The *tumorous-head-1* Locus Affects Bristle Number of the *Drosophila melanogaster* Cuticle

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ABSTRACT

The *tuh-1* maternal effect locus contains two naturally occurring isoalleles, *tuh-1*^{h} and *tuh-1*^{g}. Until recently there has been no possibility to distinguish between the *tuh-1*^{h} and the *tuh-1*^{g} maternal effects other than evaluating their effect on the Bithorax-Complex (BX-C) *Abdominal B* (*Abd-B*) mutant *tuh-3*. However, in this report we identify a bristle phenotype associated with the *tuh-1* locus that has very interesting evolutionary implications. Females homozygous for *tuh-1*^{h} always produce adult offspring with more bristles than females homozygous or heterozygous for *tuh-1*^{g}. The effect is global. Increased bristle number occurs in the head, the thorax, and the anterior and posterior abdomen. Females totally deficient for the *tuh-1* gene produce offspring with high bristle number. Thus, the bristle phenotype results from the absence of the maternally contributed *tuh-1*^{g} factor. Genetic evidence shows that the bristle phenotype is caused by the *tuh-1* locus and that *tuh-1*^{h} is completely recessive to *tuh-1*^{g}. The *tuh-1* locus is located at the euchromatin-β-heterochromatin junction near the centromere of the *X* chromosome and deficiency analysis places the locus between the lethal genes *extra organs* (*eo*) and *lethal B20 (lB20)*. The variance in bristle number attributable to the *tuh-1* locus in nature is approximately 10.1%, an indication that the bristle phenotype is most likely a neutral, pleiotrophic side effect of *tuh-1*.

TUMOROUS-HEAD-1 (*tuh-1*) is a maternal effect locus with two isoalleles, *tuh-1*^{h} and *tuh-1*^{g} (Gardner and Woolf 1949). *tuh-1* interacts with the *tuh-3* gene located in the distal Bithorax-Complex (BX-C). In the presence of the *tuh-1*^{h} maternal effect, homozygous and heterozygous *tuh-3* flies show homeotic defects that transform portions of the eye, the antenna, and the rostralhaut regions into tergite, leg, and genital structures in the adult (Postlethwait *et al.* 1972; Kuhn *et al.* 1979; Kuhn and Packert 1988). Penetrance of these head abnormalities is increased by subjecting embryos to high temperature during the first 24 hr of development (Gardner and Woolf 1950). A different phenotype can be observed in the presence of the *tuh-1*^{g} maternal effect allele. Flies homozygous for the *tuh-3* mutation now show genital disc defects resulting in undeveloped testes in males and missing or abnormal external genitalia in males and females (Woolf 1960, 1966, 1968b; Kuhn *et al.* 1981).

Until recently, no phenotype could be attributed to the non-essential *tuh-1* locus and the only possible way to distinguish the two isoalleles was based on their interaction with the *tuh-3* mutation. However, during an extensive genetic study of *tuh-1*, an increase in bristle number was noted on abdomens of offspring from females homozygous for the *tuh-1*^{h} maternal effect (Packert 1995).

This study evaluates (1) the effect the maternal effects exert on the bristle number on the abdomen, the thorax, and the head of the fly and (2) the effects of temperature on bristle number and the developmental rate of Drosophila.

MATERIALS AND METHODS

Drosophila stocks: (1) *v tuh-1*^{h}/*Y*;+;+, has an attached *X* chromosome carrying the *tuh-1*^{h} allele, the recessive mutant marker gene *vermilion* (*v*) and is homozygous for the *Canton-S* 2nd and 3rd chromosomes. (2) *FMA3 y*^{2}/*Y*;+;+, has an attached *X* chromosome carrying the *tuh-1*^{g} allele and the recessive mutant *yellow* (*y*^{2}) and is homozygous for the *Canton-S* 2nd and 3rd chromosomes. (3) *y*$^+$*Y mal*^{106} is a *Y* chromosome carrying sections of the base of the *X* chromosome (regions 19 and 20) and the *yellow* locus (*y*$^+$). (4) *FM6*;+;+, carries an *X*-chromosome balancer containing multiple inversions, the dominant *Bar* mutation and *tuh-1*^{g} and is homozygous for the *Canton-S* 2nd and 3rd chromosomes. (5) *Df GA37*;+;+ is deficient for polytene chromosome bands 19E2 to 19F5-6 and homozygous for the *Canton-S* 2nd and 3rd chromosomes. (6) *Df JC4*;+;+ is deficient for bands 20A1 to 20E-F and homozygous for the *Canton-S* 2nd and 3rd chromosomes. (7) *Canton-S* is a wild-type strain homozygous for *tuh-1*^{g}. (8) *Amherst-56* is a *tuh-1*^{g} strain used as the background strain for many of the *X* chromosome deficiencies described above. (9) *tuh-1*^{h};+;+ is a wild-type strain homozygous for *tuh-1*^{h} and homozygous for the *Canton-S* 2nd and 3rd chromosomes. (10) *Oregon-R* is a wild-type strain homozygous for *tuh-1*^{h}. (12) *v tuh-1*^{h}/*Y;I127B* and *FMA3 y*^{2}/*Y; I127*^{B} carry a mutant within the BX-C *Abdominal-B* (*Abd-B*) gene. (13) *Miami* are wild type females collected

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from Miami Shores, Florida, May 1997. Drosophila strains listed are described further in Lindsey and Zimm (1992) and Miklos *et al.* (1987).

Stock maintenance: Fly strains were raised at room temperature (RT) on standard Drosophila medium consisting of agar, cornmeal, yeast extract, sucrose, and dextrose (Lewis 1960). To inhibit fungal growth, proprionic and phosphoric acid were added.

Temperature studies: For the temperature studies flies were raised at 18° or at 25° on standard Drosophila medium.

Preparation of abdomens: Abdomens were dissected free from the thorax in water, cut along the dorsal midline, internal soft tissue was removed, the cuticle flattened, and mounted in a drop of Hoyer's mounting medium following the procedure of Kuhn and Packert (1988). Heads were dissected; the occipital region was removed and mounted in a drop of Hoyer's mounting medium. Wings were mounted in a drop of Hoyer's mounting medium.

Statistical analyses: Confidence limits of population means were determined by using the formula for the standard error of the mean σ^2/n. An Analysis of Variance, completely randomized design - Model 1 parametric test was performed on bristle number on tergite 7 of Drosophila (Woolf 1968a). The phenotypic variance of bristle number was partitioned into its genotypic and environmental components (Falconer 1981).

RESULTS

The number of bristles on the posterior abdomen increase in the presence of the *tuh-1^h* maternal effect: During a genetic analysis of the *tuh-1* locus, an increase in bristle number was observed on abdomens of offspring from females carrying the *tuh-1^h* maternal effect. Figure 1, A–G presents the average bristle number on tergite 7 of females derived from the following mothers: (A) *v* *tuh-1^h*/*Y*, (B) *tuh-1^h*/*tuh-1^h*, (C) *Oregon-R*, (D) *Canton-S*, (E) *FMA3* *y^2*/*Y*, (F) *Amherst 56i*, and (G) *tuh-1^h*/*FM6*. The first three strains (A–C) are homozygous for *tuh-1^h*, D through F are homozygous for *tuh-1^g* and G is heterozygous for *tuh-1^g*/*tuh-1^h*. The results demonstrated a statistically significant increase in bristle number of all strains homozygous *tuh-1^h*. The bristle number for offspring from *tuh-1^h*/*FM6* heterozygotes were similar to results obtained for offspring of *tuh-1^g* homozygous mothers. Figure 1 gives the number of abdomens scored (n); mean bristle number ($\bar{x}$) and the 95% confidence limits. The mean number of bristles on tergite 7 was significantly higher in all *tuh-1^h* strains.

To further evaluate the influence of the *tuh-1* locus on bristle number, a series of crosses was designed utilizing the free-*X* *tuh-1^h* chromosome and *FM6*. Figure 2 outlines the crosses for this study. The number of abdomens analyzed (n); the mean ($\bar{x}$) and 95% confidence are shown in Figure 3 for all generations. The maternal and zygotic genotypes are also indicated for all generations.

Offspring from *tuh-1^h*/*tuh-1^h* homozygous females showed a statistically significant increase in the number of 7th tergite bristles in comparison to offspring from *tuh-1^g*/*tuh-1^h* heterozygous females. The results presented in Figure 3 show that the parental stock (P) and the F_1 generation both have a high bristle number even though the parental stock is homozygous for *tuh-1^h*, and the F_1 generation is heterozygous for *tuh-1^h*/*tuh-1^g*. These findings indicate that the maternal genotype was responsible for the high bristle number in the F_1 gener-

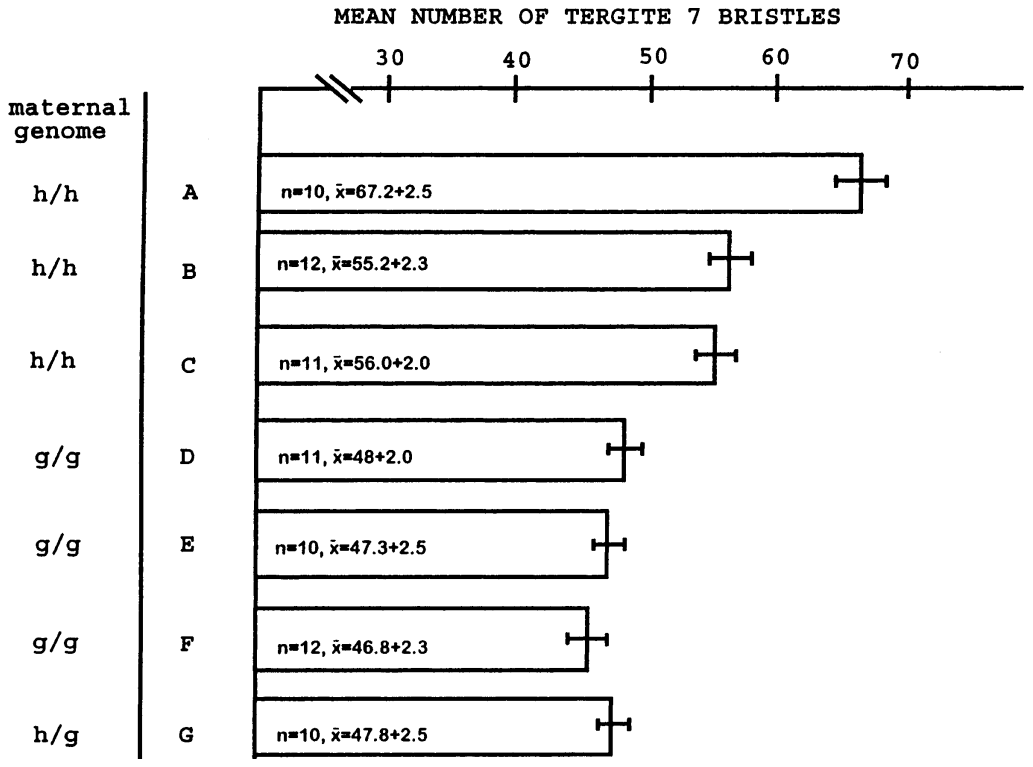

Figure 1.—Analysis of bristle number on tergite 7 in females of various genetic backgrounds. (A) *v* *tuh-1^h*/*Y*; (B) *tuh-1^h*/*tuh-1^h*; (C) *Oregon-R*; (D) *Canton-S*; (E) *FMA3* *y^2*/*Y*; (F) *Amherst 56i*; (G) *tuh-1^h*/*FM6*. n = sample size; $\bar{x}$ = mean number of bristles ± 95% confidence limits. The maternal genotype is indicated. h denotes *tuh-1^h*, g denotes *tuh-1^g*.

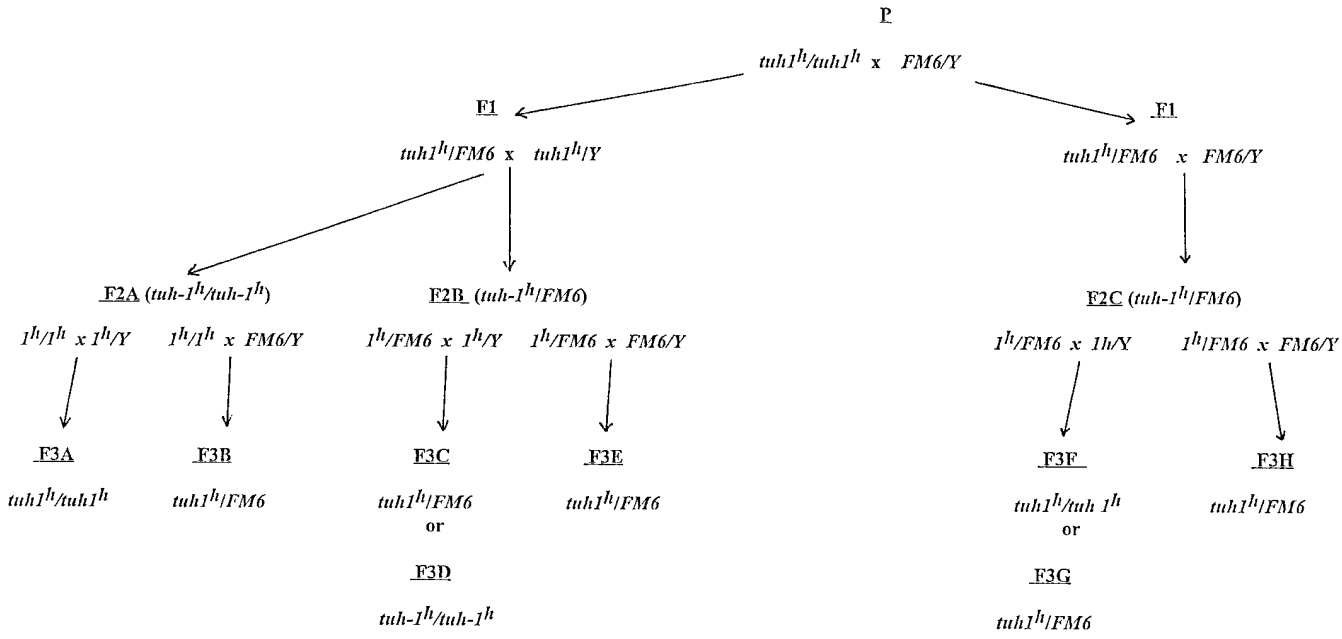

Figure 2.—Genetic crosses utilizing the *tuh-1*^{h} and the *tuh-1*^{g} isoalleles to evaluate the *tuh-1* maternal effect. P = Parental strain, F1 = first generation, F2A through F2C designate F_2 generation, F3A through F3G designate F_3 generation. *1h* denotes *tuh-1*^{h}, *FM6* carries *tuh-1*^{g}. For each cross females are given on the left, males on the right. Only female offspring were analyzed.

ation. The F_2 results show that offspring from mothers carrying the *tuh-1*^{g} maternal effect allele have a reduction in bristle number, regardless whether their offspring are heterozygous *tuh-1*^{h}/*tuh-1*^{g} or homozygous *tuh-1*^{h}/*tuh-1*^{h}.

The F3A and F3B offspring in Figure 3 were produced by mothers homozygous for *tuh-1*^{h} who showed low bristle numbers (F2A). All their offspring (F3A and F3B) showed high bristle numbers. The F2B and F2C females (heterozygous for *tuh-1*^{h}/*tuh-1*^{g}) produced the F3C-F3H offspring. All of their offspring have a low bristle number, regardless of their being *tuh-1*^{h}/*tuh-1*^{h} (F3D, F3F) or *tuh-1*^{h}/*tuh-1*^{g} (F3C, F3E, F3G, F3H).

Extensive genetic analysis as well as a deficiency analysis mapped *tuh-1* between *lethal B20* (*lB20*) and *extra organs* (*eo*) at the base of the *X* chromosome in salivary chromosome band 20A1 (Pyati 1976; Miklos *et al.* 1988; these data). *Df GA37* and *Df JC4* both uncover the *tuh-1* locus. They are homozygous lethal and balanced over *FM6*. In the presence of the BX-C mutant *tuh-3* their offspring show head defects (Kuhn *et al.* 1993). Thus, one would predict that offspring from deficiency heterozygous mothers should show the increased bristle number as seen in the presence of the *tuh-1*^{h} allele.

Figure 4 outlines the genetic crosses utilizing *Df JC4* and Figure 5 provides the results of the *Df JC4* analysis, while Figure 6 summarizes the *Df GA37* analysis. The *Df JC4*/*FM6* stock females show a low bristle number (Figure 5, P generation) due to the presence of the *tuh-1*^{g} maternal effect allele present in the *FM6* chromosome. All their offspring show a low bristle number (Figure 5, F_1). The Df *JC4*/*tuh-1*^{h} F_1 mothers with low bristle number produce offspring that have high bristle number counts irrespective of their being *Df JC4*/*tuh-1*^{h} or *Df JC4*/*FM6* (Figure 5, F2A and F2B). These data show that *Df JC4* acts like *tuh-1*^{h}. Note that the bristle number for *Df JC4*/*FM6* (P) is significantly lower than the number for the F_1 (*Df JC4*/*tuh-1*^{h}). These results have been obtained consistently and we believe that these differences are probably not due to *tuh-1* but may be due to the loss of ribosomal genes in *Df JC4* having an additional suppressive effect on bristles number (Frankham *et al.* 1980).

Offspring designated F3A and F3B in Figure 5 are offspring from females of the genotype *tuh-1*^{h}/*tuh-1*^{h} or the genotype *Df JC4*/*tuh-1*^{h} (F2A), and once again show high bristle numbers, while offspring designated F3C and F3D are offspring from females heterozygous *Df JC4*/*FM6* or *tuh-1*^{h}/*FM6* (F2B) and show lower bristle numbers. This is expected of mothers hemizygous or heterozygous for *tuh-1*^{g}. Although the differences are not significant, there is a tendency for F_3 females to have more bristles if their fathers are *tuh-1*^{h}/*Y* rather than *tuh-1*^{g}/*Y*. This tendency was shown in Figure 5 where the *tuh-1*^{h}/*Y* fathers (F2A) yielded offspring with higher bristle number than offspring from *tuh-1*^{g}/*Y* fathers (F2B). Because the deficiencies and the free-*X* *tuh-1*^{h} maternal effect chromosome carry no additional markers it was not possible in these crosses to determine homozygous *tuh-1*^{h} females from females of the genotype *Df JC4*/*tuh-1*^{h}.

The results of the *Df GA37* analysis are shown in Figure 6. *Df GA37* was substituted for *Df JC4*. The crosses are not shown since they were the same as outlined for

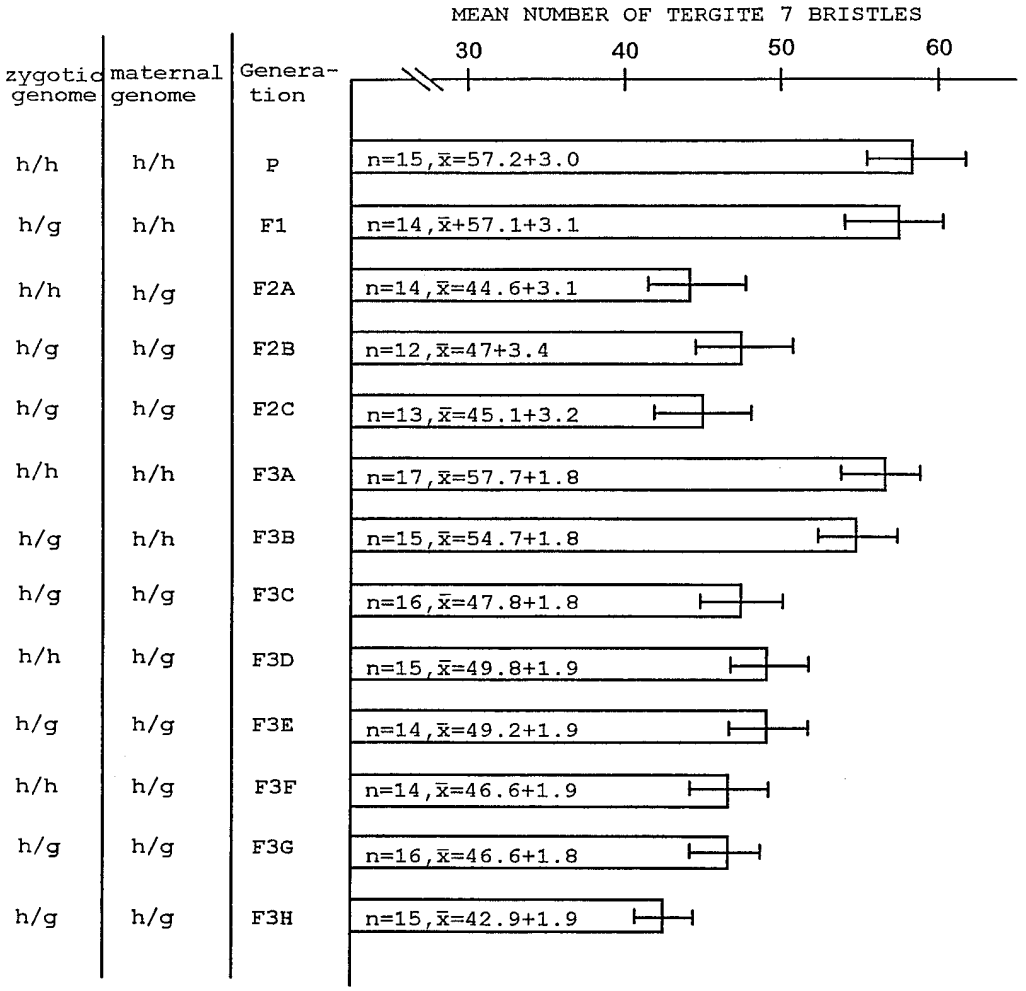

Figure 3.—Analysis of bristle number on tergite 7 in females through 3 generations. See Figure 2 for crosses. P = parental strain, F1 = first generation, F2A through F2C designate F_2 generation, F3A through F3H designate F_3 generation. n = sample size, $\bar{x}$ = mean number of bristles ± 95% confidence limits. The maternal and the zygotic genotype are indicated. h denotes *tuh-1^h*, g denotes *tuh-1^g*.

Df JC4 (Figure 4). Figure 6 presents the statistical comparison between the P, F_1, F_2, and F_3 generation females. The *Df GA37/FM6* strain shows a low bristle number due to the presence of the *tuh-1^g* maternal effect allele present in the *FM6* chromosome. *Df GA37/tuh-1^h* F_1 mothers with low bristle number produce offspring with high bristle numbers irrespective of their being *Df GA37/tuh-1^h* or *Df GA37/FM6* (F2A and F2B).

Offspring designated F3A and F3B in Figure 6 are offspring from mothers either homozygous *tuh-1^h/tuh-1^h* or heterozygous *Df GA37/tuh-1^h* (F2A). These offspring show a high bristle number in comparison to offspring designated F3C and F3D from mothers heterozygous *tuh-1^h/FM6* or *Df GA37/FM6* (F2B). As in the *Df JC4* analysis, there was a tendency for higher bristle numbers when the father was *tuh-1^h/Y*.

In the final series of crosses, *Df GA37/Df JC4* females were tested (Figure 7). Deficiency heterozygotes are viable and fertile but do not carry any genetic markers that would allow a distinction among themselves and *tuh-1^h* females in the F_2 and F_3 generations. To construct a deficiency strain, *Df JC4/FM6* females were crossed to *Df GA37/y$^+$Ymal^{106}* males. Once again the P (*Df JC4/FM6*) and the F_1 (*Df JC4/Df GA37*) generation show a low bristle number as expected since in each case their mothers were hemizygous for the *tuh-1^g* maternal effect allele (Figure 7). Note the extremely low bristle number for the *Df JC4/FM6* female parents. The F_1 *Df GA37/Df JC4* females are deficient for *tuh-1* and their offspring (F2A and F2B) show high bristle numbers. These results were expected since the *Df GA37/Df JC4* F_1 females should behave like *tuh-1^h* homozygotes.

Offspring designated F3A and F3B in Figure 7 are offspring from females of the genotype *tuh-1^h/tuh-1^h* or *Df GA37/tuh-1^h* or *Df JC4/tuh-1^h* (F2A) and show high bristle numbers. Offspring designated F3C, F3D, and F3E are offspring from females heterozygous *tuh-1^h/FM6* or *Df GA37/FM6* or *Df JC4/FM6* (F2B) and show low bristle numbers, as expected of mothers hemi- or heterozygous for *tuh-1^g*. These data demonstrate that offspring of females that are deficient for the *tuh-1* locus behave like offspring of *tuh-1^h* homozygous females. As in the previous studies, there was a tendency of higher bristle number in the presence of *tuh-1^h/Y* males. However, there was one exception (Figure 7) where *tuh-1^h/Y* fathers (F3A) did not produce offspring with more bristles than offspring from *tuh-1^g* fathers (F3B).

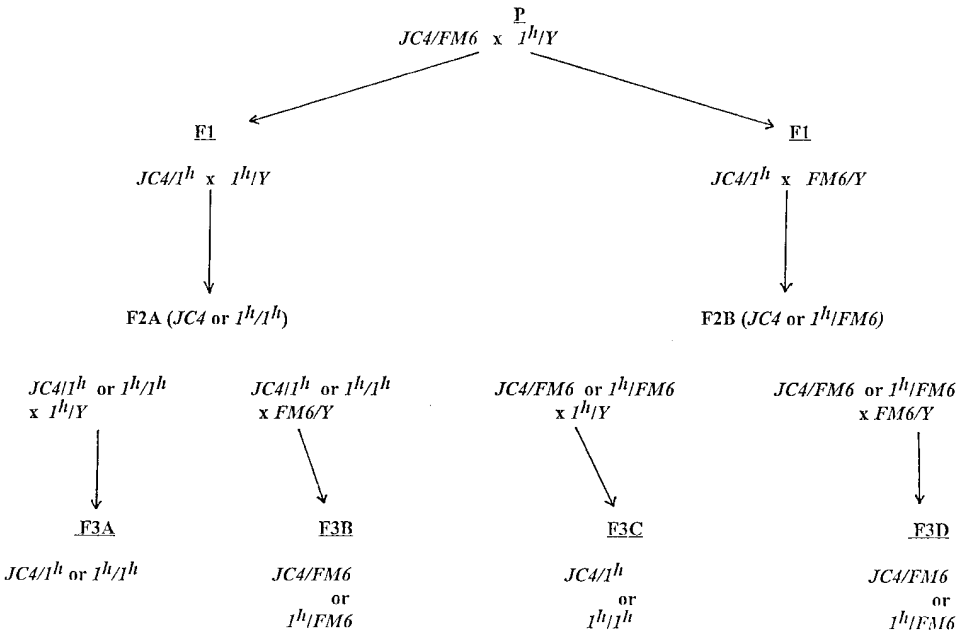

Figure 4.—Genetic crosses utilizing *Df JC4* to evaluate the *tuh-1* maternal effect. P = parental strain, F1 = first generation, F2A and F2B designate F_2 generation, F3A through F3D designate F_3 generation. *1h* denotes *tuh-1*^{h}, *FM6* carries *tuh-1*^{g}. For each cross females are given on the left, males on the right. Only female offspring were analyzed.

Evaluation of bristle number on the head, the wing blade, and anterior and posterior abdomen: To determine whether the effect of the *tuh-1* locus on bristle number is restricted to the posterior abdomen or has global effects influencing bristle number on the entire adult cuticle, an additional study was initiated. Two attached *X* chromosomes, *v tuh-1*^{h}/*Y* and *FMA3 y*^{2}/*Y* (*tuh-1*^{g} maternal effect) were utilized in this study. Both strains were homozygous for the *Canton-S* 2nd and 3rd chromosomes. The statistical analysis is presented in Figure 8. The results clearly demonstrate that T7, T3, and the occipital bristles (posterior head) are dramatically increased among offspring from *v tuh-1*^{h}/*Y* mothers when compared to offspring from the *FMA3 y*^{2}/*Y* mothers. Although the tendency is in the same direction for the wing blade, the differences were not statistically significant.

Effect of temperature on the *tuh-1* bristle phenotype: Studies to determine the frequency of *tuh-1*^{h} and *tuh-1*^{g} alleles in wild-type populations showed an increase of *tuh-1*^{h} in colder climates (Johnson and Gardner 1965). It is therefore of interest to determine the effect temperature has on bristle numbers of *tuh-1*^{h}/*tuh-1*^{h} homozygotes and *tuh-1*^{g}/*tuh-1*^{g} homozygotes. Two fly strains, *v tuh-1*^{h}/*Y;I127B* and *FMA3 y*^{2}/*Y;I127B* were utilized in this study. For each strain, stocks were maintained at 18° and 25°. Adult females were collected at random

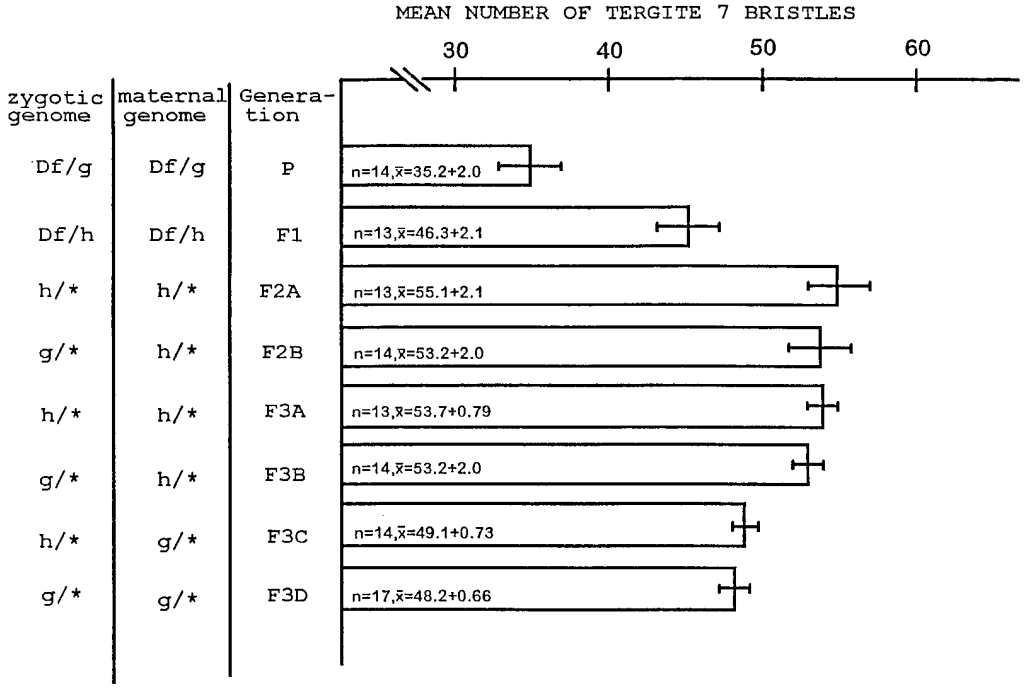

Figure 5.—Analysis of bristle number on tergite 7 in females utilizing *Df JC4* to evaluate the *tuh-1* maternal effect. See Figure 4 for crosses. P = parental strain, F1 = first generation, F2A and F2B designate F_2 generation, F3A through F3D designate F_3 generation. n = sample size, $\bar{x}$ = mean number of bristles ± 95% confidence limits. Df denotes *Df JC4*, h denotes *tuh-1*^{h}, g denotes *tuh-1*^{g}, * indicates *tuh-1*^{h} or *Df JC4*.

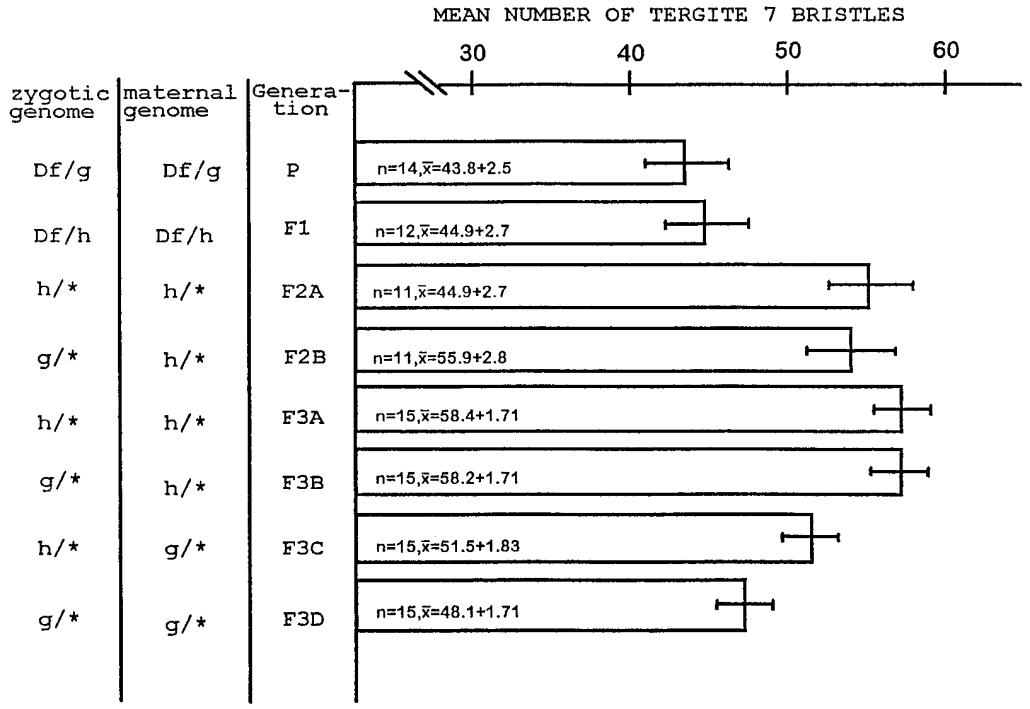

Figure 6.—Analysis of bristle number on tergite 7 in females utilizing *Df GA37* to evaluate the *tuh-1* maternal effect. See Figure 4 for crosses. P = parental strain, F1 = first generation, F2A and F2B designate F_2 generation, F3A through F3D designate F_3 generation. n = sample size, $\bar{x}$ = mean number of bristles ± 95% confidence limits. Df denotes *Df GA37*, h denotes *tuh-1*^{h}, g denotes *tuh-1*^{g}, * indicates *tuh-1*^{h} or *Df GA37*.

and bristle number on tergite 7 was determined. At 18° *tuh-1*^{h} homozygotes possess significantly more bristles on tergite 7 than the *tuh-1*^{g} homozygotes (data not shown). At 25° the difference in bristle number among the strains is still maintained although the *tuh-1*^{g} homozygotes show a slight increase in bristle number when compared with homozygotes raised at 18° (data not shown).

Effect of temperature and *tuh-1* on Drosophila development: During the course of this study it became apparent that flies homozygous or hemizygous for *tuh-1*^{h}

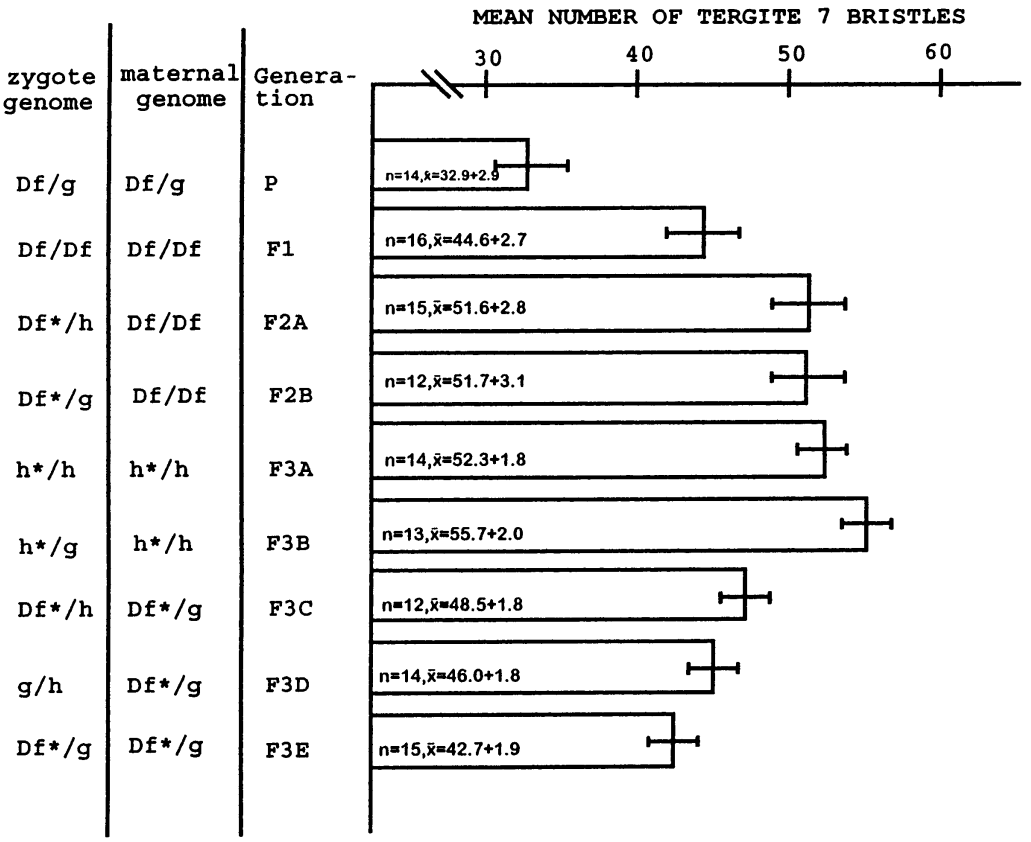

Figure 7.—Analysis of bristle number on tergite 7 in females utilizing *Df JC4* and *Df GA37* to evaluate the *tuh-1* maternal effect. P = parental strain, F1 = first generation, F2A and F2B designate F_2 generation, F3A through F3E designate F_3 generation. n = sample size, $\bar{x}$ = mean number of bristles ± 95% confidence limits, Df denotes *Df JC4* or *Df GA37*, h denotes *tuh-1*^{h}, g denotes *tuh-1*^{g}, * indicates *tuh-1*^{h} or *Df JC4* or *Df GA37*.

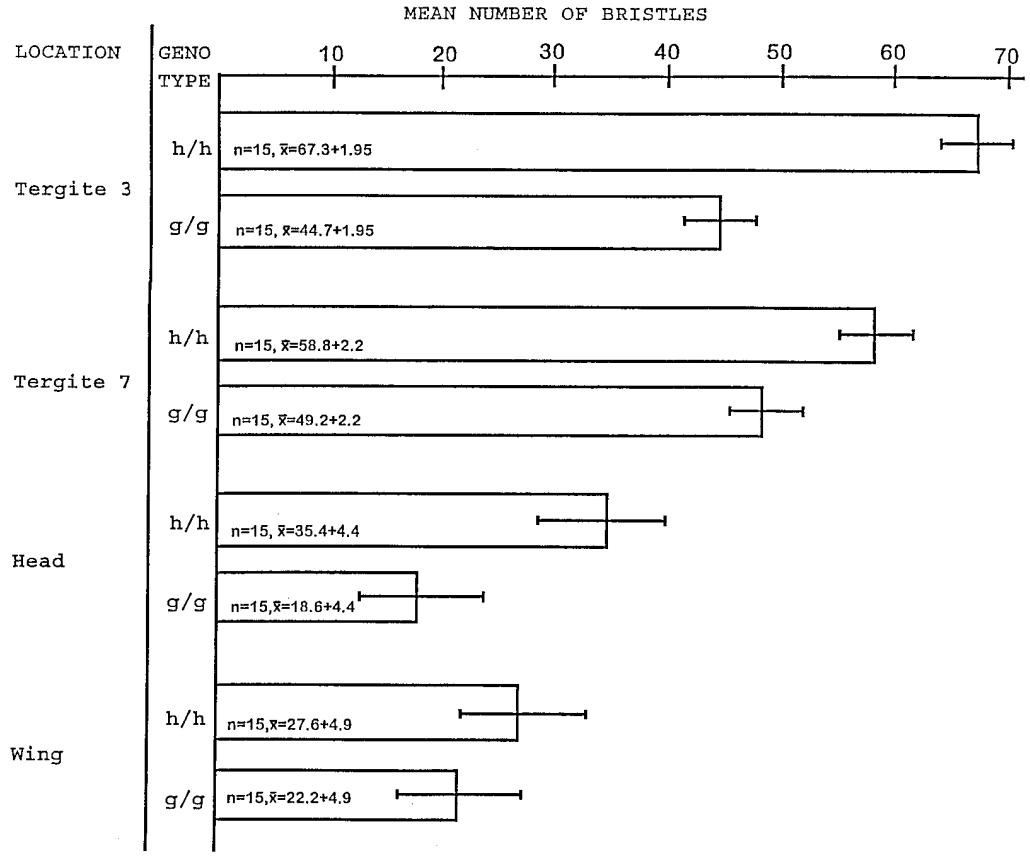

Figure 8.—Evaluation of bristle number on anterior and posterior abdomen, head and wing blade. n = sample size, $\bar{x}$ = mean number of bristles ± 95% confidence limits. h denotes *tuh-1^h*, g denotes *tuh-1^g*.

developed faster than flies hetero- or homozygous for *tuh-1^g*. To test this observation eggs were collected from *v tuh-1^h/Y*, *tuh-1^h/tuh-1^h*, *FMA3 y^2/Y* and *Canton-S* females and placed at 18° and RT. Bottles were examined daily for larval activity. At RT larval activity was observed beginning on day 2 in all bottles. Pupae were visible on day 6 and on day 11 flies began to emerge and continued to emerge to day 13. No differences were observed among the four fly strains. In the bottles maintained at 18° larval activity was observed on day 2 in all bottles. Pupal cases appeared on day 8 and 9 in the *v tuh-1^h/Y* and *tuh-1^h/tuh-1^h* bottles and on days 12 and 13 in the *Canton-S* and the *FMA3 y^2/Y* bottles. Adults started to emerge on day 18 in the *v tuh-1^h/Y* and *tuh-1^h/tuh-1^h* bottles and by day 21 all flies had emerged. In the *Canton-S* and the *FMA3 y^2/Y* bottles flies slowly started to emerge on day 20 and continued to emerge until day 24. While there was no significant difference in developmental time among the four strains at RT, the *tuh-1^h* strains developed faster at 18°.

Variance of bristle number on tergite 7 in females attributable to the *tuh-1* locus in the wild: The phenotypic variance (V_P) for the *tuh-1^h* laboratory strain was calculated as 12.66. The mean for tergite 7 bristles was 56 and the standard deviation was 3.55. At Miami Shores 17 adult females were trapped and analyzed for tergite 7 bristles. The V_P was calculated as 1.14. The mean number of tergite 7 bristles was 43 and the standard deviation was 2.3. The genotypic and environmental components of the variance were determined (Falconer 1981). The results showed that 10.1% of the genetic variance for the bristle phenotype can be attributed to the *tuh-1* locus in the wild.

DISCUSSION

The goal of this study was to identify an observable phenotype for the two isoalleles at the *tuh-1* locus. Originally the *tuh-1* locus was identified as a modifier locus affecting expression of the *tuh-3* mutant gene at the BX-C (Gardner and Woolf 1950; Woolf 1966; Kuhn *et al.* 1981; Kuhn and Packert 1988; Kuhn *et al.* 1993). *tuh-1^h/tuh-1^h*; *tuh-3/tuh-3* females produce offspring with head defects, while *tuh-1^g/tuh-1^g* or *tuh-1^h/tuh-1^g*; *tuh-3/tuh-3* females produce offspring showing genital disc defects (Woolf 1966; Kuhn *et al.* 1981). Here we have shown that the two isoalleles at the *tuh-1* locus have a spectrum of allelic effects that are totally independent of the BX-C mutant *tuh-3*.

The *tuh-1^h* maternal effect causes an increase in bristle number on the adult cuticle: All Drosophila strains homozygous for *tuh-1^h* show an increase in bristle number as compared to flies from strains homozygous for *tuh-1^g*. Backcross data collected over a three generation period supported the above results demonstrating that (1) bristle number is increased in the presence of the *tuh-1^h* maternal effect; (2) the *tuh-1^h* maternal effect allele is completely recessive to *tuh-1^g*; and (3) the *tuh-1*

maternal effects are temperature sensitive, with respect to developmental time.

Two deficiencies, *Df GA37* and *Df JC4* have been shown to behave genetically like *tuh-1*^{h} in the presence of *tuh-3* (Kuhn *et al.* 1993). Offspring from hemizygous mothers, *Df*/*tuh-1*^{h} showed the high bristle number, while offspring from mothers hemizygous for *tuh-1*^{g}, *Df*/*FM6*, consistently showed the lower bristle number. The data suggest that both deficiencies behave like *tuh-1*^{h}. These results are in accordance with a genetic analysis of the deficiencies in the presence of *tuh-3*. Offspring from flies hemizygous for either deficiency showed head defects in the presence of the *tuh-1*^{h} maternal effect allele (D. T. Kuhn, unpublished data). Females *Df GA37*/*Df JC4* are homozygous viable, fertile and behave genetically like females homozygous for the *tuh-1*^{h} allele in the presence of *tuh-3*. Offspring from females *Df GA37*/*Df JC4* have a high bristle number. These results also suggest that *tuh-1*^{h} is either a hypomorph or an amorph of *tuh-1*^{g}. Further analysis of the bristle phenotype showed that the elevated bristle number in offspring from *tuh-1*^{h}/*tuh-1*^{h} mothers was not confined to the posterior abdomen. A significantly higher number of bristles were observed on the occipital region of the head and the anterior abdomen. The same trend was not present in the wing blade. However, some bristle patterns have been highly conserved during evolutionary time and it is possible that this is true for bristle number on the wing blade. On the other hand, it is also possible that *tuh-1* does not influence the wing. The former explanation is currently favored since the BX-C *Ultrabithorax* (*Ubx*) mutant phenotype is extreme in the presence of *tuh-1*^{g}, yet in the presence of the *tuh-1*^{h} maternal effect the mutant phenotype is partially rescued, as are other BX-C regulatory mutants. (G. Packert and D. T. Kuhn, unpublished data).

Bristle number on the adult cuticle of Drosophila is a quantitative trait that has been studied extensively (Robertson 1967; Shrimpton and Robertson 1988a,b; Falconer 1981; Slatkin and Frank 1990; Barton 1990; Long *et al.* 1995; Mackay 1996). The ultimate goal of many of these studies was to identify loci responsible for quantitative variations, to determine how newly arising mutations in these loci affect bristle number, to determine the consequences of pleiotrophy and stabilizing selection on quantitative trait loci (QTLs), and to determine long-term as well as short-term selection responses. The studies generally were based on evaluations of highly selected laboratory strains. They were intended to determine relationships between genetic variations of quantitative traits relative to fitness, or to test effects of natural selection on quantitative traits. In this study none of the stock cultures were selected at any time and yet offspring from females homozygous for *tuh-1*^{h} consistently showed a higher bristle number. The phenotype was independent of the genetic background, suggesting that it can be attributed to the presence of the two naturally occurring isoalleles of *tuh-1* rather than newly arising mutations.

A large number of genes responsible for the proper spacing and morphogenetic development of bristles on the Drosophila cuticle have been isolated and characterized at the molecular level. Alleles of *bobbed* (*bb*) (Frankham *et al.* 1980), *scute* (*sc*) (Yoo 1980), *scabrous* (*sca*) (Mlodzik *et al.* 1990), *Delta* (*Dl*) (Parks and Muskavitch 1993) and insertional mutations in the *achete-scute* region (Mackay and Langley 1990) have all been associated with quantitative variation in abdominal bristle number. Mutations at these loci all have pleiotrophic effects involving various developmental pathways. *sca* also effects eye morphogenesis (Mlodzik *et al.* 1990) and more recently, Lai *et al.* (1994) showed that variation in bristle number was correlated to DNA sequence polymorphism at the *sca* locus. Graba *et al.* (1992) speculate that direct interactions of BX-C genes with *cis*-acting elements in *sca* negatively regulate this gene. Interestingly, like homozygotes deficient for *tuh-1*, the *sca* homozygotes and deficiencies are viable and fertile with no apparent abnormal phenotype. *Dl* is a neurogenic locus that effects viability and development of adult bristles and is also expressed during oogenesis. Temperature studies utilizing *Dl* mutations showed that the phenocritical periods for the development of bristles are during third instar and puparium formation (Parks and Muskavitch 1993).

It is not clear how *tuh-1* interacts with *tuh-3* to cause head defects or genital disc defects nor how it affects bristle number. Since *tuh-1* is a maternal effect locus it is difficult to envision a direct role for *tuh-1* in specification of a morphogenetic trait or neurogenesis. Because of the appearance of head defects and an increase in bristle number in the presence of *tuh-1*^{h}, the recessive isoallele, it could be envisioned that *tuh-1* negatively effects transcription of BX-C genes by interacting with genes that affect the assembly of chromatin complexes. This theory requires the assumption that a functional product is only produced by *tuh-1*^{g}.

Temperature influences the *tuh-1* bristle phenotype: Quantitative traits, such as bristle number on the adult cuticle, are often influenced by environmental factors. Caligari and Mather (1975) showed that chromosome 3 carried many modifiers for sternopleural bristle number. However, chromosome 2 had the greatest influence upon the response to temperature shifts. In another study Schnee and Thompson (1984) showed that genes that determine the response to one temperature are not always linked to genes that determine the response to another temperature. In their study chromosome 3 had the largest effect on mean sternopleural bristle number and also carried genes responding to 18° and 25°, while at 29° chromosome 2 was the most important.

Our results demonstrated that the *tuh-1* maternal effect gene influences bristle number on the adult cuti-

cle. The phenotype is temperature sensitive and the biggest mean for bristle number on the 7th tergite in females is obtained in the presence of the *tuh-1*^{h} maternal effect at 18°. However, in contrast to the two studies cited previously, in our case, both the increase in bristle number and the temperature effect are due to the *tuh-1* maternal effect, since the 2nd and 3rd chromosomes of the strains tested were identical.

The ability of a species to adapt to a change in environment is often determined by its ability to maintain flexible control of its phenotype. Temperature dependent expression of polygenic loci may be an important tool in maintaining genetic variability. Johnson and Gardner (1965) demonstrated that the gene frequency for the *tuh-1*^{h} allele is favored at lower temperatures in natural populations and that a balanced polymorphism exists between the two isoalleles. The *tuh-1* locus therefore appears to be a prime candidate for a locus that plays an important role in interactions between genotype and environment. These implications are manifested further by the observation presented here that flies homozygous for the *tuh-1*^{h} maternal effect allele develop to adulthood significantly faster at 18° than flies heterozygous or homozygous for the *tuh-1*^{g} allele. These results suggest a selective advantage for *tuh-1*^{h} in colder climates and fit well with the observations by Johnson and Gardner (1965) that there is an increase in the presence of the *tuh-1*^{h} allele in wild-type populations as seasons progress and at higher latitudes. Further, we know from this study that only 10.1% of the genetic variance for the bristle phenotype can be attributed to the *tuh-1* locus suggesting that the bristle phenotype has only a minor effect on the maintenance of this polymorphism and may just be a neutral but pleiotrophic side effect of *tuh-1*.

Gardner and Woolf (1950) have shown that in the presence of *tuh-1*^{h} the expression of the *tuh-3* phenotype increases progressively when temperature is increased from 18° to 30° while viability decreases considerably. Accumulation of BX-C transcripts are elevated in embryos produced by *tuh-1*^{h}; *tuh-3* homozygous mothers (Mack 1994; Kuhn *et al.* 1993). One could envision a selective advantage for increased transcription in colder climates. In this sense, *tuh-1*^{h} should have a selective advantage.

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