Expression of *cis*-regulatory mutations of the *white* locus in metafemales of *Drosophila melanogaster*

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Summary

At the *white* eye colour locus, there are a number of alleles that have altered expression between males and females. To test these regulatory mutations of the *white* eye colour locus for their phenotypic expression in metafemales (3X; 2A) compared to diploid females and males, eleven alleles or transduced copies of *white* were analysed. Two alleles that exhibit dosage compensation between males and females (*apricot, blood*) also exhibit dosage compensation in metafemales. *White-ivory* and *white-eosin,* which fail to dosage compensate in males compared to females, but that are distinct physical lesions, also show a dosage effect in metafemales. Two alleles with greater expression in males than females (*spotted, spotted-55*) exhibit even lower expression in metafemales. Lastly, five transduced copies of *white* carrying three different lengths of the *white* promoter, but that all exhibit higher expression in males, show reduced expression in metafemales, exhibiting an inverse correlation between the level of expression and the dosage of the X chromosome. Because these alleles of *white* respond to dosage compensation in metafemales as a continuum of the male and female responses, it is concluded that the same basic mechanism of dosage compensation is involved and that the dosage of the X chromosome conditions the sexually dimorphic expression.

1. Introduction

At the *white* eye colour locus in *Drosophila*, there are a number of alleles that have an altered sexually dimorphic expression (see Hazelrigg, 1987, for review). The physical analysis of the alleles indicates that these are *cis*-regulatory lesions in the gene. These alleles include the *spotted* alleles that have a mottled appearance and that have greater expression in males than in females despite fewer copies in the former. Another type of regulatory allele involves those that fail to exhibit dosage compensation between males and females – in this case the males have less pigment than females. There are also a series of transformed truncated isolates that exhibit greater expression in males than in females when inserted in the autosomes. We are interested in the regulation of the *white* locus so we wished to examine further the basis for these phenotypes. On the one hand, the altered response of these alleles might be a sex-limited effect; there being one mode operating in males and a second in females.

Secondly, their response might involve a difference in pairing or transvection – a phenomenon that has been documented at *white* with its interaction with *zeste* and with the w^{DZL} allele. Lastly, the sexual difference could be a response to the dosage of the X chromosome, involving varying types of altered response to the dosage compensation mechanism.

The phenomenon of dosage compensation of X-linked genes in *Drosophila* was formulated using a hypomorphic allele of the *white* locus, *white-apricot* (w^a) (Muller, 1932, 1950). A dosage series of w^a in females produces a directly proportional amount of pigment in the eyes, but males with one copy are nearly equivalent to females with two. Likewise, males with two copies of w^a are even darker than the three copy females. These and similar observations at other loci led to the concept that most genes on the X chromosome are doubled in expression per copy in the male, relative to the female. Numerous studies have addressed the problem of dosage compensation since that time (for review see Lucchesi, 1983; Lucchesi & Manning, 1987).

Stern (1960) was the first to address experimentally the question of whether dosage compensation ex-

tended to metafemales (3X; 2A). He produced a metafemale homozygous for w^a, and found that the eye colour was basically identical to her diploid sisters. Subsequently, the expression of X-linked genes in metafemales has been investigated by several individuals, with varying conclusions concerning whether compensation occurs depending on the type of analysis and treatment of the data. The majority of studies conclude that compensation does occur (see Lucchesi, 1983). Birchler *et al.* (1989) re-examined the problem with standardization to DNA levels and found that the absolute expression of the X-linked genes was basically equivalent in total amount to diploid females but that autosomal gene expression was reduced. This finding could account for the great inviability of metafemales despite the fact that the total output of the three X's is equivalent to diploid females.

The compensation of the X and the autosomal reductions could be due to the inverse effect. This phenomenon has been observed in organisms as diverse as maize, *Drosophila*, mouse and human (Birchler, 1979; Birchler & Newton, 1981; Devlin *et al.* 1988; Birchler *et al.* 1990; Klose & Putz, 1983; Reichert, 1986; Whatley *et al.* 1984). The observation is that a dosage series for a particular chromosomal segment will inversely affect the quantitative expression of genes encoded elsewhere in the genome. Segmental dosage compensation in maize (Birchler, 1981) and autosomal dosage compensation in *Drosophila* (Devlin *et al.* 1982, 1988) are brought about by a cancellation of the opposing structural gene dosage effect and an inverse effect simultaneously produced in a dosage series (Birchler, 1981; Birchler *et al.* 1990).

Structural gene localization studies reveal that basically every segmental trisomic comprised of just a few percent of the total cytological length of the genome will produce an inverse effect on one or more gene products (Detwiler & MacIntyre, 1978; Hall & Kankel, 1976; Hodgetts, 1975; Moore & Sullivan, 1978; O'Brien & Gethman, 1973; Oliver, Huber & Williamson, 1978; Pipkin *et al.* 1977; Rawls & Lucchesi, 1974). Six of seven segmental aneuploid series examined in maize produced an inverse or direct effect upon a subset of proteins expressed in the particular tissue studied (Birchler & Newton, 1981). The data of Devlin *et al.* (1988) on whole arm trisomy in *Drosophila* shows that the phenomenon is operative on all sixteen loci examined. This suggests that there are many regulatory genes in any one species that produce an inverse effect upon groups of structural genes.

The widespread occurrence of the inverse effect and autosomal dosage compensation raises the possibility that X chromosomal dosage compensation has evolved as a refinement of the same process (Birchler, 1979). The pre-existing regulatory system that manifests itself as the inverse effect could have been modified to account for dosage compensation in males and metafemales. One could postulate, however, that a completely novel mechanism has evolved for X chromosomal dosage compensation. This would bring about the compensation of the genes responsible for the inverse effect, which would then not produce any response in a sex chromosomal dosage series. A variation of this hypothesis would be that a novel mechanism is operative only in males, which is, in fact, the chromosomal situation subjected to natural selection. In this view, dosage compensation in metafemales would be brought about by a distinct mechanism but one analogous to that producing autosomal dosage compensation. The experiments described in this paper were designed to address these possibilities.

The rationale for the experiments described here was to examine the expression of representative alleles of each type of regulatory lesion of the *white* eye colour locus, for their expression in metafemales. The alleles were recognized over the course of *Drosophila* history for their response in males versus females; therefore a determination of their relative expression in metafemales would provide valuable information about the mechanism of dosage compensation and *white* locus expression in general. Mutant alleles that are lacking, blocked or altered with regard to male dosage compensation would give the complementary response in metafemales if the mechanism is the same.

The results demonstrate that the expression in metafemales is a continuum of that found in males and females. That is, those alleles, that do not compensate in males versus females, do not compensate in metafemales either; those alleles that are dosage compensated between males and females are dosage compensated in metafemales; and those alleles that condition greater expression in males than females have reduced expression in metafemales. The fact that the observed response in metafemales is a continuum to that found in males suggests that the expression of the *white* locus responds to the dosage of the X chromosome by a similar mechanism in males and metafemales.

2. Materials and methods

(i) *Construction of attached X chromosomes*

The construction of the various attached X chromosomes carrying different alleles was conducted as follows. First, the selected alleles were recombined with the *yellow* (*y*) body colour marker at the tip of the X. This was accomplished by crossing a *y w* stock by males from w^{bl} (blood), w^{sp} (spotted), w^i (ivory), w^{sp55} (spotted-55), and w^e (eosin). The F$_1$ from these crosses were allowed to mate *inter se* and the F$_2$ were screened for males that recombined *y* with the respective hypomorph. The exceptional males were mated to $C(1)DX$, *y f*/ *Y* females to establish stocks. A deletion allele of *white*, w^{118}, was recombined to
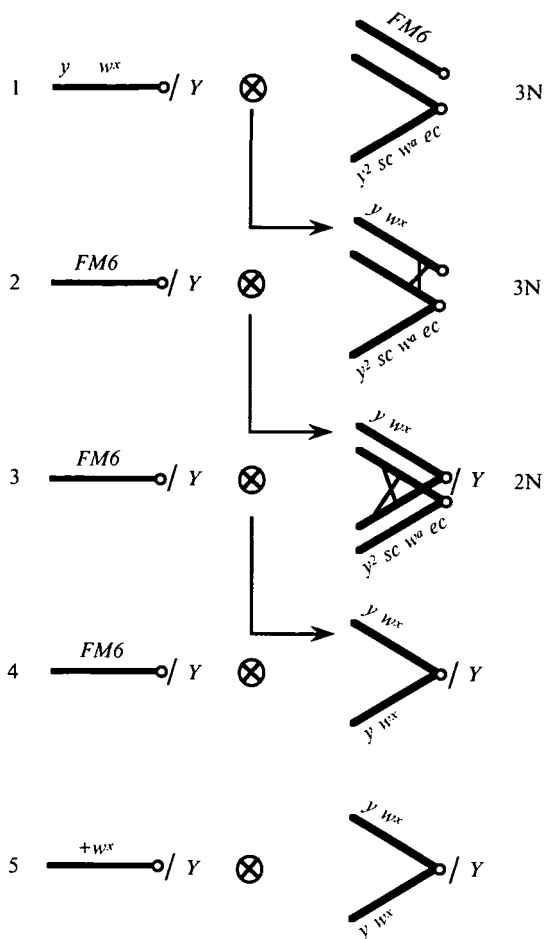
Fig. 1. Genetic protocol for construction of compound metacentric attached X chromosomes homozygous for *yellow* and various *white* alleles. 1, The respective *white* (w^x) alleles, linked with yellow (y) were crossed to triploid females carrying a reversed metacentric X chromosome and the balancer, FM6. 2, The triploid females among the progeny are heterozygous for the attached X and the free X carrying the *white* allele of interest. A recombination event proximal to w will transfer the new allele to the attached X. The triploid females were crossed by *FM6*, which is marked by the dominant *Bar* (*B*) mutation to distinguish diploid and triploid progeny. 3, Among the diploid females are heterozygous recombinants. These can be recognized as y^2 sc^+ ec^+. Proximal recombination in the attached X (shown at the four-strand stage) will result in homozygosis of either *white* allele and associated markers. 4, Homozygous attached X females were selected and crossed by *FM6/Y* males to perpetuate the chromosome. 5, A stock homozygous for the respective *white* allele but with *yellow* females and y^+ males was produced by mating attached X females by the appropriate males. Metafemales were selected from these stocks as y^+ *females*.

y by crossing to a y w^{bt} stock and selecting y w^{1118} males in the F_2.

To generate attached X chromosomes homozygous for each of the above *white* alleles in combination with y, the following five-generation protocol was used. The procedure involves recombination of the various alleles onto a compound reversed metacentric X within triploid females. Each y and *white* allele

combination chromosome was crossed to triploid females with the X chromosome constitution $C(1)RM$, y^2 sc w^a $ec/FM6$, y^{31d} sc^8 dm B. Triploid females in the F_1 can be selected as those heterozygous for $C(1)RM$, y^2 sc w^a ec and the respective *white* allele. These triploid females were crossed by $FM6/Y$ males. The non Bar females among the progeny carry the attached X and are diploid. If recombination has transferred the respective *white* allele to the attached X, then the flies will have an eye colour indicative of a heterozygote with w^a as well as normal for *scute* (*sc*) and *echinus* (*ec*). These recombinants were again mated to $FM6/Y$ males. Because of the distal location of *white* and *yellow* on the X, recombination in the attached X will generate approximately one-quarter of the female progeny that will be homozygous for the two markers due to recombination proximal to *white*. Once a true breeding stock was established with $FM6/Y$ males, virgin attached X females were collected and mated to males of the respective *white* alleles but carrying the normal allele of *yellow*. This produced stocks with $C(1)RM$ attached X females homozygous for y and each *white* allele and with males homozygous for y^+ and the same *white* allele. The protocol for construction of these chromosomes is given in Fig. 1.

(ii) *Construction of stocks carrying autosomal transduced copies of white*

The stock of $C(1)RM$, y w^{1118} described above was crossed by males of five stocks of transduced *white* genes at autosomal sites present with a y^+ w^{1118} X chromosome. Backcrosses resulted in homozygous or balanced stocks. Four of the insertions are described by Hazelrigg *et al.* (1984) and Levis *et al.* (1985). All have truncated 5′ regulatory sequences that partially impair the function of the gene. Two of these are Kpn to *Bgl* II fragments from Canton S wild type, namely E6(57B) and E8(47D), with cytological position noted. The other two are *Kpn–Sca* fragments and are designated Q211(64B) and Q20(50A). The *Kpn* to *Bgl* II fragments contain all of the structural portion of the *white* gene and 1039 bp 5′ to the start of transcription. The presumptive enhancer of the *white* locus lies outside this fragment (Davison *et al.*, 1985); hence it is greatly reduced in level of expression. The other pair of transduced genes has 360 bp present 5′ to the start of transcription and are reduced further in the level of expression. The fifth construct has been described previously (Birchler *et al.*, 1990). This isolate has the 5′ regulatory sequences of the *alcohol dehydrogenase* (*Adh*) gene fused to the *white* locus structural gene in the respective 5′ non-translated leader sequences of the messenger RNAs. It was constructed by Janice Fischer and kindly made available to the author. The transduced copy shows linkage to the third chromosome. All five stocks,

including the *Adh* promoter driven construct, exhibit higher levels of expression in males than in females. Since the copy number is the same in both sexes it is concluded that they all exhibit dosage compensation.

Fly culture. In accordance with Dobzhansky (1928) and our previous studies (Birchler *et al.* 1989), flies were grown at 20 °C to maximize survival of meta-females, which are found only at a frequency of approximately 1×10^{-3}.

Metafemale screening. All of the eleven stocks carry an attached X marked by *yellow* in the females; the males carry the same *white* allele but have y^+ on their X chromosome. Therefore, the rare exceptional metafemales will have both X chromosomes and will be homozygous for the *white* allele under consideration but will carry y^+. Metafemales can be distinguished from triploids or diploid females of the same phenotype that result from detachment of the $C(1)RM$ in the maternal parent by several criteria; most consistently for flies grown at 20 °C are defects in the abdominal tergites, sterility, and occasionally crumpled wings. Nicked wing margins and disarrayed eye facets, common in metafemales grown at 25 °C, are rarely observed at 20 °C.

Cultures were screened for metafemales until at least two examples were found for each allele. The vials were screened daily. Metafemales, diploid sisters and brothers were aged for 48 h before photography. An exception involved the flies in the w^{sp55} stock, which were aged for 1 week before photography, because of the low level of pigment in this mutant. Vials, in which a presumptive metafemale and several males were held, were incubated further to confirm the sterility. Detachment y^+ females are fertile and have an eye colour equivalent to the *y* females. Detachment females (and detachment *y* males) occurred often as clusters and therefore at very different frequencies among the different cultures.

3. Results

To test the response of various *white* alleles in metafemales, it was necessary to construct genetic stocks that would facilitate recognition of metafemales homozygous for each allele. For this, attached X chromosomes were generated that were marked by the *yellow* (*y*) body colour mutant as well as the respective *white* allele. When such females are crossed by males with X chromosomes with the same *w* allele but y^+, the triple X metafemale individuals will have normal body colour among yellow sisters. These stocks were generated by first recombining each *white* allele used with *yellow* and then recombining the resulting chromosome onto an attached X in triploid females in a five-generation procedure. Further details are given in Materials and methods and the protocol is outlined in Fig. 1.

Although the screened stocks were grown under conditions that favour the survival of metafemales,

their recovery is still quite low. It was deemed necessary to observe two examples of each of the eleven isolates and confirm each as being sterile (as one criteria to distinguish them from detachments of the respective X chromosomes that also give y^+ females). It is estimated that approximately 40000 female flies were screened to achieve this goal.

The results for each *white* allele assayed in metafemales will be discussed individually in the context of any knowledge of the molecular lesion and the relative expression in males and females.

(i) *Dosage compensating alleles*

white-apricot (w^a). This allele exhibits dosage compensation between males and females with the male being subtly darker in most backgrounds. The molecular lesion is an insertion of a copy of the retrotransposon, *copia*, into the second intervening sequence (Bingham *et al.* 1981; Bingham & Judd, 1981; Goldberg *et al.* 1982). Metafemales homozygous for w^a exhibit dosage compensation of pigment levels (Table 1).

white-blood (w^{bl}). This allele is slightly darker than *apricot*. The mutation is due to an antiparallel insertion of a retrotransposon into the second intron of *white* (Bingham & Chapman, 1986). The males of w^{bl} are dosage compensated but routinely darker than females. The metafemales observed are very near the intensity of normal females, but subtly lighter (Table 1, Fig. 2).

(ii) *Transduced, truncated constructs*

E6 w^+ construct. This transduced truncated copy of normal *white* from a Canton-S strain (Hazelrigg *et al.* 1984; Levis *et al.* 1985) is inserted on the second chromosome at 57B. The construct is maintained as a heterozygote balanced against *Cy0*. The construct has approximately one kilobase of *white* sequence 5′ to the start of transcription. Males of this strain have darker eyes than females indicating that the construct is dosage compensated. Metafemales have lighter eye colour than females indicating that this construct responds to the dosage compensation function in these flies as well (Table 1). There is an inverse correlation between the dosage of the X chromosome and the level of expression.

E8 w^+ construct. This is a second insertion of the same construct as above (Hazelrigg *et al.* 1984; Levis *et al.* 1985). This insert is located on the second chromosome at 47D. It also has greater expression in males than females and metafemales are lowest of the three (Table 1).

Q20 w^+ construct. This transduced truncated construct is derived from Canton-S wild type and is inserted on the second chromosome at 50A (Hazelrigg *et al.* 1984; Levis *et al.* 1985) and balanced against *Cy0*. There are only 360 bp of 5′ *cis*-regulatory
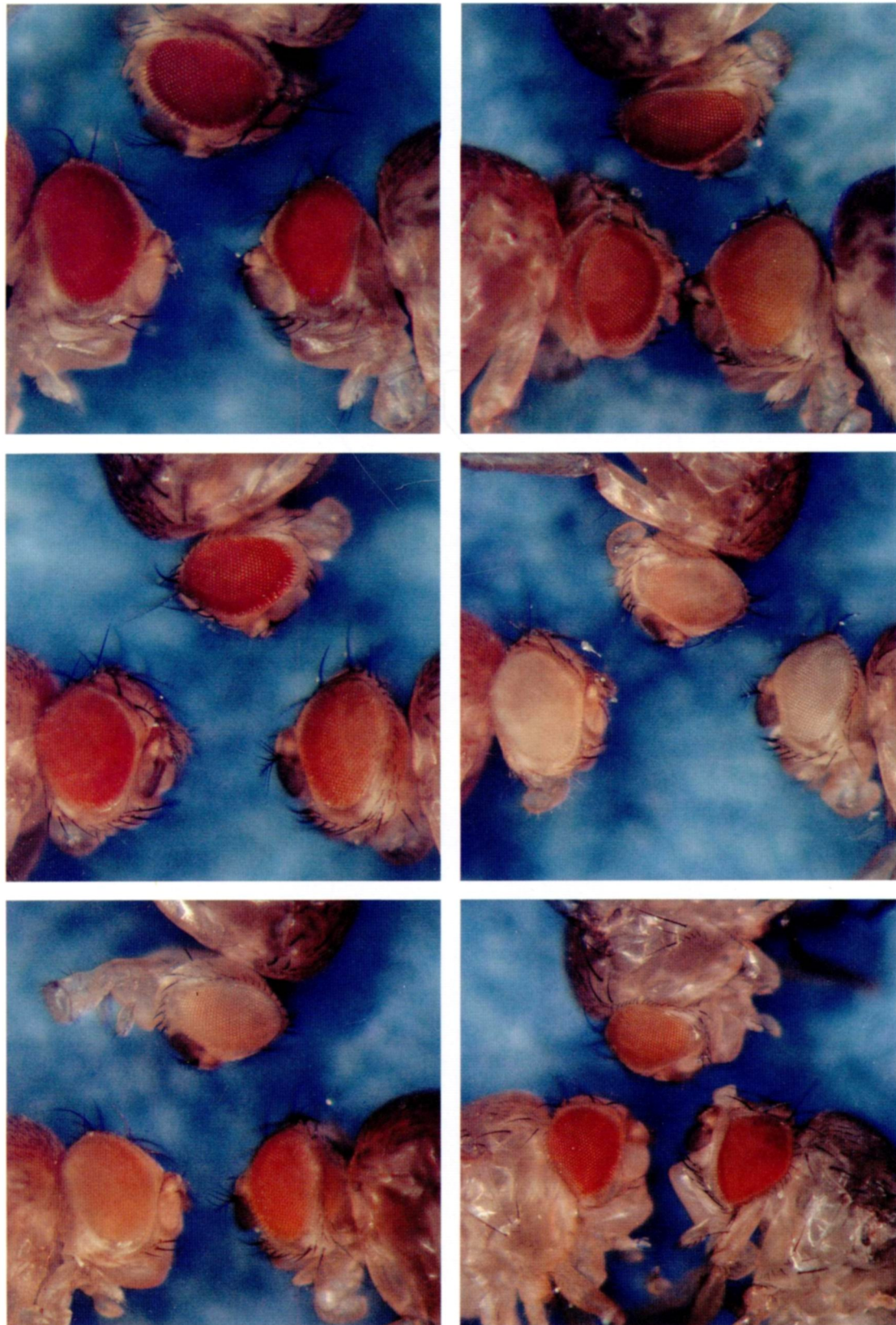
FIGURE 2. Expression of *white* alleles in males, females and metafemales. In each panel the male is at the top, normal female at the left and metafemale at the right. The genotype of all flies is given in Table 1. Top left panel, w^{bl}, a dosage compensating allele; Top right, *Q2ll*, a representative autosomal transformant; Middle left, w^{sp}, a male overexpressing allele; Middle right, w^{sp55}, a male overexpressing allele; Bottom left, w^i, a non-dosage compensating allele; Bottom right, w^e, a non-dosage compensating allele.

Table 1. *Genotypes and relative expression in males, females and metafemales*

white allele and genotype	Dosage of w	Dosage of X	Relative expression
$y^+ w^a/Y$; 2A	1	1	
$C(1)RM$, $y w^a/Y$; 2A	2	2	♂ = ♀ = m♀
$y^+ w^a/C(1)RM$, $y w^a$; 2A	3	3	
$y^+ w^{bl}/Y$; 2A	1	1	
$C(1)RM$, $y w^{bl}/Y$; 2A	2	2	♂ = ♀ = m♀
$y^+ w^{bl}/C(1)RM$, $y w^{bl}$; 2A	3	3	
$y^+ w^{1118}/Y$; E6/CyO	1	1	
$C(1)RM$, $y w^{1118}/Y$; E6/CyO	1	2	♂ > ♀ > m♀
$y^+ w^{1118}/C(1)RM$, $y w^{1118}$; E6/CyO	1	3	
$y^+ w^{1118}/Y$; E8/E8	2	1	
$C(1)RM$, $y w^{1118}/Y$; E8/E8	2	2	♂ > ♀ > m♀
$y^+ w^{1118}/C(1)RM$, $y w^{1118}$; E8/E8	2	3	
$y^+ w^{1118}/Y$; Q211/TM3	1	1	
$C(1)RM$, $y w^{1118}/Y$; Q211/TM3	1	2	♂ > ♀ > m♀
$y^+ w^{1118}/C(1)RM$, $y w^{1118}$; Q211/TM3	1	3	
$y^+ w^{1118}/Y$; Q20/CyO	1	1	
$C(1)RM$, $y w^{1118}/Y$; Q20/CyO	1	2	♂ > ♀ > m♀
$y^+ w^{1118}/C(1)RM$, $y w^{1118}$; Q20/CyO	1	3	
$y^+ w^{1118}/Y$; Adh-w#2/Adh-w#2	2	1	
$C(1)RM$, $y w^{1118}$; Adh-w#2/Adh-w#2	2	2	♂ > ♀ > m♀
$y^+ w^{1118}/C(1)RM$, $y w^{1118}$; Adh-w#2/Adh-w#2	2	3	
$y^+ w^{sp55}/Y$; 2A	1	1	
$C(1)RM$, $y w^{sp}/Y$; 2A	2	2	♂ > ♀ > m♀
$y^+ w^{sp}/C(1)RM$, $y w^{sp}$; 2A	3	3	
$y^+ w^{sp55}/Y$; 2A	1	1	
$C(1)RM$, $y w^{sp55}/Y$; 2A	2	2	♂ > ♀ > m♀
$y^+ w^{sp55}/C(1)RM$, $y w^{sp}$; 2A	3	3	
$y^+ w^e/Y$; 2A	1	1	
$C(1)RM$, $y w^e/Y$; 2A	2	2	♂ < ♀ < m♀
$y^+ w^e/C(1)RM$, $y w^e$; 2A	3	3	
$y^+ w^t/Y$; 2A	1	1	
$C(1)RM$, $y w^t/Y$; 2A	2	2	♂ < ♀ < m♀
$y^+ w^t/C(1)RM$, $y w^t$; 2A	3	3	

Further descriptions of the *white* alleles are given in the text.

sequences remaining. The expression in males indicates a response to dosage compensation and this trend is followed in metafemales (Table 1).

Q211 w⁺ construct. A second insertion of the construct above, this one is present on the third chromosome at 64B (Hazelrigg *et al.* 1984; Levis *et al.* 1985). This construct is maintained as a heterozygote balanced against *TM3*. The order of pigment intensity from greatest to least is male, female, metafemale (Table 1, Fig. 2).

Adh promoter-white structural gene. In this construct, the 5′ regulatory sequences of *white* are replaced with those of *alcohol dehydrogenase* (J. Fischer, described in Birchler *et al.* 1990). The pigment level in the eyes is very low at eclosion but increases with age. This construct is borne on the third chromosome and exhibits dosage compensation, the eyes of males being darker than females. Despite the fact that all of the

white promoter sequences are replaced by those of an autosomal gene, compensation is observed. The assay of metafemales homozygous for the *Adh-w* construct shows that they have lighter eye colour than normal females (Table 1).

(iii) *Male overexpressing alleles*

white-spotted (w^{sp}). This allele results from the insertion of a B104 element into the 5′ *cis*-acting regulatory sequences of *white* (Zachar & Bingham, 1982; O'Hare *et al.* 1984). The site of insertion is believed to be in the enhancer of *white*, which is also the sequence that interacts with the regulatory gene, *zeste* (Davison *et al.* 1985). It is typical of a series of alleles with lesions in this segment of the gene (Davison *et al.* 1985), which are characterized by a mottled appearance that is darker in males than in females.

Metafemales homozygous for w^{sp} are lighter than normal females (Table 1, Fig. 2).

white-spotted-55 (w^{sp55}). This allele has a very low level of expression, which is darker in males than in females. The molecular lesion is an insertion of an unspecified retrotransposon near the 5' start of transcription (Zachar & Bingham, 1982). Metafemales homozygous for w^{sp55} have a lighter eye colour than normal females (Table 1, Fig. 2).

(iv) *Non-compensating alleles*

white-eosin (w^e). This allele fails to exhibit dosage compensation, i.e. males are lighter than females. It is a reversion of the original *white* mutant (w^I), which is a *Doc* insertion in the 5' leader sequence of the messenger RNA. The *eosin* reversion is associated with a second insertion (pogo element) into the original one causing w^I (Hazelrigg, 1987; Driver *et al.* 1989; O'Hare *et al.* 1991). It has not been determined whether the functional message is promoted from *w* or the transposable element (Levis *et al.* 1984; Pirrotta & Brockl, 1984). In metafemales, it shows a dosage effect compared to normal w^e females (Table 1, Fig. 2).

white-ivory (w^i). This allele is a tandem duplication of sequences from the first intron and a portion of the second and third exons (Karess & Rubin, 1982; O'Hare *et al.* 1984). The allele fails to show dosage compensation: the males are lighter than females. In metafemales, this failure of dosage compensation is extended such that they exhibit more pigment than normal w^i females (Table 1, Fig. 2).

4. Discussion

For all of the eleven *white* alleles or constructs examined in metafemales, the complementary result was found relative to male expression. The two classical hypomorphic mutations, *white-apricot* and *blood*, both exhibit dosage compensation in metafemales. Both, of course, exhibit compensation between males and females.

Copies of *white* that are cloned and reintroduced in the genome via P-element-mediated transformation exhibit dosage compensation whether they insert into X or autosomal sites (Hazelrigg *et al.* 1984). This is the case for full length constructs as well as those reduced in quantitative expression because of truncated promoters. Within the context of the present study, only the constructs, in which the *white* gene product is limiting for pigment deposition, can be assayed phenotypically in metafemales. For both kinds of construct, metafemales have a reduction in eye pigment. Whether *white* is inserted in the X or the autosomes, it appears to respond to dosage compensation signals, in contrast to xanthine dehydrogenase (Spradling & Rubin, 1983) and alcohol dehydrogenase (Goldberg *et al.* 1983, Laurie-Ahlberg & Stam, 1987), which only respond on the X. Because

of this difference it could perhaps be argued that *white* is influenced by a sex-limited system that creates a sexual dimorphism independent of dosage compensation. The observation in the present study that these constructs are reduced in expression in metafemales suggests that the elevation in males is a reflection of the X chromosomal dosage. The dosage of the X chromosome produces an inverse effect on the expression of transduced *white* constructs.

In addition to truncated promoters of *white*, there are presently two constructs available that exchange the *white* promoter with one from an autosomal gene. The first is an *hsp70* promoter driving *white* structural sequences (Pirrotta *et al.* 1985). The eye colour of these flies is saturated due to the constitutive promotion of *hsp70*. The second construct has the promoter region of alcohol dehydrogenase replacing the *white* promoter. This strain was produced by J. Fischer and T. Maniatis. The pigment levels are low; but the males are darker than the females. Because of the reduced pigment levels, this construct was used in the phenotypic test in metafemales. As with the constructs described above, the pigment levels exhibit an inverse correlation with the dosage of the X chromosome.

The *white-spotted* allele is representative of *cis*-regulatory lesions in the presumptive enhancer of *white* (Davison *et al.* 1985), which is also the sequence that interacts with the regulatory gene, *zeste*. These alleles are characterized by reduced pigment that is uneven over the surface of the eye and that is darker in males than in females. The intensity difference extends to metafemales, which are lightest of the three genotypes. This suggests that this aspect of the spotted phenotype is conditioned by the dosage of the X chromosome.

The *white-spotted-55* allele is unique in that the females of this stock are nearly bleach white at eclosion but develop pigment as they age whereas males are much darker. This over-expression also appears to be influenced by the dosage of the X chromosome because it extends to metafemales, which are still basically bleach white even after ageing.

There are two additional classes of mutations at *white* that have altered dosage compensation. In these cases, the male expression is less than the female. The 'eosin' series are partial revertants of the original w^I mutation. A representative of this class, *white-eosin*, was examined in metafemales and was found to have a dosage effect, being darker than normal eosin females. One interpretation might be that this allele fails to respond to dosage compensation because it is promoted from the transposable element. Alternatively, it could be that sequences required for dosage compensation are disrupted (see discussion in O'Hare *et al.* 1991). Whichever is the case, it is a mutant allele with no response to dosage compensation and serves as a control on the ability to recognize a dosage effect in metafemales.

The last type of mutant examined was *white-ivory*. The DNA lesion is a tandem duplication spanning most of the first intron of *white* and the second and third exons. The promoter region is intact but phenotypically the allele does not exhibit dosage compensation, the male being lighter than the female. This dosage effect extends to metafemales.

Taken together, the observations of this study suggest that the various sexual dimorphisms exhibited by alleles of *white* are all reflections of a response to the dosage of the X chromosome, rather than to sex differences *per se*.

Metafemales rarely survive to pharate adult stage so it perhaps could be argued that the escapers of lethality represent rare cases in which compensation has occurred and that normally the X exhibits a dosage effect in metafemales. However, it is found that all of the lesions at *white* that alter dosage compensation in males do also in metafemales. This observation suggests that there is dosage compensation of the *white* gene in metafemales and that the mechanism is similar to that operative in males.

The X/A balance must condition gene expression such that the activity of each copy of X-linked genes is inversely proportional to the degree of chromosomal imbalance in order to explain the relevant facts of dosage compensation, i.e. equivalence of total expression in males, females and metafemales. This condition is illustrated directly in the case of the autosomal constructs that are maintained at constant dosage but inversely affected as the dosage of the X chromosome changes.

The present study puts the following constraints on models of dosage compensation. The data indicate that dosage compensation does indeed occur at the *white* locus in metafemales. The recognition of compensation, dosage effect and overcompensation in metafemales as well as males argues that the phenotypes are not obscured in metafemales by secondary consequences of aneuploid syndromes. Concepts of dosage compensation must account for several levels of X chromosomal expression as opposed to a binary switch. Lastly, the complementary response of *cis*-regulatory mutants of *white* in males and metafemales argues that the basic mechanism of compensation in the two types of flies is the same, although additional processes that discriminate between the X and the autosomes are not ruled out.

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