# Genetic Interactions of Modifier Genes and Modifiable Alleles in *Drosophila melanogaster*

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ABSTRACT

We have examined the effects of mutations in the six allele-specific modifier genes *su(Hw)*, *e(w$^e$)*, *su(f)*, *su(s)*, *su(w$^a$)*, and *su(pr)* on the expression of 18 modifiable alleles, situated at 11 loci. Ten of the modifiable alleles are associated with insertions of the *gypsy* retrotransposon and the others include alleles associated with insertions of *copia* and *412*. We tested or retested 90 of the 108 possible combinations and examined the expression of modifiable alleles in flies mutant for pairs of modifier genes in various heterozygous and homozygous configurations. Our principal findings are: (1) a screen of 40,000 mutagenized *X* chromosomes yielded three new mutations in known modifier genes, but revealed no new modifier genes; (2) the modification effects of different mutations in a given modifier gene were qualitatively similar; (3) each of the six modifiers suppressed some modifiable alleles, enhanced others, and had no noticeable effect on still others; (4) the modifier genes could be placed in four classes, according to their effects on the *gypsy*-insertion alleles; and (5) the effects of mutations in different modifier genes combined additively. Implications of these results for models of modifier gene action are discussed.

CERTAIN modifier genes in Drosophila alter the expression of specific alleles at other loci, making the phenotypes of such alleles more nearly wild type or more extremely mutant. The former effect is known as suppression and the latter as enhancement. Such allele-specific modifier genes are of special interest, since the alleles they modify generally result from the insertion of retrovirus-like transposable elements. For example, nearly all of the alleles suppressed by mutations of the gene *suppressor of Hairy-wing*, *su(Hw)*, are associated with insertions of the *gypsy* transposable element (MODOLELL, BENDER and MESELSON 1983). Other such elements associated with modifiable alleles include *copia* and *412* (GEHRING and PARO 1980; BINGHAM and JUDD 1981; SEARLES and VOELKER 1986; WALKER, HOWELLS and TEARLE 1986).

In addition to *su(Hw)*, other genes that modify the expression of retrotransposon-insertion alleles include *suppressor of sable*, *su(s)*; *suppressor of white-apricot*, *su(w$^a$)*; *enhancer of white-eosin*, *e(w$^e$)*; *suppressor of forked su(f)*; and *suppressor of purple*, *su(pr)* (LINDSLEY and GRELL 1968). The designation of each modifier gene as the suppressor or enhancer of a particular allele refers to the effect first described in the literature for mutations of the gene. In fact, a given modifier may act on additional alleles at various loci, suppressing some and enhancing others. For example, *su(f)* suppresses the *gypsy*-insertion allele *f$^1$* and enhances the *copia*-insertion allele *w$^a$* (GREEN 1959). Also, a given insertion allele may respond differently to different modifiers, being suppressed by some and enhanced by others. An example is the *gypsy*-insertion allele *Hw$^1$*, which is suppressed by *su(Hw)* but enhanced by *su(pr)* (LINDSLEY and GRELL 1968).

These and other observations reveal the existence of complex but specific interactions by which modifier genes regulate the effects of retrotransposon insertion on gene expression. An understanding of their action seems likely to reveal novel aspects of genetic regulation. We therefore carried out a systematic study of the genetic interactions of 6 allele-specific modifier genes and 18 modifiable alleles.

**This paper is dedicated to E. B. LEWIS on the occasion of his seventieth birthday.**

## MATERIALS AND METHODS

**Stocks:** Stocks were kept on a yeasted cornmeal-sucrose-agar medium at 25° and 50% relative humidity, except for larvae used for hybridization *in situ*, which were kept at 18°. Mutations used in this study are described in Table 1. One of the two chromosomes used for mutagenesis, *y$^2$ w$^a$ ct$^6$ f$^1$*, was derived from *y$^2$ w$^a$ ct$^6$ lz$^{bg}$ v$^1$ f$^1$* by double exchange with wild-type Oregon R, removing *lz$^{bg}$* and *v$^1$*, thereby facilitating the scoring of *w$^a$*. This *lozenge* allele was designated *lz$^1$* by the Bowling Green stock center. It differed, however, from the *lz$^1$* obtained from the Cal Tech stock center and described by LINDSLEY and GRELL (1968) and by MODOLELL, BENDER and MESELSON (1983), in that it was

**TABLE 1**

**Stocks and mutations used**

| Locus | Alleles | Map positions | Relevant phenotype |
|---|---|---|---|
| *yellow* | $y^2$ | 1–0.0 | Yellow cuticle and wings |
| *Hairy-wing* | $Hw^1$ | 1–0.0 | Extra bristles along wing veins, on head and thorax |
| *scute* | $sc^1$ | 1–0.0 | Loss of specific head and thoracic bristles |
| *white* | $w^a$, $w^e$ | 1–1.5 | Yellowish-orange eyes |
| *cut* | $ct^6$, $ct^K$ | 1–20.0 | Wings altered in shape. $ct^6$: pointed wings, with rough edges. $ct^K$: scalloped wing tips and margins |
| *lozenge* | $lz^1$, $lz^3$, $lz^{34}$, $lz^{37}$, $lz^{46}$, $lz^k$ | 1–27.7 | Eyes roughened and reduced in size |
| *vermilion* | $v^1$ | 1–33.0 | Bright orange eyes |
| *sable* | $s^1$ | 1–43.0 | Darker cuticle than wild type |
| *forked* | $f^1$, $f^5$ | 1–56.7 | Gnarled or bent bristles |
| *purple* | $pr^1$ | 2–54.5 | Reddish eyes |
| *bithorax* | $bx^{34e}$, $bx^3$ | 3–58.8 | Partial transformation of 3rd thoracic segment into 2nd. $bx^{34e}$: halteres slightly enlarged, with some marginal wing bristles present. $bx^3$: halteres much enlarged, with long row of marginal wing bristles |
| *su(s)* | $su(s)^3$, $su(s)^{44}$ | 1–0.0 | No visible mutant phenotype |
| *su($w^a$)* | $su(w^a)^1$, $su(w^a)^{20}$ | 1–0.1 | No visible mutant phenotype |
| *e($w^e$)* | $e(w^e)^S$ | 1–32 | No visible mutant phenotype |
| *su(f)* | $su(f)^1$, $su(f)^{20}$ | 1–65.9 | No visible mutant phenotype |
| *su(Hw)* | $su(Hw)^2$, $su(Hw)^{69k}$, $su(Hw)^{70}$, $su(Hw)^f$, $su(Hw)^{f3}$ | 3–54.8 | No visible mutant phenotype |
| *su(pr)* | $su(pr)^{e3}$, $su(pr)^{e4}$, $su(pr)^B$ | 3–95.5 | No visible mutant phenotype |

not suppressible by *su(Hw)* or *su(f)* and was not associated with a *gypsy* insertion, as determined by hybridization *in situ* (data not shown). We designated it $lz^{bg}$ and did not investigate it further.

**Mutagenesis and screening:** Three-day-old $y^2\ w^a\ ct^6\ lz^{bg}\ v^1\ f^1$ and $y^2\ w^a\ ct^6\ f^1$ males were treated with ethyl methanesulfonate for 18–24 hr (LEWIS and BACHER 1968) and mated with attached-*X* virgin females. Male progeny were screened for modification of the *X*-linked markers. Putative modifier mutants were crossed to females heterozygous for the balancer FM7 and to attached-*X* females, in order to establish stable stocks. This screen permits recovery of *X*-linked or dominant autosomal modifier mutations in the first generation but does not recover highly deleterious mutations.

**Hybridization *in situ*:** Hybridization *in situ* was done with biotinylated nick-translated DNA probes (LANGER-SAFER, LEVINE and WARD 1982) and was followed by binding of streptavidin-horseradish peroxidase and reaction with 3,3′-diaminobenzidine tetrahydrochloride (Enzo Biochem, Inc.). The probes used for hybridization were full-length copies of the transposable elements *gypsy* (pbx-gyp; MODOLELL, BENDER and MESELSON 1983), *copia* (cDm5002; DUNSMUIR *et al.* 1980) and *412* (cDM2042; G. RUBIN, personal communication).

**Forked bristle counts and eye pigment analysis:** Five males and five to ten females were kept 3 days in half-pint bottles with fresh medium. Male progeny were collected daily and aged in fresh vials for 4 days before scoring forked bristles. Males prepared in the same way were stored at −70° for subsequent pigment extraction.

The forked phenotype was quantified by examining 20 thoracic bristles and 12 head bristles on each fly. These were the four anterior and posterior scutellars, the two anterior post-alars, the four anterior and posterior dorsocentrals, the four anterior and posterior supra-alars, the four anterior and posterior notopleurals, the two presuturals, the two ocellars, the two postverticals, the four anterior and posterior verticals, and the four anterior and posterior orbitals. Bent or forked bristles were given a score of one and straight bristles were scored zero. Twenty flies of each genotype investigated were scored independently by the same two persons and the scores were averaged.

Pteridine eye pigments were analyzed by one-dimensional thin-layer chromatography (TLC) on 160 μm Kodak chromagram cellulose TLC plates according to WILSON and JACOBSON (1977). Pigments were extracted from 60 heads (approximately 6 mg) by 20 strokes in a 1 ml Dounce homogenizer in 240 μl of 2:1 propanol-3.5% $NH_4OH$ with 0.5% 2-mercaptoethanol. After centrifugation, 40 μl of supernatant were loaded per lane and chromatography was conducted for 4 hr with 50% isopropanol, 1% ammonium acetate, 0.1% 2-mercaptoethanol. Pigment extractions and chromatography were done under a yellow safe-light.

## RESULTS

**New mutations of modifier genes:** Approximately 20,000 progeny from each of the two mutagenized *X* chromosomes $y^2\ w^a\ ct^6\ lz^{bg}\ v^1\ f^1$ and $y^2\ w^a\ ct^6\ f^1$ were screened for new modifier gene mutations. Three such mutations were recovered. These were designated $su(s)^{44}$ and $su(f)^{br}$ from the former chromosome and $su(w^a)^{20}$ from the latter. Numerous other mutations were found, including approximately ten each with the phenotypes of mutations at *white* and *rudimentary*. The three modifier mutations were first noted by their effects on eye color and/or bristle shape and were characterized by allelism tests and genetic mapping. The mutation $su(f)^{br}$ is closely linked to or inseparable from a variable recessive abnormality giving small misshapen eyes. Each of the three new modifier mutations gave qualitatively the same pattern of suppression and enhancement as did the already known mutations we examined at these loci, although the enhancement of $w^a$ by $su(f)^{br}$ was seen only in $su(f)^1/\ su(f)^{br}$ heterozygotes.

**Modifiable alleles:** Ten of the modifiable alleles

**TABLE 2**

**Effects of modifier mutations on modifiable alleles**

| | | *gypsy* | | | | | | | | | | ? | | *copia* | ? | | | 412 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Modifier | Alleles | $y^2$ | $Hw^1$ | $sc^1$ | $ct^6$ | $ct^K$ | $lz^1$ | $f^1$ | $f^5$ | $bx^{34e}$ | $bx^3$ | $lz^{34}$ | $lz^k$ | $w^a$ | $w^e$ | $lz^{37}$ | $s^1$ | $v^1$ | $pr^1$ |
| *su(Hw)* | *2/2, 2/f* | $\underline{S}^a$ | $\underline{S}^b$ | $\underline{S}^c$ | $\underline{S}^d$ | $\underline{S}^c$ | $\underline{S}^b$ | $\underline{S}^f$ | $\underline{S}$ | $\underline{S}^g$ | (S) | $\underline{0}$ | 0 | $\underline{0}$ | 0 | E | 0 | $\underline{0}^g$ | $\underline{0}$ |
| *e($w^e$)* | *S* | 0 | 0 | 0 | 0 | | $\underline{S}$ | $\underline{S}$ | S | S | | 0 | S | $\underline{0}$ | $\underline{E}$ | 0 | 0 | | |
| *su(f)* | *1* | $0^h$ | 0 | 0 | $0^h$ | S | $\underline{S}^h$ | $\underline{S}^h$ | $\underline{S}$ | S | | $\underline{0}$ | 0 | $\underline{E}^h$ | $\underline{0}$ | $\underline{E}^h$ | 0 | 0 | |
| *su(s)* | *3, 44* | $\underline{0}^i$ | | (0) | 0 | $E^i$ | $\underline{E}$ | E | | $\underline{E}^i$ | (E) | $E^j$ | $E^j$ | $0^i$ | | E | S | $\underline{S}$ | (S) |
| *su($w^a$)* | *1* | $0^k$ | | 0 | $0^k$ | $E^k$ | E | $E^k$ | | E | | E | E | $\underline{S}^k$ | $\underline{0}$ | 0 | 0 | 0 | |
| *su(pr)* | *e3/e3* | 0 | $E^l$ | $0^l$ | $0^l$ | $E^m$ | S | $S^n$ | S | (E) | E | S | S | 0 | 0 | 0 | 0 | $0^l$ | S |

S = suppression, E = enhancement, 0 = no clear effect, underlined letters = result published or communicated to us by others and reproduced by us, ( ) = result published or communicated to us by others not tested by us.

The reports and citations published or communicated to us by others are LEWIS (1949, 1967, 1981); LEE (1973); and MODOLELL, BENDER and MESELSON (1983) for *su(Hw)*; GREEN (1957 and 1959) and MODOLELL, BENDER and MESELSON (1983) for *e($w^e$)*; GREEN (1955, 1959) and SCHALET (1970) for *su(f)*; SCHULTZ and BRIDGES (1932), E. B. LEWIS (personal communication) and MODOLELL, BENDER and MESELSON (1983) for *su(s)*; GREEN (1959) for *su($w^a$)*; and SCHULTZ and BRIDGES (1932) and E. B. LEWIS (personal communication) for *su(pr)*. The modifier mutations with which we tested each combination are designated in the table, with additional modifier mutations denoted by superscripts, as follows: a = $su(Hw)^{f3}/su(Hw)^{f3}$, $su(Hw)^2/su(Hw)^{f3}$, $su(Hw)^{69k}/su(Hw)^f$ and $su(Hw)^f/su(Hw)^{f3}$; b = $su(Hw)^{69k}/su(Hw)^f$; c = $su(Hw)^{f3}/su(Hw)^{f3}$, $su(Hw)^{69k}/su(Hw)^{70}$, $su(Hw)^{69k}/su(Hw)^f$ and $su(Hw)^{70}/su(Hw)^f$; d = $su(Hw)^{69k}/su(Hw)^{70}$, $su(Hw)^{69k}/su(Hw)^f$ and $su(Hw)^{70}/su(Hw)^f$; e = tested only with $su(Hw)^2/+$ and $su(Hw)^f/+$; f = $su(Hw)^{f3}/su(Hw)^{f3}$, $su(Hw)^2/su(Hw)^{70}$, $su(Hw)^2/su(Hw)^{f3}$, $su(Hw)^{69k}/su(Hw)^f$, $su(Hw)^{70}/su(Hw)^f$ and $su(Hw)^f/su(Hw)^{f3}$; g = tested only with $su(Hw)^2/su(Hw)^2$; i = tested only with $su(s)^{44}$; j = tested only with $su(s)^3$; k = $su(wa)^{20}$; l = $su(Pr)^{e4}/su(pr)^B$; m = tested only with $su(pr)^{e3}/+$ and $su(pr)^{e4}/+$; n = $su(pr)^B/su(pr)^B$, $su(pr)^{e4}/su(pr)^{e4}$, $su(pr)^B/su(pr)^{e3}$ and $su(pr)^B/su(pr)^{e4}$; o = $su(pr)^{e3}/su(pr)^B$.

($y^2$, $Hw^1$, $sc^1$, $ct^6$, $ct^K$, $lz^1$, $f^1$, $f^5$, $bx^3$, and $bx^{34e}$) are associated with *gypsy* insertions (MODOLELL, BENDER and MESELSON 1983). The extreme allele $f^5$ is associated with two *gypsy* insertions in the same orientation, approximately 5 kb apart (MCLACHLAN 1986). The *gypsy* insertions in $bx^3$ and $bx^{34e}$ are also parallel to each other and are 6.5 kb apart (BENDER *et al.* 1983), while the *gypsy* elements in $ct^6$ and $ct^K$ are 73 kb apart and are oriented oppositely to each other (JACK 1985). Another modifiable allele, $w^a$, results from an insertion of *copia* (GEHRING and PARO 1980; BINGHAM and JUDD 1981), and $w^e$ is associated with a *doc* element into which additional DNA is inserted (K. O'HARE, cited in HAZELRIGG 1987). The *412* element is associated with the $v^1$ allele and may also be responsible for $pr^1$ (SEARLES and VOELKER 1986; WALKER, HOWELLS and TEARLE 1986).

We tested $lz^1$ and five additional spontaneous *lz* alleles ($lz^3$, $lz^{34}$, $lz^{46}$, $lz^{37}$, and $lz^k$) for the presence of *gypsy*, *copia*, and *412* by hybridization *in situ*. Although each of these elements hybridized elsewhere in the genome of each stock, the only hybridization at 8D, the chromosomal subdivision of the *lozenge* locus, was that expected in the $lz^1$ stock with the *gypsy* probe. The copia probe hybridized in the $lz^k$ stock proximal to this site, at 8DE. Homologous sequence less than approximately a kilobase, such as a transposon long terminal repeat (LTR), could have gone undetected.

**Summary of interactions:** Table 2 summarizes the effects of the six modifiers on the expression of 18 modifiable alleles at 11 loci. Of the 108 possible combinations, 95 have been tested. We tested or retested 90 combinations, many of them with at least two different mutations of the corresponding modifier, as noted in the table. An exception was *enhancer of white-eosin*, for which only one mutation was available. All tests were done in stocks multiply marked with modifiable alleles, so that the expression of two or more such alleles could be seen under identical conditions in individual flies.

The six modifier genes could be placed in four classes, according to their effects on the *gypsy*-insertion alleles. The modifier *su(Hw)* suppressed all ten *gypsy*-insertion alleles. For all combinations tested, the four modifiers *e($w^e$)*, *su(f)*, *su(s)*, and *su($w^a$)* modified the same six *gypsy*-insertion alleles ($ct^K$, $lz^1$, $f^1$, $f^5$, $bx^3$, and $bx^{34e}$) and were without effect on the other four ($y^2$, $Hw^1$, $sc^1$, and $ct^6$), with *e($w^e$)* and *su(f)* acting as suppressors and *su(s)* and *su($w^a$)* acting as enhancers. Finally, *su(pr)* suppressed some *gypsy*-insertion alleles, enhanced others, and was without effect on the rest.

The alleles $lz^{34}$ and $lz^k$ were not modified by *su(Hw)*. Like certain *gypsy*-insertion alleles, however, both $lz^{34}$ and $lz^k$ were enhanced by *su(s)* and *su($w^a$)*, and $lz^k$ was suppressed by *e($w^e$)*.

**Effects of heterozygous modifiers:** The effects of mutations of the six modifiers on most modifiable alleles are recessive, although an exception had been noted in the suppression of $ct^K$ by *su(Hw)/+* (LEE 1973). We also observed the suppression of $ct^K$ by *su(Hw)/+* and in addition found this allele to be enhanced by *su(pr)/+*. We found no heterozygous effects of any of the six modifiers on the phenotypes produced by any of four modifiable alleles $y^2$, $w^a$, $ct^6$, and $f^1$. We also tested the expression of these modifiable alleles in flies heterozygous for two different

**TABLE 3**

**Bristle counts**

| Genotype | Average no. of forked bristles | Modification |
|---|---|---|
| Oregon R | 0.0 | |
| $su(f)^{br}$; $su(Hw)^2/su(Hw)^f$ | 0.8 | S + S |
| $e(w^e)^S$; $su(Hw)^2/su(Hw)^f$ | 1.7 | S + S |
| $su(f)$ | 1.8 | S |
| $su(Hw)^2/su(Hw)^f$ | 2.1 | S |
| $su(f)^{br}$ | 2.4 | S |
| $su(w^a)$; $su(Hw)^2/su(Hw)^f$ | 2.5 | E + S |
| $su(w^a)^{20}$ $su(f)$ | 2.8 | E + S |
| $su(s)^{44}$; $su(Hw)^2/su(Hw)^f$ | 3.5 | E + S |
| $e(w^e)^S$ | 4.5 | S |
| $su(pr)^{e3}$ | 9.1 | S |
| No suppressor | 14.0 | |
| $su(w^a)$ | 14.9 | E |
| $su(s)^{44}$ | 15.8 | E |
| $su(w^a)^{20}$ | 16.2 | E |

For each modifier the number of forked bristles was significantly different ($P < 0.05$) from the control strain, as determined by the $\chi^2$ value for the corresponding 2 × 2 contingency table. In the case of double modifiers $su(w^a)$;$su(Hw)^2/su(Hw)^f$ and $e(w^e)^S$;$su(Hw)^2/su(Hw)^f$ compared to $su(Hw)^2/su(Hw)^f$ the corresponding $\chi^2$ values were 2.97 and 3.54, respectively, with $0.10 > P > 0.05$. With the three other double modifier combinations, $P < 0.01$. In separate experiments, each of the five double modifier combinations listed in the table consistently showed the same rank order relative to the corresponding single modifiers.

modifiers. No effect of any of the 15 possible pairs of heterozygous modifiers was observed. In particular, even though $f^1$ was suppressed by $su(Hw)$, $e(w^e)$, $su(f)$, and $su(pr)$, no doubly heterozygous combination of these modifiers had any noticeable effect.

**Additivity of modifier gene effects:** Six of the 15 possible pairs of the six modifiers were tested as double hemi- and/or homozygotes for their effects on the modifiable alleles $y^2$, $w^a$, $ct^6$, and $f^1$. The pairs tested were $su(Hw)^2/su(Hw)^f$ with $e(w^e)^S$, $su(f)^1$, $su(f)^{br}$, $su(s)^{44}$, and $su(w^a)^1$; and $su(f)^1$ with $su(s)^{44}$ and $su(w^a)^{20}$. The effects were additive in each case. Modifiers which singly were without effect on an allele had no effect on that allele in combination with any other modifier. Modifiers that suppressed an allele, when combined, suppressed more strongly. Finally, modifiers that acted oppositely on an allele gave an intermediate effect when combined. For example, $w^a$ flies with the double mutant combination $su(wa)^{20}$ $su(f)^1$ had eyes indistinguishable in pigmentation from eyes of $w^a$ flies, while $w^a$ flies with $su(wa)^{20}$ or $su(f)^1$ alone had eyes much darker or much lighter, respectively.

**Bristle counts and pigment analysis:** The above qualitative observations of the additivity of pairs of modifiers were supported by counts of forked bristles and by chromatographic analysis of eye pigments. The mutation $f^1$ affects many hairs and bristles, making them bent or gnarled. As described in MATERIALS AND METHODS, we scored a specific set of 32 bristles on male flies of various genotypes. The proportion of affected bristles was paralleled by the severity of the effect on individual bristles. As may be seen in Table 3, the average number of forked bristles on $f^1$ flies ranged from 0.8 for $su(f)^{br}$; $su(Hw)^2/su(Hw)^f$ to 16.2 for $su(w^a)^{20}$, with 14.0 for flies with no modifier mutations. Wild-type flies had no forked bristles. Modifier effects appeared to be additive in each of the five double mutant combinations listed in the table. Additivity was also observed in a separate experiment in which we scored forked bristles on $f^1$ flies with the modifier combinations $su(f)^1$; $su(Hw)^2/su(Hw)^f$ and $su(s)^{44}$ $su(f)^1$ (data not shown).

The designation of *suppressor of white-apricot* and *suppressor of sable* as enhancers of $f^1$ rests on the severity of the effect on individual bristles and on bristle counts. As may be seen in Table 3, enhancement was indicated by the bristle counts for flies having these modifiers both alone and combined with other modifiers that suppressed $f^1$.

Mutations in the *white* gene affect the deposition of pteridines and ommochromes in pigment granules (reviewed by PHILLIPS and FORREST 1980). The effects of modifiers on the amounts of pteridines in heads of $w^a$ flies were examined by TLC. At least six pteridines were evident (data not shown). They were all increased or decreased coordinately by modifier gene mutations. The effects may be ranked in order of increasing amount of pteridines as $su(f)^1 < su(f)^{br} \approx su(Hw)^2/su(Hw)^f \approx e(w^e)^S \approx su(w^a)^{20}$ $su(f)^1 \approx su^+ \approx su(s)^{44} < su(w^a)^1 \approx su(w^a)^{20}$, in agreement with visual inspection of eye color. While all of the pteridines were apparently reduced in $w^a$ flies, the pteridines most prominent in wild-type flies, the drosopterins, were reduced to a much greater extent. Suppression and enhancement of $w^a$ appeared to affect all of the pteridines. Suppressed $w^a$ had nearly wild-type amounts of pteridines except for drosopterins, which, although increased, remained far below the wild-type level.

**Properties of individual modifiable alleles:** Most of the 16 alleles that showed suppresion were suppressed completely or almost completely by one or, in some cases, two modifiers. These alleles include $y^2$, $Hw^1$, $ct^6$, $lz^1$, $lz^k$, $v^1$, $s^1$, $f^1$, $bx^3$, $bx^{34e}$, and $pr^1$. Some alleles were suppressed to differing degrees, depending on the modifier. For example, the bristle and eye morphology phenotypes of $f^1$ and $lz^1$, respectively, were suppressed almost completely by $su(f)$ and $su(Hw)$ but only partially by $e(w^e)$ and $su(pr)$. The female sterility of $lz^1$ was suppressed by $su(f)$ and $su(Hw)$; the effects of $e(w^e)$ and $su(pr)$ were not investigated. The modifiers $su(f)$ and $su(Hw)$ also suppressed $f^5$ more strongly than did $e(w^e)$ and $su(pr)$, although in each case the forked phenotype was more extreme than seen for $f^1$ with the same

modifiers. The $bx^{34e}$ allele was fully suppressed by *su(Hw)* but its suppression by $e(w^e)$ and *su(f)* was restricted to the removal of anterior wing margin bristles from the capitellum/wing of the homeotically transformed haltere (see Table 1). The alleles $w^a$ and $lz^{34}$ were suppressed only partially. Suppressed $sc^1$ had a variable phenotype, ranging from partial to complete restoration of the wild-type bristle pattern, together with occasional duplication of scutellar bristles. Finally, the wing phenotype of $ct^K$ was fully suppressed by heterozygous *su(Hw)*, while the bristle phenotype was suppressed only partially.

The enhancement of $Hw^1$ by *su(pr)* placed adventitious bristles on the capitellum and on the dorsal metathorax but had no clear effect on the number of extra bristles on the wing blade. Seven of the modifiable alleles we examined were not enhanced by any modifier. Two of these alleles, $y^2$ and $sc^1$, are less extreme than others at their respective loci, so that significant enhancement would presumably have been detectable. The phenotypes of the remaining five alleles ($ct^6$, $v^1$, $s^1$, $f^5$, and $pr^1$) are among the most extreme associated with these loci and therefore might not be capable of enhancement.

A particular study was made of modification at the *lozenge* locus. Since most *lz* alleles cause female sterility, they were maintained in attached-*X* stocks and examined in males. Six spontaneous alleles were studied: $lz^3$, $lz^{34}$, $lz^{46}$, $lz^1$, $lz^{37}$, and $lz^k$, in order of decreasing severity of effect on eye morphology. Two of the alleles, $lz^3$ and $lz^{46}$, were not affected by any of the six modifiers. No two of the four modifiable *lz* alleles displayed the same pattern of modification. Enhancement of $lz^{37}$ by *su(Hw)* was observed in both males and females with each of the two combinations tested, $su(Hw)^2/su(Hw)^2$ and $su(Hw)^2/su(Hw)^f$. This is the first reported instance of enhancement of any allele by *su(Hw)*.

Observations of the modification of *lozenge* alleles by *su(s)* in females gave unexpected results. In contrast to the results in males (Table 2), the eye morphology phenotype of $lz^1$ and $lz^{37}$ females appeared suppressed when either homozygous or heterozygous for $su(s)^3$. We do not know whether this effect is allele specific or locus specific, nor whether it is definitely attributable to mutation at *su(s)*.

**Properties of individual modifier mutations:** At least two different mutations of each modifier gene except *enhancer of white-eosin* were included in this study, as specified in Table 2. Five different mutations of *suppressor of Hairy-wing* were tested: $su(Hw)^2$, $su(Hw)^{69k}$, $su(Hw)^f$, $su(Hw)^{f3}$, and $su(Hw)^{70}$. No clearly significant differences in the degree of suppression or enhancement were seen with any of the homozygous or *trans*-heterozygous combinations listed in the table, except for combinations with $su(Hw)^{70}$, which suppressed less strongly. The two $su(Hw)$ mutations tested, $su(Hw)^2$ and $su(Hw)^f$, showed dominant partial suppression of $ct^K$. The same degree of dominant partial suppression was shown by the deficiency *Df(3R)su(Hw)*, indicating that $su(Hw)^2$ and $su(Hw)^f$ are nulls with regard to modification.

The original $e(w^e)$ mutation isolated by GREEN (1959) is lost. The mutation $e(w^e)^S$ is almost certainly allelic to it since both mutations enhance $w^e$ and suppress $f^1$ and both map to the same region of the *X* chromosome, between $lz^1$ and $v^1$. The female sterility and plexate-wing phenotype of $e(w^e)^1$ were not observed in $e(w^e)^S$ flies.

There was a difference in the effects of the two mutations of *suppressor of forked*. The mutation $su(f)^1$ suppressed $f^1$ and enhanced $w^a$. The mutation $su(f)^{br}$ also suppressed $f^1$ but did not noticeably enhance $w^a$. Enhancement of $w^a$ was seen, however, in $su(f)^1/su(f)^{br}$ flies, the eyes of which were intermediate in color between those of $su(f)^1$ and $su(f)^{br}$ homozygotes (not shown). No clear differences were noted in the degree of modification by $su(s)^3$ and $su(s)^{44}$, by $su(w^a)^1$ and $su(w^a)^{20}$ or by $su(pr)^B$, $su(pr)^{e3}$, $su(pr)^{e4}$, and $su(pr)^B/su(pr)^{e3}$.

## DISCUSSION

The effects of the six modifier genes on the 18 modifiable alleles we studied are summarized in Table 2. Each modifier had dual effects, suppressing some alleles and enhancing others. Also, many of the modifiable alleles were both suppressed and enhanced, depending on the particular modifier. That the suppression and enhancement effects are due to specific interactions between the modifier genes and the transposon-insertion alleles is indicated by the fact that the same results were obtained using several genetic backgrounds and with different alleles of modifier genes, including three mutations induced in this study.

**Groups of modifiers:** The pattern of interaction of modifiers with gypsy-insertion alleles is clearly not random. In every case in which modification of such alleles was observed, *su(Hw)*, $e(w^e)$, and *su(f)* acted as suppressors and *su(s)* and $su(w^a)$ acted as enhancers. All ten *gypsy*-insertion alleles were suppressed by *su(Hw)*. In contrast, $e(w^e)$, *su(f)*, *su(s)*, and $su(w^a)$ acted only on a particular subset, consisting of $ct^K$, $lz^1$, $f^1$, $f^5$, $bx^3$, and $bx^{34e}$. The modifier *su(pr)* suppressed some *gypsy*-insertion alleles, enhanced others and did not affect the rest. The six modifiers may therefore be placed in four groups with respect to their effects on *gypsy*-insertion alleles: *su(Hw)*; $e(w^e)$ and *su(f)*; *su(s)* and $su(w^a)$; and *su(pr)*.

Since most of the *gypsy*-insertion alleles tested were first identified as such on the basis of their suppressibility by *su(Hw)* (MODOLELL, BENDER and MESELSON

1983), it might be thought that the wider specificity of *su(Hw)* is an artifact. Indeed, not all *gypsy*-insertion alleles are suppressed by *su(Hw)*; for example, there are three nonsuppressible *su(s)* alleles associated with *gypsy* insertions (CHANG *et al.* 1986). However, many *gypsy*-insertion alleles not preselected for suppressibility are nevertheless suppressed by *su(Hw)*. Cloning of mutant alleles of the *bithorax* complex without regard to their modifiability has identified 11 different *bx* and *bxd gypsy*-insertion alleles at unique sites and in both orientations in the *bx* and *bxd* transcribed regions. All of them are suppressed by *su(Hw)* (PEIFER and BENDER 1986). Similarly, *su(Hw)* suppresses all of 14 different *gypsy*-insertion alleles at the *cut* locus (JACK 1985). It appears that *su(Hw)* has a genuinely broad spectrum of action on *gypsy*-insertion alleles.

The failure of the modifiers $e(w^e)$, *su(f)*, *su(s)*, and $su(w^a)$ to affect the alleles $y^2$, $Hw^1$, $sc^1$, and $ct^6$ may reflect a polymorphism of the inserted *gypsy* elements that renders these alleles insensitive to these modifiers. Another possibility is that the narrower specificity of the four modifiers is determined by the site or orientation of the *gypsy* insertion within the locus. A third possibility is that the nonresponsiveness of these alleles is locus-specific, perhaps because of developmental specificity in the action of the four modifier genes. In some cases modification effects may be insufficient to alter the phenotype. For example, $ct^K$ was dominantly suppressed by *su(Hw)*, while suppression of $ct^6$ by *su(Hw)* was recessive. It may be that both *gypsy*-insertion alleles are affected, but that $ct^K$ simply provides a more sensitive phenotypic assay.

The pattern of interaction of modifiers with the eight non-*gypsy* alleles is less regular. Four of these alleles ($w^a$, $lz^{34}$, $lz^k$, and $lz^{37}$) were modified by two or more of the five modifiers *su(Hw)*, $e(w^e)$, *su(f)*, *su(s)*, and $su(w^a)$. This allowed a test of whether, for these alleles, the modifiers *su(Hw)*, $e(w^e)$ and *su(f)* comprise a similarly acting group, which acts oppositely to the group consisting of modifiers *su(s)* and $su(w^a)$. As was the case with *gypsy*-insertion alleles, the results agreed with such a classification of modifiers, with one exception, the enhancement of $lz^{37}$ by *su(s)*.

**Modifier gene action:** The modification effects of mutation in the six modifier genes are recessive, indicating that they result from the absence or reduced activity of the modifier gene products rather than from altered functions. The wild-type *su(Hw)* product appears to intensify the disruption of gene expression caused by *gypsy* insertion. Likewise, the wild-type products of the modifiers $e(w^e)$ and *su(f)* appear to intensify the disruption associated with a particular subset of *gypsy*-insertion alleles, and the products of *su(s)* and $su(w^a)$ to prevent or alleviate it. Remarkably, when both *gypsy*- and non-*gypsy*-insertion alleles are taken into account, it appears that each of the six modifier gene products can act both to disrupt the expression of some alleles and to restore the expression of others.

If the product of one modifier gene is required for the function of another, either sequentially, as in a pathway, or as a component in the structure of a molecular complex, mutation of one of these genes should have no effect in flies deficient for the other. For example, if modification by *su(f)* requires the presence of the *su(Hw)* product, the modification phenotype of the double mutant should be no different from that of a *su(Hw)* null mutation alone. However, in tests with the $f^1$ allele, the double mutation suppressed more strongly than did either one alone. In addition, each of the three other modifiers tested with *su(Hw)* showed additive effects in the corresponding double mutant. While this additivity of modifier effects could result from leakiness of the modifier mutations, this would be difficult to reconcile with the evidence cited earlier that $su(Hw)^2$ and $su(Hw)^f$ are null alleles for modification. The additivity data indicate that the product of *su(Hw)* acts independently of the products of $e(w^e)$, *su(f)*, *su(s)*, and $su(w^a)$. Similarly, *su(f)* appears to act independently of *su(s)* and $su(w^a)$. Results with double heterozygotes support the hypothesis that none of the six modifiers acts competitively or interchangeably with another modifier gene product. For example, while homozygous *su(Hw)*, $e(w^e)$, and *su(f)* each suppressed $f^1$, none of the three double heterozygotes had any effect.

**Models of suppression and enhancement:** Studies of the *copia*-insertion allele $w^a$ indicate that the insertion affects *white* gene expression by causing premature termination of transcription, which is alleviated in a $su(w^a)$ background (PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984; ZACHAR *et al.* 1985). Transcriptional analysis of the dominant *gypsy*-insertion allele $Hw^1$ also revealed truncated transcripts terminating within the transposable element, although these transcripts were more abundant than the corresponding long transcripts in wild-type strains (CAMPUZANO *et al.* 1986). In a *su(Hw)* background these truncated transcripts were less abundant (CAMPUZANO *et al.* 1986). Truncated transcripts were not observed in the *gypsy*-insertion alleles $f^1$ and $f^5$, although the amount of *forked* transcripts was severely reduced, a result attributed to "promoter interference" (PARKHURST and CORCES 1985).

Although the genetic data do not specify the molecular nature of the interactions of modifier genes and modifiable alleles, they do set constraints on their general characteristics. The genetic findings are consistent with a model in which *su(Hw)* acts at one type of site, disrupting the expression of *gypsy*-insertion

alleles, while the products of the groups $e(w^e)$, $su(f)$ and $su(s)$, $su(w^a)$, act at one or more other sites to disrupt or prevent disruption of such expression, respectively. Since $su(pr)$ suppresses some *gypsy*-insertion alleles and enhances others, it may be that it mediates the effects of two or more modifiers that act oppositely on *gypsy*-insertion alleles. Whatever the detailed mechanism of modification, however, it must provide for the groupings of modifier genes defined by their effects on *gypsy*-insertion alleles and for the remarkable duality in the effects of each modifier gene.

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## LITERATURE CITED

BENDER, W., M. AKAM, F. KARCH, P. A. BEACHY, M. PEIFER, P. SPIERER, E. B. LEWIS and D. S. HOGNESS, 1983 Molecular genetics of the bithorax complex in *Drosophila melanogaster*. Science **221:** 23–29.

BINGHAM, P. M., and B. H. JUDD, 1981 A copy of the *copia* transposable element is very tightly linked to the $w^a$ allele at the *white* locus of *D. melanogaster*. Cell **25:** 705–711.

CAMPUZANO, S., L. BALCELLS, R. VILLARES, L. CARRAMOLINO, L. GARCIA-ALONSO and J. MODOLELL, 1986 Excess function *Hairy-wing* mutations caused by *gypsy* and *copia* insertions within structural genes of the *achaete-scute* locus of Drosophila. Cell **44:** 303–312.

CHANG, D.-Y., B. WISELY, S.-M. HUANG and R. A. VOELKER, 1986 Molecular cloning of suppressor of sable, a *Drosophila melanogaster* transposon-mediated suppressor. Mol. Cell. Biol. **6:** 1520–1528.

DUNSMUIR, P., W. J. BRORein, JR., M. A. SIMON and G. M. RUBIN, 1980 Insertion of the Drosophila transposable element *copia* generates a 5 bp duplication. Cell **21:** 575–579.

GEHRING, W. J., and R. PARO, 1980 Isolation of a hybrid plasmid with homologous sequences to a transposing element of *Drosophila melanogaster*. Cell **19:** 897–904.

GREEN, M. M., 1955 Phenotypic variation and pseudo-allelism at the *forked* locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **41:** 375–379.

GREEN, M. M., 1957 Report of M. M. GREEN. Drosophila Inform. Serv. **31:** 81.

GREEN, M. M., 1959 Spatial and functional properties of pseudo allleles at the white locus in *Drosophila melanogaster*. Heredity **13:** 302–315.

HAZELRIGG, T., 1987 The *Drosophila white* gene: a molecular update. Trends Genet. **3:** 43–47.

JACK, J. W., 1985 Molecular organization of the *cut* locus of Drosophila melanogaster. Cell **42:** 869–876.

LANGER-SAFER, P. R., M. LEVINE and D. C. WARD, 1982 Immunological method for mapping genes on *Drosophila* polytene chromosomes. Proc. Natl. Acad. Sci. USA **79:** 4381–4385.

LEE, G. L. G., 1973 The phenogenetics of a super-suppressor in *Drosophila melanogaster*. III. Suppression at individual loci. Aust. J. Biol. Sci. **26:** 903–909.

LEVIS, R., K. O'HARE and G. M. RUBIN, 1984 Effects of transposable element insertions on RNA encoded by the *white* gene of Drosophila. Cell **38:** 471–481.

LEWIS, E. B., 1949 Report of E. B. LEWIS. Drosophila Inform. Serv. **23:** 59–60.

LEWIS, E. B., 1967 Genes and gene complexes. pp. 17–47. In: *Heritage from Mendel*, Edited by A. BRINK. University of Wisconsin Press, Madison.

LEWIS, E. B., 1981 Developmental genetics of the bithorax complex in *Drosophila*. pp. 189–208. In: *Developmental Biology Using Purified Genes*, Edited by D. D. BROWN and C. F. FOX. Academic Press, New York.

LEWIS, E. B., and F. BACHER, 1968 Method of feeding ethyl methane sulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43:** 193.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627.**

MCLACHLAN, A., 1986 *Drosophila* forked locus. Mol. Cell. Biol. **6:** 1–6.

MODOLELL, J., W. BENDER and M. MESELSON, 1983 *Drosophila melanogaster* mutations suppressible by the suppressor of Hairy-wing are insertions of a 7.3-kilobase mobile element. Proc. Natl. Acad. Sci. USA **80:** 1678–1682.

PARKHURST, S. M., and V. G. CORCES, 1985 *forked*, gypsys, and suppressors in Drosophila. Cell **41:** 429–437.

PEIFER, M., and W. BENDER, 1986 The anterobithorax and bithorax mutations of the bithorax complex. EMBO J. **5:** 2293–2303.

PHILLIPS, J. P., and H. S. FORREST, 1980 Ommochromes and pteridines. pp. 541–623. In: *The Genetics and Biology of Drosophila*, Vol. 2d, Edited by T. R. F. WRIGHT and M. ASHBURNER. Academic Press, New York.

PIRROTTA, V., and CH. BROKL, 1984 Transcription of the Drosophila *white* locus and some of its mutants. EMBO J. **3:** 563–568.

SCHALET, A., 1970 Some observations on the proximal euchromatic-heterochromatic region of the *X* chromosome of *Drosophila melanogaster*. Genen Phaenen **14:** 16–17.

SCHULTZ, J., and C. B. BRIDGES, 1932 Methods for distinguishing between duplications and specific suppressors. Am. Nat. **66:** 323–334.

SEARLES, L. L., and R. A. VOELKER, 1986 Molecular characterization of the *Drosophila* vermilion locus and its suppressible alleles. Proc. Natl. Acad. Sci. USA **83:** 404–408.

WALKER, A. R., A. J. HOWELLS and R. G. TEARLE, 1986 Cloning and characterization of the *vermilion* gene of *Drosophila melanogaster*. Mol. Gen. Genet. **202:** 102–107.

WILSON, T. G., and K. B. JACOBSON, 1977 Isolation and characterization of pteridines from heads of *Drosophila melanogaster* by a modified thin-layer chromatography procedure. Biochem. Genet. **15:** 307–319.

ZACHER, Z., D. DAVISON, D. GARZA and P. M. BINGHAM, 1985 A detailed developmental and structural study of the transcriptional effects of insertion of the *copia* transposon into the white locus of *Drosophila melanogaster*. Genetics **111:** 495–515.

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