score.

In this study and a previous study (Long *et al.* 1998), principal component scores were used so that bristle counts from different genetic backgrounds could be jointly tested for associations with molecular markers. Under the assumption that there is no quantitative trait nucleotide (QTN)-by-background interaction (*i.e.*, an additive model of QTN gene action), combining information from different genetic backgrounds will increase the power of detecting marker/phenotype associations. If the QTN is epistatically interacting with other genes, then analyses carried out on principal component scores are analogous to measures of the "general combining ability" of the QTN. But principal component scores may obscure important differences between backgrounds. As a result, we also applied the permutation testing procedure separately to both the whole chromosome and backcross genetic backgrounds. The results of these analyses are presented in Table 3. The test of large inserts as a class was significant for sternopleural bristle number in the whole chromosome background and significant for abdominal bristle number in the backcross chromosome background. Permutation testing involving the 14 polymorphic markers (plus the large insert dummy variable) failed to result in any markers being significant in the whole chromosome background. But two tests almost reached significance in the whole chromosome background: MC-22 for sternopleural bristle number and In R for an abdominal bristle

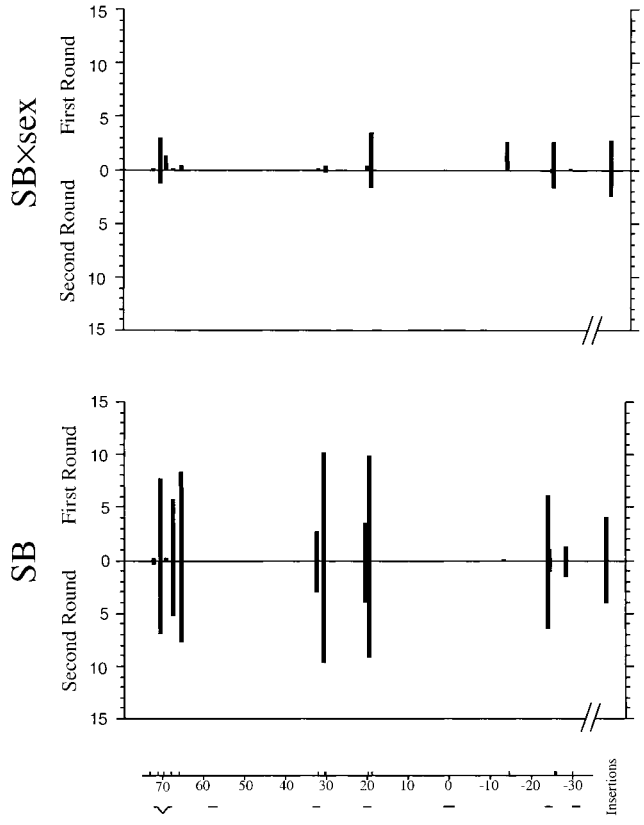

Figure 4.—Plots of the values of the *F* statistics of models testing for molecular markers that are associated with variation in the first principal component score derived from assessing bristle number in two genetic backgrounds for sternopleural bristle number. *F* statistics are plotted as a function of the estimated position of the marker site in the *ASC* gene region, with the *F* statistic associated with a model testing for the effect of large insertions as a class separated from the rest of the sites. The sites are the same as those shown in Figure 2. *F* statistics are presented from the first round of the permutation testing above the line at zero, and those from the second round of the permutation testing (*i.e.*, after the effect of In R is statistically removed from the data) below the line at zero. The top shows *F* statistics associated with a model that tests for a marker-by-sex association with the phenotype, and the bottom shows a model testing only for the main effect of the molecular marker.

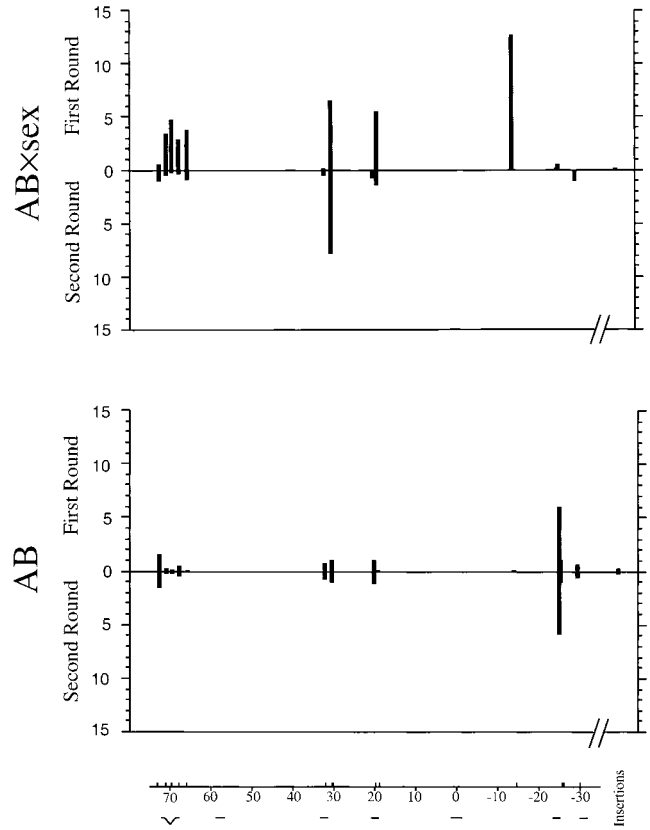

Figure 5.—Plots of the values of the *F* statistics of models testing for molecular markers that are associated with variation in the first principal component score derived from assessing bristle number in two genetic backgrounds for abdominal bristle number. From Figures 4 and 5 it is evident that the effect of In R on abdominal bristle number has little bearing on the effect of MC-22 on sternopleural bristle number.

number-by-sex interaction at $P < 0.058$ and $P < 0.072$, respectively. The permutation tests involving the 14 polymorphic markers (plus the large insert dummy variable) were different in the backcross background. Although In R was significant for an abdominal bristle number-by-sex interaction, MC-22 did not approach significance for sternopleural bristle number in any round of the permutation testing. In addition, the second round of the permutation testing identified MC-21 as being significant for an abdominal bristle number-by-sex interaction, and the third round of the permutation testing resulted in E-25.7 showing suggestive evidence for an association with variation in sternopleural bristle number ($P < 0.073$).

Line 2, an outlier for abdominal bristle number (Figure 2), has a large insertion of ~8 kb between the map coordinates −5.0 and −6.7 (In N). This was the largest insertion variant observed in the survey, and its position places it between 3.0 and 4.7 kb 5′ to the transcriptional start site of *sc* β. It is conceivable that insertion N is the direct causative agent of the reduction in abdominal bristle number associated with line 2. This insertion is likely to be rare in natural populations of *D. melanogaster* as it was not observed in a previous survey (Aguadé *et al.* 1989).

Figures 4 and 5 are plots of the *F* statistics associated with the first principal component for each of the molecular markers in this study as a function of their positions, as well as the *F* statistics associated with large insertions as a class (excluding In R). These *F* statistics are presented separately for sternopleural and abdominal bristle number in Figures 4 and 5, respectively, with each figure showing the *F* statistics for a model that tested the main effect of each marker over the two sexes (labeled SB and AB) or for a model that tested for the effect of a marker-by-sex interaction (labeled SB × sex and AB × sex). The tall bar in Figure 5 associated with an AB × sex interaction in the first round of the

permutation testing is In R, the marker with the largest *F* statistic in the study. After the statistical removal of In R from the data, many of the large *F* statistics associated with AB × sex are reduced in magnitude (*i.e.*, the bars below the zero line), whereas the large *F* statistics associated with the effect of SB in Figure 4 are unchanged. This trend indicates that the large *F* statistics throughout the *ASC* region associated with an AB × sex effect are merely large because of their correlation with In R, whereas the large *F* statistics for SB are likely due to independent associations. In the second round of the permutation test, MC-22 is significantly associated with variation in sternopleural bristle number. When the effect of MC-22 is statistically removed none of the large *F* statistics associated with SB remain (not shown). An examination of Figures 4 and 5 shows that the *F* statistics associated with large insertions as a class are largely unaffected by statistical removal of the In R (below the zero line) and MC-22 (not shown), indicating that this effect is largely independent of the effects associated with polymorphic markers. The *F* statistics associated with the effects of insertions as a class are much smaller than those associated with the polymorphic markers, small enough to only be statistically significant when they are considered separately from the other markers.

The top of Figure 6 gives the effects of MC-22 and In R on abdominal and sternopleural bristle number variation in both sexes and genetic backgrounds. MC-22 primarily has an effect on sternopleural bristle number, which is consistent across sexes and genetic backgrounds. However, In R affects abdominal bristle number almost exclusively in females in both genetic backgrounds. The lower panel of Figure 6 gives the effects of in/del polymorphisms >50 bp in size on bristle number variation, both including and excluding In R. In/del polymorphisms as a class appear to reduce abdominal and sternopleural bristle numbers equally in both sexes and both genetic backgrounds.

Table 4 gives the variance attributable to MC-22, In R, large insertions as a class, and the total genetic variance observed in the two genetic backgrounds of this study. MC-22 primarily affects sternopleural bristle number and accounts for a large proportion of the total genetic variation in sternopleural bristle number due to *X* chromosomes (25%) or backcross segments (15%). Very little of the total genetic variation in abdominal bristle number is associated with MC-22. In R accounts for 22% of the total genetic variation in *X* chromosomes and 41% of the variation in backcross chromosomes in females, but has very little effect on sternopleural bristle number. The presence of large insertions as a class excluding In R is significantly associated with variation in sternopleural bristle number and approaches statistical significance for abdominal bristle number ($P < 0.074$). The proportions of variation accounted for by insertions as a class vary greatly over genetic backgrounds. However, large insertions as a class appear to account for a great deal of variation in sternopleural bristle number in whole *X* chromosomes, but only a small portion in backcross chromosomes. On the other hand, large insertions as a class appear to account for a great deal of the variation in abdominal bristle number variation among backcross chromosomes, but not entire *X* chromosomes. These differences in effects across genetic backgrounds may imply epistatic interactions between large insertions in the *ASC* and other loci on the *X* chromosome, a conclusion consistent with previous quantitative complementation testing results (Long *et al.* 1996). But, as the variances associated with variance

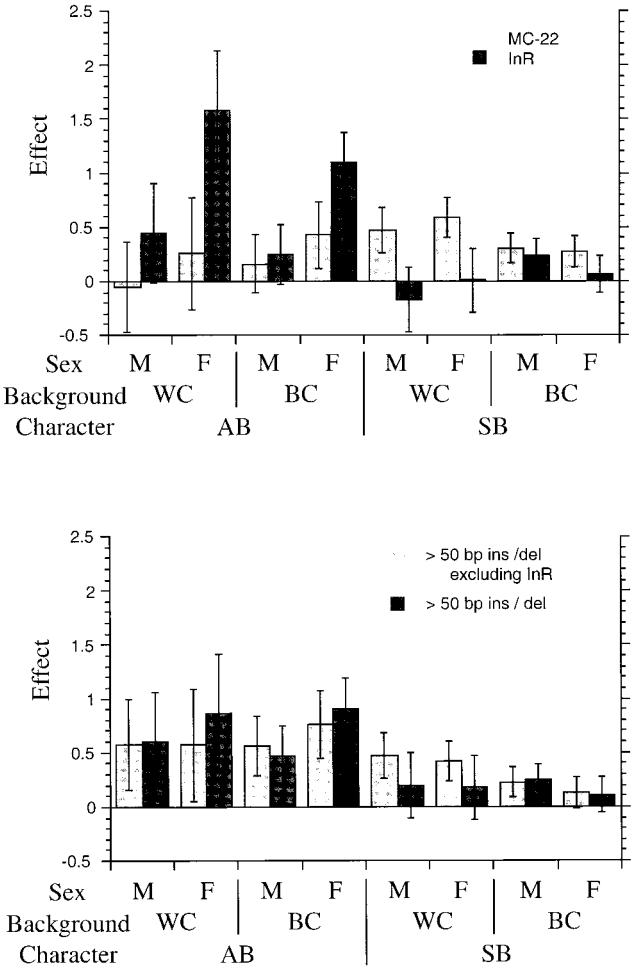

Figure 6.—(Top) Bristle effects associated with MC-22 and In R for both abdominal and sternopleural bristle number in both whole chromosome and backcross chromosome genetic backgrounds and for both male and female flies. (Bottom) Bristle effects associated with large insertions as a class, either including or excluding In R in the catagorization. Error bars represent one standard error of the effect (see text). The presence of large insertions as a class is associated with a decrease in both abdominal and sternopleural bristles. The presence of In R is associated with a reduction in abdominal bristle number in females, and the presense of a 6-bp insertion (MC-22) is associated with a decrease in sternopleural bristle number.

TABLE 4

Variance components associated with significant markers in two genetic backgrounds

	Whole chromosome		Backcross chromosome	
Variance component[a]	SB	AB	SB	AB
MC-22 (both sexes)				
Marker	0.124	0.003	0.047	0.030
Total genetic	0.492	3.495	0.307	0.855
Percentage	25.2	0.1	15.2	3.5
In R (females only)				
Marker	0.000	0.988	0.024	0.536
Total genetic	0.371	4.407	0.250	1.299
Percentage	0.0	22.4	9.7	41.3
Large insertions (averaged over sexes)				
Marker	0.077	0.039	0.015	0.157
Total genetic	0.477	3.544	0.297	0.951
Percentage	16.1	1.1	5.1	16.5

[a] Variance components were estimated using the VARCOMP procedure of SAS and then set equal to their expectations under a strictly additive model of quantitative variation. Negative variance components were set equal to zero to calculate variance due to each marker and the total genetic variance.

component estimates (and their ratios) are large, any biological explanation attached to differences in variance component estimates over genetic backgrounds should be cautiously interpreted.

DISCUSSION

The maintenance of quantitative genetic variation: Two polymorphic markers in the *ASC* gene region, as well as large insertions as a class, are significantly associated with variation in bristle number, as assessed by a permutation testing procedure. In R, a 3.4-kb insertion at a frequency of 14% is associated with abdominal bristle number variation in females and MC-22, a PCR-RFLP at a frequency of 45%, is associated with sternopleural bristle number variation. These two sites appear to be independent in their effects on sternopleural and abdominal bristle number. These results are remarkably similar to a study that detected two sites in the *Delta* gene region associated with variation in bristle number: one with effects on sternopleural bristle number and the other affecting abdominal bristle number in females (Long *et al.* 1998). Both of these association studies are consistent with a previous study that has dissected naturally occurring variation in *Adh* expression levels to at least three sites in the *Adh* gene region (Stam and Laurie 1996).

In addition to the two intermediate frequency polymorphisms associated with variation in bristle number, large insertions as a class are associated with variation in bristle number. Insertions as a class, excluding In R, are significantly associated with variation in sternopleural bristle number. Large insertions are not associated with a bristle-by-sex interaction. The association between large inserts as a class at the *ASC* and variation in bristle number replicates the results of an earlier study of Mackay and Langley (1990), but the previous finding that large inserts were also associated with sex-by-bristle character interaction effects was not observed in this study. The most parsimonious explanation for the discrepancy between this study and the earlier study of Mackay and Langley (1990) regarding the association of large inserts with bristle number variation in a sex-mediated manner is that the earlier sample contained a different set of insertions that presumably differed in their phenotypic effects.

The large insertions observed in this work tended to be rare. As a result they are unlikely to be observed in additional samples from the same population and are a different set of insertions than those examined by Mackay and Langley (1990). In Drosophila large insertions are often transposable elements and as such are expected to have mildly deleterious effects on fitness. The observation of bristle number effects associated with large insertions in the *ASC* gene region is consistent with models of mutation-selection balance for the maintenance of quantitative genetic variation (Barton and Turelli 1989). However, MC-22 and In R are at intermediate frequency (45 and 14%, respectively) and have large bristle effects associated with them ($\sim$0.5 sternopleural bristle and 1.25 abdominal bristle in females for MC-22 and In R, respectively). It is difficult to explain the observation of common polymorphisms associated with large allelic effects by postulating linkage disequilibrium with a rarer alleles of much larger effect (Long *et al.* 1998). Thus, the variances attributable to In R and MC-22 are too large to be consistent with either Gaussian allelic effects or house of cards models for the maintenance of quantitative genetic variation (Barton and Turelli 1989). Statistical tests tailored to associa-

tion study data that could be used to formally reject models for the maintenance of quantitative genetic variation via mutation-selection balance would be of heuristic value. One class of models that is capable of explaining the presence of variants of large effect at intermediate frequency assumes that the variants are neutral (Keightley and Hill 1990), but these models are difficult to reconcile with the observed levels of stabilizing selection acting on bristle number (Linney *et al.* 1971; Nuzhdin *et al.* 1995; Garcia-Dorado and Gonzalez 1996). Possibly some form of balancing selection or antagonistic pleiotropy is responsible for the maintenance of these polymorphisms. Selective models are of particular interest as they apply to In R, a polymorphism showing sex-limited expression.

The estimated bristle effects associated with MC-22 (0.39 and 0.44 sternopleural bristles averaged over backgrounds in males and females, respectively) are comparable to effects estimated in an earlier study, which mapped a QTL to a genetic interval that included the *ASC* (0.62 and 0.19 sternopleural bristles in males and females, respectively; Long *et al.* 1995). The effects associated with In R (0.34 and 1.35 abdominal bristles averaged over backgrounds in males and females, respectively) differ from those identified in the QTL mapping study (2.81 and 5.12 abdominal bristles in males and females, respectively; Long *et al.* 1995). Neither the high nor low selection lines used in the earlier QTL mapping experiments contain large insertions in the *ASC* gene region (not shown), so an explanation based on rare insertions with large bristle number effects that were fixed by artificial selection can be rejected. It is possible that the effects measured in the QTL mapping experiment include the effects of linked loci that are in linkage equilibrium with the *ASC* complex. *Notch*, a member of the same signaling pathway that includes *Delta* and *ASC*, and a strong candidate gene for contributing to bristle variation, is only 3 cM proximal to the *ASC*. Another hypothesis that may be able to explain the discrepancy is that epistatic interactions contribute to estimates of effects associated with mapped QTL, whereas in the association study these effects are minimized by introgressing fragments into a common genetic background. Epistatic effects were observed in the earlier QTL mapping study of Long *et al.* (1995).

The molecular genetics of the *ASC* and naturally occurring variation attributable to the *ASC* gene region: Genetic analyses of the *achaete-scute* gene region concluded that the region "seemed to contain several independent *achaete* and *scute* functions" (Garcia-Bellido 1979). This conclusion was based on the observation that different mutant alleles in the *achaete-scute* region resulted in stereotypic losses of bristles from different parts of the adult fly. Subsequent cloning and characterization of the region showed that it encoded multiple transcripts spread over >100 kb and that many of the mutants used in the genetic characterization of the complex were associated with physical lesions throughout this region, but not in the transcripts themselves (Campuzano *et al.* 1985). Given the proneural role played by the *ASC*, naturally occurring variants of small effect in the *cis* regulatory regions of the *ASC* could conceivably result in slightly larger or smaller regions in which sensory bristles could form and as a result contribute to standing variation in bristle number. The observation of large inserts as a class tending to reduce bristle number is consistent with loss-of-function mutants in the *ASC* (Campuzano *et al.* 1985). It should be noted in this regard that In N (the large insertion in line 2 associated with a large reduction in abdominal bristle number) mapped to within a few hundred base pairs of three lesions associated with *sc* mutant alleles (sc^{3B}, sc^{1744}, and sc^{7}; Campuzano *et al.* 1985).

In R is a common large insertion that was also present at high frequency in a previous six-cutter survey of the *ASC* (Aguadé *et al.* 1989). In R is common and old enough that it is also present in a number of laboratory mutant strains of Drosophila [$In(1)sc^{29}$, $In(1)sc^{7}$, $In(1)sc^{9}$, $In(1)ac^{3}$, $T(1;3)sc^{KA8}$, $In(1)sc^{L8}$, $In(1)sc^{8}$, sc^{6}, and sc^{+} gt^{1} w^{a}; Campuzano *et al.* 1985]. Although it is difficult to say with much certainty how old this insertion is, In R is closely linked to w^{a}, suggesting that it may have been present on the original chromosome on which w^{a} was induced in 1923 (Lindsley and Zimm 1992)!

The molecular nature of quantitative genetic variation: The significant association observed between two polymorphic markers in the *ASC* and variation in bristle number suggests that there are bristle number QTNs that are in disequilibrium with markers in the *ASC*. In Drosophila linkage disequilibrium is rarely observed over large distances in sets of inversion-free chromosomes (Aquadro *et al.* 1986; Miyashita and Langley 1988; Long *et al.* 1998). Although long-range disequilibrium can be associated with inversions in *D. melanogaster*, this is not a reasonable explanation for the associations observed in this study, as there are no common inversions found on the *X* chromosome (Lemeunier and Aulard 1992). Thus, the significant associations of this article are likely the result of QTNs that are physically very close to or in the *ASC*.

The power of detecting associations between molecular markers and phenotypic variation is dependent on the number of markers used relative to size of the region examined in recombinational units, not the absolute number of markers. Thus, although the size of the region covered was much larger, and the number of markers employed much fewer, than earlier association studies (of the *Delta* and *scabrous* gene regions), the marker coverage in terms of the recombinational size of the region is approximately the same. Because the number of lines examined is similar in all three studies, the power of detecting associations between markers and QTNs is comparable (A. D. Long and C. H. Langley, unpublished simulation data). Given the observed pat-

terns of linkage disequilibrium in the *ASC*, it seems unlikely that the QTN associated with marker MC-22 has been localized with much accuracy. An examination of Figures 3 and 4 shows strong linkage disequilibrium between this marker and other markers throughout the *ASC* gene region. On the other hand, In R shows little disequilibrium with other markers in the *ASC* (Figures 3 and 5), suggesting that a QTL may be localized to this region. Given that In R is a fairly large insertion in a region known to harbor insertions associated with *sc* mutant phenotypes, it is quite possible that In R itself is a QTN. Further experimentation will be required to directly test this hypothesis.

The significance of observed associations to theoretical population genetics: On the basis of a more thorough six-cutter survey than the one carried out in this study, Aguadé *et al.* (1989) estimated θ from a Raleigh sample of chromosomes for the *ASC* to be 0.00128/bp. From population genetics theory, 2θ is approximately the probability of a site being a "common" polymorphism (Crow and Kimura 1970). Thus the total number of common polymorphisms in the *ASC* can be estimated to be ~300. Mutants in the *ASC* are known to independently affect a number of bristle characters in *D. melanogaster* (Garcia-Bellido 1979), and *ASC* expression is observed in many different parts of the imaginal disc (Cubas *et al.* 1991). In addition, genes of the *ASC* are expressed at different times in development and in different tissues (Martin-Bermudo *et al.* 1991; Skeath and Carroll 1991, 1992; Ruiz-Gomez and Ghysen 1993). This study observed two common polymorphisms in the *ASC* to be associated with detectable variation in abdominal and sternopleural bristle number, but only examined a fraction of the total "characters" that genes of the *ASC* are likely to affect. Current population genetic theories attempting to explain standing levels of molecular variation generally assume that most polymorphic sites are likely to be neutral or have very small (unmeasurable) selection coefficients associated with them (Kimura 1983; Kaplan *et al.* 1989; Charlesworth *et al.* 1995). The possibility that a nontrivial proportion of all polymorphic sites in the *ASC* may have phenotypic effects associated with them, and are perhaps maintained by some form of balancing selection, has important implications.

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APPENDIX

Phenotypic and polymorphic restriction site data for the *ASC* region

	Abdominal Bristles				Sternopleural Bristles																	
	W		B		W		B															
	M	F	M	F	M	F	M	F	P+73.0	MC-5	MC-7	MC-12	P+65.9	MC-18	MC-21	MC-22	P+19.8	InK	InR	E-25.7	MC-33	MC-40	Inserts	
Line																								
1	17.40	19.25	16.78	19.20	17.05	18.50	15.48	16.23	1	0	0	0	1	0	1	0	1	0	0	1	0	0	0	
2	6.75	8.35	10.50	12.38	16.50	18.35	16.58	17.50	0	1	0	0	1	0	0	0	1	1	0	1	0	0	1	InN
3	18.00	17.45	15.83	17.28	16.75	17.25	17.05	17.48	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	
4	17.05	16.85	16.55	18.03	18.20	18.30	18.18	17.88	0	0	0	0	1	0	0	0	1	0	0	1	1	0	1	DelM
5	16.95	17.35	16.50	17.25	17.00	17.75	17.40	17.80	0	1	1	1	0	0	0	1	1	1	1	1	0	0	1	InF
6	17.65	20.00	16.08	18.00	18.45	19.05	17.40	17.63	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0	
7	17.45	19.20	16.00	17.63	17.50	18.20	16.83	17.40	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
9	17.40	19.00	16.80	17.30	18.80	19.20	17.03	17.80	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	
10	18.55	20.10	17.88	19.80	18.45	19.05	17.85	17.95	0	1	0	1	1	0	1	0	1	0	0	1	1	0	0	
11	17.65	17.00	15.63	16.03	18.15	18.05	17.30	17.40	0	1	0	1	0	0	0	1	1	1	1	1	0	0	1	DelB, DelI
12	18.20	20.15	16.13	18.30	18.55	19.75	17.00	18.55	0	0	0	0	1	0	0	0	1	0	0	1	1	0	1	DelH
13	17.90	20.55	16.83	19.30	18.10	18.65	17.53	18.15	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
14	17.85	19.30	17.00	18.23	17.80	17.10	17.18	18.55	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	DelQ
15	17.45	18.90	16.78	18.58	17.05	17.85	16.78	17.80	0	1	0	1	0	0	0	1	1	1	0	0	0	0	0	
16	17.85	19.40	16.55	18.50	17.95	19.05	17.45	18.23	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	DelQ
17	17.05	17.95	15.68	17.33	18.95	19.50	17.50	18.33	0	1	0	0	1	0	1	0	1	1	1	1	0	0	0	
18	15.30	14.80	15.48	16.68	16.90	17.25	16.58	18.63	0	1	1	1	0	0	0	1	1	1	1	1	0	0	1	InE
19	19.10	19.90	17.70	19.03	17.85	18.05	17.50	18.05	0	1	0	0	1	0	0	0	1	1	0	1	0	0	0	
20	16.45	18.30	16.98	18.15	17.70	18.60	17.03	17.60	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	DelD
21	16.80	17.95	16.35	17.88	17.15	17.60	17.18	18.53	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	
22	16.80	19.85	16.18	18.08	18.15	18.95	17.13	18.23	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	
23	18.30	19.40	16.05	17.90	19.20	19.50	17.73	18.05	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
24	17.15	17.45	17.15	18.50	16.95	18.10	17.13	17.85	0	0	0	0	0	0	0	0	0	1	0	1	0	0	-99	
25	17.55	18.65	17.80	19.53	17.60	17.90	17.73	18.60	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
28	16.10	17.20	14.10	16.25	19.80	20.30	17.90	17.95	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	DelS
29	18.35	19.05	16.95	18.08	18.30	18.55	16.90	17.88	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
30	16.10	18.10	15.40	16.20	18.20	18.55	17.05	17.90	0	0	0	0	1	1	0	0	1	0	0	1	0	1	1	InJ
31	17.50	19.60	16.38	18.90	18.10	18.90	17.45	18.70	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
32	17.65	20.35	16.50	19.80	18.00	18.70	17.35	18.60	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	
33	17.85	17.20	16.83	17.05	18.55	19.15	17.18	17.80	0	1	1	1	0	0	0	0	1	1	1	1	0	0	0	
34	16.55	19.65	16.40	18.33	19.10	18.95	17.60	17.15	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	
35	17.95	18.95	17.23	18.83	16.95	17.85	17.60	18.68	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
36	17.05	18.00	15.73	17.50	17.25	17.95	18.05	17.85	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
37	16.70	19.00	16.78	19.30	16.45	17.90	17.10	18.33	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
38	17.55	18.40	16.10	17.88	18.40	19.00	17.88	18.13	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	
39	17.25	17.45	17.20	18.25	18.25	18.85	18.10	18.45	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
40	17.55	19.30	17.28	18.28	17.80	18.60	17.20	17.98	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
41	15.35	17.65	16.53	17.90	18.85	19.75	18.20	18.50	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	
44	17.15	18.65	17.10	19.40	17.65	18.40	17.60	18.35	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
45	18.70	19.95	17.85	19.05	18.05	18.70	18.95	18.70	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	
46	16.55	18.30	16.25	17.28	17.35	17.60	16.95	16.65	0	1	0	1	0	0	0	1	1	1	0	0	0	0	1	DelG
47	17.00	19.25	17.48	18.80	16.10	17.25	16.75	17.35	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	InC
49	17.80	19.15	16.40	19.00	18.75	18.45	17.75	18.13	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
50	17.35	16.20	17.03	18.00	19.30	20.05	17.95	18.78	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	
51	17.40	20.45	17.48	19.73	17.75	18.85	18.55	18.65	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	DelL
53	18.25	20.10	16.80	18.35	19.20	19.35	18.03	18.45	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	
54	15.55	17.80	17.38	19.50	18.05	18.35	18.00	18.58	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	
55	17.45	18.45	17.15	17.85	19.10	19.35	18.10	18.75	0	0	0	0	1	0	0	0	1	1	0	1	0	1	0	
56	20.10	21.35	16.98	19.73	18.15	18.90	17.63	18.68	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	InA
57	18.60	19.85	17.23	18.55	18.35	18.55	18.13	18.40	0	0	0	0	1	0	0	0	1	1	0	1	1	0	1	InO
58	15.00	15.75	17.15	17.53	17.80	18.75	17.63	18.08	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	
59	17.30	19.40	16.98	18.20	16.70	17.30	17.43	18.58	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
61	18.40	21.25	17.13	18.88	17.85	18.85	18.33	19.10	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	InA
62	17.10	14.35	15.23	16.05	17.90	18.00	17.23	17.83	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
63	17.35	17.95	16.28	18.05	18.50	18.55	17.45	18.53	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	
64	17.85	20.00	15.78	17.75	17.25	18.00	17.43	18.08	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	InP
Frequency									0.04	0.46	0.13	0.46	0.46	0.27	0.14	0.45	0.27	0.45	0.14	0.07	0.21	0.29	0.29	

The left half shows mean abdominal and sternopleural bristle numbers in both males and females for each of the lines surveyed in both the whole *X* chromosome and backcross chromosome backgrounds. The right half shows molecular marker genotypes for the 14 markers scored throughout the *ASC* region. The number 1 indicates the presence of a site or insertion/deletion, and 0 indicates the absence of a site or insertion/deletion. The site name takes one of three forms: a one-letter designation indicates the restriction enzyme used to detect that site followed by a number indicating the position of the site (E = *Eco*RI, P = *Pst*I); "MC" followed by a number refers to PCR-RFLP polymorphisms previously identified in Martin-Campos *et al.* (1992); or "In" followed by a letter indicates a common insertion deletion variant. All positions of all sites can be inferred from Figure 2. The next to right-most column indicates whether or not that line contains a large insertion/deletion (see text), and the right-most column specifies the insertion or deletion event. Line 24 was not scored for the entire *ASC* region, so its status with respect to insertions/deletions is ambiguous. As a result it is treated as missing in analyses relating to insertional status.