

Dosage dependent modifiers of *white* alleles in *Drosophila melanogaster*

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Summary

As part of a study to identify dosage-sensitive modifiers of the *white* eye colour locus and the retrotransposon, copia, a segmental aneuploid screen was conducted. It surveys the autosomal complement of the genome for dosage dependent modifiers of *white*, including ones effective upon retrotransposon insertion-induced alleles. Several regions were found which, when present as a segmental trisomy, affected one or more of the alleles tested in a strong and consistent fashion. Two of these regions have been identified as containing previously described modifiers, *Darkener-of-apricot* and *Inverse regulator-a*. The remainder identify new *white* allele modifiers. Selected segmental monosomics were also tested where possible for regions exhibiting a trisomic effect. At least three regions were found to have a dosage-dependent effect in one, two and three copies.

1. Introduction

We have initiated a line of investigation to identify regulatory genes that alter the expression of the *white* eye colour locus and the retrotransposon, copia. The approach is to identify mutations that affect *white* as well as to screen dosage series for additional modifiers. The objective of our screen was to make an approximation of the number, type, and cytological location of regions that affect *white*, or its individual mutants, in a dosage-sensitive fashion. In particular, we are interested in determining the degree to which genes are subjected to dosage control. The impetus for this approach comes from the observation, in a variety of multicellular eukaryotes, of an inverse or direct modifying dosage effect on gene expression. This effect (see Birchler, 1979, 1992; Birchler *et al.* 1990; Rabinow *et al.* 1991) involves some mechanism or mechanisms whereby a gene responds to a segmental aneuploid for specific regions (linked or unlinked) in a manner that is inverse or direct, quantitatively, to the dosage of that region. A complementary approach underway is to screen for dominant mutants that mimic a lesion in a gene responsible for these dosage effects (e.g. Rabinow *et al.* 1991). This approach, however, might be un-

successful in identifying all sites that operate on *white* if any are haplo-insufficient, or if they are too subtle to be recognized during mutagenesis screens. The present study overcomes these problems by screening for dosage effects in trisomics. Previous studies of large trisomies in maize and *Drosophila* (Birchler, 1979, 1981; Devlin *et al.* 1988; Kennison & Russell, 1987; Birchler *et al.* 1990) demonstrate this effect by quantitative analysis of particular gene products, or by subjecting physical traits to numerical quantitation. Our approach is more qualitative: eye colour of diploid females is compared to that of their segmental trisomic sisters, in a nearly uniform genetic background, and scored for enhancement or suppression.

The original screen was done for trisomies as a practical measure. Viability is far higher for trisomies than for the corresponding monosomies; in addition, several haplo-lethal loci exist, while only one triplo-lethal locus is known. For those regions, which reacted strongly, and which could be examined in the monosomic state, the corresponding deficiencies were also tested. Numerous modifiers, having various allele specificities at *white*, were discovered by this procedure. This paper is a record of our observations.

2. Materials and methods

(i) *The white-apricot* (w^a) and *white-blood* (w^{bl}) alleles

The basic screen was done with w^a , which is caused by a parallel copia insertion in the second intron, and

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with w^{bl} , which is caused by an antiparallel retrotransposon in the second intron. These alleles dosage compensate and interact with *zeste*. The mutant w^a phenotype is caused by termination of *white* transcripts, with attached *copia* sequences, in the 3'-long terminal repeat (LTR) of *copia* (Pirrotta & Brockl, 1984; Levis *et al.* 1984; Zachar *et al.* 1985). This phenotype is dependent on internal *copia* sequences as well as the LTR termination site, as deduced from the fact that revertants are found which retain only one LTR (Zachar *et al.* 1985; Carbonare & Gehring, 1985). Fused *white/copia* RNAs are without *white* function; residual expression in w^a is due to the small proportion of wild-type RNA still present, presumably derived from readthrough of the *copia* termination signals, followed by appropriate splicing out of the element. As several concerted functions of *copia* and *white* are necessary for the w^a mutant phenotype, this screen could be expected to detect second site modifiers that work on many aspects of *white* or *copia* expression. The other allele used for a complete screen was w^{bl} (Zachar & Bingham, 1982), which is caused by an antiparallel retrotransposon insertion in the second intron of *white* (Bingham & Chapman, 1986).

(ii) *The alleles white-eosin (w^e), white-ivory (w^i), white-spotted (w^{sp}), and white-spotted 55 (w^{sp55})*

Selected regions reacting with w^a or w^{bl} strongly (in either direction) were further tested with the *white* alleles, *sp*, sp^{55} , *e* and *i*, which exhibit regulatory phenotypes. The null mutant w^i and partial revertant w^e are, respectively, a 5.7 kb *Doc* element insertion near the transcriptional start point, and a secondary insertion of a pogo element into the *Doc* element (Driver *et al.* 1989; O'Hare *et al.* 1991). w^e does not dosage compensate or interact with *zeste*.

The *white-ivory* (w^i) allele is a 2.9 kb duplication of part of the first intron, second exon, small second intron and part of the third exon. It produces a product that does not properly splice, and is subject to reversion by further insertions which alleviate this lesion (Karess & Rubin, 1982). It does not dosage-compensate. As the name implies, it is a lightly pigmented allele.

The w^{sp} allele is caused by insertion of a *B104* retrotransposon in the 5' *cis*-regulatory sequences (Davison *et al.* 1985). Its phenotype is a dark red-on-yellow mottling. The w^{sp} allele is a *cis*-regulatory mutation, that does not dosage-compensate properly or react with *zeste* (Lindsley & Grell, 1968).

The w^{sp55} retrotransposon insertion (Zachar & Bingham, 1982) is near the 5' start of transcription. It is of particular interest because it has been found to interact with some of the same modifiers as w^a , in the absence of a response from any other *white* mutants. Notably, it is enhanced by an inversely acting suppressor of apricot, *Darkener-of-apricot*, that does not react with any of 40 other *white* mutants (Rabinow

& Birchler, 1989). This allele is an overcompensator, with the male being darker than the female. The lightest of the alleles used (in females), sp^{55} is, nevertheless, scorable for enhancement as well as suppression.

(iii) *Stocks and crosses*

Two types of translocations were used. Most of the work was done with reciprocal *Y;A* translocations, as described in Lindsley *et al.* (1972). Those used are shown in Table 1. Females bearing a compound *X* with one of the described *white* alleles present (synthesis described in Birchler, 1992), and a balancer for either autosome studied were crossed to *Y*-autosome translocation-bearing males. Selected female progeny were crossed to the appropriate translocation to create the desired segmental aneuploid. The final cross was, then: female $C(1)RM\ y\ w = x$; $T(Y;A)/Bal$ by males $T(Y;A)/B'$, where $x = a, bl, e, i, sp$ or sp^{55} , where *Bal* = *CyO*, *Cy Roi* for the second chromosome and *TM3*, *Sb Ser* for the third chromosome, and where *B'* is any dominantly marked chromosome other than the one present in the female. Males carried a compound *XY* to assure male fertility in the event of possible lesions in the translocated *Y*. Terminal trisomics were generated in the first cross for translocations with autosomal breakpoints located near the distal end of each chromosome.

The second type of translocation used is insertionals (Table 2). The $T(Y;2)$'s were balanced over $In(2LR) SM1, al^2\ Cy\ cn^2\ sp^2$; the $T(Y;3)$'s over $In(3LR) TM6, ss^- bx^{34e} Ubx^{67be}$. The same compound-*X*'s with the desired *white* alleles were crossed to the insertional translocations, and female progeny were scored.

Finally, segmental monosomics were tested in a few regions that were first narrowed to more precisely identify an effective trisomic region.

(iv) *Controls*

As noted, the main control was internal to the experiment, that is, the use of genetically similar siblings in the trisomic-diploid comparison. A control was made for the effect of the different balancer types on the various *white* mutants tested by observing the number of regions affecting a given allele, and the direction of the effect. Balancer effects were suspected in those cases where (a) a preponderance of the regions tested had an effect, and (b) the effect was always in the same direction. In addition, we compared the terminal trisomics for balancer effects as well as for segmental aneuploid effects, where possible. Combinations of regions and alleles which were deemed suspect were tested for balancer effects by repeating the above crosses without *CyO*, *Cy Roi* or *TM3*, *Sb Ser*. Scoring was then done by the B^S and y^+ markers in the translocation stocks, or by the paternal marker. In addition, a selection of the crosses

Table 1. *Y-autosome translocations used to generate segmental trisomics*

T(Y;2)		T(Y;3)	
Designation	Breakpoints	Designation	Breakpoints
<u>G146</u>	23BC; Y ^S	B21	62E; Y ^S
<u>G120</u>	24A; Y ^S	G11	63CD; Y ^L
<u>H116</u>	25A; Y ^L	<u>B141</u>	64E; Y ^S
<u>J96^c</u>	25A; Y ^L	B234	65B; Y ^L
<u>D6</u>	25CD; Y ^S	B186	65E; Y ^S
<u>H52</u>	27E; Y ^S	R86	66A; Y ^S
<u>L52</u>	30F; Y ^L	<u>G122</u>	67C; Y ^L
<u>R15</u>	35BC; Y ^S	<u>A31</u>	70AC; Y ^S
<u>P58</u>	35D; Y ^S	J112	71BC; Y ^S
<u>B4</u>	36C; Y ^S	B207	72D; Y ^S
<u>H174</u>	37D; Y ^S	B96	73AB; Y ^L
<u>B110</u>	38C; Y ^L	<u>D228/3</u>	74A; Y ^L
<u>L138</u>	39C; Y ^S	L131	75D; Y ^S
<u>D20</u>	40; Y ^L	R153	78A; Y ^L
<u>G10</u>	41; Y ^S	<u>J162^c</u>	79D; Y ^S
<u>B135</u>	42AB; Y ^S	<u>J1^c</u>	81F; ?
<u>R155</u>	43C; Y ^L	<u>J17</u>	82A; Y ^D
<u>L23</u>	45F; Y ^S	<u>L132^a</u>	83D; Y ^S
<u>A24</u>	46A; Y ^S	<u>G8</u>	85F; Y ^S
<u>B107^c</u>	47E; Y ^L	<u>R36</u>	86B; Y ^L
<u>L110</u>	50C; Y ^L	A78	87B; Y ^L
<u>R14</u>	52E; Y ^L	D226	87E; Y ^L
<u>H149</u>	54F; Y ^S	H172	87F; Y ^L
<u>L139</u>	56E; Y ^S	<u>G48^b</u>	88C; Y ^S
<u>L107</u>	57B; Y ^L	<u>B116</u>	90E; Y ^L
<u>A120</u>	57E; Y ^S	R135	91B; ?
<u>R104</u>	58A; Y ^S	<u>B93</u>	93F94A; Y ^S
<u>B202</u>	59AB; Y ^S	<u>D100</u>	94A; Y ^L
<u>P59</u>	59B; Y ^S	A117	96A; Y ^S
<u>J131</u>	59D; Y ^L	<u>G73</u>	96A; Y ^L
<u>L11</u>	60C; Y ^S	R87	97A; Y ^S
		B158	97B; Y ^L
		<u>R128</u>	97F; Y ^S

Translocations used in the original screen are underlined.

^a Denell (1976).

^b Kennison & Russell (1987) suggest a breakpoint proximal to the *trx* [or *Rg(bx)*] locus at 99B1–3.

^c J96 is a complex T(Y;2;4); B107 and J162 both contain an inversion; and J1 contains an insertion. For purposes of this screen, we have assumed that this has no effect on their properties.

Table 2. *Insertional Y-autosome translocations used to generate segmental trisomics*

T(Y;2)		T(Y;3)	
Designation	Breakpoints	Designation	Breakpoints
B231	27D; 31E	G130	61E; 66F
J64	56F; 57A	A81	75D; 80

performed with insertional translocations were done using stocks bearing a balancer (for either chromosome) so as to create two sets of segmental trisomics and two of diploids, one with and one without the suspect balancer. Finally, for those few regions which were examined as segmental monosomics, an extra control was present, in that the balancer was in the diploid, rather than the aneuploid, class. Thus, if the

trisomic were found to work in the opposite direction from the monosomic, the balancer was clearly not causing the effect.

The only balancer effects found were, for the second chromosome, a moderate suppression in the *Cy Roi*-marked diploid relative to the trisomic for *w^f* and *w^{sp}*; and, for the third chromosome, a stronger suppression in the *Sb Ser*-marked diploid relative to the trisomics

for w^{sp} . In the former case, tests done while using the insertional translocations suggest that the actual effect was due to the paternal marker chromosome bearing *Sco*, which was acting as an enhancer in the trisomic. In all three cases, the minimum threshold for scoring an enhancement effect was adjusted to compensate, and Table 3 shows these adjusted judgements. Though a very weak effect on w^{sp} or w^i could be lost in this way, we are confident that we have been able to score consistently for all moderate to strong effects encountered.

(v) *Distinguishing progeny classes*

Difference in balancer markers was used as the primary means in distinguishing trisomic and monosomic versus diploid progeny classes generated by the reciprocal translocations. Where possible, the system described in Lindsley *et al.* (1972) of distinguishing classes by scoring B^S and y^+ in zero, one or two copies was used. Due to the high reversion frequency of B^S , translocations without B^S now are predominant. As eye colour is more difficult to score in the presence of the *Bar* phenotype, and the detection of *Rough eye* can be difficult in *Bar* flies as well, the absence of *Bar* was considered as a positive factor in selecting translocations for use. Unfortunately, this created the problem of being unable to separate terminal trisomics based on genetic markers in those crosses where they were not identified by being marked by y *vs.* y^+ . For most crosses except the more distal in each arm, terminal trisomics are expected to be rare or absent, allowing adequate scoring on the basis of balancer type alone.

(vi) *Rearing, scoring and recording*

Flies were raised on Formula 4-24 instant *Drosophila* medium (Carolina Biological Supply Co.) at 25 °C. Progeny to be scored were collected within 8 h of eclosion and stored on fresh medium for 2.5–4 days (unless otherwise noted). Flies were photographed with Kodak Kodachrome KPA 40 colour slide film.

3. Results

The rationale for the use of the particular *white* mutants used in this screen is based on the range of lesions and insertions that determine their phenotype. Several sets of control mechanisms, each one necessary for wild-type expression, exist at the *white* locus. Any one of these regulatory regions might be accessible to regulation by some autosomal gene or genes in a dosage dependent fashion. Retrotransposon insertion mutants in introns were used because they are particularly sensitive indicators for a large range of gene interactions and combine the recognition sequences of *white* with those of the specific transposon. They also are apparently sensitive to changes in

splicing and termination efficiency (Birchler *et al.* 1989; Birchler & Hiebert, 1989; Peng & Mount, 1990). Mutants deficient for specific *cis*-acting control regions are also expected to identify modifiers specific for those sequences by being unique among the alleles in their lack of response. Finally, a duplication such as w^i might be expected to screen particularly strongly for those modifiers which affect aberrant splice patterns.

Twenty-five dosage-dependent second-site modifiers for w^a , w^{bl} or both were found. Additional modifications specific for other alleles were identified. Because they are not really divisible into types or categories, we will discuss them in order of their position on the autosomes. The cytological regions are given below, but the translocations used are also noted in Table 3.

(i) *Chromosome two, left arm*

The terminal segments of 2L (up to 30F) give no effect on either w^{bl} or w^a as trisomics. However, a smaller region internal to this, 25A–25CD, produces a weak effect on w^{bl} . Suppressing w^{bl} as a monosomic, enhancing as a trisomic, the gene produces an inverse response on the pigment levels of w^{bl} .

Strong effects were observed for segmental trisomy of 30F to 35BC. In addition to a slight enhancement of w^a and w^{bl} , w^{sp} is enhanced, and w^i and w^e are strongly suppressed. Enhancement of w^{bl} by the insertional translocation covering 27D to 31E suggests that the active region can be placed in 30F–31E.

Enhancement of w^a , w^{sp55} and w^i by the trisomic covering 35BC to 38C is clear, though not strong. The *e*, *bl*, and *sp* alleles gave no clear effect. Though the entire region does not affect w^{sp} above the average for chromosome two, a sub-region, 36C to 37D, shows a very strong enhancement effect, seemingly limited to the red pigments. It is possible that this enhancer is itself masked by the action of an adjoining gene, accounting for the lack of effect in the larger region.

The trisomy for 38C to 39C influences one of the alleles tested in a patterned manner: the trisomy affects the sp^{55} allele such that several small, darker spots appear on an otherwise phenotypically sp^{55} eye. The region does not act upon w^{bl} or w^e , but strongly enhances w^{sp} .

The region 39C to 40 showed poor viability as a trisomic. Two alleles, *bl* and *e*, are suppressed, *e* distinctly so. No effect is seen on *sp*. A few flies were recovered for the larger trisomy, 38C to 40, for which w^e is clearly suppressed, as in the smaller region.

(ii) *Chromosome two, right arm*

The segmental trisomic from 41 to 43C enhances w^a , w^{bl} , w^{sp55} , w^{sp} and w^i , but not w^e .

The next region, 43C to 45F, affects three alleles: w^{bl} , w^i and w^e are all clearly enhanced in the segmental trisomic. No effect was seen on w^a or w^{sp} .

Table 3. Summary of genomic trisomic screen

Cytological interval	Translocations	white allele					
		w ^a	w ^{bl}	w ^{sp55}	w ^{sp}	w ^e	w ⁱ
21A-24A	G120	0	0				
23BC-25A	J96/G146	0	0				
25A-27E	H52/J96	0	0				
25A-25CD	H116/D6	N.D.	-				
27E-30F	L52/H52	0	0				
30F-35BC	R15/L52	-	-	0	-	+	+
27D-31E	Insertional B231	N.D.	-				
35BC-38C	B110/R15	-	0	-	0	0	-
36C-37D	B4/H174				-		
38C-39C	L138/B110	N.D.	0	+	-	0	
39C-40	L1381/D20	N.D.	+		0	+	
38C-40	B110/D20	0	0			+	0
40-41	G10/D20	0	+				
41-43C	G10/R155	-	-	-	-	0	-
43C-45F	R155/L23	0	-		0	-	-
45F-46A	L23/A24	0	-				
46A-47E	A24/B107	-	-				
47E-50C	B107/L110	-	+				
50C-52E	L110/R14	-	-		0	0	0
52E-54F	R14/H149	0	+	0	0	+	-
54F-57B	H149/L107	+	-				
56F-57A	Insertional J64	0	0	0	-		-
57B-59B	P59/L107	-	0	-	0	0	0
59A-60F	B202	0	-				0
57E-60F	A120		-				
58A-60F	R104		-				
58A-59B	R104/P59		0				
59B-60C	P59/L11		-				
60C-60F	L11		0				
61A-64E	B141	0	0	+	0	0	
61A-62E	B21			+			
62E-63CD	B21/G11			0			
63CD-64E	G11/B141			0			
64E-67C	G122/B141	-	+		0		
64E-66A	B141/R86		+				
66A-67C	R86/G122		0				
64E-65B	B141/B234		+				
67C-70AC	A31/G22	-	0	-	0	-	N.D.
70AC-74A	D228/A31	+	+	0	N.D.	N.D.	0
74A-75D	L131/D228	N.D.	+				
75D-78A	R153/L131	-	+				
78A-79D	J162/R153	N.D.	+				
79D-81F	J1/J162	0	0				
81F-83CD	L132/J1	0	-				
86B-88C	G48/R36	+	+	0	0	+	0
86B-87B	R36/A78		0				
87B-87E	A78/D226		0				
86B-87F	R36/H172		+				
87E-87F	D226/H172		+				
87F-88C	G48/H172		0				
88C-90E	B116/G48	-	-	0	-		0
90E-93F/94A	B93/B116	0	+				
93F/94A-94A	B93/D100	0	0				
93F	A117/D100	0	0				
94A-96A	B93/G73	0	0				
96A-97F	R128/G73	-	0	0	-	0	0
97F-100F	R128	-	0		+		

The cytological region is the trisomic segment between the breakpoints of the translocations listed, that was used in the comparisons to euploid controls. The level of pigment in the trisomic versus the diploid is given under the respective allele designation; + signifies an increase in pigment, - signifies a reduction in pigment and 0 signifies an equivalent eye colour between the segmental trisomic and the euploid. N.D. denotes those cases in the w^a screen in which the comparison was not determined due to no trisomic progeny from the attempted cross. Blanks indicate no attempted cross.

The region 45F-46A has no effect upon w^a but enhances w^{bl}. The adjacent region 46A-47E enhances both w^a and w^{bl}. This is the site of *Inr-a*, which

inversely affects white expression (Rabinow *et al.* 1991).

The enhancement effect on w^a from trisomy for 47E

to 50C is not particularly pronounced. This same region suppressed w^{bl} .

The next region, 50C to 52E, is also a trisomic enhancer of w^a . Also enhanced, though not strongly, is w^{bl} . No effect was seen in w^{sp} , w^e , or w^i .

Trisomy for 52E to 54F suppresses both w^{bl} and w^e , both quite strongly. Enhancement occurs for w^i . The other alleles are unaffected.

Trisomy for 54F to 57B enhances w^{bl} and slightly suppresses w^a .

Insertional translocation-produced trisomies for 56F to 57A show enhancement of w^{sp} and w^i . No effect is seen for the alleles sp^{55} , bl , or a in this region.

Trisomy for 57B shows enhancement for w^a and $w^{sp^{55}}$ but no effect for other tested alleles. Trisomy for the tip of 2R (59AB–60F) shows an enhancement of w^{bl} . Three different terminal trisomics act as enhancers of w^{bl} . Subdivision of this region with overlapping translocations narrowed the responsible site to 59B–60C.

(iii) Chromosome three, left arm

A gene lying between 61A and 62E, when trisomic, suppresses sp^{55} intensely. No effect attributable to this region is seen on any other allele.

Trisomic suppression of w^{bl} occurs in the region 64E–67C. Subdivision of this segment gives trisomic suppression in 64E–66A but no effect from 66A–67C. The responsible region can be narrowed further to 64E–65B. The corresponding deficiency for the strongly suppressing trisomic clearly enhances w^{bl} , giving a full-dosage series for one, two and three copies.

Three alleles are enhanced by three copies of 67C to 70AC, with w^a being most strongly affected, w^e and $w^{sp^{55}}$ less so.

Only w^a and w^{bl} are affected by the trisomy for 70AC to 74A. They are slightly suppressed.

w^{bl} is suppressed by trisomy of the adjoining region, covering 74A to 75D. The region from 75D–78A enhances w^a and suppresses w^{bl} . The adjoining region from 78A–79D suppressed w^{bl} .

(iv) Chromosome three, right arm

The region from 81F–83CD acts as an enhancer of w^{bl} . The strongest effect in the region of 86B to 88C can be localized to the sub-region 87E to 87F, which, as a trisomic, suppresses w^{bl} particularly strongly. Conversely, the segmental monosomic (87E–87F) is a clear enhancer of w^{bl} . It also suppressed w^a and w^e . No significant effect is seen for the sp , sp^{55} or i alleles.

The trisomy for 88C to 90E enhances w^a , w^{bl} and w^{sp} . Of the alleles tested, only w^{bl} is affected by trisomy for 90E to 93F–94A, being slightly suppressed. The next region with any effects is from 96A–97F which enhances w^a and w^{sp} .

The terminal trisomic distal to 97F enhances w^a . This is probably due to the modifier gene *Doa*, which lies in this region (Rabinow & Birchler 1989). *Doa* has been determined to suppress w^a as a mutant, and enhance it in three copies of the wild-type gene. *Doa* has also been found to enhance $w^{sp^{55}}$ as a mutant, and to have no effect on 40 other *white* alleles.

In summary, we note that many segments of the genome produce a dosage dependent effect upon *white-apricot* or *white-blood* or both, when assayed in segmental trisomics. In a few cases, it was possible to assay corresponding monosomics, when the effective trisomic region could be narrowed. A consistent dosage effect on w^{bl} was observed in three cases (25A–CD, 64E–65B and 87E–87F) in which all three doses could be assayed. Undoubtedly, other regions would give a complete dosage effect, if it were possible to test the corresponding monosomics.

4. Discussion

(i) The white locus and its mutants

The *white* gene regulates deposition of pigment in the eyes, ocelli, testes and Malpighian tubules. The DNA sequence indicates it is a membrane transport protein (O'Hare *et al.* 1984; Dreesen *et al.* 1989; Hazelrigg, 1987). Mutations in *white* may be divided into proximal regulatory mutations and distal structural mutations in the transcribed region (Zachar & Bingham, 1982), roughly following earlier divisions into pattern mutants and non-pattern mutants (Judd, 1976). Partial complementation is observed between these groups.

Different kinds of mutational events occurring within *white* can be distinguished, roughly, by the type of effect that they have on gene expression. Colourless *white* mutants can be produced by large deletions in coding or non-coding regions of the gene; smaller deletions, point mutations, and insertions generally also produce bleach-white eyes if they fall within exons. A mottled or spotted phenotype is correlated with disruption of the 5' transcriptional enhancer sequences (see Davison *et al.* 1985). A large percentage of coloured *white* mutants, however, derive specifically from insertion within introns of transposable elements (Zachar & Bingham, 1982), primarily retrotransposons.

A large number of modifiers were identified in this study. One reason for this is that several molecular mechanisms can be modulated by dominant modifiers and not only could *white* be modulated but also each of the respective transposable elements. Our initial studies on *apricot* modifier loci supports the conclusions that many such genes exist and that they mediate their effects in a variety of ways (Rabinow & Birchler, 1990). While there are many regions identified in this study, we note the possibility of interactions that would prevent recognition of all such

modifiers. The simultaneous inclusion of an enhancer and suppressor within the same trisomic would go unnoticed.

The large number of modifiers is notable in two respects. First, such modifiers could contribute to and be the specific basis of much of quantitative variation. Variation in structural genes certainly contributes but variation in second site modifiers does so on a more global level. Secondly, the great number of modifiers that show specificity for retrotransposon induced alleles raises the possibility that a particular insertion allele might exhibit a significantly different phenotype from background to background. McDonald (1990) has pointed out how numerous suppressor or enhancer loci of retrotransposon insertions could contribute to speciation mechanisms. The data presented here demonstrates there is indeed a great number of modifiers of retrotransposon induced alleles.

Muller (1950) described dosage dependent modification of *white-apricot* in aneuploids along the length of the X chromosome. The present study demonstrates that a similar array of effects occurs also with autosomal aneuploids. The context of Muller's study was with regard to dosage compensation and concentrated on the X. If such modifiers are involved in compensation, genes on both the X and the autosomes are implicated by the fact that both the X and the autosomes have such effects. The dosage sensitive modifiers identified by Muller, while interpreted differently, are undoubtedly coincident with the modifiers identified more recently to affect gene expression, inversely or directly (Birchler, 1979; Devlin *et al.* 1988; Rabinow *et al.* 1991).

(ii) Patterns of allele specificity

The effective regions could be the result of a general modifier of gene expression acting upon a regulatory site of the *white* gene. The sequences of *white* that are responsible for interaction are implicated by the lesion in those mutants not affected. Conversely, the modifiers might regulate proper splicing or alter proper termination. Any allele whose phenotype is caused by improper termination, inserted sequences, or improper splicing would be open to this form of modification. Except for w^{sp} , for which the lesion is outside the structural portion of the gene, any allele could be a candidate for interaction with this sort of modifier, and allele specificity of different modifiers could be expected to vary greatly. While some regulators of *white* would be expected not to interact with w^{sp} , those that do are candidates for regulatory effects on the *white* locus itself.

We examined the relationship of these *white* modifier regions with previously studied dosage-dependent second-site modifiers for other genes. While there is no consistent correlation between the regions containing second site regulators of *white* and those affecting the homoeotic mutants *Polycomb* and *Anten-*

napedia (data from Kennison & Russell, 1987; Kennison & Tamkun, 1988) in a dosage-dependent fashion, a number of overlaps do exist. As this screen has only mapped modifiers in a gross fashion, such overlap is unremarkable.

The results of the present study are consistent with related studies involving enzyme activity measurements in segmental trisomics (Birchler, 1979; Devlin *et al.* 1988; Birchler *et al.* 1990, and refs cited therein). For any one structural gene, there are multiple regions that affect its expression. The most frequent result is an inverse correlation of the varied segment and the expression of the structural gene. In the present study, there are both direct and inverse effects observed. This, however, must be interpreted in view of the fact that some fraction of modifiers are interacting with the inserted retrotransposon and may therefore cause the opposite phenotypic effect from *white*. The *apricot* and *blood* alleles involve retrotransposon insertion in the second intron but in opposite orientation. *Apricot* is a copia insertion in parallel to *white*; whereas *blood* has an anti-parallel insertion ('blood' element). Regions that modify both *apricot* and *blood* in the same direction might be considered as candidates for modifiers of *white* itself, given that the two elements are inserted in opposite orientations. The majority of these similar effects on both alleles are inverse correlations between the chromosomal dosage and the level of *white* pigment.

Also, in parallel with the enzyme data, is the observation that in ploidy comparisons of diploid and triploid, the phenotypic intensity of the eye colour is the same. In the attached X stock of *white-blood*, a triploid female spontaneously arose so that it was possible after mating her to w^{bl} males to observe the eye colour in diploid and triploid females homozygous for this allele. They were indistinguishable. The same is true of *white-apricot* (Rabinow *et al.* 1991). Taken together, these results are similar to enzyme activity and messenger RNA comparisons which are modulated by multiple aneuploidies but that do not appear to be additive in ploidy comparisons (Birchler *et al.* 1990). As with the enzyme data, this suggests a stoichiometric interaction or 'balance' among the modifiers.

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