Genetic Screens for Factors Involved in the Notum Bristle Loss of Interspecific Hybrids Between *Drosophila melanogaster* and *D. simulans*

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ABSTRACT

Interspecific cross is a powerful means to uncover hidden within- and between-species variation in populations. One example is a bristle loss phenotype of hybrids between *Drosophila melanogaster* and *D. simulans*, although both the pure species have exactly the same pattern of bristle formation on the notum. There exists a large amount of genetic variability in the *simulans* populations with respect to the number of missing bristles in hybrids, and the variation is largely attributable to *simulans X* chromosomes. Using nine molecular markers, I screened the *simulans X* chromosome for genetic factors that were responsible for the differences between a pair of *simulans* lines with high (H) and low (L) missing bristle numbers. Together with duplication-rescue experiments, a single major quantitative locus was mapped to a 13F–14F region. Importantly, this region accounted for most of the differences between H and L lines in three other independent pairs, suggesting segregation of H and L alleles at the single locus in different populations. Moreover, a deficiency screening uncovered several regions with factors that potentially cause the hybrid bristle loss due to epistatic interactions with the other factors.

ARTIFICIAL selection for quantitative characters changes the phenotype far beyond the range of variation in the original base population in most cases (Falconer 1960). Environmental stresses (phenocopy, Waddington 1953; Gibson and Hogness 1996) and mutant backgrounds (Rendel 1959; Rutherford and Lindquist 1998) also uncover a surprising amount of hidden variation, and selection for abnormal phenotypic characters in such conditions increases the frequencies of affected individuals. What is more, after several generations of selection, anomalous characters continue to be expressed without environmental stresses or in wild-type background conditions (Waddington 1953; Rutherford and Lindquist 1998). Thus, cryptic variation and selection may provide a scenario for discontinuous evolutionary changes in morphological characters.

Cryptic variation exists probably because of stabilizing selection and genetic buffering ability. Although these two factors can act independently, stabilizing selection may also contribute to development of genetic buffering ability. The ability to resist genetic and environmental perturbation is known as developmental canalization (Waddington 1942). Canalization, in turn, largely depends on functional redundancy in several forms. Multiple regulatory sites in a gene or multiple genes may exert the same function individually, or cells may substrate for some others (*e.g.*, developmental regulation and equivalence groups). Interactions among molecules, genes, and cells are clearly very common. While we are deepening our understanding of interactions and genetic networks (for Drosophila, Sanchez *et al.* 1999), the mechanisms underlying observable and hidden variation in populations and their roles in adaptive evolution and in morphological evolution are old issues that are still poorly understood.

Interspecific-hybrid analysis is another tool to reveal hidden between-species differences among closely related species. In Drosophila, postmating reproductive isolation, namely, sterility and inviability, has been an intensive focus of various studies (*e.g.*, Dobzhansky 1970 for review of early works; Watanabe 1979; Wu and Beckenbach 1983; Coyne 1984; Hutter *et al.* 1990; Orr 1992; Pantazidis *et al.* 1993; Perez *et al.* 1993; True *et al.* 1996b). Morphological anomalies, the focus of this study, are also manifested in many hybrids (*e.g.*, Sturtevant 1920; Weisbrot 1963; Dobzhansky 1970; Coyne 1985; Khadem and Krimbas 1991; Papaceit *et al.* 1991). One example is a bristle loss phenotype in the *Drosophila melanogaster-D. simulans* hybrids, although both the pure species have exactly the same pattern of bristle formation on the notum (Sturtevant 1920; Biddle 1932; Takano 1998). This means that the genetic architecture of bristle formation can change in local populations without any obvious phenotypic alternation.

Takano (1998) found a large amount of genetic variability in the *simulans* populations with respect to the missing bristle numbers and significant *simulans X* chromosome effects in hybrids with *melanogaster*. I present here results of the screens of the *simulans X* chromo-

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some for genetic factors affecting the bristle phenotype in *melanogaster-simulans* hybrids. Hybrid bristle loss anomaly is potentially caused by several distinct classes of genetic factors: (1) factors responsible for the within-*simulans* variation in the number of missing bristles, (2) fixed *melanogaster-simulans* differences involved in the hybrid bristle loss, and (3) factors that show dose-sensitive effects, especially in a hybrid background, without a between-species functional difference. Because the pattern of notum bristles is fixed within each species and identical between species, these factors do not cause the anomaly in the original pure species—only the combination of the factors in hybrids does. Thus, we have to make interspecific hybrids to screen for genetic factors responsible for the bristle loss phenotype. On the other hand, both male and female hybrids between the two species are completely sterile (but see Davis *et al.* 1996 for an exception), allowing only F_1 hybrid screening. In this article, I used two screening methods: (i) quantitative trait loci (QTL) mapping of *X* chromosome factors affecting the hybrid bristle number by using a pair of *simulans* lines with high (H) and low (L) missing bristle numbers and (ii) deficiency screening. The former is one method for identifying class 1 factors; the second method is based on phenotypic effects in *melanogaster* deficiency-carrying hybrids. It has the potential to identify factors of the three classes.

This QTL mapping, together with duplication rescue experiments, recovered a single major *X* chromosome QTL (or block of QTL). It is important to note that most differences between H and L *simulans* lines were attributed to the region including this QTL in three independent pairs of H and L lines. This suggests segregation of H and L alleles (but not necessarily the same alleles) at the same locus in different populations. On the other hand, a deficiency screening uncovered several regions with factors that potentially cause the hybrid bristle loss due to epistatic interactions with the other factors (class 2 or 3 factors).

MATERIALS AND METHODS

Drosophila strains: Inbred and isofemale *D. simulans* lines used in this study are given in Table 1. I classified these lines into high missing bristle (H) lines and low missing bristle (L) lines on the basis of the numbers of missing bristles in the interspecific F_1 male hybrids with *C(1)RM, y w*^{a} [*Basc/C(1)RM, y w*^{a}, TT-35; Takano 1998] females of *D. melanogaster*. In the deficiency-screening experiments, I also used one *D. mauritiana* inbred strain, Mau-g76 (G20; provided by the Genetic Strain Research Center, National Institute of Genetics, Mishima, Japan), which had no bristle anomaly in the hybrids with TT-35 *melanogaster* females (0.1 ± 0.1).

DNA markers on the *X* chromosome: Single-strand conformation polymorphism (SSCP; Orita *et al.* 1989) and length polymorphism markers on the *simulans X* chromosome were

TABLE 1

***D. simulans* lines used in this study**

Class[a]	*D. simulans* line	Inbred or isofemale	Collection location and date	Experiments[b]
H	Sim-5 (G20)[c]	Inbred	Brazzaville, Congo, 1983[d]	*X*; *Df*; *Dp*; Map; SSCP
	A-1 (G20)[c]	Inbred	Australia, 1986[d]	*X*; *Df*
	T-6 (G20)[c]	Inbred	Tunisia, 1983[d]	*X*
	CH-1 (G15)[c]	Inbred	North Carolina, provided by T. K. Watanabe	*Dp*
	Sim-3 (G20)[c]	Inbred	Raleigh, North Carolina[d]	Dm1911
	Shira-2 (G13)[c]	Inbred	Kofu, Japan, 1995[e]	Dm1911
	Sim-16	Isofemale	Zimbabwe[d]	*Dp*; Dm1911
	Shira-1	Isofemale	Kofu, Japan, 1995[d]	*Dp*
	Shira-2	Isofemale	Kofu, Japan, 1995[d]	*Dp*
	Shira-8	Isofemale	Kofu, Japan, 1995[d]	*Dp*
	Man-15	Isofemale	Kofu, Japan, 1995[d]	*Dp*
L	Tananarive (G20)[c]	Inbred	Madagascar, 1984[d]	*X*; Map; SSCP
	Sim-11 (G20)[c]	Inbred	Ethiopia[d]	*X*; Dm1911
	Sim-12	Isofemale	Tsimbazaza, Madagascar[d]	Dm1911
	Sim-14	Isofemale	Zimbabwe[d]	Dm1911

[a] Based on the number of missing bristles in the interspecific F_1 male hybrids with *C(1)RM, y w*^{a} [*Basc/C(1)RM, y w*^{a}, TT-35; Takano 1998] females of *D. melanogaster*, *D. simulans* lines were classified into two classes: high missing bristle number (H) lines and low missing bristle number (L) lines.

[b] A list of experiments for which each line was used. *X*, *X* chromosome effects (Figure 2); *Df*, deficiency screening (Table 3); *Dp*, duplication effects (Table 4); Map, genetic map construction of *simulans X* chromosome; QTL, QTL mapping; and Dm1911, Dm1911 genotype effects (Table 5).

[c] The number following the letter G in parentheses indicates the number of generations of half-sib mating.

[d] Fly sources are described in Takano (1998).

[e] Shira-2 (G13) was developed by sib-mating of an isofemale Shira-2 line.

TABLE 2

Summary of the molecular makers on the *X* chromosome

Gene[a]	Primers	Typing method
sgg (3B1, 1.3)	5′-TCCAGCAATCACAGCAAACT-3′ and 5′-ACTTCATCTTCGCCTCCTCT-3′	Length variation
norpA (4B6, 6.5)	5′-CCGCTCGGAAGTTGTGGCAG-3′ and 5′-TCTGTCCCCCGTCGCTCGCT-3′	Length variation
sqh (5D6, 13)	5′-ACGAACTGAACTCCCAAAGG-3′ and 5′-ACCGCAGTTGAAGCACCCA-3′	Length variation
G01498 (8A1-2)	5′-GTGGTATGTTGGACACCTTCC-3′ and 5′-AAGTGAGCGTTCTCCTTCTCC-3′	SSCP
*Pp1*β*-9C* (9C1-2, 31)	5′-GCGATAGTAGAGAAAGGGCAA-3′ and 5′-GACTTAACTATGGGCCTCTGA-3′	SSCP
Dm1865 (10F2-11)	5′-TACAACGTCGTCATGTTTGG-3′ and 5′-GCCCACATAGCTATGGGTATT-3′	SSCP
Dm0478 (13A8-9)	5′-AGACTCAGCTCGTTGGTCAC-3′ and 5′-GGATAGTGCCATACTGCTCAA-3′	SSCP
f (15F2-4, 56.7)	5′-CAGGAATCTCAAGCGGGTCTC-3′ and 5′-CTCAATGGTGGAGGTAGTGGT-3′	SSCP
run (19E2, 65)	5′-CAGTGGGATAGAAGGATAAAC-3′ and 5′-ATGAGTCCGAGCATAAAACTT-3′	SSCP

[a] The cytogenetic and genetic map positions in *D. melanogaster* are given in parentheses (FlyBase 1999).

developed at the nine loci including three Berkeley Drosophila Genome Project sequence tagged sites (STSs): *shaggy* (*sgg*; 3B1, its genetic map position in *D. melanogaster* is 1.3 from FlyBase 1999), *no receptor potential A* (*norpA*; 4B6, 6.5), *spaghetti squash* (*sqh*; 5D6, 13), G01498 [designed from a published sequence of accession no. G01498 in the GenBank, European Molecular Biology Laboratory, and DNA Data Bank of Japan sequence databases (STS name = Dm1977), 8A1-2], *Protein phosphatase 1*β *at 9 C* (*Pp1*β*–9C*; 9C1-2, 31), Dm1865 (10F2-11), Dm0478 (13A8-9), *forked* (*f*; 15F2-4, 56.7), and *runt* (*run*; 19E2, 65). Table 2 summarizes the primer sequences and the genotype-determining methods. The length polymorphisms in *sgg* and *norpA* were analyzed on 3% MetaPhor agarose gels (FMC, Rockland, ME) and that in *sqh* on 5–20% gradient polyacrylamide gels. SSCP analyses were done as in Takano-Shimizu (1999).

Experimental design to study *X* chromosome effects (F_1 experiment): Reciprocal G_0 crosses between Sim-5 (G20; abbreviated to S5) and Tananarive (G20; Ta) were done to produce F_1 males carrying the different *X* chromosomes but, on average, the same autosomes (S5 × Ta and Ta × S5; throughout this article, the first strain indicates the female parent). The interspecific crosses were made between about 20 pairs of TT-35 females of *D. melanogaster* and the above F_1 males as well as between TT-35 females and males of Sim-5 (G20) and Tananarive (G20) with six replications each. All parental flies were transferred to new vials after 3 days. This was done twice, and the transfer set was cleared after another 3 days (on the 9th day of cross). In sum, each parental set was allowed to lay eggs in three different vials. Five male progeny were sampled from each vial, making a total sample size of 15 males (5 males × 3 vials) per cross. The number of missing bristles for these hybrid males was studied for 13 pairs of macrochaetae on the notum and humeri as described in Takano (1998; Figure 1).

The same experiment was done simultaneously for a pair of T-6 (G20) and Sim-11 (G20) and a pair of A-1 (G20) and Tananarive (G20) with two replications for each of *simulans* parental males and with four replications for F_1 male progeny of each cross, except for one replication for Tananarive (G20) males in this experimental set and three replications for F_1 males of Sim-11 (G20) (♀♀) × T-6 (G20) (♂♂). Because of a small number of replications, I made an effort to examine 10 males from each vial.

As mentioned above, each parental set was allowed to lay eggs in three different vials. Before pooling the data from different vials, a two-way analysis of variance for each male parent [*e.g.*, individually for Sim-5 (G20) males, Tananarive (G20) males, the F_1 males of S5 × Ta cross, and the F_1 males of Ta × S5 cross] was done for the number of missing bristles of hybrids in a mixed model. When there were missing data, the sample sizes were reduced in such a way as to have an equal sample size for all subclasses. The model for the analysis is $Y_{ijk} = \mu + C_i + V_j + (CV)_{ij} + \varepsilon_{k(ij)}$, where C_i is the random effect of the ith cross (replication), V_j is the fixed effect of the jth vial, $(CV)_{ij}$ is the cross-by-vial interaction, and $\varepsilon_{k(ij)}$ is the residual. Only 3 of 33 tests [the cross-effect in F_1 males of Sim-5 (G20) (♀♀) × Tananarive (G20) (♂♂) ($P < 0.05$; this added variance component is 12% of the overall variation), the cross-effect ($P < 0.05$; 14% of the overall variation) in Sim-11 (G20) males, and the cross-by-vial interaction effect ($P < 0.01$, 43% of the overall variation) in Sim-11 (G20) males] were significant. Because of the low frequency of significant tests and the small effects of separate crosses (replication) and different vials except for one case, the data from different vials and crosses were pooled.

Using the pooling data, I made planned comparisons of two means of the missing bristle numbers for different types of *simulans* males individually in each pair of *simulans* males, because, as seen in Figure 2, an equality of variances was rejected in many comparisons. I did a *t*-test for two samples when there was no significant difference in their variances, but I did Welch's approximate *t*-test when there was a significant difference in variances of two samples (Sokal and Rohlf 1995). The comparisons made are, for example, in the Sim-5 (G20)-Tananarive (G20) pair, the F_1 male progeny of the S5 × Ta cross *vs.* the F_1 males of Ta × S5, the Sim-5 (G20) males *vs.* the F_1 males of S5 × Ta cross, and the Tananarive (G20) males *vs.* the F_1 males of Ta × S5.

Deficiency screening: The deficiency and duplication stocks

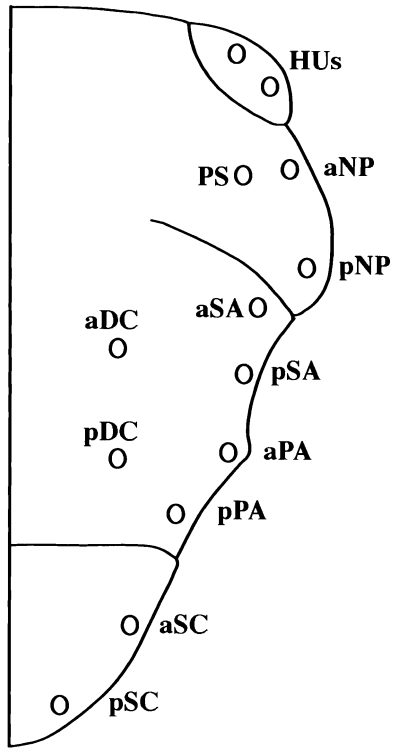

FIGURE 1.—Diagram shows the macrochaete positions on a heminotum and humerus with their nomenclature. PS, presutural; uHU and lHU, humerals; aNP and pNP, notopleurals; aSA and pSA, supraalars; aPA and pPA, post-alars; aDC and pDC, dorsocentrals; and aSC and pSC, scutellars.

used in this study and their origins are summarized in Tables 3 and 4. *Dp(1;Y)shi*$^+$*3* was given as a *Dp(1;Y)shi*$^+$*1* stock [*Df(1)sd72a/Df(1)FM7* + *Df(1)sd72a/Dp(1;Y)shi*$^+$*1*] by the Bloomington Drosophila Stock Center (Bloomington, IN), but it turned out to carry *Dp(1;Y)shi*$^+$*3*. As in the other experiments, about 20 females heterozygous for a deficiency were crossed independently to about 20 males of three strains: two *simulans* [Sim-5 (G20) and A1 (G20)] and one *mauritiana* [Mau g76 (G20)] inbred strain. The experimental design was the same as that for the *X* chromosome-effect study and one cross for each combination of line and deficiency chromosome was made for most cases. I made an effort that the bristle numbers were counted for five hybrid females carrying a deficiency and five females carrying a balancer chromosome from each of three vials, making a total sample size of 30 females. Because the stocks were maintained over different balancer chromosomes and the background of different deficiency stocks was not isogenized, the absolute numbers of missing bristles could not be simply compared among the different deficiency stocks. As a simple indication of deficiency effects, the statistical tests for the number of missing bristles per fly were done between deficiency-carrying and balancer chromosome-carrying female hybrids. When the sample variances of these two classes were significantly different, Welch's approximate *t*-test was done (SOKAL and ROHLF 1995). As usual in many deficiency screenings, different origins of deficiency chromosomes require consistent significance of multiple independent deficiencies covering a region on bristle phenotype to prove its effect in hemizygous condition.

Besides the deficiencies, several duplications were studied for rescue action of the bristle loss. The compound-*X* females carrying duplications were crossed to the *simulans* males, and male hybrids were examined for the bristle number as in the other experiments. I used a molecular marker (Dm1944 from Berkeley Drosophila Genome Project STS, 14A3-5) to detect the presence of a duplication on the fourth chromosome, *Dp(1;4)r*$^+$. The amplified fragments with these primers in *D. melanogaster* and *D. simulans* had different lengths.

Genetic map construction of the *D. simulans X* chromosome: Using Sim-5 (G20) and Tananarive (G20), I studied crossover frequencies among the nine markers on the *X* chromosome described above. F_1 females from the G_0 crosses in the above experiment (F_1 experiment), S5 × Ta and Ta × S5, were backcrossed to the two parental stocks, making four different G_1 crosses (S5 × Ta) × S5, (S5 × Ta) × Ta, (Ta × S5) × S5, and (Ta × S5) × Ta. Each type of cross was done in 10 vials and each vial contained about 10 pairs of females and males. Genotypes at the nine loci in Table 2 were determined for 2 to 5 males from each vial (35 males each for four different crosses) except for *sgg* genotype of one male. Crossover frequencies were converted to map distances by the map function of FOSS *et al.* (1993; with $m = 4$) as in TRUE *et al.* (1996a).

QTL mapping: Twenty F_2 males from each of 40 G_1 crosses in the genetic map construction experiment were collected and crossed to 20 TT-35 females of *D. melanogaster* in a vial. The number of missing bristles on the notum and humeri for these hybrid males was studied as in the F_1 experiment. Six hundred hybrid males were examined for missing bristles [4 (G_1 different crosses) × 10 (replications) × 3 (vials) × 5 (males)]. Genotypes at the nine loci in Table 2 were determined for two or three hybrid males randomly selected from the first and second vials for each cross [in sum, 4 (G_1 crosses) × 2 (vials) × 24 males from 10 vials = 192 males].

The F_2 males from four different crosses between Sim-5 (G20) and Tananarive (G20) [(S5 × Ta) × S5, (S5 × Ta) × Ta, (Ta × S5) × S5, and (Ta × S5) × Ta] have the different autosomal composition: the F_2 males from (S5 × Ta) × S5 and (Ta × S5) × S5 carry, on average, larger amounts of the Sim-5 (G20) genome than the F_2 males from (S5 × Ta) × Ta and (Ta × S5) × Ta. To examine this autosomal effect, I did a *t*-test for the difference in the mean numbers of missing bristles between the F_2 males from (S5 × Ta) × S5 and those from (S5 × Ta) × Ta, and between the F_2 males from (Ta × S5) × S5 and those from (Ta × S5) × Ta.

I did the composite interval mapping (ZENG 1994) on two populations of different genetic backgrounds [96 F_2 males each from (S5 × Ta) × S5 and (Ta × S5) × S5 crosses (Sim-5 backcross) and from (S5 × Ta) × Ta and (Ta × S5) × Ta ones (Tananarive backcross)] separately. I used QTL Cartographer programs (BASTEN *et al.* 1996) to calculate the likelihood ratio (LR) test statistic for H_1/H_0 in Model 6 (with a window size of 5 cM) of Zmapqtl and to obtain estimates of additive effects, assuming a random-mating F_2 population. The likelihood test statistic is $-2\ln(L_0/L_1)$. L_0 is the maximum likelihood under the null hypothesis (H_0) of a (additive effect of a putative QTL) = 0 and d (its dominance effect) = 0, and L_1 the maximum likelihood under the alternative hypothesis (H_1) of $a \neq 0$ and $d = 0$. Because I studied only the male hybrids, the dominance effects could not be estimated. Using the FB method (BASTEN *et al.* 1996), I made the stepwise regression analyses, which selected four markers for the Sim-5 backcross (*sqh*, G01498, *Pp1*β*–9C*, and *f*) and two markers for the Tananarive backcross (G01498 and *f*) to control genetic background. The 5% critical value of the likelihood ratio test statistic was calculated by $\chi^2_{0.05/8=0.00625}$(d.f. = 2) (ZENG 1994).

I also examined the effect of the Dm0478 (13A8-9)–*f* (15F2-4) region on bristle loss, using three different pairs of H and L strains: Sim-16 and Sim-14; Sim-3 (G20) and Sim-12; and Shira-2 (G13) and Sim-11 (G20). The crosses were done in the same way as the crosses between Sim-5 (G20) and Tananarive

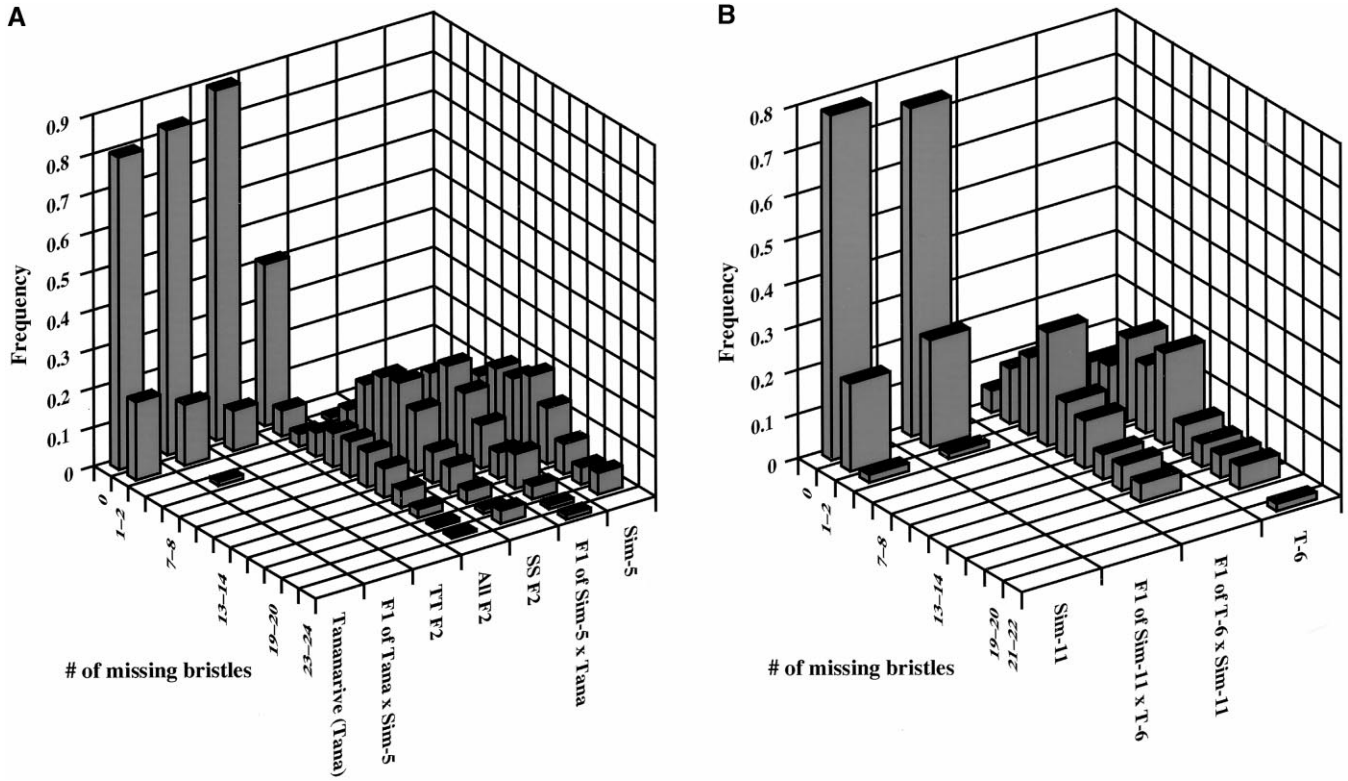

FIGURE 2.—Distributions of the number of missing bristles in *melanogaster-simulans* hybrids. (A) Bar diagram showing distributions of the number of missing bristles in hybrids of Sim-5 (G20), Tananarive (G20), F_1 males of Sim-5 (G20) (♀♀) × Tananarive (G20) (♂♂), and those of the reciprocal crosses with *C(1)RM/Y* females of *D. melanogaster* as well as those of hybrids between the F_2 males [all F_2 males, F_2 males that carried the Sim-5 (G20) alleles both at Dm0478 and at *f* (SS F_2), and F_2 males with the Tananarive (G20) alleles both at Dm0478 and *f* (TT F_2)] and *C(1)RM/Y* females in the QTL mapping experiment. The mean number of missing bristles (sample size) was 12.76 ± 0.41 (SEM; 90) for Sim-5 (G20); 0.22 ± 0.05 (90) for Tananarive (G20); 11.16 ± 0.47 (90) for F_1 males of Sim-5 (G20) (♀♀) × Tananarive (G20) (♂♂); 0.22 ± 0.08 (90) for F_1 males of Tananarive (G20) (♀♀) × Sim-5 (G20) (♂♂); 5.45 ± 0.24 (600) for all F_2 males; 9.92 ± 0.45 (88) for SS F_2; and 0.10 ± 0.03 (87) for TT F_2. (B) Distributions of the number of missing bristles in hybrids of T-6 (G20) males, Sim-11 (G20) males, and the F_1 males with *C(1)RM/Y* females. The mean number of missing bristles per fly (sample size) was 8.21 ± 0.61 (58) for T-6 (G20) males; 0.27 ± 0.08 (55) for Sim-11 (G20) males; 6.32 ± 0.36 (120) for F_1 males of T-6 (G20) (♀♀) × Sim-11 (G20) (♂♂); and 0.37 ± 0.08 (78) for F_1 males of Sim-11 (G20) (♀♀) × T-6 (G20) (♂♂).

(G20): 10 crosses of 20 TT-35 females with 20 F_2 males for each of 4 different G_1 crosses and 5 crosses between 20 TT-35 females and 20 males for each of two parental strains. In addition, 5 crosses were made between TT-35 females and males for each of two different F_1 crosses, Shira-2 (G13) × Sim-11 (G20) and Sim-11 (G20) × Shira-2 (G13). There were three vials of no progeny: one of the crosses of Sim-14, one of Sim-11 (G20), and one of F_2 males from [Shira-2 (G13) × Sim-11 (G20)] × Shira-2 (G13) with TT-35. All the crosses for each pair of H and L lines were done simultaneously. As in other experiments, each parental set (20 females and 20 males) was allowed to lay eggs in three different vials. I counted the number of missing bristles of five hybrid males from each vial and determined the genotype at Dm1911 (14B1-4) for two hybrids randomly chosen from the five hybrid males from the first vial of each cross. Thus, the data from only the first vial of crosses are given in Table 5.

RESULTS

***X* chromosome effects:** TAKANO (1998) revealed that *melanogaster-simulans* hybrid males carrying the *simulans X* chromosomes exhibited much larger numbers of missing bristles than hybrid males carrying the *melanogaster X* chromosomes. That study also showed that there exists much variation in the *simulans* strains at the level of hybrid bristle loss. Using Sim-5 (G20; S5) as a high missing bristle number line and Tananarive (G20; Ta) as a low missing bristle number line, I examined the effects of the *simulans X* chromosomes on the hybrid bristle loss. Figure 2A illustrates the distributions of the number of missing bristles in *simulans X* chromosome-carrying male hybrids. Although the mean number of missing bristles for F_1 males of S5 × Ta cross was significantly smaller than that for Sim-5 (G20) ($P < 0.05$, $t = 2.6$ with d.f. = 178), the difference [11.16 ± 0.47 for S5 × Ta and 12.76 ± 0.41 for Sim-5 (G20)] was small. The sample variances among hybrid flies were not significantly different from each other [19.9 for S5 × Ta and 15.2 for Sim-5 (G20)]. Indeed, the distribution for the F_1 males from the S5 × Ta crosses was similar to that for Sim-5 (G20). The distribution for the F_1 males from the Ta × S5 crosses was also very similar to that

TABLE 3

Deficiency screening

	Origin	Breakpoints	Sim-5 (G20)	A1 (G20)	Mau-g76 (G20)
Df(1)260-1	C	1A1; 1B4-6	8.0 ± 0.5/2.0 ± 0.3***	4.2 ± 1.0/2.0 ± 0.4*	1.7 ± 0.3/0.0 ± 0.0***
In(1)y^{3PL}sc^{8R}	D		2.8 ± 0.4/2.7 ± 0.3	2.7 ± 0.6/5.3 ± 1.2	0.0 ± 0.0/0.1 ± 0.1
In(1)sc^{8L}sc^{4R}	D	Deleted for 21 kb in *y–sc*	6.8 ± 0.8/4.4 ± 0.7*	7.1 ± 0.8/4.2 ± 0.8*	2.5 ± 0.3/0.1 ± 0.1***
Df(1)sc10-1 = In(1)sc$^{10.1}$	D	1B1-2; 1B2-3	10.5 ± 0.8/4.7 ± 0.4***	5.0 ± 0.7/0.7 ± 0.7**	1.3 ± 0.3/0.1 ± 0.1**
Df(1)S39	B	1E1; 2B5-6	1.5 ± 0.3/2.1 ± 0.4	0.8 ± 0.3/0.6 ± 0.3	0.2 ± 0.1/0.2 ± 0.1
Df(1)A94	B	1E3-4; 2B9-10	2.9 ± 0.6/2.2 ± 0.4	2.1 ± 0.7/1.9 ± 0.4	0.0 ± 0.0/0.0 ± 0.0
Df(1)Pgd35	A	2C2-4; 2E2-F1	2.0 ± 0.5/2.9 ± 0.6	1.3 ± 0.5/0.0 ± 0.0	0.1 ± 0.1/0.0 ± 0.0
Df(1)64c18, g^{1} sd^{1}	A	2E1-2; 3C2	8.1 ± 0.5/5.2 ± 0.5***	10.5 ± 0.7/5.0 ± 0.6***	0.1 ± 0.1/0.0 ± 0.0
Df(1)JC19	A, B	2F6; 3C5	12.5 ± 0.7/4.8 ± 0.5***	16.7 ± 0.9/9.4 ± 1.1***	0.1 ± 0.1/0.0 ± 0.0
Df(1)62g18, y^{1}	A	3A1-2; 3A4-5	3.9 ± 0.5/3.5 ± 0.5	4.6 ± 0.4/4.6 ± 0.7	0.1 ± 0.1/0.2 ± 0.1
Df(1)w258-11, y^{1}	A	3A2-3; 3C3-5	11.7 ± 1.0/6.5 ± 0.6***	11.5 ± 0.9/6.1 ± 0.8***	0.1 ± 0.1/0.1 ± 0.1
Df(1)65j26, y^{1}	A	3A3; 3A4-6	3.0 ± 0.5/4.5 ± 0.5	3.9 ± 0.7/4.8 ± 0.9	0.0 ± 0.0/0.1 ± 0.1
Df(1)w258-45, y^{1}	A	3B2-3; 3C2-3	2.5 ± 0.2/1.1 ± 0.3**	1.3 ± 0.3/1.8 ± 1.2	0.0 ± 0.0/0.1 ± 0.1
Df(1)N-8	A	3C2-3; 3E3-4	7.9 ± 1.2/5.1 ± 0.7*		0.7 ± 0.3/0.1 ± 0.1*
Df(1)HF366	B	3E8; 5A7	3.3 ± 0.6/3.4 ± 0.5	3.3 ± 0.7/5.0 ± 0.7	0.1 ± 0.1/0.0 ± 0.0
Df(1)RC40	B	4B1; 4F1	4.5 ± 0.8/3.5 ± 0.7	3.3 ± 0.4/2.6 ± 0.5	0.0 ± 0.0/0.0 ± 0.0
Df(1)C149	B	5A8-9; 5C5-6	5.7 ± 0.8/2.4 ± 0.7**	1.0 ± 0.4/0.6 ± 0.2	0.3 ± 0.2/0.0 ± 0.0
Df(1)N73	B	5C2; 5D5-6	4.0 ± 0.6/1.8 ± 0.4**	1.8 ± 0.6/0.6 ± 0.4	0.2 ± 0.1/0.3 ± 0.1
Df(1)sqh, y^{1}	A	5D1-2; 5E	2.2 ± 0.9/2.1 ± 0.3	2.8 ± 1.0/3.1 ± 0.3	0.1 ± 0.1/0.2 ± 0.1
Df(1)G4e^{1}H24i^{R}, M f^{1}	A	5E3-8; 6B	3.1 ± 0.5/4.3 ± 1.0	0.7 ± 0.2/6.6 ± 0.9***	0.3 ± 0.1/0.1 ± 0.1
Df(1)JF5, f^{1} car^{1}	B	5E3-5; 5E8	2.2 ± 0.3/3.7 ± 0.5*	1.3 ± 0.2/0.7 ± 0.6	0.2 ± 0.1/0.1 ± 0.1
Df(1)HA32	B	6E4-5; 7A6	8.3 ± 0.7/4.6 ± 0.6***		0.3 ± 0.2/0.1 ± 0.1
Df(1)ct4b1, y oc ptg	B	7B2-4; 7C3-4	5.3 ± 0.8/3.4 ± 0.6	2.1 ± 0.4/3.1 ± 0.7	0.5 ± 0.2/0.1 ± 0.1
Df(1)C128	A	7D1; 7D5-6	4.5 ± 0.7/1.8 ± 0.3**	1.1 ± 0.3/1.1 ± 0.3	0.3 ± 0.2/0.1 ± 0.1
Df(1)RA2	A	7D10; 8A4-5	8.2 ± 0.8/6.0 ± 0.7*	2.0 ± 0.4/0.5 ± 0.5	0.2 ± 0.1/0.0 ± 0.0
Df(1)KA14	A	7F1-2; 8C6	5.8 ± 0.6/4.1 ± 0.7	4.7 ± 1.1/4.0 ± 0.8	0.1 ± 0.1/0.0 ± 0.0
Df(1)lz-90b24, y^{1} w^{a}	A	8B5-8; 8D8-9	4.4 ± 0.7/5.6 ± 1.4	1.6 ± 0.3/4.9 ± 0.7***	0.0 ± 0.0/0.1 ± 0.1
Df(1)C52	B	8E; 9C-9D	8.1 ± 0.7/3.8 ± 0.3***	4.5 ± 1.2/4.3 ± 1.0	0.1 ± 0.1/0.0 ± 0.0
Df(1)v-L15	A	9B1-2; 10A1-2	1.2 ± 0.3/3.4 ± 0.5**	0.3 ± 0.2/1.3 ± 0.3*	0.0 ± 0.0/0.0 ± 0.0
Df(1)HA85	A, B	10C1-2; 11A1-2	14.1 ± 0.5/5.5 ± 0.8***	6.7 ± 0.9/5.2 ± 0.5	0.0 ± 0.0/0.2 ± 0.1*
Df(1)KA6	B	10E1; 11A7-8	14.7 ± 0.8/2.7 ± 0.4***	11.1 ± 1.3/4.7 ± 0.6***	0.1 ± 0.1/0.1 ± 0.1
Df(1)RA47	A	10F1; 10F9-10	13.1 ± 0.5/2.6 ± 0.3***	6.7 ± 0.9/2.1 ± 0.6***	0.1 ± 0.1/0.1 ± 0.1
Df(1)N105	B	10F7; 11D1	4.9 ± 0.7/1.3 ± 0.2***	0.8 ± 0.3/1.1 ± 0.3	0.1 ± 0.1/0.0 ± 0.0
Df(1)JA26	B	11A1; 11D-11E	7.5 ± 0.6/4.3 ± 0.4***	2.5 ± 0.4/3.5 ± 1.0	0.0 ± 0.0/0.0 ± 0.0
Df(1)HF368	B	11A2; 11B9	6.8 ± 0.4/3.1 ± 0.4***		0.0 ± 0.0/0.1 ± 0.1
Df(1)C246	B	11D-11E; 12A1-2	7.0 ± 0.8/3.1 ± 0.6**	3.3 ± 0.5/1.1 ± 0.3**	0.0 ± 0.0/0.1 ± 0.1
Df(1)N12, ras^{1} v^{1}	A	11D1-2; 11F1-2	5.3 ± 0.4/2.4 ± 0.3***		0.1 ± 0.1/0.0 ± 0.0
Df(1)HA92	B	12A6-7; 12D3	6.5 ± 0.7/5.1 ± 0.5		0.1 ± 0.1/0.1 ± 0.1
Df(1)g, f^{1} B^{1}	B	12A3-10; 12E9	4.4 ± 0.5/4.3 ± 0.7		0.0 ± 0.0/0.0 ± 0.0
Df(1)RK2	B	12D2-E1; 13A2-5	7.6 ± 1.1/8.3 ± 1.0	5.0 ± 1.3/6.0 ± 1.2	0.3 ± 0.3/0.1 ± 0.1
Df(1)KA9	B	12E1; 13A5	6.0 ± 0.9/4.2 ± 0.7		0.3 ± 0.2/0.6 ± 0.5

(*continued*)

TABLE 3

(Continued)

	Origin	Breakpoints	Sim-5 (G20)	A1 (G20)	Mau-g76 (G20)
Df(1)RK4	A	12F5-6; 13A9-B1	6.2 ± 0.4/4.5 ± 1.0	2.3 ± 0.3/1.9 ± 0.3	0.0 ± 0.0/0.0 ± 0.0
Df(1)4b18, y^1 cv^1 v^1 $nonA^{4b18}$ f^1 car^1	A	14B8; 14C1	6.5 ± 0.7/3.9 ± 0.6**		0.6 ± 0.3/0.0 ± 0.0*
Df(1)B	E	15F9-16A1; 16A6-7	7.6 ± 0.7/4.8 ± 0.4**		0.1 ± 0.1/0.5 ± 0.4
Df(1)N19	B	17A1; 18A2	3.9 ± 0.5/1.7 ± 0.4**	3.4 ± 0.7/2.7 ± 0.7	0.0 ± 0.0/0.1 ± 0.1
Df(1)JA27	B	18A5; 18D	8.2 ± 0.9/3.9 ± 0.5**	4.7 ± 0.5/2.7 ± 0.8*	0.1 ± 0.1/0.1 ± 0.1
Df(1)16-2-19, y mei-9 mei-41	B	19A5; 19D3	6.0 ± 0.7/5.3 ± 1.2	0.5 ± 0.3/0.2 ± 0.1	0.2 ± 0.1/0.2 ± 0.1
Df(1)DCB1-35b	A	19F1-2; 20E-20F	1.6 ± 0.7/1.7 ± 0.2	2.0 ± 0.3/1.5 ± 0.2	0.3 ± 0.1/0.1 ± 0.1
Df(1)bb, y sl^2	B	deficient for bobbed	1.6 ± 0.5/1.6 ± 0.3		0.0 ± 0.0/0.2 ± 0.1

Hybrids were made by crosses between *melanogaster* deficiency lines and three inbred lines: two *simulans* [Sim-5 (G20) and A-1 (G20)] and one *mauritiana* [Mau-g76 (G20)] line. The average number of missing bristles ± standard error of mean for deficiency-carrying hybrids/those for balancer chromosome-carrying hybrids and results of statistical tests of equality of these two means (***$P < 0.001$; **$P < 0.01$; and *$P < 0.05$) are given. Origin A stands for the Bloomington Drosophila Stock Center (Bloomington, IN), B for the Genetic Strain Research Center, National Institute of Genetics (Mishima, Japan), C for the European Drosophila Stock Center at Umeå (Umeå, Sweden), D for J. Modolell, and E for R. S. Hawley.

for Tananarive (G20). No difference existed in the mean number of missing bristles between F_1 males of Ta × S5 (0.22 ± 0.08) and Tananarive (G20; 0.22 ± 0.05). By contrast, the difference between the mean number of missing bristles for the F_1 males of the S5 × Ta crosses and that for the F_1 males of the Ta × S5 crosses was highly significant ($P < 0.001$, Welch's $t' = 22.9$ with d.f. = 89). The same findings were obtained from the crosses between T-6 (G20) as an H line and Sim-11 (G20) as an L line (Figure 2B) and the crosses between A-1 (G20) as an H line and Tananarive (G20) as an L line. In the latter case, the mean number of missing bristles per fly (sample size) was 5.57 ± 0.49 (60) for A-1 (G20) males, 0.05 ± 0.05 (20) for Tananarive (G20) males, 4.87 ± 0.28 (120) for F_1 males of A-1 (G20) (♀♀) × Tananarive (G20) (♂♂), and 0.40 ± 0.08 (114) for F_1 males of Tananarive (G20) (♀♀) × A-1 (G20) (♂♂).

These findings strongly suggest that most of the difference in the number of missing bristles in hybrids between the H and L lines was attributable to their *X* chromosomes. Therefore, the subsequent experiments focused on the *X* chromosome.

Deficiency screening: I screened the *melanogaster X* chromosome for genes whose absence affects the bristle number of interspecific hybrid females. Females of *melanogaster* deficiency stocks were crossed to *D. simulans* and *D. mauritiana* males. Table 3 shows the results with the number of missing bristles in balancer chromosome-carrying hybrid females from the same vials as a control. Between-species differences were very clear. No deficiency *mauritiana* hybrid showed, on average, more than one missing bristle per fly except for *Df(1)260-1*, *In(1)$sc^{8L}sc^{4R}$*, and *Df(1)sc10-1*. The great degree of *simulans-mauritiana* differences in the bristle numbers of hybrids with *D. melanogaster* is consistent with the previous study, in which the loss of bristles was examined for hybrid males carrying the *X* chromosomes of *simulans* or *mauritiana* (Takano 1998).

Many deficiency chromosomes exhibited significantly greater numbers of missing bristles than the balancer chromosomes. The numbers of missing bristles in deficiency-carrying hybrids were significantly greater than those in balancer-carrying hybrids for both the two *simulans* strains in the following five regions, assuming the minimum number of loci involved: (1A1; 1B4-6), (3A4-5; 3C2), (10F1; 10F7), (11D-E; 11F1-2), and (18A5; 18D). Particularly, two regions, (3A4-5; 3C2) and (10F1; 10F7), were covered by three different deficiency chromosomes each and showed the greatest numbers of missing bristles.

The deficiency chromosomes, *Df(1)260-1*, *In(1)$sc^{8L}sc^{4R}$*, and *Df(1)sc10-1*, had significant effects in all three hybrids. The missing region contains the *achaete-scute* complex (*AS-C*). Many *AS-C* mutants exhibit loss of macrochaetae and microchaetae because of the failure of emergence of sensory mother cells (García-Bellido 1979). The important difference between the *AS-C* defi-

TABLE 4

Mean number of missing bristles (± standard error of mean) in hybrids carrying duplication chromosomes

Female parent	Origin	Breakpoints	Male parent	*Dp*-carrying hybrids	Non-*Dp* (balancer-carrying) hybrids
C(1)DX/Dp(1;Y)w⁺Y	F	2D2; 3D3	Sim-5 (G20)	7.6 ± 0.5***	14.0 ± 0.8[a]
C(1)DX/Dp(1;Y)w⁺Y	F		Shira-1	4.6 ± 0.6***	8.5 ± 1.0[a]
C(1)DX/Dp(1;Y)w⁺Y	F		Shira-8	6.1 ± 0.9*	9.5 ± 0.8[a]
C(1)DX/Dp(1;Y)w⁺Y	F		Man-15	5.2 ± 1.0*	8.7 ± 1.0[a]
C(1)RM/Y; TM3/Dp(1;3)W^VCO	A	2C1; 3C5	Sim-5 (G20)	5.9 ± 1.1***	11.8 ± 0.9
C(1)DX/Y; TM3/Dp(1;3)f⁺^71b	E	15A4; 16C2-3	Sim-5 (G20)	13.3 ± 1.5	11.0 ± 1.1
C(1)DX; Dp(1;4)r⁺/+	A	14A1-2; 16A7-B1	Sim-5 (G20)	11.9 ± 1.1**	16.3 ± 0.8
C(1)RM/Dp(1;Y)W39, y⁺	A	16F1-3; 18A5-7; 19E5-7; 20F; h1-h25	Sim-5 (G20)	9.9 ± 1.0	—
C(1)RM/Dp(1;Y)W73, y^31d f⁺B^l, B^S	A	15B1-D; 16F; h1-h25	Sim-5 (G20)	18.2 ± 0.9	—
C(1)RM/Dp(1;Y)shi⁺1, y⁺ B^S	G	13F1-4; 14A	Sim-5 (G20)	9.2 ± 0.5	—
C(1)RM/Dp(1;Y)shi⁺3	A	13F1-4; 14F4-6	Sim-5 (G20)	5.7 ± 0.4	—
C(1)RM/Dp(1;Y)shi⁺3	A		Sim-16	1.9 ± 0.5***	8.4 ± 1.0[a]
C(1)RM/Dp(1;Y)shi⁺3	A		CH-1 (G15)	2.7 ± 0.5**	6.1 ± 0.9[a]
C(1)RM/Dp(1;Y)shi⁺3	A		Shira-2	2.5 ± 0.4**	6.0 ± 0.9[a]

The results of statistical tests of equality of the mean bristle numbers of duplication-carrying hybrids and nonduplication (balancer chromosome-carrying) ones are given (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). Origin F represents B. Williams, and origin G means C. W. Bazinet; origins A and E are given in Table 3.

[a] The number of missing bristles in hybrids with the *C(1)RM/Y melanogaster* females, in which the crosses were done, as a control, in the same sets as the crosses making the duplication-bearing hybrids in the same row.

ciencies and the other strong-effect deficiencies is that both the *melanogaster-mauritiana* and the *melanogaster-simulans* hybrids showed a significant reduction in the bristle number in *AS-C*-deletion heterozygotes. On the other hand, interspecific hybrids between wild-type strains of *D. melanogaster* and *D. mauritiana* did not show the bristle anomaly, but *melanogaster-simulans* hybrids did. It seems unlikely that the *AS-C* region contains the locus responsible for the *mauritiana-simulans* difference, and thus this region alone cannot explain the high degrees of bristle anomaly in *melanogaster-simulans* hybrids.

The three deficiencies, *Df(1)64c18*, *Df(1)JC19*, and *Df(1)w^258-11*, had great effects on the bristle numbers of the *melanogaster-simulans* hybrids, but not on those of *melanogaster-mauritiana* hybrids. To study the region affected by these three deficiencies in more detail, the bristle numbers were examined for the hybrid males carrying the following duplication chromosomes: *Dp(1;Y)w⁺Y* chromosome carrying a duplication of the 2D2; 3D3 region and *Dp(1;3)w^vco* carrying a duplication of the 2C1; 3C5 region. Table 4 shows the significant rescue action of these duplications.

Taken together, the deficiency screening identified several regions of factors that potentially cause the hybrid bristle loss due to interactions with the other factors.

Genetic map of *simulans X* chromosome: Before the QTL mapping of genetic factors responsible for the bristle loss phenotype of *melanogaster-simulans* hybrids, I constructed a genetic map of the *simulans X* chromosome by using two *simulans* strains [Sim-5 (G20) and Tananarive (G20)] and nine markers (Table 2). The polytene *X* chromosome banding patterns of the two species are identical except for the small inversions (1E1-2; 1E3 and 3A1-2; 3A5) and the chromosome tip (Horton 1939; Lemeunier and Ashburner 1976). The nine markers covered most of the *X* chromosome (cytogenetic location 1.32 at the *sgg* through the location of 65.8 at the *run* in *D. melanogaster*). The map distances between consecutive markers (those in *D. melanogaster* are given in parentheses) were as follows: 10.80 cM (5.2) between *sgg* and *norpA*, 7.14 (6.5) between *norpA* and *sqh*, 15.79 between *sqh* and G01498, 9.29 between G01498 and *Pp1*β*–9C*, 6.43 between *Pp1*β*–9C* and Dm1865, 10.01 between Dm1865 and Dm0478, 7.86 between Dm0478 and *f*, and 8.57 (8.3) between *f* and *run*. The total map length between the *sgg* and *run* loci was 75.89 cM, which was 1.2 times longer than the standard *melanogaster* map (65). The longer map of the *simulans* chromosome is consistent with previous reports that showed that the *simulans* map is about 1.3 times longer than the *melanogaster* map as a whole (Sturtevant 1929; Ohnishi and Voelker 1979; True *et al.* 1996a).

QTL mapping: F_2 males from four different crosses

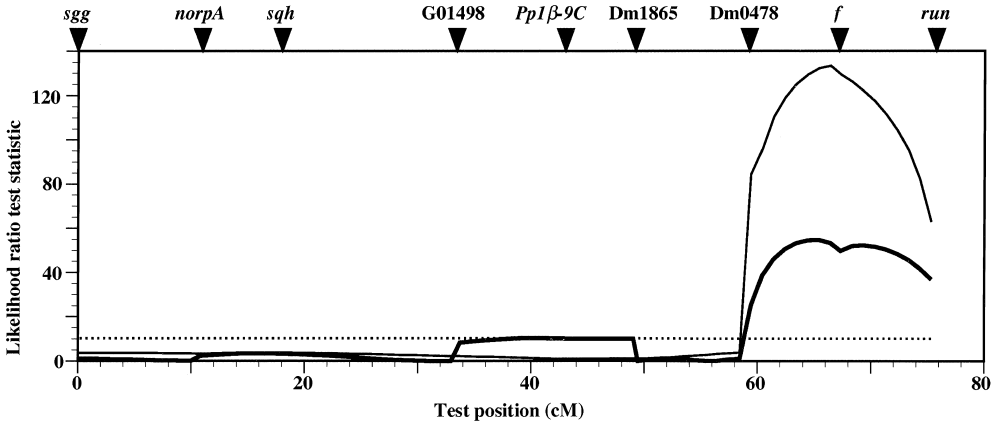

FIGURE 3.—Results of QTL mapping on F_2 males between Sim-5 (G20) and Tananarive (G20) for bristle loss phenotype in hybrids with *D. melanogaster*. The analysis was done separately for two populations of different genetic backgrounds [96 F_2 males from (S5 × Ta) × S5 and (Ta × S5) × S5 crosses (Sim-5 backcross, thick line) and 96 F_2 males from (S5 × Ta) × Ta and (Ta × S5) × Ta ones (Tananarive backcross, thin line)]. Likelihood ratio test statistic (ZENG 1994) is plotted at every 1-cM position. The likelihood ratio test statistic = 2 × (ln 10) × LOD (= 4.61 × LOD). We may take $\chi^2_{0.05/8}$ (d.f. = 2) = 10.2 as the 5% critical value (dashed line, ZENG 1994).

between Sim-5 (G20) and Tananarive (G20) [(S5 × Ta) × S5, (S5 × Ta) × Ta, (Ta × S5) × S5, and (Ta × S5) × Ta] were crossed to TT-35 females of *D. melanogaster*. The distribution of the number of missing bristles in a total of 600 male hybrids is illustrated in Figure 2A. The mean number of missing bristles per fly was 5.5 ± 0.2. Significant differences among the four crosses existed. The average number of missing bristles per fly was 6.0 ± 0.5 for 150 males from the (S5 × Ta) × S5 cross, 4.5 ± 0.4 for 150 males from the (S5 × Ta) × Ta, 6.5 ± 0.5 for 150 males from the (Ta × S5) × S5, and 4.8 ± 0.4 for 150 males from the (Ta × S5) × Ta. Both the difference between the (S5 × Ta) × S5 and (S5 × Ta) × Ta and that between (Ta × S5) × S5 and (Ta × S5) × Ta were statistically significant at the 5% level (Welch's $t' = 2.2$ for the former comparison and Welch's $t' = 2.5$ for the latter), but no significant difference existed between (S5 × Ta) × S5 and (Ta × S5) × S5 and between (S5 × Ta) × Ta and (Ta × S5) × Ta. Thus, males carrying larger amounts of the Sim-5 (G20) genome had greater numbers of missing bristles in hybrids.

I determined the genotypes at the nine marker loci for 192 hybrid males and did a QTL analysis using a composite interval mapping method (ZENG 1994). Given the significant background effects, the data were analyzed separately for the two different backcrosses [Sim-5 backcross includes (S5 × Ta) × S5 and (Ta × S5) × S5 crosses; and Tananarive backcross (S5 × Ta) × Ta and (Ta × S5) × Ta ones], and the results are graphically presented in Figure 3. The maximum LR scores lay between Dm0478 and *f* both in the two backcrosses. Their estimated additive effects and positions are 4.52 bristles at 65 cM position from the *sgg* for the Sim-5 backcross and 4.43 at 66 cM for the Tananarive backcross, showing a very good concordance of the two results.

The LR test statistic profile gave evidence for additional QTL; for instance, another QTL may exist between the *f* and *run* loci particularly in the Sim-5 background. However, because estimates and positions for putative QTL within adjacent intervals are interdependent, I was not able to further dissect the possible effects of QTL around *f* with the present amount of data. In addition, LR test statistic scores within the G01498 and *Pp1*β*–9C* interval slightly exceeded the 5% critical value in the Sim-5 backcross, but the evidence for a QTL in this interval depended on the analytical model. When all the markers are used to control the genetic background (model I in ZENG 1994), the maximum LR score in this interval was only 1.7. In sum, although more than one QTL may be involved, the current QTL analysis found a putative QTL between the Dm0478 and *f* loci. Its average additive effect was about 4.5 bristles, and this QTL explains 72% of one-half the difference in mean number between the parental strains. Indeed, the number of missing bristles per fly for 88 males with the Sim-5 (G20) alleles both at Dm0478 and at *f* (SS genotype) ranged from 2 to 22 with the mean of 9.9 ± 0.5; the number of missing bristles per fly for 87 males with the Tananarive (G20) alleles at Dm0478 and *f* (TT genotype) never exceeded 1 (the mean was 0.1 ± 0.0; Figure 2A).

As mentioned above, the comparisons of mean number of missing bristles between the two different backcrosses provided evidence for the autosomal effects of the two strains. The same tendency was also found in the F_2 males of SS genotype. The average number of missing bristles per fly was 10.93 ± 0.71 for 44 SS males from the Sim 5 backcross and 8.91 ± 0.52 for 44 SS males from the Tananarive backcross, and the difference was statistically significant at the 5% level (Welch's $t' = 2.3$). This difference was not due to a segregation bias of the Sim-5 (G20) and Tananarive (G20) genomes at the other loci on the *X* chromosome between the two backcrosses, because the frequencies of Sim-5 (G20) alleles in the Tananarive-backcross SS-genotype F_2 males were higher than or equal to those in the Sim-5-backcross SS males at all the seven loci excluding Dm0478 and *f*. Thus, the results are best explained by the autosomal effects. By contrast, no difference in the average number

of missing bristles of TT-genotype males existed between the two backcrosses, 0.14 ± 0.05 for 44 TT males from the Sim 5 backcross and 0.07 ± 0.04 for 43 TT males from the Tananarive backcross. This suggests an epistatic interaction between the QTL in the Dm0478–*f* region and an autosomal putative QTL (or multiple QTL). The autosomal factor(s) on Sim-5 (G20) functioned as a dominant enhancer in the SS genotype hybrid males.

The average number of missing bristles of the SS genotype males from the Sim-5 backcross was 2.0 greater than that from the Tananarive backcross. The probability that the hybrid males from the former backcross carried the Sim-5 (G20) allele at the autosomal loci is 3/4 and that in the latter is 1/4. Consequently, the autosomal effects between Sim-5 (G20) and Tananarive (G20) was estimated as two times the difference between the two different backcrosses, that is, about 4. An alternative approach is to use the estimates from F_1 male data. The difference between the parental Sim-5 (G20) and the S5 × Ta males was 1.6 and this difference was statistically significant. This gave 3.2 as the estimate of the autosomal effects between the two parental strains. At any rate, no matter which estimate we take, the sum of the Dm0478–*f* QTL and autosomal effects could explain nearly all difference between the two parental lines, 12.5 ($4.5 \times 2 + 3.2 = 12.2$).

The major QTL responsible for the difference between Sim-5 (G20) and Tananarive (G20) is not necessarily responsible for the variation in the bristle loss phenotype of hybrids of different *simulans* strains with *D. melanogaster*. Different loci may be involved in bristle losses of different hybrids. The effects of the Dm0478–*f* region on bristle loss phenotype was examined in three different pairs of H and L lines. In this experiment, I determined the genotypes only at Dm1911 (14B1-4), which lies halfway between Dm0478 (13A8-9) and *f* (15F2-4). Table 5 clearly shows the significant effects of Dm1911 genotypes on the number of missing bristles. However, in the Sim-3 (G20)-Sim-12 pair, the difference between two Dm1911 genotypes of the F_2 males was much smaller than that between the parental lines, although the mean number of missing bristles in Sim-3 (G20) males was not significantly larger than that in F_2 males carrying the Sim-3 (G20) allele at Dm1911. This result was largely due to two outliers in the TT-35 × Sim-3 (G20) crosses, namely, 12 and 16. The others ranged from 1 to 7, with a mean of 3.6 ± 0.4. The mean number of missing bristles for each parental line in Table 5 was obtained from five hybrids from the first of three vials in which each parental set was allowed to lay eggs. If we pool the data from the three vials, the average number of missing bristles for Sim-3 (G20) is only 3.3 ± 0.3, including the above two outliers. Thus, the Dm1911 genotypes accounted for most of the differences between the parental strains in all three cases. In sum, most of the variability in bristle loss phenotype in hybrids of different *simulans* strains with *D. melanogaster* may be attributed to a single locus.

The F_1 males between Shira-2 (G13) and Sim-11 (G20) were also crossed with TT-35 males, and the average numbers of missing bristles in hybrids were 8.8 ± 0.7 for F_1 males of Shira-2 (G13; as female parents) × Sim-11(G20; as male parents) and 0.4 ± 0.2 for those of the reciprocal cross. This finding indicates significant *X* chromosome effects again for this pair of lines, but also a significantly greater number of missing bristles for F_1s of Shira-2 (G13) × Sim-11 (G20) than that for the parental Shira-2 (G13) ($P < 0.001$, $t = 3.7$, d.f. = 48). Though the difference is not statistically significant, the Dm1911-Shira-2 (G13) F_2 males have a greater number of missing bristles than Shira-2 (G13) males (Table 5). This may be due to an epistatic interaction between the Shira-2 (G13) *X* chromosomal factor around Dm1911 and Sim-11 autosomal factor(s).

In conclusion, the QTL mapping analysis provided

TABLE 5

Significant effects of Dm 1911 genotypes on the hybrid bristle number in three pairs of H and L lines of *D. simulans*

		Mean number of missing bristles per fly ± SEM (sample size)			
H line	L line	H line	L line	F_2 carrying H-line alleles at Dm1911	F_2 carrying L-line alleles at Dm1911
Sim-16	Sim-14	4.9 ± 0.8*** (25)	0.2 ± 0.1 (20)	4.0 ± 0.6*** (41)	0.5 ± 0.2 (39)
Sim-3 (G20)	Sim-12	4.4 ± 0.7*** (25)	0.1 ± 0.1 (25)	2.9 ± 0.3*** (31)	0.2 ± 0.1 (49)
Shira-2 (G13)	Sim-11 (G20)	5.2 ± 0.6*** (25)	0.1 ± 0.1 (20)	6.7 ± 0.7*** (32)	0.7 ± 0.3 (46)

Statistical tests (Welch's approximate *t*-test) were done between H and L lines and between F_2 males carrying H-line Dm 1911 alleles and those carrying L-line alleles, finding highly significant differences for all tests. ***$P < 0.001$.

evidence for the disproportionately large effect of a single QTL (or block of QTL) and the epistatic interactions between *X*-linked and autosomal factors of *D. simulans*.

DISCUSSION

Limitation of deficiency- and duplication-chromosome study and QTL mapping: The results of the deficiency screenings should be treated with caution because there was no good control to evaluate the effects of different deficiencies and duplications. Comparisons of the mean numbers of missing bristles between deficiencies and balancer chromosomes serve only the purpose of uncoupling *X* chromosome effects from autosomal contributions. Multiple independent deficiencies are required to test a region. In addition to the *AS-C* region, the two regions (3A4-5; 3C2) and (10F1; 10F7) met this criterion, where three different deficiency chromosomes showed great numbers of missing bristles (larger than 10 missing bristles per fly in most cases) for each region. On the other hand, many deficiencies showed relatively small numbers of missing bristles. For instance, 21 of 49 deficiency chromosomes studied had less than five missing bristles in hybrids with Sim-5 (G20). Though the small number of missing bristles by itself cannot rule out any effects of the region involved, the data clearly suggest the disproportionate effects of the above two regions. Importantly, the QTL mapping analyses did not find a QTL in these two regions.

Duplication study also requires multiple tests by independent duplications because of a lack of ability to control their genetic backgrounds. The recipient chromosomes of six duplications in Table 4, however, are the *Y* [*Dp(1;Y)*] or fourth [*Dp(1;4)*] chromosomes. Both of these chromosomes of *D. melanogaster* carry only very small numbers of genes except for the tandemly repeated copies of the ribosomal RNA genes on the *Y* of *D. melanogaster*. The copy number of the ribosomal RNA genes, however, cannot explain the great reduction of the bristle number in Sim-5 *melanogaster* hybrids (Takano 1998), and an accumulation of deleterious mutations are not allowed on the *Y* chromosome because of its hemizygosity except for the ribosomal RNA genes (the *X* chromosome also carries the copies of the ribosomal RNA genes). Therefore, the significant effects of several *Dp(1;Y)* and *Dp(1;4)* duplications are likely to be attributable to the duplication segments. At any rate, the duplication study complemented the deficiency screening and QTL mapping, and its results supported the presence of genetic factor in the (3A4-5; 3C2) region found in the deficiency screening and the QTL in the (13F; 14F) region (discussed below).

The composite interval mapping potentially has high power to detect a QTL under the condition of no epistatic interaction between QTL, and we may apply a joint mapping method to pool the two backcross populations. However, the small number of individuals studied in the current analysis and the large sampling variance in missing bristle numbers as seen in the hybrids of parental inbred Sim-5 (G20) males with *D. melanogaster* females did not allow a precise mapping of QTL, and the results of the QTL mapping alone seemed not to determine whether a major QTL (or block of QTL) lies in the Dm0478–*f* interval or in the *f*–*run* or whether a QTL lies in each of the two intervals. Hybrids of *simulans* L lines with *D. melanogaster* always showed no reduction in the bristle number; large sampling variances in missing bristle numbers seem to be a common character of H lines (Takano 1998). Therefore, a large sampling variance is likely to have existed in the hybrids of Sim-5 (G20)-Tananarive (G20) F_2 males with TT-35 females. To be more exact, hybrids of certain genotypes had large error variances and the others did not. The large sampling variance is problematic in the QTL mapping.

Alternatively, a cutoff point for the missing numbers may be set, say 2 or more *vs.* 0 or 1, to classify hybrids into H and L types. In total, 34 recombinants between Dm0478 and *run* (14 from the Sim-5 and 20 from the Tananarive backcross) were recovered from 192 hybrids studied. In a population of these recombinants, all 13 hybrids carrying the Sim-5 (G20) allele at *f* showed 4 or more missing bristles except for 1 hybrid (only 1 missing bristle), which carried the Tananarive (G20) Dm0478 allele and the Sim-5 (G20) *run* allele (a crossover between Dm0478 and *f*, TSS genotype). On the other hand, the number of missing bristles of the 21 hybrids carrying the Tananarive (G20) allele at *f* did not exceed 1 except in 2 hybrids. One exceptional hybrid carried the Sim-5 (G20) alleles both at Dm0478 and *run* (double crossovers, STS genotype) and showed 13 missing bristles. The other carried the Sim-5 (G20) allele at Dm0478 and the Tananarive (G20) allele at *run* (STT genotype) and lost 12 bristles. Despite the relatively low resolution of the QTL map for the hybrid bristle loss in the current analysis, the results strongly suggest the proximity of a QTL to the *f* locus, and the genotypes and the missing bristle numbers of these exceptional recombinants were consistent with the presence of a QTL between Dm0478 and *f*.

Factors involved in hybrid bristle loss: Hybrid bristle loss anomaly is potentially caused by several distinct genetic factors. The Dm0478–*f* QTL is the factor responsible for the within-*simulans* variation in the number of missing bristles (class 1 factor). It is important to note that the origins of the Dm1911 (14B1-4) region accounted for most of the differences between H and L lines in the three other different pairs, suggesting that the within-*simulans* variation largely comes from a single major locus or block of loci.

The deficiency screening, however, failed to find significant effects of the Dm0478 (13A8-9)–*f* (15F2-4) region. There are several possible explanations. Not all

the regions were surveyed in the deficiency screening. Particularly, the 14-15 region could not be studied completely because the strong *Minute* locus (haplo-insufficient locus and very low viability of the deficiencies including this region in heterozygotes), *Minute(1)15D*, existed. Alternatively, sex-dependent effects may explain the differences between the two screenings. The bristle loss was studied in hybrid females in the deficiency screening, and the bristle loss was studied in hybrid males in the QTL mapping. I previously showed that the male hybrids are more susceptible to bristle loss than are the female hybrids (Takano 1998). Therefore, I did the duplication screening in this region (Table 4), where the rescue action of duplications was studied in male hybrids carrying the *simulans X* chromosome and duplications on the *Y* or autosomes. Although there were large background effects among the different *melanogaster* strains, the *Dp(1;Y)shi*$^{+}$*3* chromosome had the greatest rescue effects on the bristle formation in hybrid males. Thus, the results supported the large effect of the Dm0478–*f* region and the failure of the deficiency analysis may be due to the sex specificity in genotypic effect.

On the other hand, a few regions, especially 3A4-5; 3C2 and 10F1; 10F7 regions, manifested significant effects on the bristle number in *melanogaster* deficiency-carrying hybrid females. Moreover, the duplications, *Dp(1;Y)w*$^{+}$*Y* covering 2D2; 3D3 and *Dp(1;3)W*^{VCO} covering 2C1; 3C5, rescued the bristle development significantly. These regions are likely to include fixed *melanogaster-simulans* differences involved in the hybrid bristle loss (class 2) or factors that show dose-sensitive effects, especially in a hybrid background, without a between-species functional difference (class 3). Thus, they do not necessarily have a between-species difference. It is not easy to distinguish between these two possibilities without molecular cloning of the factor even if a duplication of the region restores bristle formation.

Special attention may be paid to the *AS-C* gene because the insertion polymorphism in this region significantly affects the number of sternopleural bristles (Mackay and Langley 1990). However, the *AS-C* deficiencies in heterozygous condition significantly increased the number of missing bristles not only in the *melanogaster-simulans* hybrids but also in the *melanogaster-mauritiana* ones. In addition, this complex is located on the tip of the *X* chromosome, and the within-species DNA variation in this region is extremely low in *D. simulans* (Begun and Aquadro 1991; Martín-Campos *et al.* 1992). Taken together, functional *melanogaster-simulans* differences at the *AS-C*, if any, are presumably categorized as class 2 not 1, though there is no compelling evidence of between-species differences at this complex so far.

The estimate of Dm0478–*f* QTL homozygous effect (9.0) was about two times larger than that of the autosomal effects (3.2–4) for the Sim-5 (G20) and Tananarive (G20) pair. However, because of the "homozygosity effects" of the *X* chromosomes, the *X* chromosomal effects cannot be compared with the autosomal effects (Wu and Davis 1993; True *et al.* 1996b). The autosomal factors in homozygous condition may have the same levels of effect as the *X* factor. Interestingly, I found epistatic interactions in the hybrid bristle loss phenotype between the *simulans X* and autosomal factors for two pairs of H and L lines. A significant effect of Sim-5 (G20) autosomes in the SS genotype and a lack of autosomal effects in the TT genotype were observed for the Sim-5 (G20)-Tananarive (G20) pair. Additionally, I found a significantly greater number of missing bristles of F_1 males between Shira-2 (G13) and Sim-11 (G20) than the parental Shira-2 (G13) males. These findings suggest the presence of within-*simulans* variation on the autosome(s). Because the heterozygotes between H and L strains have the normal bristle pattern (Takano 1998), hybrid bristle loss must further depend on a *melanogaster* autosomal factor(s) that may be a fixed difference(s) (class 2).

Variations in pure species and hybrid backgrounds: A comparison of within-species and between-species sequence variations is a powerful method to gain insights into the evolutionary processes and has been used to distinguish between selective and neutral changes (Hudson *et al.* 1987; Sawyer *et al.* 1987; McDonald and Kreitman 1991; Akashi 1995; Eyre-Walker 1999). This method is applicable to quantitative characters as well, if they can be described in molecular terms.

Unfortunately, only a few studies have examined within-species variation for interspecific-hybrid traits (Johnson *et al.* 1993; Wade *et al.* 1997; Takano 1998). Wade *et al.* (1997) found significant within-species variation in family size, sex ratio, and morphological anomalies in hybrids of two flour beetle species, but not a significant correlation in those traits between interspecific hybrids and conspecific offspring. This suggests that the factors responsible for the variation in hybrids are different from those in the pure species background or that the same factors act differently in the two backgrounds.

With respect to the number of missing bristles in hybrids with *D. melanogaster*, Takano (1998) showed a large amount of between-population genetic variability in the *simulans* populations: very low levels of anomaly in the *simulans* strains collected in Madagascar and the Seychelles and a wide range of degrees of bristle defects in the strains from Japan, the United States, and Africa (Zimbabwe and Congo). As mentioned above, conspecific crosses of the *simulans* strains produce flies with the normal bristle number, so that the within-*simulans* variation found in interspecific hybrids is hidden at the level of macrochaetae number in natural populations. On the other hand, sternopleural and abdominal bristle numbers in *D. melanogaster* have long been used as a

model for study of the genetic basis of quantitative variation and of its maintenance mechanisms in natural populations (Mackay 1995 for review). DNA polymorphisms at candidate loci uncovered significant associations with naturally occurring phenotypic variation in bristle number (Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998). The QTL involved in the within-*simulans* variation in notum-bristle number of hybrids are not known to have an effect on the other bristle numbers in the pure *simulans* background.

The hybrid bristle defect does not lie in cell fate decisions during bristle development, but in the maintenance of neural fate or differentiation of the descendants of sensory mother cells (SMCs) or both (Takano 1998). The loci known to affect the sternopleural and abdominal bristle numbers, the *AS-C*, *scabrous*, and *Delta* (Mackay and Langley 1990; Lai *et al.* 1994; Long *et al.* 1998), are all involved in the emergence and regulation of sensory mother cells, suggesting variations in the SMC numbers for sternopleural and abdominal bristles. Thus, like flour beetles, the genetic basis of the hybrid bristle loss may be different from that of the bristle-number variation in pure species background.

By contrast, sex specificity of genotypic effects are interestingly common both in the QTL affecting sternopleural and abdominal bristle numbers in flies of pure *melanogaster* background (Gurganus *et al.* 1999) and in the factors responsible for the hybrid bristle defect (Takano 1998). Sex-specific action of the factors probably plays a much bigger part in shaping within- and between-species variation than has been thought.

Factors of large effects: Quantitative traits are genetically dissected in terms of the number of genes involved and their effects. The classical model of the genetic basis of within- and between-species quantitative variation has assumed a large number of genes with small effects (Fisher 1930; Lande 1981, 1983; Charlesworth *et al.* 1982). However, this view is questioned by recent results of theoretical (Orr 1998) and experimental studies (*e.g.*, Paterson *et al.* 1988; Doebley and Stec 1991; Hilbert *et al.* 1991; Jacob *et al.* 1991; Lai *et al.* 1994; Bradshaw *et al.* 1995; Long *et al.* 1995; Paterson *et al.* 1995; Liu *et al.* 1996). In agreement with these findings, this study also identified the single major QTL on the *simulans X* chromosome. This means that the number of factors involved in the hybrid bristle loss presumably is not so large. In other words, genetic interactions of a small number of factors caused a great degree of bristle anomaly in hybrids. Thus, the defect in the maintenance of neural fate or differentiation of the descendants of SMCs is likely to be attributed to failures of activation of some key genes but not to an accumulation of small effects like slight reductions of gene activities.

In conclusion, although distinct factors potentially cause the bristle loss due to their epistatic interactions, this study identified the single small *X* chromosomal region that accounted for most of the differences between the H and L *simulans* lines. This finding is encouraging, and there is reason to investigate the molecular basis of this hidden variation because it may become a future target of natural selection (Waddington 1953; Rutherford and Lindquist 1998).

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