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Interactions among modifiers of retrotransposon-induced alleles of the *white* locus of *Drosophila melanogaster*

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Summary

Mutations in five loci that modify the phenotype of $white^{apricot}$ (w^a), caused by the retrotransposon, *copia*, were examined in two-way combinations to determine whether their effects were additive or epistatic. All two-way combinations of mutations in these five loci, *mottler of white* (*mw*), *suppressor of forked* (*su(f)*), *suppressor of white apricot* (*su*(w^a)), *Enhancer of* $white^{apricot}$, (*E*(w^a)) and *Darkener of apricot* (*Doa*), are additive in their effects on w^a, implying that each second-site modifier locus affects a different process. Three other *copia*-induced mutations, Hw^{Ua}, $w^{hd81b25}$ and ct^{ns} were also examined for responsiveness to mutations in these modifier loci. None clearly responded. Mutations associated with *B104* insertions, including *Gl*, vg^{ni}, ct^{n} and w^{ric} were also examined for responsiveness to *mw* mutations, which have specificity for this element as well. Both vg^{ni} and w^{ric} respond to mutations in *mw*. The former interaction demonstrates that *mw* is capable of interacting with *B104* elements in loci other than *white*. The significance of the results with respect to the nature of second-site modifier loci is discussed.

1. Introduction

Spontaneous mutations in a variety of organisms are frequently caused by the insertion of a transposable element, which includes mammalian retroviruses and LINE sequences as well as retrotransposons such as those described in *Drosophila*, tobacco, maize, *Arabidopsis* and yeast (Bender *et al.* 1983; Campuzano *et al.* 1986; Grandbastien *et al.* 1989; Johns, Mottinger & Freeling, 1985; Modolell, Bender & Meselson, 1983; Morse *et al.* 1988; Nusse, 1986; Voytas & Ausubel, 1988). Retrotransposons, as a class, transpose through RNA intermediates, and resemble each other to varying degrees in sequence homology and the overall organization of their genomes. Retrotransposon insertions have caused mutations by inserting within exons, introns, non-translated leader sequences, and upstream of transcription start-sites.

Why the insertion of transposable element sequences causes mutations when inserted in non-coding sequences (e.g. intron) is not immediately obvious, since this should not necessarily interfere with translation of a mature, normally spliced RNA to form an active protein. One explanation for the mutant phenotype, in such cases, is that the introduction of the transposon alters expression of the host gene, resulting in premature termination of transcription, improper patterns of its expression, or incorrect splicing (Cullen, Lomedico & Ju, 1984; Dorsett *et al.* 1989; Morishita *et al.* 1988; Parkhurst & Corces, 1985; Zachar *et al.* 1985).

Several lines of evidence support this concept. First, revertants of insertion mutations restoring full or partial function of the host gene have in several instances been characterized as secondary insertions into the original transposon (Campuzano *et al.* 1986; Geyer, Green & Corces, 1988; Mount, Green & Rubin, 1988; Sang *et al.* 1984; Williams & Bell, 1988; Zachar & Bingham, 1982). These revertants may be thought of as insertion mutations in the first transposon causing the original mutation. Secondly, the transcriptional activity of the inserted element has been hypothesized to be the causative factor for the mutation in several of these instances. For example, at the *Notch* locus of *Drosophila*, the type of inserted element, rather than the position of the insertion, correlates with the mutant phenotype produced (Kidd & Young, 1986). Partial excision of retrotransposon insertions can result in almost complete reversion of the original mutation, indicating that the simple interruption of host sequences is often not sufficient for the mutant phenotype (Bender *et al.* 1983; Mount *et al.* 1988; Searles & Voelker, 1986; Zachar *et al.* 1985).

Insertion mutations are also known which are due to increased or ectopic expression of the host gene,

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indicating that new patterns of expression have been imposed on it by the inserted element. Examples include the *Hairy-wing* locus (*Achaete-scute* complex) of *Drosophila*, where insertions of different transposons have yielded mutant phenotypes due to improper stage-specific regulation of the gene (Campuzano *et al.* 1986), and the activation of cellular oncogenes by retroviral insertion (Morishita *et al.* 1988; Morse *et al.* 1988; Nusse 1986).

In *Drosophila*, second-site modifier loci altering the phenotypes of transposon insertions have been described, with varying degrees of specificity for affected transposons and mutant alleles (Chang *et al.* 1986; Green, 1959; Modolell *et al.* 1983; Rutledge *et al.* 1988). They have also been found in other eukaryotic organisms, and are well documented in yeast, (Clarke-Adams *et al.* 1988; Clarke-Adams & Winston, 1987; Fassler & Winston, 1988; Neigeborn, Celenza & Carlson, 1987; Winston *et al.* 1984*a*, *b*, 1987). In mammals, host loci affecting the expression of retroviruses have also been identified (Levy, Lerner & Wilson, 1982, 1985; Traina-Dorge *et al.* 1985). Modifiers can enhance (increase) or suppress (decrease) the severity of the mutations they affect, and in some cases a single mutation does both, to different mutant alleles at different loci. In other instances, both enhancers and suppressors of a single mutant allele have been described. The exact molecular mechanisms of phenotypic modification remain poorly defined, although some clues are beginning to emerge, through genetic and molecular characterization of modifier genes, as well as the analysis of transcripts from affected alleles and transposons. The results imply that several different mechanisms are at work, including potential changes in splicing and transcription. It is hypothesized that second-site modifier loci are genes whose products play a role in the functions of the mutation-causing transposable element, and which, in modifying its activity, result in an alteration of the mutant phenotype.

The *white*^{apricot} (w^a), allele of the *white* locus *Drosophila* is due to the insertion of a *copia* retrotransposon in the second intervening sequence of the gene (Bingham & Judd, 1981; Gehring & Paro, 1980; O'Hare *et al.* 1984; Pirotta & Brockl, 1984). Several modifiers of the w^a phenotype have been described, some with overlapping effects on transposon-induced mutations at other loci, and others with effects on additional transposon-induced alleles at *white* (Birchler, Hiebert & Rabinow, 1989; Birchler & Hiebert, 1989; Green, 1959; Rabinow & Birchler, 1989; Rutledge *et al.* 1988). These modifiers comprise one of the most extensive sets affecting a single transposon-induced allele known. In order to further characterize the diverse mechanisms of suppression and enhancement acting on a single transposable element-induced allele, we undertook a study of the previously described second-site modifiers of w^a in combinations, reasoning that two mutations altering a single step in expression of the inserted transposon would interact in an epistatic, rather than additive fashion. We also examined the effects of these modifiers on insertions of the *copia* transposon in other loci to determine if the position and orientation of the insertion with respect to the host gene affect its ability to be influenced by second-site modifiers in a predictable manner. Finally, we examined the effects of the w^a modifier, *mw*, on mutations at several other loci caused by the *B104* retrotransposon, since *mw* also enhances *white* mutants caused by this element.

2. Materials and methods

(i) Drosophila *culture*

Drosophila melanogaster stocks and crosses were reared on Carolina Biological Instant Drosophila medium at 25 °C. Eye colours were scored at eclosion and after 3 days.

(ii) *Stocks*

The source and nature of *copia*- and *B104*-induced mutations examined are described below. Modifier alleles and stocks are presented in Table 1. *D. melanogaster* transposable elements and mutations were reviewed by Finnegan & Fawcett (1986).

(iii) Copia *insertions*

The structure and expression of *copia* has been extensively characterized (Flavell *et al.* 1980, 1981; Mount & Rubin, 1985; Parkhurst *et al.* 1988; Schwartz, Lockett & Young, 1982).

white^{apricot} (w^a). Bingham & Judd (1981), Gehring & Paro (1980) and O'Hare *et al.* (1984) describe the genomic structure of w^a. Levis, O'Hare & Rubin (1984), Pirrotta & Brockl (1984) and Zachar *et al.* (1985), describe w^a RNA products, which include those originating in the *white* promoter and terminating in either LTR of *copia*, as well as a small amount of wild-type-sized mRNA, accounting for the low level of pigment observed. Other, less well defined RNA species have also been observed (Birchler *et al.* 1989; Mount *et al.* 1988; Pirrotta & Brockl, 1984; Zachar *et al.* 1985). Zachar *et al.* 1985) describe effects of the *copia* insertion on transcription from the *white* promoter. (Source: Bowling Green Stock Center.)

white^{hd81b25} ($w^{hd81b25}$). (Rubin, Kidwell & Bingham, 1982). Two or three independent *copia* anti-parallel insertions into the *white* locus, $w^{hd18b6,11}$ and ^{25}, occurred at the same location. The exact position of the insert was determined by sequencing $w^{hd81b11}$ (O'Hare *et al.* 1984). The $w^{hd81b25}$ allele was used in the present study. The phenotype is bleach-white eye colour, and it is thus a null. (Source: Dr Margaret Kidwell, University of Arizona.)

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Table 1. *Modifiers of* w^a: *allele specificity at affected loci*

Modifier	Specificity/ Inserted element	en or su	Stock(s)	Reference
mw	white^a/*copia*	en	w^a mw^2	Birchler *et al.* (1989)
	white^{a4}/*BEL**	en	and	
	white^{bf}/*B104*	en	w^a *ct mw f*	
	white^{sp55}/unnamed	en		
	zeste^1 white^{zm}/*BEL**	en		
	white^h/*B104* into *Doc*†	en (slight)		
E(w^a)	*white^a*/*copia*	en	w^a; *E*(w^a)/*CyO*	Birchler & Hiebert (1989)
	white^{a4}/*BEL**	en	and	
	white^{sp55}/unnamed	en	*y* w^a; *Frd E*(w^a) *sp Pin^2*/*SM1*	
	white^h/*B104* into *Doc*†	en		
Doa	*white^a*/*copia*	su	w^a; *Doa^{HD1}*/	Rabinow & Birchler (1989)
	white^{sp55}/unnamed	en	*TM3 or TM6*	
			and	
			w^a; *Doa^{105}*/*CyO*	
			and	
			w^a; *Doa^{CC}*/*SM6*	
			and	
			y w^a; *Doa^{EMS1}*/*TM3*	
			and	
			w^a; *Doa$^{1-5}$*/*TM3*	
su(f)	*white^a*/*copia*	en	w^a *su(f)*	Bowling Green/ Rutledge *et al.* (1988)
	cut^k/*gypsy*	su		
	lozenge^1/*gypsy*	su		
	forked^1/*gypsy*	su		
	forked^5/*gypsy*	su		
	bithorax^{34e}/*gypsy*	su		
su(w^a)	*white^a*/*copia*	su	*y^2 sc su*(w^a) w^a	Bowling Green/ Rutledge *et al.* (1988)
	cut^k/*gypsy*	en		
	lozenge^1/*gypsy*	en		
	forked^1/*gypsy*	en		
	bithorax^{34e}/*gypsy*	en		

en, enhancement, or increase of mutant severity; su, suppression, or decrease of mutant severity.
The effects of *su*(w^a) and *su(f)* on many insertion mutations were recently reviewed in Rutledge *et al.* (1988), from which all of the presented data on loci other than w^a is summarized for these two modifier loci.

* The *BEL* element in w^{a4} has the same restriction map as, and hybridizes to the *3S18* element in w^{zm} (K. Peterson and R. Levis, personal communications).
† The w^h allele is a revertant of w^1, and is due to the insertion of a *B104* element (K. O'Hare, personal communication).

Hairy-wing^{Ua} (*Hw^{Ua}*) (Campuzano *et al.* 1986). This mutation is a hypermorph, caused by an anti-parallel *copia* insertion in an exon generating a truncated RNA. This RNA yields a functional protein, and is present at elevated levels and inappropriate developmental times relative to normal. (Source: Dr Juan Modollel, Universidad Autonoma de Madrid.)

cut^{ns} (*ct^{ns}*). This allele is due to the anti-parallel insertion of a *copia* element approximately 70 kb. 5′ from the start of the known coding region in several cDNAs (Blochlinger *et al.* 1988; Jack, 1985). Although there are no known *cut* exons in this region, transcript mapping of the gene is not yet complete. The *copia* insertion lies in a region where a number of other insertion events have caused mutations with similar phenotypes (Jack, 1985). Since this allele arose on an inversion chromosome (*op cit.*; Lindsley & Grell, 1968), no effort was made to test for interactions between it and mutations at the other X-linked w^a modifiers, *su*(w^a), *mw*, and *su(f)*. Tests were scored by visually estimating the degree of wing-scalloping. (Source: Dr Joseph Jack, Memorial Sloane–Kettering Cancer Center.)

(iv) B104 (*or* 'roo') *insertions*

B104 structure and expression was characterized by Scherer *et al.* (1982).

Glued (*Gl*) (Swaroop, Paco-Larson & Garen, 1985). This *B104* insertion is located in the transcribed region of the *Gl* locus, and is oriented in the 'sense' direction, with respect to the transcription of the host gene. The mutation is a recessive lethal, and produces a truncated RNA. The eyes are malformed and the

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facets rough (Lindsley & Grell 1968). (Source: Bowling Green Stock Center.)

vestigial^{nicked} (*vg^{ni}*) (Williams & Bell, 1988). A *B104* insertion occurred into the *412* element which caused *vg^{BG}*, reverting the mutant phenotype. The *412* insertion probably occurred in an intron (John Bell, personal communication). The *vg^{ni}* allele does not show any mutant phenotype when homozygous, and only shows occasional wing-nicking when heterozygous with a strong *vg* allele (Lindsley & Grell, 1968). (Stock source: Dr John Bell, University of Alberta.)

white$^{roo\text{-}in\text{-}copia}$ (*w^{ric}*) (Davis, Shen & Judd, 1987). This allele is the result of *B104* insertion into the central region of the *copia* element in *w^a*, although the phenotypes of the two alleles are indistinguishable. The direction of transcription of the inserted *B104* is the same as that of *copia* and *white*. (Source: Dr Burke Judd, NIEHS.)

cut^n (*ct^n*) (Jack 1985). This insertion of a *B104* element is in the same region as that of *ct^{ns}*, i.e. approximately 70 kb, 5′ to the closest cDNA yet characterized, and in the same transcriptional orientation as the host gene. This region is of importance to normal *ct* expression, however, since several different insertion events have caused mutations with related phenotypes (Jack, 1985). (Source: Dr Jo Jack.)

(v) Gypsy *insertions*

The *gypsy* element and second-site modifiers affecting it have been extensively studied (Chang *et al.* 1986; Dorsett *et al.* 1989; Freund & Meselson, 1984; Geyer *et al.* 1986, 1988; Parkhurst & Corces, 1985, 1986*a*, *b*, 1987; Parkhurst *et al.* 1988; Peifer & Bender, 1988).

cut^k (*ct^k*) (Jack 1985). This *gypsy* insertion is located near the 5′ end of cDNAs recovered for the *ct* locus (Blochlinger *et al.* 1988), and is particularly sensitive to modifiers of *gypsy* mutations (Rutledge *et al.* 1988; J. Jack, personal communication). (Source: Dr Joseph Jack.)

(vi) *Crosses to score interactions*

Interactions among the modifier loci were determined by crossing balanced stocks of the autosomal modifiers and comparing the effects on *w^a* in balancer and modifier classes of siblings. X-linked modifiers were scored for interaction with autosomal modifiers in males only. Interaction among the X-linked modifiers on *w^a* was previously reported elsewhere (Rutledge *et al.* 1988; Birchler *et al.* 1989). All of the *copia* insertion alleles tested were X-linked. Autosomal modifiers (*Doa* and *E*(*w^a*)) were tested as heterozygotes by crossing males of a balanced stock of the modifier to females of the *copia* insertion allele, and comparing F_1 male progeny of the two segregating classes, balancer versus modifier. *E*(*w^a*) homozygotes were also tested in each case, by crossing F_1 females heterozygous for the *copia* insertion allele and *Frd E*(*w^a*) *sp Pin^2* with males of the genotype *y w^a*; *E*(*w^a*)/*CyO*. F_2 males hemizygous for the *copia* insertion allele and homozygous for *E*(*w^a*) were compared with their heterozygous and wild-type siblings. *Doa* homozygous escapers of lethality were tested for interaction with *Hw^{Ua}*, *ct^{ns}*, *w^{hd81b25}* and *w^{ric}* in males of the genotype *Doa^{HD1}*/*Doa^{l05}*, generated as described (Rabinow & Birchler 1989). X-linked modifiers were tested for interaction with *copia*, *B104*, or other insertion alleles by recombining the appropriate modifier onto the chromosome to be tested, and scoring the recombinant male progeny versus non-recombinant siblings. Alternatively, they were analyzed by constructing females homozygous for the insertion mutation and heterozygous for the modifier, followed by scoring segregating male progeny for the appropriate markers. These tests were performed in a *w^a* background, and appropriate genetic markers were used to allow unambiguous scoring of the modifier class. Interaction of *mw^2* with the autosomal *B104* insertions in *vg^{ni}* and *Gl^l* were performed by constructing females heterozygous for *w^a mw^2* and the autosomal mutation, and backcrossing them to either *vg^{ni}* or *Gl*. F_1 male sibling progeny were compared to score the results.

(viii) *Construction of a population segregating for* su(f) *and* su(w^a)

Females heterozygous for either *y+ su(f)* or *y^2 su(w^a)*, and homozygous for *w^a*, the non-modified *w^a* chromosome carrying *y* were produced in standard crosses. These females were backcrossed to *y w^a* males. Female F_2 progeny were then scored for phenotypic effects of the *su(f)* or *su(w^a)* as heterozygotes, and compared to *w^a* siblings of the same sex, age and, aside from the X-chromosome, the same genetic background. The presence of *y+* or *y^2* was used to indicate the presence of the modifier chromosome.

3. Results

The experiments reported here were designed to extend the genetic analysis of *Doa*, *mw* and *E*(*w^a*), three recently described modifiers of *w^a*. Characterizations included examining interactions among these, as well as *su(f)* and *su(w^a)*, two previously described modifiers of *w^a*, and the susceptibility of a selected set of other retrotransposon-induced insertions to modification.

(i) *Action of modifier loci in combinations*

Combining two mutations which affect the same step in any process should produce a phenotype no more severe than the stronger of the two. Although other possibilities exist, as a general rule mutations affecting different processes are expected to produce additivity in their phenotypes. It was reasoned that a systematic study of the effects of *w^a* modifiers in pairs would be

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Table 2. *Interactions among modifiers of* w^a

Modifier loci	Phenotype	Reference
$su(f)+su(w^a)$	= w^a	Rutledge *et al.* (1988)
$su(f)+mw^2$	$\ll w^a$	Birchler, *et al.* (1989)
$su(f)+E(w^a)$	$\ll w^a$	This report
$mw^2+E(w^a)$	$\ll w^a$	This report
$mw^2+su(w^a)$	= w^a	Birchler, *et al.* (1989)
$Doa^{HDI}+su(w^a)$	$\gg w^a$	This report
$Doa^{HDI}+mw^2$	= w^a	This report
$Doa^{HDI}+su(f)$	= w^a	This report
$Doa^{HDI}+E(w^a)$	= w^a	This report
$su(w^a)+E(w^a)$	= w^a	This report

= w^a is a phenotype of approximately w^a, i.e. light orange in colour.
$\gg w^a$ is a phenotype substantially darker than w^a, and either of the two modifiers alone, i.e. a dark ruby red in colour.
$\ll w^a$ is a phenotype substantially lighter than w^a, and of either modifier alone, i.e. bleach white in colour.
Crosses were peformed by crossing males of a balanced stock of either autosomal modifier, $E(w^a)$ or *Doa* to females of a homozygous stock of a w^a chromosome carrying one of the X-linked modifier mutations and comparing progeny classes in F_1 males.

informative as to whether one, or several processes were being affected.

To summarize our results, the effect of combining any two mutations modifying the expression of w^a is additive (Table 2). If mutations at two loci suppressing w^a, such as *Doa* and $su(w^a)$ are combined, then the double mutant is darker than either mutation alone. The opposite applies for two mutations enhancing (lightening) w^a, such as *mw* and $E(w^a)$, which when combined, yield essentially white eyes. This also holds for mutations affecting w^a in opposite directions, i.e. the combination of an enhancer with a suppressor, which yields a colour approximately equivalent to the original w^a phenotype. Biases are seen, however, based upon the relative strengths of the enhancers and suppressors used. For example, *Doa* as a heterozygote is a qualitatively 'weaker' suppressor than *mw* is an enhancer. Combining the two mutations results in a phenotype slightly lighter than w^a, yet darker than *mw* alone.

(ii) *Dominant and recessive modifier mutations*

Mutant alleles of the X-linked modifier loci $su(f)$ and $su(w^a)$ have dramatic effects on w^a when hemizygous or homozygous. Mutations in two dominant w^a modifiers, *Doa* (Rabinow & Birchler, 1989), and loss of function revertants of the original neomorphic $E(w^a)$ allele (Birchler & Hiebert, 1989), have only subtle effects as heterozygotes. *Doa* mutants, for example, elevate w^a pigment levels by only two-fold as heterozygotes, based on comparisons with flies carrying a duplication of the apricot allele. The subtle effects of heterozygosity for dominant mutations in *Doa* and $E(w^a)$ revertants suggested the possibility that mutations in $su(f)$ and $su(w^a)$ might actually be dominant, but with less dramatic effects when heterozygous. This situation is in fact the case for the X-linked modifier mutation mw^2, in which mutant hemizygotes and homozygotes have nearly bleach white eyes, while heterozygous females are only slightly but reproducibly reduced in pigment levels (Birchler *et al.* 1989). However, scoring for effects of $su(f)$ and $su(w^a)$ on w^a as heterozygotes in a segregating population showed that they are in fact recessive mutations. This differentiates them from the other three modifiers of w^a examined in this study *mw*, $E(w^a)$ and *Doa*, which all behave as dominant mutations.

(iii) *Effects of* w^a *modifiers on* copia *insertions in other loci*

Copia insertions have been shown to be associated with mutations at a number of loci. These mutations are caused by *copia* insertions in various parts of the gene, and in different orientations, illustrating some of the ways in which insertions can cause disruption of normal gene expression. The tested mutant alleles, the molecular nature of the lesions, and their responsiveness to the modifiers of w^a under study are summarized in Table 3. The isolation and identification of these mutations as *copia* insertions is referenced and described in Methods and Materials.

None of the modifier loci tested had any detectable effects on $w^{ha81b25}$ or ct^{ns} implying that their action is mediated through mechanisms which could not affect the cause of the mutant phenotypes (Table 3). Even *Doa* homozygous escapers, which completely suppress the phenotype of w^a, had no effect on these two *copia* insertions.

Hw^{Ua} has been shown to be caused by the over- and aberrant expression of the host transcript, which originates in the normal location, and terminates in the 3′ LTR of the anti-parallel oriented *copia*. Unlike the analogous *copia* insertion in $w^{ha81b25}$, the gene retains activity, and it is the excessive and aberrant expression of the product which generates the mutant phenotype. The *copia* insertion in Hw^{Ua} occurred in a sc^1 background, and thus the weak *Hairy-wing* phenotype of this allele is perhaps best described as a partial suppression of the sc^1 phenotype, (Campuzano *et al.* 1986), which removes bristles from the scutellum and head (Lindsley & Grell, 1968).

Modifier effects on the Hw^{Ua} phenotype were determined by counting the number of scutellar bristles in individuals from each of the progeny classes, and comparing the number of individuals in each (Tables 3, 4). Tests with X-linked modifiers were performed with only $su(f)$, and *mw*, due to the tight genetic linkage between $su(w^a)$ and *Hw*. Mutations in $su(f)$ and *mw* had no effect on the Hw^{Ua} phenotype, in the assay described above (Table 4). $E(w^a)$ had no effect unless carried on the *Frd* $E(w^a)$ *sp* Pin^2 chromosome. Thus, we cannot attribute these effects to $E(w^a)$, but must suggest some interaction between a linked

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Table 3. *Mutations induced by* copia *insertion tested for interaction with second-site modifiers*

Locus	Allele/ chromosome	Orientation	Position	Type of mutation	Effect of modifier				
					mw	$E(w^a)$	*Doa*	*su(f)*	$su(w^a)$
white	w^a	Parallel	Intron	Hypomorph	en	en	su	en	su
	$w^{ha81b25}$	Anti-parallel	Exon	Null	0	0	0	0	0
Hairy-wing	y^2 *sc* Hw^{Ua} w^a	Anti-parallel	Exon	Hypermorph	0	0	su?	0	ND
cut	*Δ49*,	Parallel	5′ non-	Hypomorph	ND	0	0	ND	ND
	y ct^{ns} v g f		coding						

Copia insertion alleles are described and referenced in Materials and Methods. 'Orientation' refers to the direction of transcription of the inserted element relative to the host gene, which were deduced from published restriction maps accompanying the descriptions of the insertion alleles. 'Position' refers to the site of the insertion in the host gene. 'Type of mutation' refers to the degree of the phenotype, as described in the cited reference. Mutations in $E(w^a)$ were tested as both hetero- and homozygotes. X-linked mutations (*su(f)*, *mw* and $su(w^a)$) were tested in males only. *Doa* mutants were tested as heterozygotes and as homozygous escapers of lethality.

en, enhanced; su, suppressed; 0, no effect; ND, not determined. See text for details, and Materials and Methods for stock sources, descriptions and crosses.

The alleles mw^2, *su(f)*, $su(w^a)$ and $E(w^a)$ and Doa^{HD1} were used in the tests for interactions reported here. Additional *Doa* alleles tested for interaction with Hw^{Ua} are shown in Table 4. Doa^{105} was also tested, while generating homozygous escapers of lethality with $w^{ha81b25}$, Hw^{Ua} and ct^{ns}.

Table 4. *Effects of* w^a *modifier loci on scutellar bristle number of* Hw^{Ua} *in segregating populations*

Modifier	Number of individuals with scutellar bristle number				
	0	1	2	3	4
$E(w^a)$ and siblings					
CyO/+	0	2	45	68	73
$E(w^a)$/+	0	0	18	46	56
$E(w^a)$/*Frd* $E(w^a)$ *Pin* sp^2	1	3	32	19	5
su(f) and siblings					
y^2 *sc* Hw^{Ua} w^a	3	28	85	23	0
y^2 *sc* Hw^{Ua} w^a *su(f)*	9	21	88	19	2
mw and siblings					
y^2 *sc* Hw^{Ua} w^a	0	7	80	38	17
y^2 *sc* Hw^{Ua} w^a mw^2	0	0	21	11	3

	Number of individuals with scutellar bristle number			
	0	1–2	3	4
Doa and siblings				
CyO/+	—	8	37	23
Doa^{CC}/+	—	86	17	4
TM3/+	—	5	18	27
Doa^{HD1}/+	—	81	33	7
TM3/+	—	10	28	42
Doa^{EMS1}/+	—	137	44	3
TM3/+	—	7	14	45
Doa^{1-5}/+	—	96	12	2
Doa homozygous escapers of lethality (siblings not scored)				
Doa^{105}/Doa^{HD1}	6	1	0	0

Balancer chromosomes listed are derived from the balanced stock from which the modifier was derived. Four scutellar bristles is wild type.

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Table 5. B104 *insertions tested for interaction with* mottler-of-white

Locus	Allele/ Chromosome	Orientation	Position	Type of mutation	Effect of mw^2
white	w^{bf} f^5	Parallel	Intron	Hypomorph	en
white	w^{sp}	Parallel	5′ non-coding	Hypomorph	0
white	y^2 w^{ric} *spl ec*	Parallel	*copia* in w^a	Hypomorph	en
white	w^h	Parallel	in w^1	Hypomorphic	en
			Doc element in or near mRNA leader	Revertant of null	en (slight)
Glued	*Gl*	Parallel	Exon?	Null	0
vestigial	vg^{ni}	Anti-parallel	Into *412* element in intron	Revertant of Hypomorph	en
cut	w^a ct^n	Anti-parallel	5′ non-coding	Hypomorph	0

B104 insertion alleles are described and referenced in Materials and Methods. The tests with w^{sp}, w^h and w^{bf} were reported in Birchler, *et al.* (1989). Notations are the same as in Table 3. All tests were performed in males only. The mw^2 allele, a non-mottling, uniform enhancer of w^a was used in the tests for interactions reported here. The original mottling allele was also tested for interaction with w^{ric}, and mottling was observed.

gene and *Ac-sc* mutations. In contrast, all 5 *Doa* alleles tested resulted in partial suppression of the mutant phenotype. This effect is complete in the case of *Doa* homozygous escapers of lethality, in which six out of seven completely lacked scutellar bristles, and the seventh lacked three of four (Table 4). A caveat is necessary in interpreting this result, however, since it has been previously noted that approximately 50% of homozygous escapers of lethality lack one or more scutellar bristles, even in a wild-type *Ac-sc* background (Rabinow & Birchler, 1989). Thus we cannot attribute the effects seen with *Doa* on Hw^{Ua} as unambiguously due either to interaction with the inserted *copia* element, or with wild-type *Ac-sc* sequences or products.

(iv) *Specificity of second-site modifiers of* w^a *in tests with* gypsy*-induced mutations*

Both *gypsy* and *copia* are retrotransposons that share similarities of overall organization. Since two modifiers of w^a, *su*(w^a) and *su*(*f*), also affect *gypsy*-induced mutations (Dorsett *et al.* 1989; Green, 1959; Rutledge *et al.* 1988), we tested whether three recently described modifiers of the w^a phenotype also affect them. *Doa*, *mw* and *E*(w^a) failed to affect the phenotypes of y^2, sc^1 and f^1, all of which are phenotypically modifiable, and caused by gypsy insertions. To confirm and extend these results, we tested the gypsy insertion in ct^k, which is particularly sensitive to second-site modifiers (Rutledge *et al.* 1988; J. Jack, personal communication). No mutation in the three modifier loci, which affect a number of retrotransposon-induced alleles at *white*, had any effect on ct^k.

(v) *Interactions of* mw *mutations with* B104 *insertions*

Mutations in the *mw* locus affect a subset of transposon-induced *white* alleles (Birchler *et al.* 1989), including w^{bf}, which is a strong hypomorph due to the insertion of a *B104* or *roo* element into the fourth intron of *white*, in a transcriptional orientation parallel to the gene (Levis *et al.* 1984; Zachar & Bingham, 1982). $white^{honey}$ was originally described as a deletion within the *Doc* element in $white^1$, but it was subsequently shown to be a *B104* insertion into this element, partially reverting the null phenotype (K. O'Hare, personal communication). It also responds to mutations at *mw*, although a third *B104* insertion at *white*, w^{sp}, does not (Birchler *et al.* 1989). This latter insertion occurred in a region implicated in the control of *white* expression 5′ to the coding region, and its location may account for the fact that it is unresponsive to *mw* mutants. As for the *copia* insertions tested with five modifier loci, we determined if a number of additional *B104* insertion mutations interact with mw^2, a strong hypomorphic or null allele. Mutant alleles and insertions tested are described in Materials and Methods. Results are summarized in Table 5. No change of the *Gl* or ct^n phenotypes was seen in tests for interaction with mw^2. Two insertions of *B104* within other transposons, vg^{ni} and w^{ric} do respond.

When male vg^{ni} are crossed to w^a mw^2 females, and the resulting doubly heterozygous female F_1 progeny are backcrossed to vg^{ni} males, approximately half the w^a mw^2 male progeny have deeply scalloped wings, a strongly enhanced phenotype of vg^{ni} (Fig. 1). Identical control crosses showed no interaction between mw^2 and vg^{BG}, the chromosome of origin of vg^{ni}, as indicated by lack of change in wing phenotype in w^a mw^2; vg^{BG}, flies. The vg^{BG} allele is a strong hypomorph, based on the criterion that the severity of the phenotype of this allele is increased (wing size decreased), when this allele is heterozygous with a deletion for the region.

Confirmation that interaction between the *B104*

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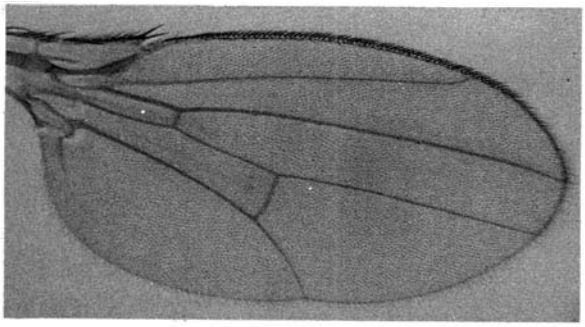
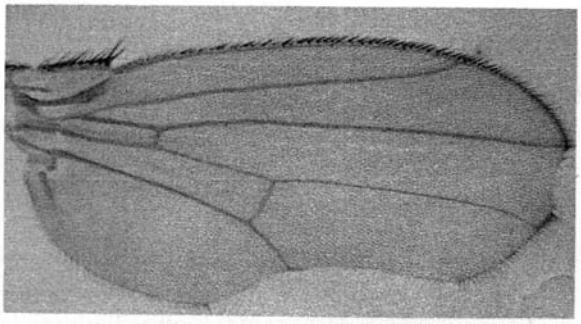
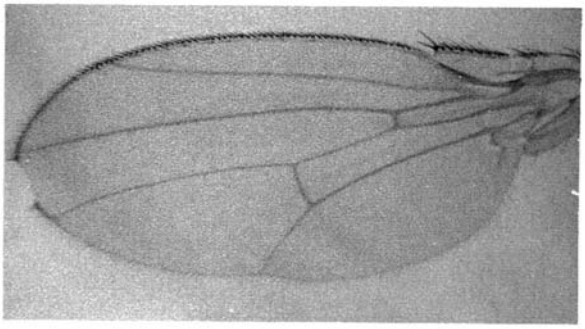
(a) $w^+ mw^+; vg^{ni}/vg^{ni}$



(b) $w^a mw^2; vg^{ni}/vg^{ni}$



(c) $w^+ mw^+; vg^{ni}/vg^{BG}$



(d) $w^a mw^2; vg^{ni}/vg^{BG}$

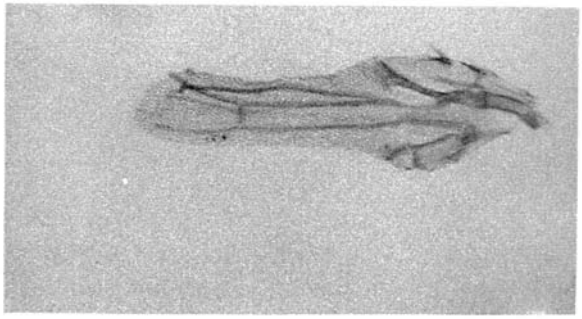


Fig. 1. Interaction of mw^2 with vg^{ni}. The genotypes are indicated above each wing, which were derived from sibling progeny of separate crosses. (*a*) $w^+ mw^+; vg^{ni}/vg^{ni}$ versus (*b*) $w^a mw^2; vg^{ni}/vg^{ni}$. Only occasional nicks are seen in the wing tips of flies wild-type for *mw*, whereas all individuals carrying mw^2 showed wing-scalloping. This effect was mapped to the *mw* locus (see text for details). (*c*) $w^+ mw^+; vg^{ni}/vg^{BG}$ versus (*d*) $w^a mw^2; vg^{ni}/vg^{BG}$. mw^+ flies heterozygous for the vg^{ni} reversion allele and the progenitor vg^{BG} (nomenclature of Williams & Bell, 1988), produce wing nicks in approximately 27% of the flies (Lindsley & Grell, 1968), while the presence of mw^2 causes the reappearance of a strong *vestigial* phenotype.

element in vg^{ni} and the mw^2 mutation causes wing-scalloping comes from a number of flies which were recovered with similarly deeply scalloped wings and wild-type eye colour. These flies were believed to be recombinants between $white^+$ and mw^2 in the heterozygous F_1 females. Since the genotype of *mw* in these flies was unknown due to w^+, they were crossed individually to w^a females. F_1 females from this cross were then backcrossed to w^a. The wing-scalloping in the w^+ males was in fact due to the presence of the mw^2 allele, since w^a mw^2 was recovered from all eight successful progeny tests. This also demonstrates that the enhancement of vg^{ni} does not recombine with mw^2.

w^{ric} (for '*roo* in *copia*') is a *B104* insertion into the *copia* element in w^a (Davis *et al.* 1987). We tested w^{ric} for interaction with *mw*, as well as $E(w^a)$ and *Doa*, since the w^a *copia* element is complete, albeit disrupted. w^{ric} is known to interact weakly with the w^a modifiers *su(f)* and $su(w^a)$ (Mount *et al.* 1988). It also responds as strongly as w^a to $E(w^a)$ mutations, as hetero- or homozygotes. *Doa* mutations, however, do not effect w^{ric} as strongly as w^a, although homozygous escapers of lethality are essentially wild-type in colour, just as w^a. Both *mw* alleles also affect w^{ric} as strongly as w^a. Since *mw* interacts with both *B104* and *copia* elements, we cannot determine whether only one or both of the retrotransposons is responsible for the interaction observed in w^{ric}.

4. Discussion

In this paper, experiments are described showing that second-site modifiers of a single transposable element insertion allele, w^a, act additively when mutant alleles at two loci are combined. Although other interpretations are not ruled out, the finding, by us and others (Rutledge *et al.* 1988), that all modifier mutations tested to date are additive in combinations, suggests that these loci define genes whose products affect diverse processes causing the mutant phenotype. This argument is strengthened by the finding that second-site modifier mutations can be either recessive or dominant in nature, implying the existence of products which are, and others which are not limiting on processes involved with the expression of the affected transposable elements. Further evidence supporting this concept comes from the fact that networks of second-site modifier loci exist with overlapping, but not identical specificities for affected transposable elements (this report; Birchler & Hiebert, 1989; Birchler *et al.* 1989; Chang *et al.* 1986; Rabinow & Birchler, 1989; Rutledge *et al.* 1988; Searles & Voelker, 1986).

This latter result also supports the idea that the modifier loci are involved in processes specific to inserted transposable elements. Although the allele specificity of several modifiers may implicate them as

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interacting specifically with only one or more insertion alleles at a given locus, it is possible to envisage models whereby it is the position of the inserted element in the locus which interferes with its expression. In such a model, second-site modifiers are involved in expression of the host locus, and would appear to be specific for the inserted element in allele specificity tests. This possibility seems unlikely for each of the modifiers examined in this study, because they all affect more than one transposable element-induced allele of *white*, or other genes, and these insertions are in different locations. An additional argument, specific to *su(f)* and *su*(w^a), is that they affect expression of a second copia-induced mutant allele, inserted in the *Adh* promoter (Strand & McDonald, 1989). *E*(w^a) mutations affect four different retrotransposon insertions at *white*, in different locations, making it unlikely that *E*(w^a) is involved directly in *white* expression. Finally, the same argument which also applies to *mw*, is reinforced by our finding that it affects an allele of *vg* caused by the insertion of a *B104* element, as well as an allele at *white*, w^{bf}, caused by the same element.

The ability of a given insertion mutation to respond to second-site modifier mutations is apparently a rare case. We examined many different insertions of *copia* and *B104* elements for their response to second-site modifier loci identified by interactions at the *white* locus. These elements were inserted in different genes, in various regions of the gene, and in various orientations. Their effects on expression of the host gene range from complete disruption of function to overexpression. The type of mutation caused by the insertion, the species of element, its orientation and its pattern of expression, as well as the intactness of the individual element, all presumably determine its ability to respond to second-site modifier loci. Results presented here indicate that for the *mw*, the orientation of the inserted element is not a determining factor in its responsiveness, since *B104* elements in parallel (w^{bf}, w^h) as well as anti-parallel orientations (vg^{ni}) with respect to the direction of transcription, interact with *mw* mutations.

Non-responsive elements may interfere with normal expression of the host locus by directly impeding the formation of a functional RNA through premature termination or by disruption of an exon, yielding a non-functional polypeptide (e.g. $w^{hd81b25}$, *Gl*). Disruption of sequences which require physical proximity, as may be the case in ct^{ns} and ct^n, is one explanation for how non-suppressible insertions might affect promoter regions. It may be coincidence, but these two insertions are located close to one another, in the same orientation, and have phenotypes of roughly the same severity and type (Jack, 1985). Other non-responsive insertion mutations may be due to inactive or otherwise defective elements.

Molecular demonstrations that diverse processes are affected by different modifier loci have recently begun to accumulate. For example, modifier loci interacting with specific sequences in transposons have been described (Geyer *et al.* 1988; Mazo *et al.* 1989; Peifer & Bender, 1988). Studies differentiating among the effects of modifiers on w^a RNA have shown different effects (Birchler & Hiebert, 1989; Birchler *et al.* 1989; Levis *et al.* 1984; Pirrotta & Brockl 1984; Rabinow & Birchler, 1989; Zachar *et al.* 1985), supporting the genetic data presented here. Finally, studies of two modifiers of *gypsy*-induced mutations have shown directly opposite effects of these modifier mutations on levels of transposon RNA (Parkhurst & Corces, 1986*a*, *b*), and on the efficiency of transcription termination within the transposon (Dorsett *et al.* 1988). Molecular analysis of two cloned modifier loci indicates that the products can act through DNA binding (Mazo *et al.* 1989; Parkhurst *et al.* 1988; Spana, Harrison & Corces, 1988), or possess RNA binding consensus sequences, implying interaction with an RNA product (Chou, Zachar & Bingham, 1987; Zachar, Chou & Bingham, 1987). In summary, the many identified second-site modifiers of w^a, and other transposon-induced mutations as well, each appear to affect a different function, allowing a thorough genetic and molecular dissection of the diverse processes involved.

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