Interaction of the *Enhancer of white-apricot* With Transposable Element Alleles at the *white* Locus in *Drosophila melanogaster*

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ABSTRACT

The *Enhancer of* w^a [$E(w^a)$] mutation was shown to interact strongly with 4 of 41 tested alleles of the *white* (w) eye color locus. All four of the affected w alleles result from the insertion of a transposable element. $E(w^a)$ was further localized cytogenetically. The locus lies between the breakpoints of *T(Y;2)L11* and *T(Y;2)H137* (section 60) in 2R. The original mutation was shown to be antimorphic on the basis of its action in the presence of additional normal copies and the ability to revert the original allele to one that mimics the effect of a deficiency for the locus. The RNA transcribed from w^a was analyzed from flies segregating for $E(w^a)$ and normal. The low level of normal functional messenger RNA present in *white-apricot* is reduced further in Enhancer homozygotes. Total *copia* RNA was also examined on Northern analyses from the segregating population but no quantitative change in the major *copia* RNA was produced by $E(w^a)$ homozygotes compared to normal.

As part of a project to identify *trans*-acting modifiers of the *white* locus, an analysis of the *Enhancer of white-apricot*, $E(w^a)$, was undertaken. This gene was discovered by Scandlyn in 1964 (see LINDSLEY and GRELL, 1968; VON HALLE, 1969) as a dominant mutation that lightened the *apricot* allele of *white*. As a heterozygote with wild-type [$E(w^a)$/+], it reduces the level of pigment in the eyes of *white-apricot* flies. As a homozygote, the color is nearly white. In order to investigate further the genetic basis of this interaction, we performed genetic crosses in which the $E(w^a)$ mutant was combined with a series of alleles at *white*. The rationale for determining the allele specificity was that it should be informative about the action of the enhancer locus. This collection of hypomorphs included representatives of lesions in the structural portion as well as in the regulatory region of the gene. Among these classes were point mutants, deletions and transposable element insertions. If it were involved in regulating the *white* locus, one would expect that at least some of the mutants in the 5′ *cis*-acting regulatory sequences would not respond. If it were involved with the retrotransposon, *copia*, which causes the *apricot* mutant, then one would expect $E(w^a)$ to be specific to the *apricot* allele. The result found, however, was that the enhancer is effective on several alleles each caused by a transposon. The affected alleles include representatives induced by several different elements, but not all transposon-induced alleles are affected.

If the enhancer is involved in the expression of the transposable element, then several different ones must share a common mechanism. Alternatively, the enhancer gene product could be involved in the splicing process that removes the transposon sequences to form the functional messenger RNA or the termination of *white* initiated transcripts within the transposable element.

A chemical mutagenesis on the original allele shows that it can be mutated to a form that mimics a heterozygous deficiency for the locus. This observation coupled with the fact that extra copies of the normal allele ameliorate but do not eliminate the mutant effect suggests that the original allele is an antimorph.

An analysis of the RNAs produced by the w^a allele shows that the normal functional messenger RNA is virtually eliminated by the homozygous Enhancer as is an RNA that initiates in *copia* and terminates in *white*. These results suggest that the gene product is involved in the 3′ *copia* termination reaction and that the product of the normal allele permits the low level of read through that gives the leaky *apricot* phenotype.

MATERIALS AND METHODS

Fly culture: Flies were grown on Instant Drosophila medium (Carolina Biological Supply Co.) at 25°.

Translocation crosses: For the localization of $E(w^a)$, a series of translocations between the *Y* chromosome and selected positions in the extreme right end of chromosome two were used. These translocations are marked by y^+ on the *Y* short arm and B^s on the *Y* long arm in a *yellow* (y) non-Bar background. Males of these stocks carry an attached *XY*, $Y^SX{\cdot}Y^L$, *In(1)EN, y* to ensure fertility. The nontranslocated second chromosome in these stocks is the balancer, *In(2LR)SM1,* al^2 *Cy* $cn^2 sp^2$.

When a male from such a translocation stock is crossed to a female with free *X*s, the male progeny will inherit their *X* chromosomes from their mothers. With regard to the translocation, the usual case is that the balanced translocation, *2;Y/Y;2* or the balancer chromosome, *SM1, Cy* plus the

Y;2 chromosome will be transmitted. The former class of gamete produces a chromosomal euploid when crossed to normal females and the latter class produces a terminal segmental trisomic.

For the production of a synthetic deficiency surrounding $E(w^a)$, the translocations *T(Y:2)L11* and *T(Y:2)H137*, which, respectively, suppressed $E(w^a)$ and had no effect as terminal trisomics, were crossed together in a background of *white-apricot*. In this cross, the second chromosomes in the two sexes were marked by different dominant markers because both of these translocations are broken in the short arm of the *Y* chromosome, thus necessitating the use of other markers to classify the various classes of progeny. Specifically, the male stock was *In(1)EN, y; T(Y:2)H137/Sco* and the female stock was *C(1)RM,y*w^a; *T(Y;2)L11*(y^+)*/In(2L)Cy*^{l}. The balanced euploid females are those that inherit *In(2L)Cy*^{l} from their mothers and the balanced H137 translocation. The deficiency females are those that inherit the *2;Y* chromosome of *T(Y;2)L11* from their mothers and the second chromosome, marked by *Scutoid, Sco*, plus the *Y;2* chromosome of H137 from their fathers.

Mutagenesis: Males were treated with ethyl methanesulfonate (EMS) as described by LEWIS and BACHER (1968). Treated males of *y* w^a; *Frd* $E(w^a)$ *Pin*^{2}/SM1 were mass mated to virgin w^a females. After 3 days the males were discarded and the females were transferred to new food. Thereafter, the females were transferred to new food every 5 days for 1 month. An estimated 10,000 progeny were scored. Potential mutants were mated again to individuals from the w^a stock to check heritability and to establish stocks. In general, flies with a mutant phenotype in both eyes transmitted the mutation while those with only one mutant eye did not. The sacrifice of the mosaic flies is offset by the fact that large numbers of flies can be screened extremely easily.

RNA isolation: RNA was extracted from males and females of w^a with various doses of $E(w^a)$ by the method of COX (1968) with the following modifications. Flies were weighed and homogenized in 10 volumes of 8 M guanidine-HCl, 0.01 M EDTA (pH 7.0) using a Tissumizer. The homogenate was centrifuged at 12,000 × *g* for 10 min and filtered through nylon mesh. After precipitation with one-half volume of absolute ethanol for 1 hr, the pellet was resuspended in 4 M guanidine-HCl, 0.01 M EDTA (pH 7.0) and precipitated with one-half volume of absolute ethanol a total of five times. The pellet remaining after the washes was extracted with sterile chelexed DEPC (diethyl pyrocarbonate)-treated water (1 ml/g original mass). After 10 min of centrifugation in a microfuge, the supernatant was retained and the pellet extracted with DEPC-treated water at 56° using 2 ml/g of original mass. Following centrifugation as before, the pellet was extracted at room temperature using the 2 ml/g ratio. The three water extractions were pooled and absorbance at 260 nm was determined to assess RNA concentration. Sodium acetate was added to a final concentration of 0.1 M and followed by two volumes of absolute ethanol. After precipitation, centrifugation and resuspension in sterile, chelexed DEPC-treated water, again the yield was determined by absorbance at 260 and 280 nm. The RNA was reprecipitated as before. After centrifugation, the pellet was dissolved at the desired concentration by the addition of sterile, chelexed DEPC-treated water.

Northern analysis: Total RNA was separated on formaldehyde 1.5% agarose gels by the method of LEHRACH *et al.* (1977) with formaldehyde at the same concentration in the tank buffer as in the gel. RNA was transferred to Nytran nylon membrane, UV cross-linked (CHURCH and GILBERT 1984; KHANDJIAN 1986) and baked at 80° for 2 hr under vacuum.

Hybridization with RNA probes: Hybridization was conducted as described by THOMAS (1980). Filters were wetted in 5 × SSC (1 × SSC = 0.15 M NaCl; 0.015 M Na citrate), 0.1% SDS, and prehybridized (150 μl/cm^2) in a solution of 50% formamide, deionized with AG 501-X8 (Bio-rad) resin, 5 × SSC, 10 mM polyvinylpyrrolidone, 1% bovine serum albumin), 0.5% sodium dodecyl sulfate and 0.2 mg/ml calf thymus DNA (Sigma) for 4 hr at 60°. Hybridization was started by addition of ^{32}P-labeled probe at 1 × 10^6 cpm/ml of solution. Hybridization was conducted for 16 hr followed by four washes consisting of 0.1 × SSC, 5 mM Na_2HPO_4, 0.015% pyrophosphate, 0.2% sodium dodecyl sulfate (pH 7.0), each for 15 min at 75°. Filters were blotted to dampness, then subjected to autoradiography with Kodak XRP-1 film at −70°.

Probe preparations: Single-stranded RNA probes were generated from T7/SP6 vectors (GREEN, MANIATIS and MELTON 1983) into which had been subcloned three different segments of the *white* gene. The first (pIBI 11.5 HB) extends from the *Bam*HI to *Hin*dIII sites of the Canton S sequence. These sites flank the first exon. The vector will transcribe to produce an RNA homologous to sequences in the first exon. The second segment (pIBI 12.5 XS) extends from the *Sal*I site near the end of the third exon to the *Xba*I site preceding the beginning of the second exon. The last (pIBI 12.3 SS) is a *Sal*I fragment that extends from exon six to within the third exon. A *copia* probe extending from the *Hin*dIII to the *Apa*I restriction sites of the element was prepared in the same vector. A cDNA antisense RNA probe from the *rudimentary* locus (SEAGRAVES *et al.* 1984) was used as a control for tests of RNA concentration per gel lane. The reaction mix consisted of the following: 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, approximately 150 μCi ^{32}P-UTP (3000 Ci/mmol) (New England Nuclear), 40 units RNAsin (Promega Biotec), 15 units T7 polymerase (Bethesda Research Labs), 0.5 μg linearized plasmid in a total volume of 20 μl. The reaction was incubated at 37° for 1 hr at which point the volume was adjusted to 50 μl with 0.01 M Tris-Cl, 0.001 M EDTA (pH 8.0). One microliter was removed and diluted to 50 μl in water. Twenty microliters of the diluted sample were spotted onto two Whatman No. 1 2.3-cm discs. One was dried directly and the second washed twice in 250 ml of 5% trichloroacetic acid plus 1% sodium pyrophosphate, once in 250 ml of absolute ethanol and finally in 250 ml anhydrous ether. The washed filter was dried under a heat lamp and both counted in a Beckman LS 1801 scintillation counter to determine the percent of incorporation of label into RNA.

The completed reaction mixture was applied to a spin column of Sephadex G-50. The RNA recovered from the column was precipitated by addition of 1 μg of *Escherichia coli* transfer RNA per 50 μl recovered from the column, one-tenth volume 2 M sodium acetate and two volumes of absolute ethanol. After centrifugation and resuspension in 100 μl of sterile DEPC-treated H_2O, the appropriate volumes were added to the hybridization bags.

RESULTS AND DISCUSSION

As a first step in the characterization of the *Enhancer of white-apricot*, the original mutation was tested with 41 alleles of the *white* locus to determine the spectrum affected. The rationale for this approach was that this collection contains representatives of *cis*-acting regulatory lesions, structural sequence lesions, point mutants, transposable element insertions and

deletions. The affected alleles should be informative as to the mechanism of enhancement.

To assay the response of each allele, males of the constitution *y* w^a; $E(w^a)/CyO$ were mated to virgin females from each of the 45 stocks tested. The F_1 males were scored for any effect by comparing those of genotype $E(w^a)/+$ to $CyO/+$. For each responsive allele, a homozygous *white* allele stock was constructed with a balanced $E(w^a)/CyO$ second chromosome constitution.

The tested alleles are listed in Table 1. The alleles that produce a strong response are *apricot, spotted-55, honey,* and *apricot-4.* To a lesser extent, *eosin, eosin-2, cherry* and *apricot-like* are reduced in expression by $E(w^a)$. All of these are transposable element insertions. The types of elements present in the strongly affected alleles are *copia,* BEL, and the element in *spotted-55.* These are distinct elements but are all retrotransposons. The weakly affected alleles are all partial revertants of w^1. It is possible that these alleles are all responsive as a result of interaction with the *Doc* transposable element sequences in this mutant.

As an additional test on the four most strongly enhanced alleles, the following crosses were made. Males of $+/Y$; $In(2L)Cy^t$, *Cy Roi/Sco* were crossed to females of w^a; $E(w^a)/CyO$. The F_1 males are of three types with regard to their second chromosome genotype: (1) $E(w^a)/In(2L)Cy^t$; (2) $E(w^a)/Sco$ and (3) CyO/Sco. Males of each of these three types were mated to females of the four major affected alleles of *white.* The progeny from each type of mating was compared for the two classes of second chromosomes present. With all four *white* alleles, the $E(w^a)$ chromosome produced a lighter eye color than either the *Sco* or $In(2L)Cy^t$, *Cy Roi.* However, in the case of w^{sp55}, *Sco* is lighter than $In(2L)$ Cy^t, *Cy Roi,* but the magnitude of the effect was not sufficient to account for the differences observed between $E(w^a)$ and either *Sco* or $In(2L)Cy^t$, *Cy Roi.*

The alleles affected have lesions at various locations within the structural portion of the gene. *White-apricot* and *apricot-4* involve the second intron, whereas *spotted-55* and *honey* are lesions near the 5′ portion of the gene. While all of the affected alleles are insertions of transposons, not all transposable element insertions are affected. *White-crimson,* which is foldback (FB) element, is not responsive. The series of *I* element insertions do not respond. Other transposon alleles that are not affected are *buff, blood, zeste-mottled, zeste-light,* and *spotted.*

A series of five partial revertants of *white-apricot* were tested. Three of these are unresponsive, w^{a58112}, w^{a57i11}, and w^{a59k1}. Two others, w^{aRM} and w^{aR84h}, are both strongly enhanced. These two revertants are insertions into the 5′ and 3′ long terminal repeats. LTRs, respectively, of the *copia* element (MOUNT, GREEN and RUBIN 1988). The w^{a59k1} allele is a revertant that retains a solo LTR of *copia* (CARBONARE and GEHRING 1985). The fact that at least certain revertants of *white-apricot* fail to respond strengthens the argument that $E(w^a)$ is involved with transposable element insertion or its consequences on the expression of *white.*

Localization of $E(w^a)$: To localize $E(w^a)$, a series of *Y;2* translocations, listed in Table 2, were crossed to *y* w^a;$E(w^a)/CyO$ virgin females and the w^a sons scored for intensity of pigment. (See MATERIALS AND METHODS for a further description.) This cross produces three major classes of male progeny. The inheritance of the $E(w^a)$ chromosome from the mother and the respective balanced translocation from the father results in males with non *Cy* wings and y^+ body color. The $E(w^a)$ chromosome could also be united with the paternal *SM1* chromosome plus the *Y;2* chromosome carrying the distal portion of the right arm of chromosome *2.* This class is trisomic for the distal segment. The transmission of the *CyO* chromosome from the maternal parent and the balanced translocation from the paternal parent produces progeny that are *Cy* and y^+ and represent the normal nonmutant situation for the $E(w^a)$ locus. Therefore, the three classes represent the chromosomal euploid heterozygous for $E(w^a)$ and normal, a segmental trisomic of $E(w^a)/+/+$ genotype and a chromosomal euploid homozygous for the normal allele of the $E(w^a)$ locus. The trisomics that produce a phenotype intermediate between the mutant heterozygote and the normal were considered to include an extra copy of the normal allele of $E(w^a)$. By this criterion, $E(w^a)$ resides between $T(Y;2)L11$ and $T(Y;2)H137$.

As a further means to localize $E(w^a)$, a stock of w^a; $E(w^a)/SM1$, $a1^3$ *Cy* sp^2 was crossed as females to four deficiency stocks, namely, $Df(2R)Px$, $Df(2R)Px^2$, $Df(2R)M\text{-}c^{33a}$ and $T(1;2)sc^{S2}$. None of these delete the normal allele.

VON HALLE (1969) mapped $E(w^a)$ to the left of *Pin.* Using a *Frd* $E(w^a)$ Pin^2 chromosome heterozygous with a normal chromosome two, the same result was found. From a total progeny of 1985, three *Frd* $E(w^a)$ + and one + + Pin^2 crossovers between the two loci were recovered.

When stocks carrying the Pin^2 mutation are crossed to those carrying the *Px* or Px^2 deficiencies, the double heterozygotes are not recovered. The published breakpoint of $T(Y:2)L11$ (60C) and the presence of a normal copy of $E(w^a)$ in the $Df(2R)Px$ and $Df(2R)Px^2$ chromosomes might suggest that the enhancer locus is distal to the deficiencies since the proximal breakpoint of the *Px* deficiency is at the border of 60B and C. However, the recombination data place it proximal to Pin^2 and the latter is not complemented by the deficiencies.

To resolve this, two sets of crosses were made. In

TABLE 1

Alleles of *white* tested with *Enhancer of white-apricot*

Allele	Interaction	Lesion	Reference
w^a (apricot)	+	*copia* insertion in second intron	GEHRING and PARO (1980), BINGHAM and JUDD (1981), GOLDBERG, PARO and GEHRING (1982)
w^{a58112} (apricot revertant)	−	Unknown	
w^{a57i11} (apricot revertant)	−	Unknown	
w^{a59k1} (apricot revertant)	−	X-ray-induced revertant-solo *copia* LTR	CARBONARE and GEHRING (1985)
w^{aRM} (apricot revertant)	+	Transposable element insertion in *copia* 5′ LTR	MOUNT, GREEN and RUBIN (1988)
w^{aR84h} (apricot revertant)	+	*I* element insertion in *copia* 3′ LTR	MOUNT, GREEN and RUBIN (1988)
w^i (ivory)	−	Duplication of intron 1 sequences	KARESS and RUBIN (1982), COLLINS and RUBIN (1982), O'HARE *et al.* (1984)
w^c (crimson)	−	*FB* transposable element revertant of w^i	COLLINS and RUBIN (1982), O'HARE *et al.* (1984)
w^{sp} (spotted)	−	*B104* insertion in 5′ regulatory region	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984), O'HARE, LEVIS and RUBIN (1983)
w^{sp4} (spotted-4)	−	Deficiency in 5′ cis regulatory region	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
w^{sp2} (spotted-2)	−	Deficiency in 5′ cis regulatory region	ZACHAR and BINGHAM (1982)
w^{sp81d5} (spotted-81d5)	−	Deficiency in 5′ cis regulatory region	DAVISON *et al.* (1985)
w^{sp55} (spotted-55)	+	Retrotransposon insertion	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
w^{bf2} (buff-2)	−	Unknown	
w^t (tinged)	−	Unknown	
w^{co} (coral)	−	Unknown	
w^{ec3} (ecru-3)	−	Unknown	
w^{mo} (mottled-orange)	−	Unknown	
w^{cf} (coffee)	−	Point	ZACHAR and BINGHAM (1982)
w^{a3} (apricot-3)	−	Point	ZACHAR and BINGHAM (1982)
w^{a2} (apricot-2)	−	Point	ZACHAR and BINGHAM (1982)
w^{sat} (satsuma)	−	Point	ZACHAR and BINGHAM (1982)
w^{col} (colored)	−	Point	ZACHAR and BINGHAM (1982)
w^{Bwx} (Brownex)	−	Point	ZACHAR and BINGHAM (1982)
w^{bl} (blood)	−	Retrotransposon insertion in intron 2	ZACHAR and BINGHAM (1982), BINGHAM and CHAPMAN (1986)
w^{bf} (buff)	−	*B104* transposable element insertion in intron 4	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984), O'HARE, LEVIS and RUBIN (1983)
w^{a4} (apricot-4)	+	*BEL* insertion into intron 2	ZACHAR and BINGHAM (1982), GOLDBERG *et al.* (1983)
w^e (eosin)	+/−	Transposable element reversion of w^1 (*Doc* element)	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984), HAZELRIGG (1987)
w^{e2} (eosin-2)	+/−	Derivative of w^1 (*Doc* element)	O'HARE *et al.* (1984)
w^{ch} (cherry)	+/−	Reversion of w^1 (*Doc* element)	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
w^h (honey)	+	Deletion reversion of w^1 (*Doc* element)	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
w^{ap1} (apricot-like)	+/−	P-M hybrid dysgenic revertant of w^1 (*Doc* element)	C. MCELWAIN (personal communication)
w^{IR1}	−	*I* element insertion	SANG *et al.* (1984)
w^{IR2}	−	*I* element insertion revertant of w^1 (*Doc* element)	SANG *et al.* (1984)
w^{IR3}	−	*I* element insertion	SANG *et al.* (1984)
w^{IR4}	−	*I* element insertion	SANG *et al.* (1984)
w^{IR5}	−	*I* element insertion	SANG *et al.* (1984)
w^{IR6}	−	*I* element insertion	SANG *et al.* (1984)
z w^{is} (isoxanthopterinless)	−	Unknown	
w^m (zeste-mottled)	−	*3S18* (transposable element insertion)	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
z w^{zm} (zeste-mottled)	−	*3S18* transposable element insertion	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
z w^{zl} (zeste-light)	−	Derivative of w^{zm}	JUDD (1963)
z^a w^a	+	*copia* insertion in second intron	GEHRING and PARO (1980), BINGHAM and JUDD (1981)
z w^a	+	*copia* insertion in second intron	GEHRING and PARO (1980), BINGHAM and JUDD (1981)
z *Dp(1;1)* w^{+61e19}	−	Duplication of *white* locus	

TABLE 2

Cytogenetic localization of $E(w^a)$

Translocation	Cytology[a]	Trisomic suppresses $E(w^a)$
A120	57F;Y^S	+
B202	59A-B;Y^S	+
J131	59D;Y^L	+
L11	60C;Y^S	+
H137	60D;Y^S	−
A146	60F;Y^S	−
bw^+ Y	58F-59A;60E-F	+
Deficiency	**Cytology**	**$E(w^a)$+**
Df(2R)Px	60B8-10;60D1-2	+
Df(2R)Px^2	60C5-6;60D9-10	+
Df(2R)M-c^{55a}	60E2-3;60E11-12	+
T(1;2)sc^{52}	60E6-8;60F5	+

[a] Cytology is from LINDSLEY *et al.* (1972) and LINDSLEY and GRELL (1968). See text for discussion.

the first, males from stocks of *T(Y;2)H137, L11* and *J131* were crossed to *y* w^a; *Frd* $E(w^a)$ *sp* Pin^2/*SM1*, al^2 *Cy* cn^2 sp^2 females and secondly, in parallel, to *Df(2R)*Px^2, *px bw mr* Px^2/*SM1*, al^2 *Cy* cn^2 sp^2. From the crosses to $E(w^a)$, it was determined that H137 had a breakpoint distal to the enhancer and L11 and J131 had proximal breaks. As before, the trisomic produced by H137 has no effect on $E(w^a)$ nor on Pin^2. In contrast, both L11 and J131 trisomics suppress both $E(w^a)$ and Pin^2. Likewise, the trisomics for L11 and J131 suppress the Px^2 phenotype.

The summation of these observations is that the breakpoint of L11 must be proximal to $E(w^a)$ and Pin^2. Genetically, $E(w^a)$ is proximal to Pin^2. The normal allele of $E(w^a)$ is present in the *Px* and Px^2 deficiency chromosomes but the normal allele of *Pin* is not. Previous reports conclude that the proximal break in *Df(2R)Px* is at 60B10 and the breakpoint of *T(Y:2)L11* is at 60C, suggesting an error, albeit potentially as trivial as a single or few bands, in the respective cytology. Reexamination of the cytology of *T(Y:2)L11* and the *Px* and Px^2 deficiencies showed that they are indeed broken at the juncture of 60B and C. *T(Y:2)H137* is broken more distal (60D). Considerable ambiguity may be involved with the translocations considering that the euchromatic break abuts heterochromatin. With these considerations, it appears that $E(w^a)$ is at 60B/C.

The strong band that demarcates the beginning of 60C (LEFEVRE 1976) is clearly visible on the *Y;2 (L11)* chromosome that protrudes from the chromocenter in salivary gland polytene spreads prepared from males carrying a balance translocation and otherwise wild type chromosomes. This major band is not deleted in *Df(2R)*Px^2 but has been in *Df(2R)Px*.

Effect of w^a of varying normal copies of $E(w^a)$. To test the effect of a deficiency for the region, the following experiment was performed. Males of the *T(Y;2)L11*(60C;Y^S) stock were crossed to females of *C(1)RM, y* w^a; *In(2L)*Cy^t/*Sco*. At the same time, males of *T(Y;2)H137*(60D;Y^S) were crossed to females of *In(1)EN, y*; In(*1*)*EN, y*; *In(2L) Cy*^{t}/*Sco*. From the progeny of the first cross, virgin females were collected that were *C(1)RM, y* w^a; *T(Y;2)L11*(y^+)/*In(2L)*Cy^t. These were mated to males from the second cross that were *In(1)EN, y*; *T(Y;2)H137/Sco*. The pertinent classes of progeny are those that have a deficiency for the region between the breakpoints of L11 and H137 and a balanced H137 euploid. The deficiency females are *Sco* and the euploid are *Cy*. These two types were compared with regard to the intensity of pigment of w^a. The deficiency heterozygotes were slightly lighter than the balanced euploid. This suggests that a reduction in the quantity of the normal product of $E(w^a)$ will enhance the mutant phenotype of w^a.

To test whether three normal copies of the enhancer locus produced an effect on w^a, the attached *X* stock was crossed by the two translocations that flank the gene, namely L11 and H137. Both of these are broken in the short arm of the *Y* chromosome. Therefore, the trisomic flies will exhibit the *y* marker while the euploids are y^+. With both translocations there was not a discernible difference between the trisomic and the euploid.

Additionally, the *bw+Y* chromosome has no phenotypic effect in males or females in the absence of the $E(w^a)$ mutation. However, when the mutant is present, the marked *Y* makes the phenotype intermediate between that of normal and $E(w^a)$/+. Thus, it is concluded that while an extra copy of the normal allele ameliorates the mutant effect, the presence of three normal copies has no phenotypic consequences beyond the normal diploid.

$E(w^a)$ is an antimorph: The results described above suggest that the original allele of *Enhancer of white-apricot* behaves as an antimorphic mutation. As a heterozygote, the level of pigment from *white-apricot* is reduced. However, the addition of a normal allele of the gene to produce an $E(w^a)$/+/+ genotype results in a phenotype intermediate between +/+ and $E(w^a)$/+. The three normal copies of the region do not result in a detectable difference in phenotype from the normal diploid. However, a deficiency for the short segment that removes the normal allele of $E(w^a)$ slightly enhances the *apricot* mutant. Taken collectively, it is proposed that at some level the original $E(w^a)$ allele is antagonistic to the normal allele, most probably at the gene product level. Clearly, the mutant is causing a greater loss of function than a mere 50% reduction of gene product.

Reversion of $E(w^a)$: If the original allele of $E(w^a)$ is an antimorph, it should be possible to induce a revertant. Accordingly, males of *y* w^a/*Y; Frd* $E(w^a)$ Pin^2/*SM1, Cy* cn^2 were treated with EMS (LEWIS and

BACHER 1968) and mated to w^a females. The progeny were screened for potential revertants among the *Frd Pin*^{2} flies. While EMS mutagenesis produces many mosaic flies, we find that a substantial fraction of mutants recognized in the F_1 are transmitted to the F_2. In a screen of this sort, a greater number of flies can be screened easily if a pair mating step for mosaic resolution is omitted.

Five potential revertants were recovered. Two of these, upon further analysis, proved to have a dominant suppressor of w^a on chromosome three that partially cancelled the $E(w^a)$ effect. The third proved to be a dominant suppressor on chromosome 2. The remaining two cases were revertants.

To test whether the revertant phenotype mapped to the Enhancer locus, as a means of confirmation, females of the constitution w^a/w^a, *Frd* $E(w^a)^R$ *Pin*^{2}/+++ were mated, respectively to w^a males. In the first potential revertant, 231 progeny were screened in which no individuals were recovered that had the original $E(w^a)$ phenotype or one suggestive of a suppressor. In the second case, 319 progeny were screened with the same result. Thus, in both cases the results are consistent with a mutation being produced in $E(w^a)$ (Figure 1).

While much darker in phenotype than the enhancer, the revertants are lighter when present as a heterozygote compared to a homozygote for a normal chromosome two. This is the expected result if the reversion of the antimorphic allele has created a "loss of function" mutation. The two new alleles differ consistently in the level of pigment present. Revertant #2 is darker than #1. This suggest that #1 is probably not a complete amorph.

To test whether the revertants could be compensated by the addition of an extra copy of the chromosomal region containing the normal allele of $E(w^a)$, males of the constitution *bw*+*Y*/*y* w^a; $E(w^a)$/*CyO* were crossed to virgin females that were w^a/w^a; *Frd* $E(w^a)^{R1}$ *Pin*^{2}/+++ and *Frd* $E(w^a)^{R2}$ *Pin*^{2}/+++. This cross results in four classes of males and four of females. The males carry an extra copy of the enhancer locus since they inherit *bw*+*Y*. The four classes are (1) *CyO*/+++, which has the normal *white-apricot* phenotype. (2) $E(w^a)$/+++, which has the enhanced phenotype. (3) *CyO*/*Frd* $E(w^a)^R$ *Pin*^{2}, which in both revertants has a phenotype intermediate between the first two as noted above. (4) $E(w^a)$/*Frd* $E(w^a)^R$ *Pin*^{2}, which has an enhanced phenotype that is no more severe than $E(w^a)$/$E(w^a)$. Recombinants between *Frd* and *Pin*, which are infrequent, were removed from consideration.

Males of class 1 above are slightly darker than the females but the difference between males and females from class 2 is easily discerned. The males of class 3 are very similar to the females of class 1 but darker than the class 3 females. Class 4 flies are the lightest with males being the darker of the sexes. The males of this class are quite similar to the females of class 2. The females of class 4, however, are not as light as $E(w^a)/E(w^a)$ flies constructed in other crosses.

The results of these crosses can be summarized as follows. The revertants heterozygous with a normal chromosome two and carrying a duplication of the region on the *Y* have a phenotype quite similar to females with two normal copies of chromosome *2*; therefore, it is concluded that the slight enhancement of w^a by the revertant is due to a haplo-insufficiency compared to wild type and the addition of an extra copy brings the quantity of gene product back to the normal diploid level. The fact that the revertants do not exhibit a completely normal phenotype is interpreted such that a reduction in the level of the normal gene product causes an enhancement of w^a. The revertants are presumed, however, to have inactivated (at least partially) the antimorphic function of the original allele. The observation that a deficiency for the region produces a slight enhancement of w^a is consistent with this view.

Analysis of RNA: The *white-apricot* mutant results from the insertion of the retrotransposon, *copia*, into the second intervening sequence of the *white* locus. The element is transcribed in the same direction as *white*. An analysis of RNAs produced from *apricot* reveals a complex array of molecular weight products (LEVIS, O'HARE and RUBIN 1984; ZACHAR *et al.* 1985; PIRROTTA and BROCKL 1984). The majority of RNAs, detected on Northerns, that initiate at the 5′ start of the *white* gene terminate in the 3′ long terminal repeat (LTR) of *copia*. A low level of transcription proceeds to the 3′ terminus of *white* and the *copia* sequences contained within the second intron are spliced out, resulting in a functional messenger RNA, which accounts for the leaky phenotype. There are also RNAs that initiate within the 5′ LTR of *copia* and terminate at the 3′ terminus of *white* and those that initiate at the 5′ start of *white* and terminate in the 5′ LTR of *copia*. Last, 5′ *white* initiated RNAs are present that are intermediate in molecular weight between the normal *white* message and the 3′ *copia* terminated one. The interpretation of the structure of these RNAs is explained in further detail in BIRCHLER, HIEBERT and RABINOW (1989).

To examine the effect of the *Enhancer of* w^a on this spectrum of RNAs, a cross was set up to produce a segregating progeny that had $E(w^a)/E(w^a)$ flies and +/+ controls. This was achieved by mating *y* w^a; *Frd* $E(w^a)$ *Pin*^{2}/*CyO* females by *y* w^a/*Y*; $E(w^a)$/*Sco* males. The males and females with a *CyO*/*Sco* constitution serve as a control and the *Frd* $E(w^a)$ *Pin*^{2}/$E(w^a)$ are homozygous for the enhancer. The other classes are heterozygous for $E(w^a)$. Total RNA was extracted from each class of flies and subjected to electropho-

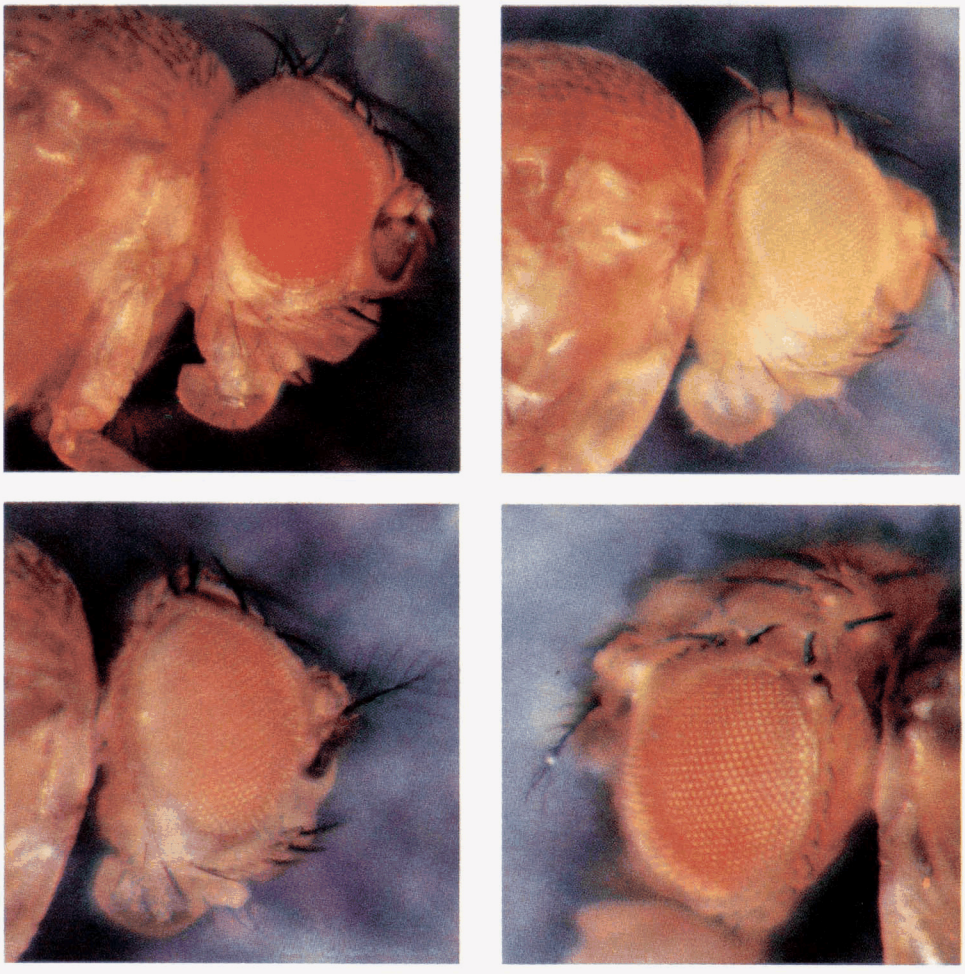

FIGURE 1.—Phenotype of normal, Enhancer and revertants. Top left, normal w^a; top right, w^a; $E(w^a)$/+; bottom left, w^a, $E(w^a)^{R1}$/+; bottom right, w^a; $E(w^a)^{R2}$/+.

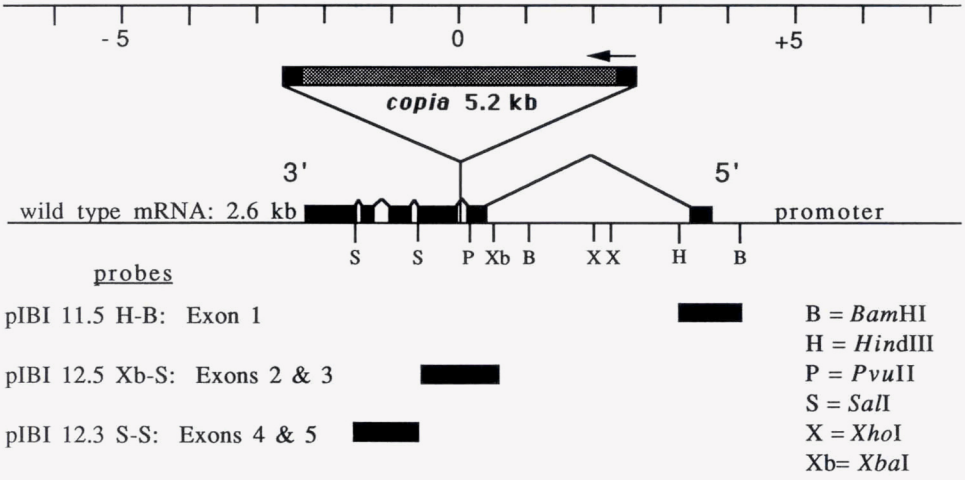

FIGURE 2.—Diagram of w^a. Selected restriction sites are shown and the positions of probes used in the Northern analysis. The scale at the top is in kilobases. Modified from RABINOW and BIRCHLER (1989).

resis through a 1.5% agarose formaldehyde gel. The RNAs were transferred onto nylon membrane and probed in three separate reactions with a single-stranded antisense RNA probe from three portions of the *white* locus. In the first case, the probe was homologous to RNA encoded in the first exon and that extends from the *Bam*HI to *Hin*dIII sites of the Canton S restriction map (Fig. 2).

The second probe extends from the *Xba*I site at the beginning of the second exon to the *Sal*I site near the end of the third exon. This probe spans the second intervening sequence, where the *copia* element is inserted. Consequently, it is homologous to all *white* transcripts whether initiated at *white* or at *copia*.

Last, the RNA was probed with labeled RNA from a vector containing a *Sal*I fragment of *white*, that extends from exon six of the messenger RNA to within the third exon. This probe will detect only those RNAs containing *white* sequences 3′ to the site of *copia* insertion. After the three blots were probed

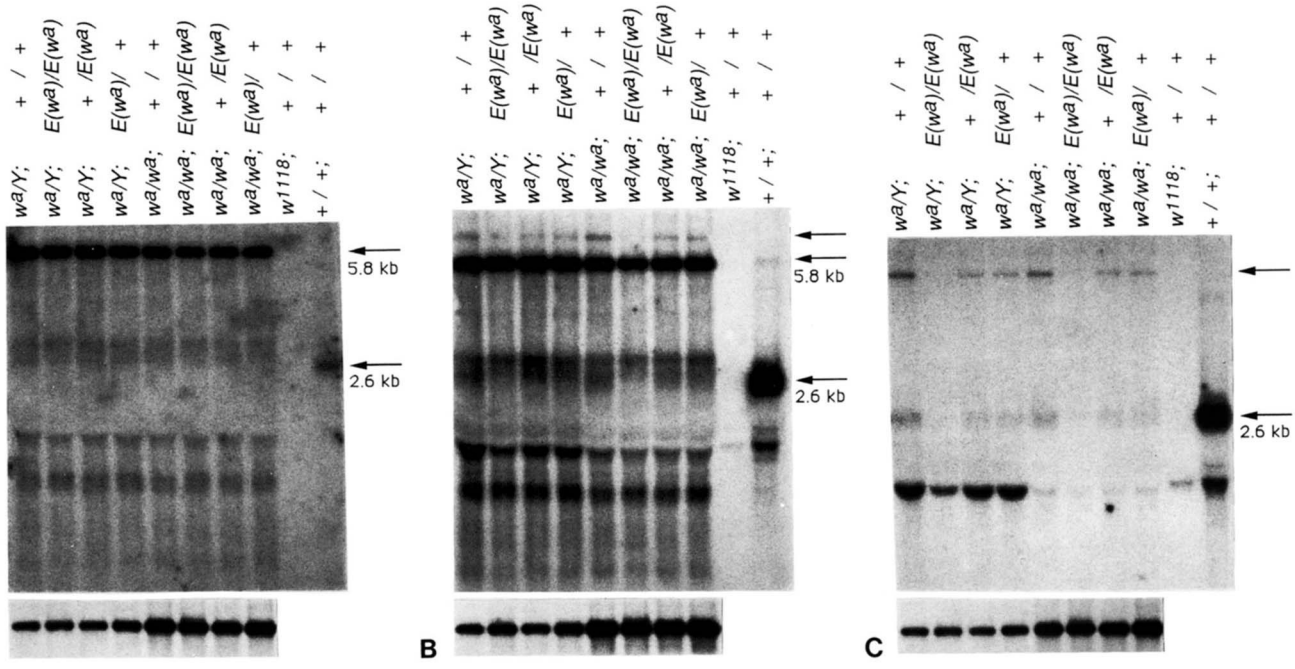

FIGURE 3.—Northern analysis of w^a RNA products from a segregating population of $E(w^a)$. RNA was isolated from flies (0–24 hr old) from a cross of *y* w^a; *Frd* $E(w^a)$ Pin^2/CyO females by *y* w^a/Y; $E(w^a)/Sco$ males. Each blot contains RNA from males and females homozygous for w^a with the following enhancer genotypes (left to right): +/+; $E(w^a)/E(w^a)$; $E(w^a)/CyO$; $E(w^a)/Sco$; males followed by females. At the extreme right are samples from the w^{1118} deficiency strain and from Canton S. The blot shown in panel A was probed with sequences homologous to the 5′ exon. The blot in panel B was probed with sequences that span the site of *copia* insertion in *white*. The blot in panel C was probed with sequences homologous to regions of *white* 3′ to *copia*. The position of the major 5.8-kb 3′ LTR termination species, the 2.6-kb normal message and the *copia-white* readthrough product are noted with arrows. At the bottom are shown the results of reprobing the respective blots with the *rudimentary* cDNA probe. The normal *white* message and the *copia-white* read through are drastically reduced in $E(w^a)$ homozygotes and intermediate in heterozygotes relative to normal.

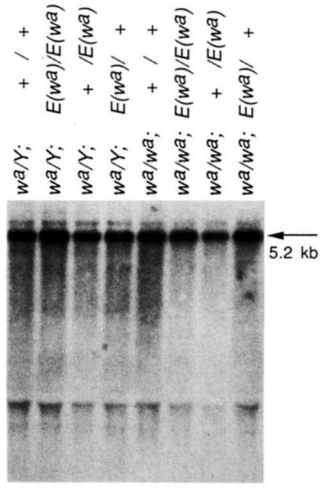

FIGURE 4.—Northern analysis of *copia* RNA in a segregating population of $E(w^a)$. The RNA samples described in Figure 3 were separated on formaldehyde agarose gels, transferred to nylon membrane and probed with sequences homologous to *copia*. With respect to Enhancer genotype, the samples (left to right) are: +/+; $E(w^a)/E(w^a)$; $E(w^a)/CyO$; $E(w^a)/Sco$ males followed by females. The major adult *copia* RNA (5.2 kb) is noted with an arrow. No detectable quantitative change could be noted in the major *copia* RNA in the presence of $E(w^a)$.

with the respective *white* sequences, each was reprobed with *rudimentary* antisense RNA to control for concentration per gel lane. The results are shown in Figure 3.

An examination of these three blots leads to the conclusion that the Enhancer appears to reduce the amount of readthrough that occurs in the 3′ LTR termination site of *copia*. This would lead to a reduction in the level of normally spliced *white* as well as the *copia-white* hybrid RNA. The reduction in the level of normally spliced RNA gives a molecular basis to the phenotypic effect of enhancement of *white-apricot*.

To test whether the $E(w^a)$ affects the levels of RNA transcribed from the active *copia* elements present in the genome, Northern blots of the genotypes described above were probed with single stranded antisense RNA transcribed from *copia* sequences. No differences in the quantity of total *copia* RNA of normal size was observed among the four types of flies (Figure 4). It is concluded that the $E(w^a)$ does not produce its effect upon w^a by modulating the transcription of *copia*. However, after extended exposure, several bands of higher molecular weight than the normal 5.2-kb *copia* RNA increased in intensity in direct proportion to the number of copies of the $E(w^a)$ mutant. In other words, these bands are lowest in quantity in *CyO/Sco*, increased but equal in $E(w^a)/Sco$ and

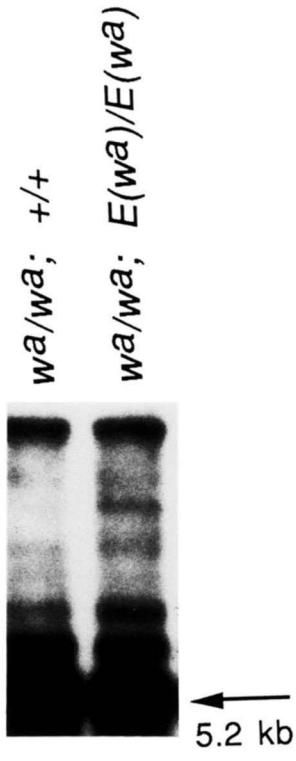

FIGURE 5.—Extended exposure of autoradiograph of RNA probed with *copia*. The RNA samples used in the blots in Figures 3 and 4 were separated for an extended time. The autoradiographs of the resulting blots were overexposed to reveal the low quantity sequences with homology to *copia* but of greater molecular weight than the full length *copia* RNA. At least four of these species show a direct quantitative correlation with the number of copies of $E(w^a)$ present. The position of the major full length *copia* RNA is marked with an arrow.

$E(w^a)/CyO$ but greatest in $E(w^a)/E(w^a)$ (Figure 5).

These RNAs are believed to be initiated or terminated outside of *copia* since their molecular weight is greater than the complete sequence of the transposon. Because the segregating population used two differently marked chromosomes, each with $E(w^a)$, and both classes of these heterozygotes showed an increase in these high molecular weight RNAs, it is believed that this response is due to the effect of the $E(w^a)$ mutation rather than a mere linkage of unique copia insertions on chromosome two that are inserted within other transcription units. This phenomenon has not been further investigated.

CONCLUSIONS

The present study has identified a function utilized by the retrotransposon, *copia*, that is encoded by a host gene. The original mutation in this locus is shown to be antimorphic, which results in a more severe phenotype than a loss of function mutant would. The loss of function alleles produce a more subtle phenotype than the antimorphic one, which accounts for the original recognition of the latter type. The allele specificity at *white* indicates that the function encoded by $E(w^a)$ is utilized by other retrotransposons distinct from *copia*. Nevertheless, there is a specificity among retrotransposon induced alleles at *white* in that *white-buff* (B104 insertion), *white-blood*, and *white-zeste mottled* are not affected by $E(w^a)$.

The $E(w^a)$ mutation modifies the types of RNAs transcribed from the *apricot* allele by reducing the level of RNA that is of normal size for *white* (2.6 kb) as well as a product that initiates in *copia* and terminates at the 3′ of *white*. In other words, the RNAs that read through the 3′ LTR of *copia* in the *apricot* allele under normal conditions are reduced in quantity by $E(w^a)$. The normal sized *copia* message is not altered in quantity, but higher molecular weight RNAs with homology to the *copia* probe increase in direct proportion to the number of copies of the mutant $E(w^a)$ allele present in the genome.

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