Genet. Res., Camb. (1993), **61**, *pp.* 177–193 *with 5 text-figures*

Mapping and characterization of *P*-element-induced mutations at quantitative trait loci in *Drosophila melanogaster*

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(*Received 8 December 1992*)

Summary

X chromosomes derived from crosses of inbred P and M *Drosophila melanogaster* strains that had extreme effects on abdominal and/or sternopleural bristle number in males, were further analyzed to determine their effects in females and to map the loci at which the mutations occurred. Seven lines that had on average 3·9 fewer sternopleural bristles than wildtype in males had average homozygous sternopleural bristle effects of −2·2. The bristle effects were partially recessive, with an average degree of dominance of −0·60. Physical mapping of the sternopleural bristle effects of these lines placed them all at approximately 24·7 cM. These mutations are apparently allelic on the basis of a complementation test, and deficiency mapping indicates they occur within chromosomal bands 8A4; 8C6. *In situ* hybridization analysis of the sites of *P* element insertions of these lines suggests that mutations probably resulted from excision of *P* elements at 8C on the original inbred P strain chromosome. Two additional lines, NDC(19) and DP(146), had reduced numbers of sternopleural and abdominal bristles. NDC(19) males had 9·7 fewer abdominal and 8·6 fewer sternopleural bristles than wildtype. The corresponding homozygous abdominal and sternopleural bristle number effects were −5·8 and −3·8, respectively; with the abdominal bristle effect completely recessive and the sternopleural bristle effect nearly additive. DP(146) males had 6·2 fewer abdominal and 4·1 fewer sternopleural bristles than wildtype, with homozygous abdominal bristle effects of −4·3 and sternopleural bristle effects of −2·0. Abdominal bristle effects of this line were partially recessive whereas the sternopleural bristle effects were additive. Physical mapping showed effects on both bristle traits segregated jointly in these two lines, with the NDC(19) mutation closely linked to *y* and the DP(146) mutation 0·17 cM from it. Complementation tests and deficiency mapping also indicate the mutations in lines NDC(19) and DP(146) are at closely linked but separate loci within chromosomal bands 1B2; 1B4-6 and 1B4-6; 1B10 respectively, with some epistatic effects. *In situ* hybridization analysis of sites of *P* element insertion suggest that the NDC(19) mutation, which may be a *scute* allele, was probably caused by a *P* element insertion in the 1B region; the DP(146) mutation is also associated with an insertion at 1B.

1. Introduction

Most economically important traits of plants and animals exhibit continuous variation resulting from the action of multiple genes modified by the environment. Understanding the nature and function of the individual genes controlling such traits has been of interest to animal and plant breeders since the beginning of this century (East, 1916). At least three approaches have been applied to detect individual quantitative trait loci (QTLs). (1) Statistical methods have been suggested for determining whether major genes affect quantitative traits (for reviews, see Hill & Knott, 1989; Mayo, 1989). These methods have rather low power and efficiency, and require very large, well-defined samples. (2) Using either inbred or artificial selection lines which differ markedly in mean performance for a quantitative trait, major determinants of the difference in scores have been mapped to specific chromosomal regions by association with linked morphological (e.g. Thoday, 1979; Shrimpton & Robertson, 1988*a*, *b*) or polymorphic molecular (Paterson *et al.* 1988; Stuber, 1989) markers. These

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methods are limited to identifying genetic 'factors' or 'chromosomal segments' affecting metric traits. (3) Spontaneous or induced mutations have been accumulated in originally inbred stocks, and those affecting the trait of interest fixed by selection (e.g. Mather & Wigan, 1942; Clayton & Robertson, 1955, 1964; Kitagawa, 1967; Hollingdale & Barker, 1971; Cabellero, Toro & Lopez-Fanjul, 1991), or maintained against a balancer chromosome (e.g. Durrant & Mather, 1954; Paxman, 1957; Mukai, 1979; Ohnishi, 1977). Mapping individual new mutations affecting quantitative traits is generally not feasible because of their small effects; instead mutational effects are quantified in terms of their overall contribution to genetic variation (Lynch, 1988). A novel modification of this method is to use transposable elements to induce mutations affecting quantitative traits.

In *Drosophila melanogaster*, *P* element transpositions, excisions, and chromosome rearrangements induced by *P* element mobility, occur at enhanced rates in dysgenic hybrids from a cross of P strain males containing multiple *P* element copies to M strain females without *P* elements (reviewed by Engels, 1989). The term dysgenic derives from a suite of aberrant traits including temperature-sensitive gonadal dysgenesis observed in F_1 hybrids of this cross. *P* element movement and hybrid dysgenic traits are much reduced in frequency in F_1 hybrids of the reciprocal, non-dysgenic, cross. Previously we used crosses of highly inbred P and M strains to assess the effects of one generation of dysgenic and non-dysgenic crosses on inducing quantitative variation for two bristle traits on *X* chromosomes of P and M strain origin, in a common but segregating autosomal background (Lai and Mackay, 1990). Twelve *X* chromosome lines were described with extreme bristle scores in males.

We wished to determine (i) hemizygous, homozygous and heterozygous effects on bristle number of these *X* chromosomes in a highly inbred genetic background; (ii) whether the extreme phenotypes of each line were determined by mutations at one or more loci; (iii) whether mutations at the same or different loci were responsible for similar phenotypic effects of the different *X* chromosome lines; and (iv) whether the mutations affecting bristle number could be associated with insertions of *P* elements. This report documents further characterization of mutational effects in females, allelic complementation tests, and localization of the mutant effects by a combination of genetic mapping relative to morphological markers, using an approach similar to that of Thoday (1979) and Shrimpton & Robertson (1988*a*, *b*), deficiency mapping, and *in situ* hybridization analysis of sites of *P* element insertion.

2. Materials and methods

(i) *Drosophila stocks*

The gene markers and chromosomes used are described in Lindsley & Zimm (1992). All flies were reared on 10 ml agar-yeast-glucose medium in shell vials at 21 °C, unless otherwise specified.

Inbred Samarkand (*Sam*): The history of this highly inbred laboratory strain is described in Lai & Mackay (1990). It is maintained by continuous full-sib inbreeding, and is a pure M strain in the P–M system of hybrid dysgenesis, containing neither complete nor defective *P* elements (Mackay, Lyman & Jackson, 1992).

C(1) DX y w f/Y; Sam (*Sam attached-X*): An attached-*X* stock in which the free *X* and *Y* chromosomes and the autosomes were derived from the inbred Samarkand strain by ten generations of backcrossing attached-*X* females to inbred Sam males. Male progeny of attached-*X*-females receive their *X* chromosome from their father and their *Y* chromosome from their mother.

FM4; Sam: The *FM4* chromosome was substituted into the Sam background by 21 generations of backcrossing to inbred Sam.

FM4; T(2,3) ap^{Xa}*; Sam*: The free autosomes are from inbred Sam.

y ct^6 *t*^{2} *v f car*: A multiply marked *X* chromosome, with autosomes of unknown origin. Male abdominal and sternopleural bristle scores average 17·8 and 20·8, respectively.

ct^6 *t*^{2} *v f car*: A multiply marked *X* chromosome derived from *y ct*^{6} *t*^{2} *v f car*. The autosomes are approximately 75% inbred Sam since it was backcrossed to *C(1) DX*; Sam for two generations before use.

'*Bristle Number Mutant*' *X chromosome lines*: Lai & Mackay (1990, Table 4) described 12 *X* chromosome lines with extreme sternopleural and/or abdominal bristle numbers in males. Lines DP(112), DP(114) and DP(187) had very low fitness and were lost during maintenance. Lines DP(98), DP(103), DP(126), DP(159), DP(164), NDP(108) and NDP(149) had lower sternopleural bristle scores of approximately 15, and wildtype abdominal bristle scores. The letters D and ND denote whether the initial cross was dysgenic or non-dysgenic, respectively, and P indicates P strain origin of the *X* chromosome. Lines DP(146) and NDC(19) had extremely low respective abdominal bristle scores of 11·5 and 10·3, and sternopleural bristle scores of 14·9 and 11·0. NDC(19), originally a non-dysgenic M strain *X* chromosome control, displayed its mutant phenotype during maintenance. Wildtype revertants of lines DP(146) and DP(164) were observed during maintenance, and lines DP(146)W and DP(164)W were derived from them. In addition line DP(146)NAB was derived from an extreme revertant of DP(146) which arose spontaneously during maintenance. It has a mean male

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abdominal bristle score of 1·6 but average sternopleural bristle number not significantly different from that of DP(146). Lines DP(168) and NDP(106) originated from the non-dysgenic cross, P strain *X* chromosome set of lines generated by Lai & Mackay (1990). They were chosen as representative wildtype control chromosomes. Line NDP(168) had average male abdominal and sternopleural bristle scores of 17·5 and 19, respectively. Male abdominal and sternopleural bristle scores of line NDP(106) were 17·4 and 19·4, respectively. The above lines were maintained for at least 10 generations by backcrossing to inbred Sam before being used in the experiments described below.

(ii) *Homozygous, heterozygous and hemizygous effects*

Lai & Mackay (1990) reported average abdominal and sternopleural bristle numbers of F_2 males derived from dysgenic and non-dysgenic crosses, and thus with segregating autosomal backgrounds. The following crosses were performed to ascertain the effects of the extreme chromosomes in hemizygous males, and homozygous and heterozygous females, in the Sam inbred genetic background. *FM4; T(2,3)ap*^{Xa}; Sam females were crossed to males from each of the extreme *X* chromosome lines described above, then F_1 females heterozygous for *FM4* and the *ap*^{Xa} marker were backcrossed to *FM4*; Sam males. In the following generation females heterozygous for *FM4* and *ap*^{Xa} were mated to *ap*^{Xa} males with the mutant bristle number phenotype. Finally homozygous females and hemizygous males for the extreme *X* chromosome were mated *inter se*. Heterozygous effects of the P-strain-derived *X* chromosome lines (all but NDC(19)) were assessed on F_1 females derived by crosses to the wildtype NDP(168) P-strain-derived *X* chromosome line, also made homozygous in the Sam inbred background. Heterozygous effects of the Samarkand-derived *X* chromosome line NDC(19) were evaluated in F_1 females obtained from crosses to inbred Sam.

With the exception of lines NDC(19) and DP(146)NAB, homozygous females of each *X* chromosome line were crossed to males of NDP(168). Females homozygous for the DP(146)NAB *X* chromosome are not viable, so heterozygous females were produced from the reciprocal cross of NDP(168) females to DP(146)NAB males. Females homozygous for the NDC(19) *X* chromosome are sterile, so Sam inbred females were crossed to males of this line to produce females heterozygous for the NDC(19) chromosomes. After two days the NDC(19) males were re-mated to *FM4*; Sam females. Heterozygous *FM4* F_1 female progeny of this cross were backcrossed to NDC(19) males to derive females homozygous for the NDC(19) *X* chromosome. Two replicate vials each containing 10 females and males were made for all crosses. Sternopleural (the sum of the number of sternopleural bristles on the right and left sternopleural plates) and abdominal (the number of bristles on the posterior abdominal sternite) bristle numbers were recorded on the 10 homozygous female parents of each replicate (and 10 from each of the replicate vials producing NDC(19) chromosome homozygotes), 40 F_1 heterozygous females from each replicate, and 15–20 males of each of the *X* chromosome lines.

Effects in females: Homozygous effects of the extreme *X* chromosomes were estimated as $a = (M_{mm} - M_{++})/2$, and heterozygous effects as $d = M_{m+} - (M_{mm} + M_{++})/2$ (Falconer, 1989), where M_{mm} and M_{m+} are the respective means of the homozygous and heterozygous extreme *X* chromosomes, and M_{++} is the mean of the homozygous wildtype *X* chromosome. Standard errors (SE) of *a* and *d* were estimated as $SE(a) = 0{\cdot}5\sqrt{(V_{mm}/n_1 + V_{++}/n_2)}$ and $SE(d) = \sqrt{[V_{m+}/n_3 + 0{\cdot}25(V_{mm}/n_1 + V_{++}/n_2)]}$ where n_1, n_2, n_3, V_{mm}, V_{++} and V_{m+} are the sample sizes and pooled variances over replicates of homozygous mutant females (mm), homozygous wildtype females (++) and heterozygous females (m+), respectively.

Effects in males: Hemizygous effects of extreme chromosomes were estimated as $\alpha = M_m - M_+$, where M_m and M_+ are the means of the males with extreme and wildtype *X* chromosomes, respectively. Standard errors were estimated as $SE(\alpha) = \sqrt{(V_m/n_1 + V_+/n_2)}$, where n_1, n_2, V_m and V_+ are the sample sizes and pooled variances over replicates of males with extreme and wildtype *X* chromosomes, respectively.

(iii) *Genetic mapping*

Males from each of the extreme *X* chromosome lines and the NDP(168) wildtype control line were crossed to *y ct*^{6} *t*^{2} *v f car* females in four replicate vials each containing 8 pairs of flies. F_1 females were backcrossed to *y ct*^{6} *t*^{2} *v f car* males, with 20 replicate vials of 10 pairs of flies for each *X* chromosome line. The procedure then differed according to whether or not the recombinant males could be classified unambiguously for the mutant bristle number phenotype.

Lines DP(98), DP(103), DP(126), DP(159), DP(164), NDP(149) and NDP(108): These lines have similar sternopleural bristle scores of approximately 15 in males. Because the *t*^{2} phenotype could not be scored reliably when flies were *y*, recombinant males were initially assigned to four classes: ++*v f car*, *y*++*f car*, *y ct*^{6}++*car* and *y ct*^{6} *v*+++. The *X* chromosome of each mutant line was thus divided into four separate 'Sections' flanked by adjacent marker loci. Section 1 is the chromosomal segment between the *y* and *ct* loci, and is 20 cM in length, similarly Section 2 is 13 cM, Section 3 is 23·7 cM, and Section 4 is 5·8 cM (Figure 1). Section 1 recombinant males were then further classified into ++*t*^{2} *v f car* and +++*v f car*. Sternopleural bristle numbers were recorded on samples of 10–20 male recombinant flies containing different sections of each mutant *X* chromosome.

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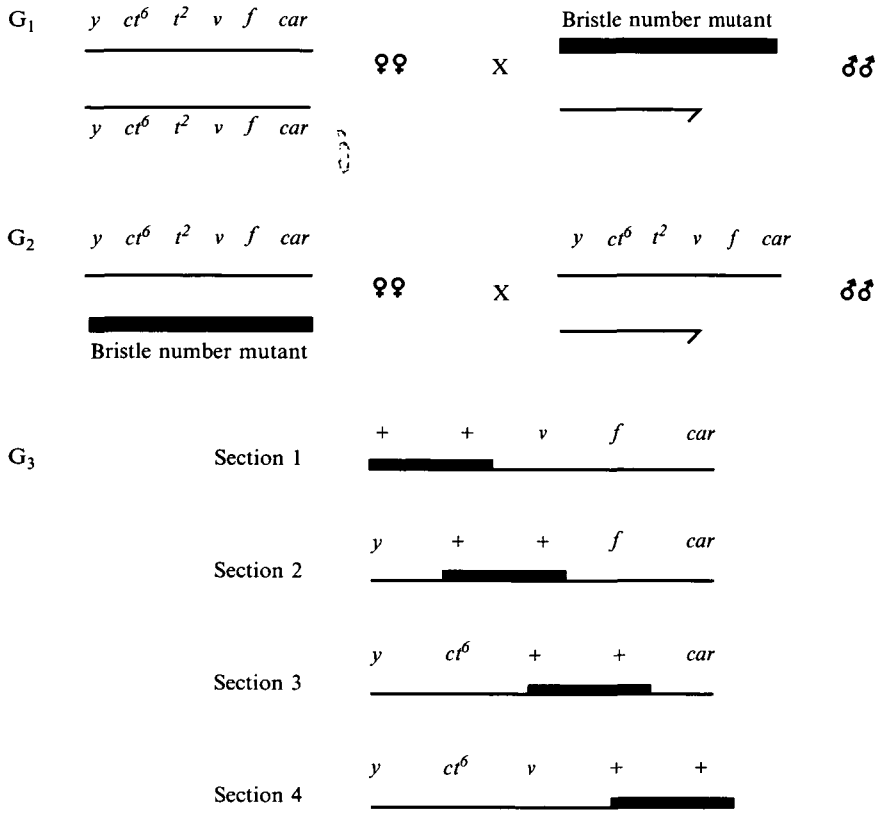

Fig. 1. Crossing scheme to map mutations affecting bristle number to *X* chromosome sections. *y ct*^{6} *t*^{2} *v f car* females were crossed to males of *X* chromosome lines with extreme bristle numbers (G_1), then female progeny were backcrossed to males of the marker stock (G_2). G_3 recombinant males were classified into four genotypes in which wildtype chromosome sections were bounded by markers (the *t*^{2} marker was ignored temporarily). Section 1 is the chromosomal segment between the *y* and *ct* loci, Section 2 is between *ct* and *v*, Section 3 is between *v* and *f*, and Section 4 is between *f* and *car*.

Control crosses were made using the wildtype stock NDP(168).

All of these extreme lines appeared to contain a mutation (or mutations) in the *ct*^{6} − *t*^{2} interval. The map positions of the mutation(s) were more precisely estimated by crossing males of the bristle number mutant *X* chromosome lines to *ct*^{6} *t*^{2} *v f car* females, then backcrossing F_1 females to *ct*^{6} *t*^{2} *v f car* males. Fifty *ct*^{6}+ and 50 + *t*^{2} recombinant males were collected for each mutant *X* chromosome in the following generation, and their sternopleural bristle numbers recorded. However, classifying single recombinant males as wildtype or mutant on the basis of their individual bristle scores could lead to a large sampling error for the estimate of map position. To derive more precise estimates, individual recombinant males were crossed to 5 *C(1) DX*; Sam females, and 10 male offspring were scored for sternopleural bristle number (Figure 2). Therefore, approximately 50 *ct*^{6}+ and 50 + *t*^{2} recombinant *X* chromosome lines were established for each mutant line. Based on the line means, the *ct*^{6}+ and + *t*^{2} recombinant *X* chromosomes were aligned in the order of minimum to maximum sternopleural bristle scores, and grouped into wildtype and mutant categories using the criterion that the variance among means within each group was minimal. The map position (p) of each mutant gene within Section 2 was estimated by the method of maximum likelihood as $p = (n_1 + n_2)/n$, with standard error $SE(p) = \sqrt{(p(1-p)/n)}$, assuming double crossovers between *ct*^{6} and *t*^{2} are negligible. n_1 is the number of mutant lines among recombinant lines of *ct*^{6}+, n_2 is the number of wildtype lines among recombinant lines of + *t*^{2}, and n is the total number of *ct*^{6}+ and + *t*^{2} recombinant lines. Since the map distance between *ct* and *t* is 7·5 cM, the approximate map distance of each mutant to the *ct* locus in centimorgans is estimated as 7·5p.

Lines NDC(19), DP(146) and DP(146) NAB: These lines can be distinguished unambiguously from the wildtype and the other mutant lines by their exceptionally low bristle scores. Recombinant males produced by the crosses shown in Figure 1 were scored directly for their bristle phenotypes and morphological markers. Map position was estimated as described above, but using bristle numbers of individual recombinant males since no error is introduced by scoring individuals.

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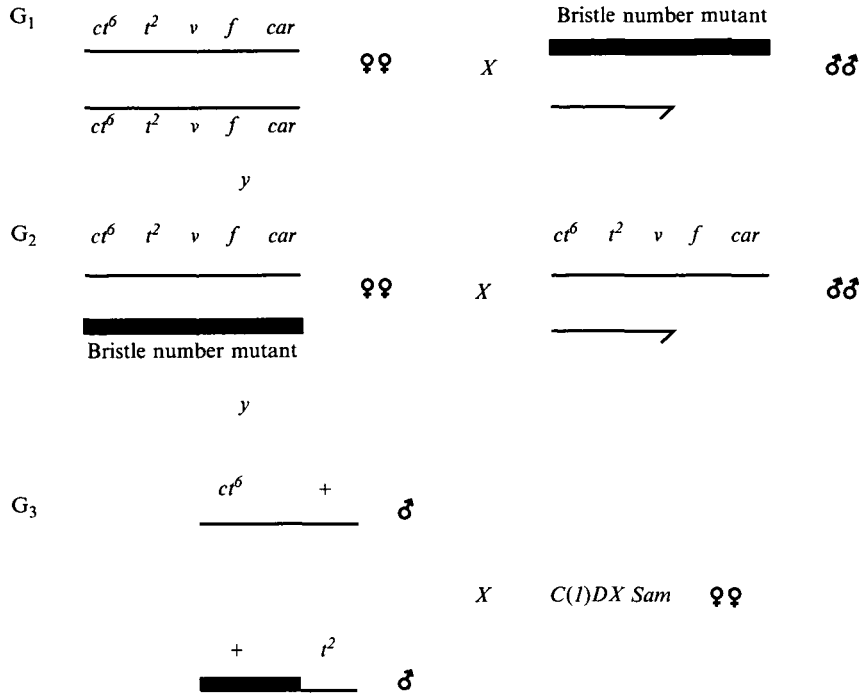

Fig. 2. Crossing scheme to estimate map distance to *ct* of bristle number mutations localized to the $ct^6 - t^2$ interval. $ct^6\ t^2\ v\ f\ car$ females were crossed to males of each of the *X* chromosome lines with extreme bristle numbers (G_1), then female progeny were backcrossed to males of the marker stock (G_2). G_3 recombinant ct^6+ and $+t^2$ males were collected and crossed individually to *C(1)DX*; Sam females to establish recombinant inbred lines for each bristle number mutant line.

Table 1. *Deficiency stocks*

Genotype	Breakpoints
Dp(1;f)107/In(1)sc⁸, Df(1)sc⁸, w⁸	1A1; 1B2
Df(1)260-1, y/FM4	1A1; 1B4-6
Df(1)svr, spl ras² fw/y²Y67g/C(1)DX, y f	1A1; 1B9-10
Df(1)64c18, g sd/w⁺ Y/C(1)DX, y w f	2E1; 3C2
Df(1)RA2/FM7c	7D10; 8A4-5
Df(1)KA14/FM7c	7F1-2; 8C6
Df(1)su(s)83, y cho ras v/y²scY/C(1)DX, y f	1B10; 1D6-E1

(iv) *Allelic complementation tests*

Tests for allelism of mutations in the different *X* chromosome lines were conducted by crossing pairs of lines in two replicates with 10 male and 10 female parents each, and recording bristle numbers of 20 F_1 females per replicate. Flies were reared on agar-glucose-yeast medium at 25 °C in shell vials.

The test of allelism of mutant effects in the extreme *X* chromosome lines proceeded in stages. Lines DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149) (Group 1) had similar sternopleural bristle numbers, and were tested for allelism by constructing all possible pairwise crosses. Only sternopleural bristle numbers were recorded on F_1 heterozygous females. Lines DP(146) and NDC(19) (Group 2) had extreme abdominal and sternopleural bristle scores, and were tested for allelism with each other. Both bristle traits were scored on F_1 females. Finally, F_1 females of the cross between DP(146) and DP(164) were scored for sternopleural bristle number to test for complementation of bristle effects between Group 1 and Group 2 lines.

(v) *Deficiency mapping*

Deficiency stocks with breakpoints spanning the regions in which genetic mapping indicated the bristle number mutations were located were obtained from Indiana University Drosophila Stock Center. They are listed in Table 1. Flies were raised on 10 ml cornmeal-agar-molasses medium at 25 °C in shell vials.

Lines DP(146) and NDC(19): Since the stocks containing *Df(1)sc⁸* and *Df(1)260–1* have deficiencies only in heterozygous females, 10 males from the mutant lines were mated with 10 females of each of these stocks. Only males from the strains with *Df(1)*

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svr, *Df(1)64c18* and *Df(1)su(s)83* contain the deficiencies, therefore 10 homozygous females of DP(146) were crossed to 10 of these deficiency males, while 10 NDC(19) males were mated with 10 heterozygous females between *FM4*; Sam females and these deficiency stock males. F_1 females without *FM4* were collected to measure abdominal and sternopleural bristle scores.

Lines DP(*98*), *DP(126)*, *DP(159)*, *DP(164)*, *NDP (108) and NDP(149)*: As the mutations in these lines are apparently allelic, only one of the lines, DP(126), was used in the test. 10 DP(126) males were crossed to females from stocks with *Df(1)RA2* and *Df(1)KA14*, and F_1 females without the *FM7c* phenotype were collected for counting sternopleural bristles.

Line NDP(168): For each deficiency stock a control cross was conducted in the same manner as the test cross using this wildtype strain.

(vi) In situ *hybridization*

Since the bristle number mutant lines were derived from crosses in which *P* elements were mobilized, *in situ* hybridization was used to correlate the presence/absence of *P* elements in the region to which the mutations map with the mutant phenotypes. Lines DP(164), DP(98), DP(126), NDC(19) and DP(146) were assessed once for sites of insertion of *P* elements within six generations of their discovery. Subsequently all lines were backcrossed to *C(1)DX*; Sam for more than 10 generations, then derived into homozygous strains and maintained for about 30 generations before assessing them for sites of insertion of *P* elements. Line NDC(19) is sterile when homozygous in females, so was maintained by backcrossing to *C(1)DX*; Sam for more than 20 generations with occasional relaxation. In addition, wildtype line DP(106), lines DP(164) W and DP(146) W, established from wildtype revertant flies from bristle number mutant lines DP(164) and DP(146), and recombinant lines NDC (19)+ct^6t^2vfcar and DP(146)+ct^6t^2vfcar derived from the mapping experiment, were also assessed for sites of *P* element insertion.

The larvae were raised at 18 °C in cornmeal-agar-molasses medium. The technique of *in situ* hybridization used was based on the method described by Shrimpton, Montgomery & Langley (1986) and Leigh Brown & Moss (1987). Plasmid pπ25·1 (O'Hare & Rubin, 1983) was used as the *P* element probe, labelled with biotinylated dATP (bio-7-dATP, BRL) by nick translation. Hybridization was detected using the ABC kit (BRL) and visualized with horseradish peroxidase (diaminobenzidine).

Cytological positions (sites) of *P* element homology were recorded only for the *X* chromosome of each line. Each slide was examined twice at magnification 630 under oil immersion, and elements assigned to bands on photocopies of Lefevre's (1976) photographic map of the salivary chromosomes. Five to ten slides were prepared and scored from each line. Fixed sites, present in all individuals sampled within a line, and polymorphic sites, which include all sites present at least once in the sample, were summarized for each line on a Bridges' (1938) polytene map.

3. Results

(i) *Homozygous, heterozygous and hemizygous effects*

Effects of the extreme *X* chromosomes in the inbred Sam genetic background on mean male and female sternopleural and abdominal bristle numbers are given in Table 2. Bristle numbers in females heterozygous for the mutant *X* chromosomes were assessed over the NDP(168) control *X* chromosome (except line NDC (19), which was assessed over the inbred Sam *X* chromosome), also in the inbred Sam autosomal background. Estimates of homozygous (a), heterozygous (d) and hemizygous (α) effects, $\pm$standard errors, are given in Table 3. Also given in Table 3 is a measure of the degree of dominance of the mutant *X* chromosomes, $-d/a$. This ranges from -1 (mutant phenotype completely recessive) through 0 (strictly additive effects) to $+1$ (mutant phenotype completely dominant) (Falconer, 1989).

Sternopleural bristle number: All lines have significant effects on sternopleural bristle number. Line NDC(19) has the largest homozygous effect of $-3{\cdot}75$ bristles, the remaining lines have similar homozygous effects that average $-2{\cdot}14$ bristles. The estimates of heterozygous effects range from 0·89 to 1·74, but are not significantly different from each other (t test, $P > 0{\cdot}05$). However, analysis of variance of sternopleural bristle scores of heterozygous females from the seven *X* chromosome lines with sternopleural bristle effects only, revealed significant heterogeneity among the line means. In particular, the mean sternopleural bristle number of lines DP(103) and DP(126) are significantly smaller ($P < 0{\cdot}05$), and the mean of line NDP(149) is significantly larger ($P < 0{\cdot}05$), than the others. Differences among the means of homozygous females of these lines for the same trait are not significant. This suggests the mutation(s) in this group of lines may have somewhat different dominance properties, although the actual estimates of d are not significantly different. Estimates of the degree of dominance for the seven lines with effects on only sternopleural bristles average $-0{\cdot}60$, indicating partially recessive mutant effects. In contrast, lines DP(146) and NDC(19), which have effects on both bristle traits, have more nearly additive effects on sternopleural bristle number (average $d/a = -0{\cdot}20$).

Baker & Belote (1983) found that Drosophila genes on the single *X* chromosome of males are regulated to produce the same amount of product as the two female copies, and this phenomenon of dosage compensation operates at the transcriptional level. As a consequence, the effects of mutations on bristle

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Table 2. *Mean (±standard error) abdominal and sternopleural bristle numbers of control and bristle number mutant X chromosome lines*

	Abdominal bristle number			Sternopleural bristle number		
		Female			Female	
Line	Male	Homozygote	Heterozygote	Male	Homozygote	Heterozygote
NDP(168)	17·30±0·273	20·40±0·380	—	19·28±0·253	19·65±0·365	—
Sam	19·30±0·298	22·35±0·357	—	20·75±0·189	21·05±0·256	—
DP(98)	17·36±0·411	20·55±0·398	19·92±0·289	15·42±0·229	15·00±0·343	18·95±0·273
DP(103)	17·08±0·571	18·39±0·593	19·92±0·291	15·40±0·289	15·05±0·322	18·24±0·236**
DP(126)	18·10±0·362	19·55±0·722	19·83±0·280	15·55±0·223	15·10±0·386	18·33±0·244*
DP(146)	11·15±0·314	11·80±0·337	17·93±0·261	15·16±0·377	15·75±0·321	18·04±0·221
DP(159)	17·82±0·685	21·45±0·629	20·01±0·285	15·40±0·221	15·30±0·431	19·04±0·229
DP(164)	17·31±0·463	21·00±0·527	19·96±0·287	15·38±0·340	15·65±0·264	18·80±0·245
NDP(108)	17·80±0·395	21·35±0·530	19·99±0·302	15·30±0·242	15·50±0·342	18·63±0·232
NDP(149)	17·18±0·325	20·05±0·591	20·43±0·258	15·35±0·209	15·65±0·366	19·38±0·262**
NDC(19)	9·57±0·369	10·75±0·481	22·54±0·324	12·14±0·261	13·55±0·530	18·16±0·225
DP(146)NAB	1·60±0·536	Lethal	17·90±0·319	15·50±0·423	Lethal	18·19±0·284

** $P < 0·01$, * $P < 0·05$.

Table 3. *Homozygous (a), heterozygous (d) and hemizygous (α) effects (±standard error) of extreme X chromosomes on sternopleural (ST) and abdominal (AB) bristle number*

		Effect			
Line	Bristle trait	$-a$	d	$-d/a$	$-\alpha$
DP(98)	ST	2·33±0·250	1·63±0·370	0·70	3·86±0·341
DP(103)	ST	2·30±0·243	0·89±0·339	0·39	3·88±0·384
DP(126)	ST	2·28±0·266	0·96±0·361	0·42	3·73±0·337
DP(159)	ST	2·18±0·282	1·57±0·364	0·72	3·88±0·336
DP(164)	ST	2·00±0·225	1·15±0·333	0·58	3·90±0·424
NDP(108)	ST	2·08±0·250	1·06±0·341	0·51	3·98±0·350
NDP(149)	ST	2·00±0·258	1·73±0·368	0·87	3·93±0·328
DP(146)	AB	4·30±0·254	1·83±0·364	0·43	6·15±0·416
	ST	1·95±0·243	0·34±0·328	0·17	4·12±0·454
NDC(19)	AB	5·80±0·300	5·99±0·441	1·03	9·73±0·474
	ST	3·75±0·294	0·86±0·370	0·23	8·61±0·322
DP(146)NAB	AB	Lethal	—	—	15·70±0·602
	ST	—	—	—	3·78±0·493

traits in males (α) are expected to equal twice the homozygous effects in females ($2a$). This is true for all sternopleural bristle effects; in no case are estimates of α and $2a$ significantly different.

Abdominal bristle number: Only three lines have significant effects on abdominal bristle number; effects not significantly different from zero are not listed. Line NDC(19) has the most extreme homozygous effects, and is completely recessive. Line DP(146) is somewhat less extreme, and is partially recessive. Males from line DP(146)NAB are nearly devoid of abdominal bristles, but this chromosome is homozygous lethal. Estimates of α and $2a$ are not significantly different for line NDC(19), in accord with expectation (Baker & Belote, 1983), but $2a$ for line DP(146) is significantly greater than the effect in males.

(ii) *Genetic mapping*

Locations of the mutations causing extreme bristle phenotypes were determined by mapping relative to standard morphological markers.

Lines DP(98), DP(103), DP(126), DP(159), DP(164), NDP(149) and NDP(108): Males of these lines have on average 4 sternopleural bristles less than wildtype, but wildtype abdominal bristle numbers; therefore only sternopleural bristle number of recombinant males was recorded. Table 4 gives the mean sternopleural bristle numbers of recombinant males of each of these lines in the four wildtype sections bounded by morphological markers into which the *X* chromosomes were initially divided. Comparison with the bristle numbers of the wildtype NDP(168) recombinants in the same intervals indicates the mutation(s)

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Table 4. *Mean (±standard error) sternopleural bristle numbers of recombinant male genotypes. Numbers of recombinant males scored are given in brackets*

	Recombinant classes			
Line	$++v\,f\,car$	$y++f\,car$	$y\,ct^6++car$	$y\,ct^6\,v++$
DP(98)	18·00±0·451 (25)	17·25±0·446 (12)	19·88±0·875 (8)	20·11±0·301 (18)
DP(103)	17·53±0·289 (38)	16·88±0·580 (8)	20·00±1·023 (7)	20·67±0·518 (18)
DP(126)	19·00±0·562 (19)	18·35±0·428 (17)	19·70±0·360 (7)	20·35±0·386 (20)
DP(159)	17·55±0·400 (19)	17·00±0·392 (13)	20·30±0·559 (10)	20·36±0·326 (27)
DP(164)	18·11±0·307 (37)	17·52±0·328 (21)	20·38±0·680 (8)	19·79±0·379 (19)
NDP(149)	18·00±0·417 (23)	17·00±0·370 (20)	19·20±0·916 (5)	20·22±0·475 (18)
NDP(108)	17·94±0·503 (17)	16·60±0·371 (10)	20·20±0·860 (5)	21·10±0·318 (18)
NDP(168) (Wildtype)	19·44±0·428 (16)	22·25±0·552 (12)	20·17±0·543 (6)	19·56±0·250 (16)

Table 5. *Mean (±standard error) sternopleural bristle numbers of recombinant males after classification of $++v\,f\,car$ recombinants according to their t^2 phenotype. N is the number of recombinant males scored*

Line	N	$++t^2\,v\,f\,car$	N	$+++v\,f\,car$
DP(98)	15	19·20±0·490	10	16·70±0·474
DP(103)	21	18·33±0·340	17	16·53±0·375
DP(126)	12	20·08±0·621	7	17·14±0·670
DP(159)	10	18·40±0·476	9	16·11±0·389
DP(164)	24	18·29±0·428	13	17·77±0·378
NDP(149)	12	19·25±0·604	9	17·67±0·624
NDP(108)	9	19·56±0·377	8	16·13±0·398

in these lines are in Sections 1 or 2 (i.e. the $y-v$ interval). Further classification of $++v\,f\,car$ recombinants into $++t^2\,v\,f\,car$ and $+++v\,f\,car$ groups suggests the mutations are in Section 2, between the ct^6 and t^2 markers (Table 5).

To estimate map positions more precisely, mean sternopleural bristle numbers were determined from approximately 50 ct^6+ and 50 $+t^2$ recombinant inbred *X* chromosome lines derived from each of lines DP(126), DP(159), DP(164), DP(108) and DP(149). (For details of the recombinant inbred line means and their grouping into wildtype and mutant bristle number categories, see Lai, 1990). The number of mutant and wildtype lines among ct^6+ and $+t^2$ recombinant classes, and map positions estimated from these numbers, are given in Table 6(*a*). The symmetrical proportions of the wildtypes and mutants within the ct^6+ and $+t^2$ recombinant classes further confirms the deduction that mutations in these five lines are present in the ct^6-t^2 interval. The estimated map distances to *ct* of these lines are not significantly different (the χ^2 statistic from the homogeneity test with 4 degrees of freedom is 0·88, $0{\cdot}90 < P < 0{\cdot}95$; Snedecor and Cochran, 1971). It is possible the mutations in each of these lines are at the same locus.

Survival of recombinant lines derived from individual ct^6+ and $+t^2$ recombinant males of line DP(98) was poor, so the map position given in Table 6(*a*) for the mutation(s) causing the low bristle number phenotype of this line was based on the phenotypes of the individual ct^6+ and $+t^2$ recombinant males. The estimated map distance to *ct* is approximately 1 cM different from the other lines in this group, and is possibly inaccurate because of errors in assigning individual males to wildtype and mutant sternopleural bristle categories. Therefore an alternative procedure to determine the location of the DP(98) bristle mutation was conducted whereby m t^2 recombinant males derived from DP(98) and the $y\,ct^6\,t^2\,v\,f\,car$ marker stock were crossed to wildtype Sam females. Then F_1 females were backcrossed to m t^2 males, and 100 male offspring were collected at random and mated individually to 5 *C(1)DX*; Sam females to give 100 recombinant lines. Sternopleural bristle scores were counted on 10 males from each line. The lines were classified into four groups according to their means and t^2 phenotype: 71 were ++, 25 were m t^2, 2 were +m and 2 were $+t^2$ (where m is the mutant DP(98) sternopleural bristle phenotype). The map distance to *ct* was estimated from these data to be 3·5 cM, in close agreement with that obtained by scoring recombinant individuals.

The DP(103) mutation was initially indicated to be in the ct^6–t^2 interval (see above). However, later mapping to localize the precise position yielded no recombinant between ct^6 and t^2. *In situ* hybridization

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Table 6. *Mapping X chromosome mutations affecting bristle number. (a) Lines with sternopleural bristle number effects; (b) Lines with effects on sternopleural and abdominal bristle number. Map distances are estimated as described in the text*

(*a*)	Recombinant genotype						
	ct^6+		$+t^2$				
Line	Mutant n_1	Wild	Mutant	Wild n_2	n	p ± S.E.	Distance to *ct* (cM)
DP(98)	24	26	27	24	101	0·475 ± 0·049	3·56
DP(126)	33	17	17	32	99	0·657 ± 0·048	4·92
DP(159)	21	17	7	20	65	0·615 ± 0·060	4·62
DP(164)	28	17	11	26	82	0·657 ± 0·052	4·94
NDP(108)	23	15	18	28	84	0·607 ± 0·053	4·58
NDP(149)	22	18	13	28	81	0·617 ± 0·054	4·63

(*b*)	Recombinant genotype						
	$y+$		$+ct^6$				
Line	Mutant n_1	Wild	Mutant	Wild n_2	n	p ± S.E.	Distance to *y* (cM)
DP(146)	1	340	242	4	587	0·008 ± 0·004	0·17
NDC(19)	0	515	72	0	587	0·000 ± 0·000	0·00

Table 7. *Abdominal bristle number phenotypes of male recombinants of Line DP(146) NAB with morphological markers. The numbers in the table are the numbers of males of each recombinant genotype with abdominal bristle numbers characteristic of the extreme revertant line DP(146) NAB, the parental line from which it was derived (DP(146)), and wildtype*

Recombinant genotype					Bristle number phenotype		
					DP(146) NAB	DP(146)	Wildtype
+	ct^6	*v*	*f*	*car*	3	35	18
+	+	*v*	*f*	*car*	1	36	15
+	+	+	*f*	*car*	0	82	30
+	+	+	+	*car*	0	4	0
y	+	+	*f*	*car*	0	1	16
y	ct^6	+	+	*car*	0	0	6
y	ct^6	*v*	+	+	0	0	36
+	ct^6	*v*	*f*	+	4	1	0
+	+	+	+	+	149	49	38

analysis of F_1 female larvae of line DP(103) males and inbred Sam females showed an inversion with breakpoints at approximately 5B and 8D (sites of *P* element insertions) on the DP(103) *X* chromosome (Lai, 1990). Evidently the inversion occurred in the four generation interval between the two mapping experiments. Line DP(103) was not included in subsequent genetic analyses.

Lines NDC(19), DP(146) and DP(146) NAB: These lines have extreme abdominal and sternopleural bristle number phenotypes (see Table 3). Nearly all recombinant males of lines NDC(19) and DP(146) from the cross depicted in Figure 1 of genotype $+ct^6\ t^2\ v\ f\ car$ displayed the mutant bristle number phenotypes, indicating the mutations carried by these lines are in the y–ct^6 interval. The mean (± S.E.) abdominal and sternopleural bristle numbers of a sample of $20+ct^6\ t^2\ v\ f\ car$ males from NDC(19) were 10·35 ± 0·284 and 12·85 ± 0·342, respectively. Twenty recombinant males of this genotype from DP(146) had respective average abdominal and sternopleural bristle scores of 9·65 ± 0·264 and 15·7 ± 0·147. More precise estimations of map position based on numbers of mutant and wildtype bristle phenotypes among $y+$ and $+ct^6$ recombinants are given in Table 6(*b*). Bristle number mutations in both lines are apparently close to the *y* locus at the tip of the *X* chromosome. The NDC(19) mutation is tightly linked to the *y* locus; no crossovers separating it from *y* occurred in 587 chromosomes. Although the map positions of the mutations in these lines are not significantly different, the two lines are phenotypically distinct with respect to both bristle phenotypes and fitness traits. Line NDC(19) has fewer sternopleural bristles than does line DP(146), and is sterile when homozygous, whereas the DP(146) homozygote is fertile.

The disproportionate number of $+ct^6$ and $y+$ recombinants is probably due to low viability associated with the bristle mutations. Based on the data of Table 6(*b*), and given mutations in both lines are closely linked to the *y* locus, their relative viabilities (v) can be estimated approximately as $v = 72/515 - 0{\cdot}140$ for NDC(19), and $v = 0{\cdot}712$ for DP(146) (Mackay, 1986). These lines have sternopleural bristle

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Table 8. *Mean (±standard error) sternopleural bristle scores of F_1 females from crosses among X chromosome lines with similar bristle number phenotypes*

	DP(98)	DP(126)	NDP(149)	NDP(108)	DP(159)	DP(164)
DP(98)	15·63±0·220	15·25±0·270	15·63±0·206	15·68±0·299	15·63±0·204	15·08±0·223
DP(126)	—	14·83±0·279	15·10±0·280	15·20±0·296	15·10±0·259	14·63±0·277
NDP(149)	—	—	15·13±0·257	15·28±0·218	15·33±0·255	15·30±0·247
NDP(108)	—	—	—	15·58±0·256	15·18±0·215	15·30±0·235
DP(159)	—	—	—	—	15·23±0·258	14·83±0·215
DP(164)	—	—	—	—	—	15·43±0·230

Table 9. *Deficiency mapping. Mean (±standard error) abdominal (AB) and sternopleural (ST) bristle numbers of females heterozygous for deficiency chromosomes and control (NDP(168)) and bristle number mutant X chromosomes. N is the number of females scored. ND = not determined*

Genotype	Breakpoints	N	AB	ST
NDP(168)/Df(1) sc^8	1A1;1B2	15	21·20±0·499	20·20±0·449
NDP(168)/Df(1) 260-1	1A1;1B4-6	17	16·88±0·283	17·00±0·332
NDP(168)/Df(1) svr	1A1;1B9-10	27	15·89±0·299	15·38±0·278
NDP(168)/Df(1) su(s)83	1B10;1D6-E1	12	19·92±0·609	21·25±0·579
NDP(168)/Df(1) 64c18	2E1;3C2	17	17·18±0·335	17·95±0·277
NDP(168)/Df(1) RA2	7D1;8A4-5	31	ND	17·71±0·246
NDP(168)/Df(1) KA14	7F1-2;8C6	26	ND	17·77±0·250
DP(146)/Df(1) sc^8	1A1;1B2	17	20·70±0·731	18·00±0·374
DP(146)/Df(1) 260-1	1A1;1B4-6	19	14·63±0·392	16·53±0·370
DP(146)/Df(1) svr	1A1;1B9-10	16	10·81±0·400	13·94±0·544
DP(146)/Df(1) su(s)83	1B10;1D6-E1	12	19·33±0·512	18·83±0·534
DP(146)/Df(1) 64c18	2E1;3C2	16	17·44±0·483	17·50±0·250
NDC(19)/Df(1) sc^8	1A1;1B2	18	20·39±0·479	16·83±0·519
NDC(19)/Df(1) 260-1	1A1;1B4-6	Lethal	—	—
NDC(19)/Df(1) svr	1A1;1B9-10	Lethal	—	—
DP(126)/Df(1) RA2	7D1;8A4-5	52	ND	16·60±0·135
DP(1326)/Df(1) KA14	7F1-2;8C6	25	ND	13·92±0·208

scores similar to those of the group of lines discussed above. However, the observation that both bristle phenotypes are extreme in $+ct^6\ t^2\ v\ f\ car$ recombinants suggests the mutations in NDC(19) and DP(146) are single gene mutations with pleiotropic effects on two bristle traits, rather than two independent mutations each affecting one bristle trait.

Line DP(146)NAB has nearly no abdominal bristle in males, and was established from a spontaneous revertant of line DP(146). The genotypes of recombinant males and their bristle phenotypes (i.e. extreme revertant phenotype, DP(146)NAB; parental DP(146) phenotype; and wildtype) are given in Table 7. The mutation(s) in this line could not be mapped reliably. The phenotype is highly unstable, and possibly is caused by two mutations, one the original DP(146) mutation in the y–ct^6 interval, and the other in the chromosomal section from f to the centromere.

(iii) *Allelic complementation*

The bristle phenotypes of females heterozygous for different extreme X chromosomes with similar mean bristle numbers were examined. If the heterozygous bristle phenotype is not significantly different from that of the two parents, then the mutations on the two X chromosomes are either at the same locus, or at different loci with strong epistatic interactions. Bristle phenotypes significantly different from both parents indicates mutations at different loci. Table 8 shows mean sternopleural bristle numbers (±standard errors) of females homozygous for X chromosomes of Group 1 lines DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149) (on the diagonal) and of females heterozygous for all possible pairs of these X chromosomes (on the off-diagonal). In no case is the heterozygous mean bristle number significantly different from the average of the two parental homozygotes. These results are consistent with those from genetic mapping; that is, the mutations causing extreme bristle numbers in this group of lines are allelic.

Females heterozygous for NDC(19) and DP(146) X chromosomes (Group 2) had abdominal bristle scores averaging 16·53±0·267 and average sternopleural bristle scores of 15·5±0·240. Abdominal bristle

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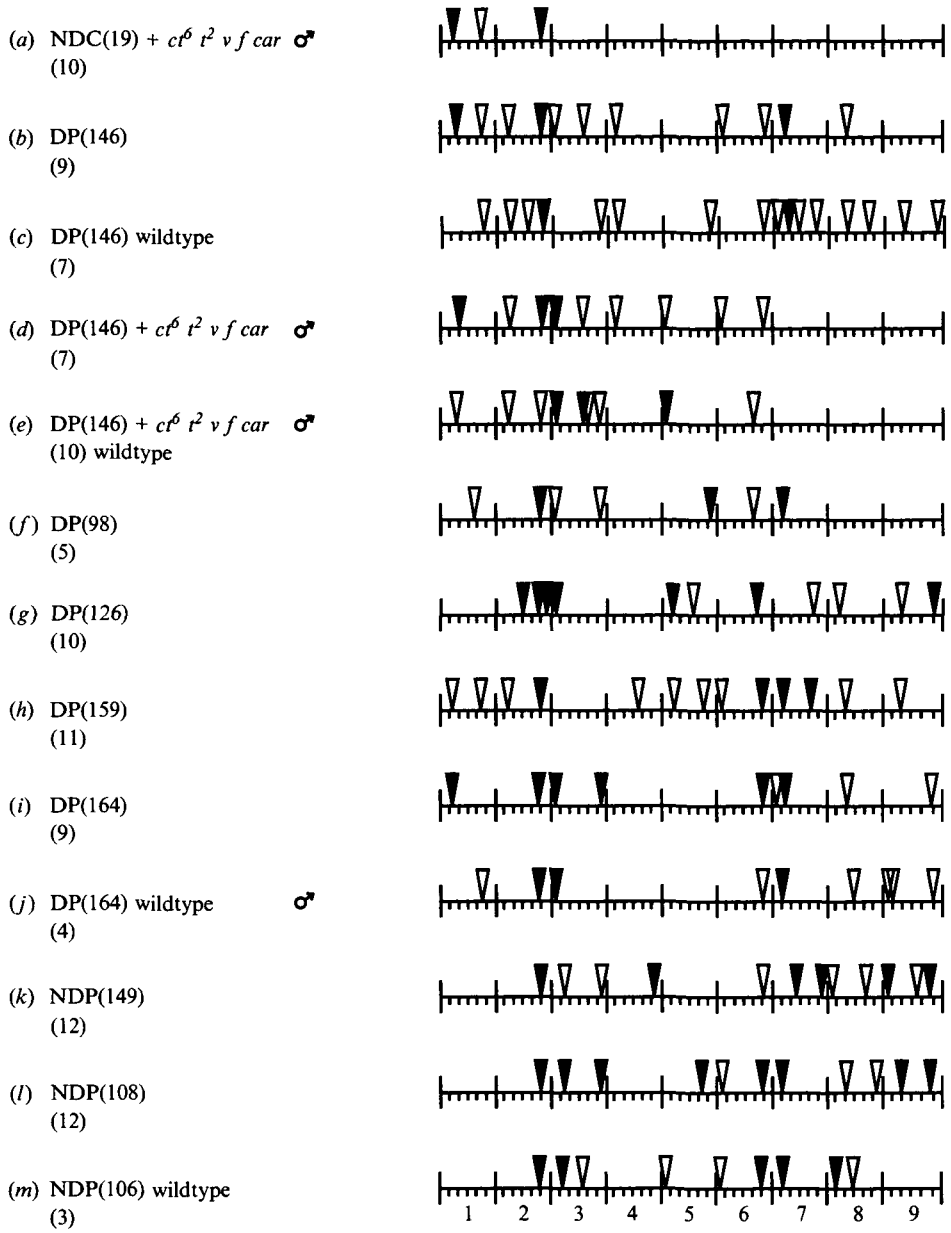

Fig. 3. Insertion sites of *P* elements on the first nine Bridges's (1938) sections of the *X* chromosome. Solid triangles and open triangles depict the fixed sites and polymorphic sites, respectively, over all salivary chromosomes assessed. '♂' indicates salivary chromosomes from male larvae only, otherwise male and female larvae were examined. Sample sizes are given in brackets.

numbers are significantly greater than those of either parent (see Table 1), indicating abdominal bristle effects at different loci. However, sternopleural bristle numbers are not significantly different from the DP(146) parent. If the mutations in lines NDC(19) and DP(146) are indeed single mutations affecting both traits but at separate, closely linked loci, as suggested by genetic mapping and non-complementing abdominal bristle scores, then the sternopleural bristle results may indicate some epistatic interaction between them.

Groups 1 and 2 lines all had similar mean sternopleural bristle numbers. Heterozygous females for DP(146) and DP(164) *X* chromosomes had a mean sternopleural bristle score of $16{\cdot}63 \pm 0{\cdot}296$; significantly greater than that of the two parental lines. Thus the lines fall into a minimum of two and probably three complementation groups on the basis of their heterozygous bristle number phenotypes.

(iv) *Deficiency mapping*

Means and standard errors for bristle traits of interest of females heterozygous for control (NDP(168)) or extreme (DP(146), NDP(19) and DP(126)) *X* chromosomes from each complementation group and *X* chromosomes from the deficiency stocks listed in Table 1 are given in Table 9. Females heterozygous for DP(146) and *Df(1)64c18*, *Df(1)sc*^{8}, *Df(1)260–1*

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Fig. 4. *In situ* hybridization of polytene chromosomes of line NDC(19), probed with pπ25·1 (O'Hare & Rubin, 1983). The arrow marks the *P* element insertion in the 1B region that may be associated with the extreme bristle number phenotype of this line.

and *Df(1) su(s) 83* have wildtype (or nearly wildtype) bristle numbers, but *DP(146)/Df(1) svr* heterozygotes display the mutant abdominal and sternopleural bristle phenotypes, suggesting the DP(146) mutation is in the region 1B4-6; 1B10, but not at 1B10. The heterozygous combinations of NDC(19) with *Df(1) svr* and *Df(1) 260–1 X* chromosomes are lethal, but *NDC(19)/Df(1) sc*^{8} heterozygous females have wildtype bristle numbers, placing the NDC(19) mutation within 1B2 to 1B4-6. The lethality of the former combinations is somewhat surprising, since NDC(19) homozygous females are viable but sterile. DP(126) was picked to represent its complementation group. The mutant bristle phenotype of *DP(126)/Df(1) KA14* but not *DP(126)/Df(1) RA2* heterozygotes assigns this mutation to the 8A4-5; 8C6 interval. Overall, the deficiency and physical mapping results are consistent.

(v) In situ *hybridization*

Results of both direct mapping and the complementation tests have shown that the mutations causing extreme bristle number phenotypes are at a minimum of two and probably three loci. NDC(19) is closely linked to *y*, and DP(146) is approximately 0·17 cM from it, whereas DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149) are at the same locus, about 4·7 cM from *ct*. Therefore, only the first nine sections of the *X* chromosome were diagnosed for insertion sites of *P* elements. The sites of *P* element insertion in this region common to all individuals sampled (solid triangle) or present in at least one individual (open triangles) for each of the *X* chromosomes of interest are presented in Figure 3.

Line NDC(19): Insertion sites of *P* elements on the tip of the *X* chromosome of recombinant line + *ct*^{6} *t*^{2}*v f car* NDC(19) are depicted in Figure 3*a*. The fixed *P* element insertion site in the 1B region (see Figure 4) is most likely associated with the mutation causing the NDC(19) bristle phenotype. This insertion also was detected in an *in situ* hybridization analysis of this line done within six generations of its discovery. The *X* chromosome of NDC(19) originated from the inbred Sam stock, which harbours no defective or autonomous *P* elements, so this site is a *de novo* insertion. The mutant bristle number phenotype of this line is stable, and so may be caused by an insertion of a defective *P* element.

Line DP(146): Figure 3*b* shows the fixed and polymorphic sites of *P* element insertions for line DP(146), combining results from both the initial and later (see Figure 5) analyses. Figure 3*c* gives the

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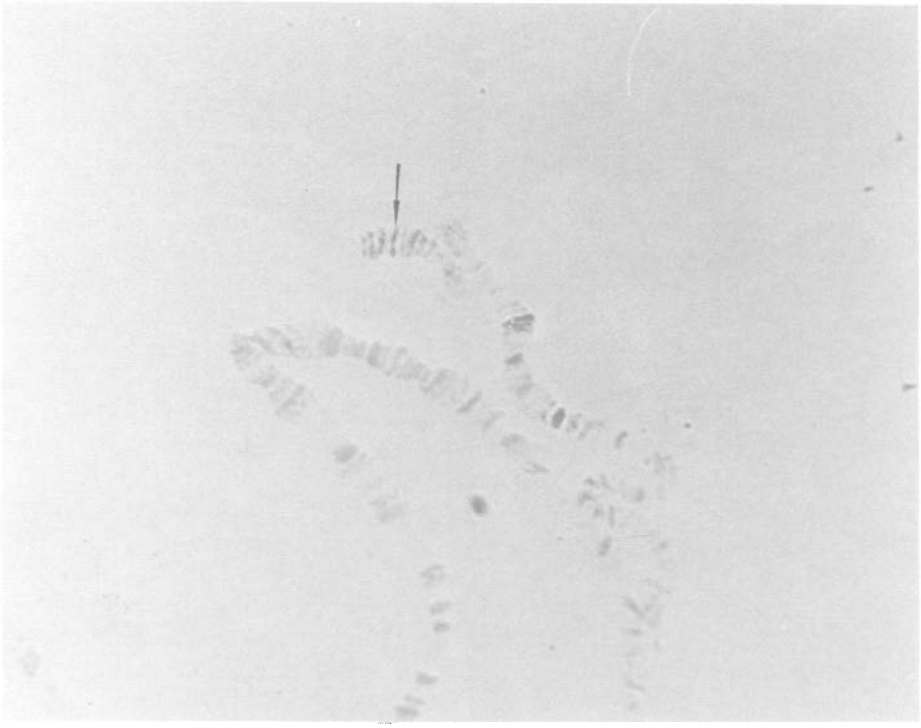

Fig. 5. *In situ* hybridization of polytene chromosomes of the DP(146) – marker recombinant line shown in Figure 3, panel d; probed with pπ25·1 (O'Hare & Rubin, 1983). The arrow marks the *P* element insertion at 1B that may be associated with the extreme bristle number phenotype of this line.

pooled sites of insertion from three wildtype lines derived independently from line DP(146) in different generations. DP(146) shares two fixed sites with its wildtype revertants, at bands 2E and 7B. However, the fixed site of insertion at band 1B (see Fig. 5) is unique to the DP(146) mutant phenotype, and is not present in the wildtype revertants. This has been repeatedly confirmed by the observation that most (nine out of ten) wildtype revertants from recombinant $+ ct^6\ t^2\ v\ f\ car$ DP(146) have no *P* element insertion at this site. This strongly suggests that the DP(146) mutation was induced by the insertion of a *P* element at band 1B; the instability of this mutant during maintenance supports this deduction. The map distance of the DP(146) mutation is 0·17 cM from *y*, but its physical position is likely to be close to the *y* locus on the basis of deficiency mapping which placed it between 1B4-6 and 1B10. The chromosome on which the mutation appeared was of P strain origin, but on the basis of comparison of insertion sites with other *X* chromosome lines derived from the same inbred P strain, it appears the site at 1B is a *de novo* insertion.

Lines DP(98), DP(126), DP(159), DP(164), NDP (108) and NDP(149): Genetic mapping and complementation tests indicate the bristle mutations in these lines are at the same locus, which deficiency mapping places within 8A4–8C6. The bristle number mutations in these lines cannot be associated with *P* element insertions; none have a fixed insertion site in this region (see Figure 3*f–l*), although polymorphic sites were found in some. Given that the mutations apparently occurred at the same locus and were derived from a *P* strain *X* chromosome which previously harboured about 12 copies of *P* elements, they are most likely caused by precise or imprecise excisions of *P* elements. Unfortunately, the *P* element insertion profile of the parent inbred P strain from which the mutant *X* chromosome lines were derived is not available, so it is not known whether there was initially a fixed *P* element site in the 8A–C region. This explanation is consistent with the stability of the lines during maintenance. Imprecise excisions leaving a small defective *P* element would not be consistently recognizable by the method of *in situ* hybridization.

4. Discussion

(i) P-*element-induced mutations at QTLs*

Hybrid dysgenesis was used by Lai & Mackay (1990) to generate mutations affecting male abdominal and/or sternopleural bristle number on highly inbred *X* chromosomes of P and M strain origin. The homozygous, heterozygous and hemizygous effects of the *X* chromosome lines from this study that had the most extreme effects on bristle number were further quantified in a highly inbred autosomal background. The mutations affecting bristle number in each were tested directly for allelism, and localized by recombination and deficiency mapping, combined with

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cytological mapping of locations of *P* elements. These genetic analyses consistently indicated the *X* chromosome mutations affecting bristle number fell into three complementation groups.

The largest group of seven lines had sternopleural bristle numbers that averaged 15·4 in males and homozygous females, compared to the control mean of 19·5. The mutant effects were partially recessive; heterozygous sternopleural bristle numbers averaged 18·8 across these seven lines, but with significant variation in heterozygous effect. One of the lines acquired an inversion that precluded localization more precisely than to the $ct^6 - t^2$ interval. The bristle effects of five lines in this group did not differ significantly in their map location of 24·7 cM, 4·7 cM from *ct*. The remaining line, DP(98), had a physical map position 3·5 cM from *ct*. However, the mutation in this line did not complement the others of this group, suggesting it is an allele of the same locus. It is quite likely that the poor viability of the DP(98) chromosome led to a biased estimate of map position. A disproportionate number of parental ++ compared to m t^2 (*71 vs.* 25) chromosomes were recovered from the alternative procedure adopted to map the DP(98) mutation, suggesting a more appropriate estimate of map distance from *ct* may be 7·5 − (2/71)100 = 4·68 cM (i.e. not significantly different from the map positions of the other mutations in this complementation group). Cytological examination of the DP(98) *X* chromosome revealed that the original chromosome on which the mutation occurred had been altered by a translocation of the 12C-13B region into 3A during maintenance (Lai, 1990). The translocation breakpoints were previous *P* element sites. The rearrangement indicates the instability of *P* elements in this line, which has possibly contributed to its reduced viability.

Deficiency mapping localized the bristle effects in this group of lines to 8A4 to 8C6, and direct complementation indicates the bristle number effects are allelic, but analysis of their *P* element sites by *in situ* hybridization did not reveal a *P* element site in this region common to all. The most plausible hypothesis explaining the origin of these alleles is that they result from independent excisions of a *P* element at 8C in the original P strain *X* chromosome. The following arguments support this hypothesis. (i) While the sites of *P* element insertion on the parental inbred strain were not determined, the outbred Harwich strain from which it was derived was polymorphic for insertions at 8C with an occupancy frequency of 0·4 (data of Shrimpton, Mackay & Leigh Brown, 1990), giving a probability of fixation by inbreeding of 0·23. (ii) Hybrid dysgenesis causes *P* element excisions more frequently than insertions (reviewed by Engels, 1989). (iii) The extreme *X* chromosomes were induced by a single generation of mutagenesis. Four lines of this group were polymorphic for *P* element insertions at 8C, possibly because of variation in the detection threshold of small inserts in different *in situ* preparations, or because 8C is a hotspot for *P* element insertion and excision. The latter explanation is consistent with the observed polymorphism for *P* element insertion at 8C in line DP(146), which is not a member of this group, and wildtype revertants of lines DP(164) and DP(146).

Line NDC(19) was extreme for both bristle characters, with only 10·2 abdominal and 12·8 sternopleural bristles (averaged over males and homozygous females), fully 10·6 abdominal and 8·1 sternopleural bristles fewer than wildtype. The abdominal bristle effects were completely recessive, whereas the sternopleural bristle effects were nearly additive. It is likely that the mutation in line NDC(19) is a *P* element insertion in the *achaete-scute* complex (*AS-C*) that causes pleiotropic effects on both bristle traits. This line has a *scute* phenotype, maps by recombination mapping (0·0 cM) and deficiency mapping (1B2; 1B4-6) to the region in which most *AS-C* mutants are located, and is possibly associated with a *P* element insertion in the 1B region. Since the progenitor *X* chromosome was from an inbred M strain, this must be a *de novo* insertion. No revertants of this line have been obtained, but the *AS-C* has been cloned (Campuzano *et al.* 1985), so it is possible to determine by restriction mapping whether or not this line contains an insert in this region.

Line DP(146) was also extreme for both bristle traits, with 11·5 abdominal and 15·5 sternopleural bristles (averaged over males and homozygous females), 7·4 abdominal and 4·0 sternopleural bristles less than wildtype. However, this line is not phenotypically *scute*. Abdominal bristle effects were partially recessive, and sternopleural bristle effects nearly additive. This line is highly unstable, frequently giving rise to wildtype and extreme bristle number revertants. Reversion to wildtype that occurred during recombination mapping will have biased the estimation of map position. The deficiency of mutants among recombinants of $y+$ compared to the number of wildtypes among recombinants of $+ct^6$ suggests the estimate of 0·17 cM for the DP(146) mutation to *y* may be an overestimate. A conservative estimate is 0·07 cM, based on the number of mutants among recombinants of $y+$(2/584 X 20 = 0·07). Deficiency mapping also places the mutation in this line proximal (between 1B4-6 and 1B10) to that of NDC(19), and direct complementation shows these mutations are non-allelic, although they have epistatic effects on sternopleural bristle number. The mutation in DP(146) is clearly associated with a *P* element insertion in 1B, since this site is fixed in the line containing the mutation and is absent in wildtype revertants and control *P* strain *X* chromosomes.

(ii) *The nature of QTLs*

Drosophila bristles and hairs are external sense organs

https://doi.org/10.1017/S0016672300031359 Published online by Cambridge University Press

of the peripheral nervous system (Jan and Jan, 1990), therefore mutations at the functionally related genes participating in the common developmental pathways of the central and peripheral nervous system might be responsible for *de novo* quantitative variation in bristle number. In this regard it is interesting that the mutation in line NDC(19) appears to be an allele of *scute*, one of the genes of the *AS-C*. The genes of the *AS-C* endow ectodermal cells with the capacity to become neuronal precursors; *achaete* and *scute* being the most important for adult sense organ development (reviewed by Campuzano & Modolell, 1992). In addition, insertional variation in the *AS-C* region is strongly associated with naturally occurring variation for abdominal and sternopleural bristle number (Mackay & Langley, 1990).

The mutation of line DP(146) maps proximal to *scute* (to 1B4-6; 1B10), and complements the abdominal bristle number phenotype of line NDC(19). Two loci associated with nervous system development have been described which map to 1B9: *ventral nervous system defective* (*vnd*) and *β Amyloid protein precursor like* (*Appl*) (Lindsley & Zimm, 1992). Mutations in the former gene are usually late embryonic lethals, with an unorganized, uncondensed ventral nervous system (White, DeCelles & Enlow, 1983). The *Appl* locus is defined by cDNA clones derived from cloned DNA from 1B. *Appl* transcript and APPL protein immunoreactivity appear confined to post-mitotic neurons (Lindsley & Zimm, 1992). Further molecular analysis will reveal whether or not the insert at 1B that apparently causes the DP(146) bristle number mutant phenotype has affected either of these loci.

The mutations in the remaining lines all map to the same location, between 8A and 8C. It is less easy to propose potential candidate loci in this region. The only identified loci from 24·3 to 25·4 cM with described bristle effects are *tiny bristleoid* at 25·0 cM, between 8A1 and 8C1; and *small* at 25·0 cM (Lindsley & Zimm, 1992). However, effects of these loci on sternopleural and abdominal bristle number are unknown, and there are no extant alleles of either available for complementation testing. Molecular analysis of this group of mutations is not straightforward in any case, since they are not associated with *P* element insertions.

(iii) *Mapping QTLs by transposable element mutagenesis*

It has been proposed that transposable element-induced mutations with effects on quantitative traits afford an attractive alternative to conventional QTL mapping designs using recombination with polymorphic morphological or molecular markers in those species in which elements can be readily mobilized (Mackay, 1985; Robertson, 1985; Soller & Beckmann, 1987). The obvious advantage is that QTLs identified by an insertional mutation can be cloned using the transposon as a molecular tag (e.g. Bingham, Levis & Rubin, 1981), with the ultimate goal of understanding the molecular basis of quantitative variation. Less obvious is the advantage that single QTLs are so identified. Traditional interval mapping methodology logically defines 'effective factors' or 'chromosomal segments' between adjacent marker brackets. Not only can one not discern whether an effective factor contains one or many loci affecting a trait, thus invalidating inferences of pleiotropy, but the existence of more than one QTL in a region between two markers can lead to serious errors in estimating effects and locations (McMillan & Robertson, 1974).

P element mutagenesis in Drosophila using hybrid dysgenic crosses has been superseded by refinements which enable transformation by single, marked, stable elements; considerably simplifying the correlation of mutant effects with presence/absence of the insertion and facilitating cloning of adjacent sequences by plasmid rescue (Cooley, Kelley & Spradling, 1988; Bier *et al.* 1989; Wilson *et al.* 1989). Identification of QTLs by insertional mutagenesis is also feasible in mice, by hybrid dysgenesis-like crosses (Jenkins & Copeland, 1985), retroviral infection of embryos (Jaenisch, 1980) or embryonic stem cells (Robertson *et al.* 1986) and direct microinjection of DNA into fertilized eggs (reviewed by Palmiter & Brinster, 1986); and in maize by mobilization of one of many endogenous transposable element systems (reviewed by Fedoroff, 1989). The efficiency of the method in these species compared to QTL analysis using closely spaced highly polymorphic microsatellite markers (Dietrich *et al.* 1992; Serikawa *et al.* 1992) will depend on how easily sublines of an inbred strain which differ in the trait(s) of interest and the locations of relatively few, new, stable, inserts can be produced. Results from *Drosophila* (this report; Mackay, Lyman & Jackson, 1992) suggest this may be easier than was predicted by Soller & Beckmann (1987).

We have demonstrated that transposable element mutagenesis is indeed a feasible method for generating mutations at and subsequently localizing loci potentially responsible for quantitative variation in *Drosophila*. The spectrum of loci at which mutations affecting quantitative traits can occur does not necessarily coincide exactly with the loci at which naturally occurring variation for these traits segregates. It is encouraging in this regard that one of the genes of the *AS-C* was identified by insertional mutagenesis, for naturally occurring insertions in the *AS-C* region have been shown to account for approximately 5% of the total genetic variation for abdominal and sternopleural bristle number (Mackay & Langley, 1990). Continued molecular and phenotypic analysis of naturally occurring variation at loci identified by transposable element mutagenesis will yield empirical distributions of effects within and among loci. Understanding quantitative variation in

terms of additive, dominance, epistatic and pleiotropic effects at individual QTLs is within reach.

We thank Tony Shrimpton, Andy Leigh Brown and Robert McMahon in Edinburgh and Michael Jackson and Richard Lyman in Raleigh for their valuable comments and technical advice during the course of this study, and Charles Langley and Tony Long in Davis for their valuable comments on the manuscript, and the Indiana University and Bowling Green Drosophila Stock Centers for providing Drosophila stocks. This work was supported by an ORS Award from the Overseas Research Student Award Committee of the U.K. to C.L. and NIH Quantitative Genetics Program Grant GM11546 and Statistical and Quantitative Genetics Program Grant GM45344.

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