THE ROLE OF SEXUALITY IN DOSAGE COMPENSATION IN DROSOPHILA[1,2]

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PROBLEMS of genetic regulation have received increasing attention in recent years and controlling mechanisms are being elucidated with great rapidity. One such problem, that of dosage compensation, was originally defined in Drosophila, and, for nearly thirty years, its mechanism of action has remained open to question. The term "dosage compensation" indicates that, for sex-linked genes which differ in dose between males and females, there is a regulatory mechanism operating such that the resultant phenotypes of these *X*-linked genes are equivalent in the two sexes.

Two main theories have been proposed to account for dosage compensation in Drosophila. The first, suggested by STERN (1929, as quoted in STERN, 1960) but later elaborated and tested by MULLER (1932, 1950) postulates that the compensatory action is brought about by modifier genes which have evolved to equalize the phenotypes of the sexes so as to eliminate any differential selection which might endanger the continued perpetuation of the species. The second theory, espoused by GOLDSCHMIDT (1954), proposed that males and females were inherently different physiological systems and that dosage compensation was brought about by this difference which was established by the sex determining genes. GOLDSCHMIDT contended that one developmental mechanism based on differing physiologies could explain not only dosage compensation but also cases of autosomal dimorphism, whereby males and females exhibit different phenotypes for genes present in the same dose in the two sexes. Although GOLDSCHMIDT was unable to unequivocably substantiate his hypothesis, recent evidence (KOMMA, 1966) has suggested that differing sex physiologies may play a role in the regulation of the sex-linked enzyme, glucose-6-phosphate dehydrogenase (G-6-PD).

In order to examine more fully the possibility that sex physiology plays a role in dosage compensation and attempt to elucidate a unifying mechanism to explain not only *X* chromosome regulation in Drosophila but also cases of autosomal dimorphism, in which the male always seems to appear more wild-type than the female (LINDSLEY and GRELL, 1968), the following experimental scheme was employed. The third chromosome sex transformation gene, doublesex (*dsx*: HILDRETH and LUCCHESI 1963; HILDRETH 1965), which converts chromosomal

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male and female zygotes into $1X$ and $2X$ intersexual adults, respectively, was used to alter sex physiologies. This gene was introduced into various partially coisogenic eye pigment mutation strains, after which the red pigment content of males, females, and diploid intersexes was determined. Eye pigment mutations were selected as the appropriate parameter of inquiry for the following reasons: 1) pigment content can be accurately quantified; 2) the system allows for the establishment of a non-dosage compensated control; 3) sexually dimorphic autosomal mutations can be investigated; and, 4) eye pigmentation was one of the characteristics originally employed by MULLER in the definition of this phenomenon.

MATERIALS AND METHODS

Mutant strains and genetic procedures: The various eye pigment mutations employed in this study have been listed in Table 1 and categorized with respect to their locus, phenotype, and relation to dosage compensation. All mutants with the possible exception of glass^3 can be considered to be hypomorphic with respect to red pigment synthesis (hypomorphs are mutants whose effect is similar but less than that of the normal allele (MULLER 1950)). Detailed descriptions of the majority of the mutants can be found in LINDSLEY and GRELL (1968).

The red pigment content of each of the experimental strains was determined for the non-isogenic condition in order to establish baseline pigment measurements and to verify their reported classification with respect to their expression between the sexes. All experimental mutant strains were then made partially coisogenic by allowing the mutant-bearing chromosome to recombine freely with a homolog derived from an isogenic Samarkand wild-type line and by replacing the other two major chromosomes in the mutant strain with corresponding chromosomes from the same wild-type line. Pigment determinations were then performed on all mutants to provide measurements in the presence of a more uniform genetic background. Finally, the sex transformation gene "doublesex" (*dsx*, 3–48.1) was introduced into each of the partially coisogenic strains and its effect on eye pigment levels was determined. All experiments were conducted at 25 ± 1°C on a standard cornmeal, agar, molasses, and brewers' yeast medium containing Tegosept and propionic acid as mold inhibitors. Live yeast was used to seed the cultures.

TABLE 1

Eye mutant strains

Mutation	Locus	Phenotype	Dosage compensation
prune	1-0.8	brownish purple	dosage compensated
white-apricot	1-1.5	yellowish orange	dosage compensated
white-apricot^2	1-1.5	orange	dosage compensated
white-eosin	1-1.5	yellowish pink	non dosage compensated
glass (P)	3-63.1	eyes reduced from normal	autosomally dimorphic
glass (BG)		size, surface rough due	
glass^2		to fused and irregular	
glass^{63a14}		facets, color orangy	
glass^{63f6}			
glass^3	3-63.1	scarlet	not dimorphic
glass^{60j}	3-63.1	very light orange	not dimorphic
glass^{60j9}			

glass (P): Pasadena strain
glass (BG): Bowling Green strain

Pigment determinations: Red pigment determinations were performed according to the method of EPHRUSSI and HEROLD (1944). To obtain the flies used for all pigment assays, ten pairs of parents were placed in half pint bottles and the females were allowed to oviposit for 48 hrs. Parents were then removed and the offspring were allowed to develop for 13 to 15 days. After emergence, the flies were transferred to fresh medium and aged for 6 to 7 days to permit full pigment deposition. Following aging, the appropriate number of flies was decapitated and their heads were bisected and placed in acidified ethanol (pH 2). After 24 hrs, the pigment extract was centrifuged (15 min at 15,000 rpm, International micro-capillary centrifuge, model MB) and pigment intensity was measured spectrophotometrically (Beckman DB-G, 0.2 ml microcell, 480 mμ).

In order that all pigment determinations from each experimental genotype would be comparable, two corrective procedures were introduced. All spectrophotometer readings were adjusted to "optical density per 100 heads per ml of acidified ethanol" and average eye areas were determined by measuring the lengths and widths of the left eye of 50 males and females of the various genotypes and applying the formula for the area of an ellipsoid, length $\times$ width $\times \pi/4$. Each experimental determination could then be expressed as "amount of pigment per 100 heads per ml of acidified ethanol per unit eye area."

Statistical treatment: Ten pigment determinations were made for males and females in each group for every genotype under study. All measurements were adjusted according to the methods previously discussed and a male/female pigment ratio was computed for each pair. From the corrected pigment determinations, mean male and female pigment concentrations $\pm$ their standard deviations were computed for each genotype studied. In addition, a mean male/female pigment ratio was calculated. For *X*-linked genes, a ratio of 1 indicated a dosage compensated mutant while a ratio approximating 0.5 indicated a non-dosage compensated one. For autosomal mutants, a ratio of 1 is expected while ratios deviating significantly from 1 indicate sexual dimorphism. In order to determine if male and female measurements within each genotype were equal, *t* tests were performed on paired observations. Comparisons of females *versus* 2*X* intersexes and males *versus* 1*X* intersexes were made by calculating similar parameters to those indicated for males *versus* females.

RESULTS

X-linked mutants: Pigment determinations for the non-isogenic wild-type and *X*-linked mutant strains are recorded in Table 2. Recall that measurements on prune, white-apricot and white-apricot^2 represent measurements on dosage com-

TABLE 2

Pigment determinations: non-isogenic X-linked mutant strains*

Strain	Female	Male	Ratio male/female†	Significance at 95%‡
Wild type	1064.24 ± 46.05	1137.27 ± 56.02	1.07 ± .02	S
prune	183.72 ± 31.97	201.27 ± 24.41	1.11 ± .12	S
white-apricot	18.63 ± 3.11	19.13 ± 4.77	1.03 ± .21	NS
white-apricot^2	57.44 ± 6.76	60.46 ± 8.67	1.05 ± .10	NS
white-eosin	44.07 ± 2.34	19.04 ± 1.82	0.43 ± .03	S

* All pigment determinations are expressed as pigment/100 heads/ml acidified ethanol/unit eye area $\times 10^4$.

† All ratios are mean ratios calculated by averaging the ratios computed for each paired observation and are not ratios of the means given in the columns to the left. This method is advantageous because it allows for the calculation of a standard deviation for the ratio.

‡ Significant differences were determined by pairing individual determinations and employing the *t* test.

TABLE 3

Pigment determinations: partially coisogenic X-*linked strains*

Strain	Female	Male	Ratio male/female	Significance at 95 %
Wild type	988.08 ± 96.33	1090.91 ± 88.49	1.11 ± .10	S
prune	216.04 ± 14.49	245.55 ± 20.85	1.14 ± .06	S
white-apricot	11.03 ± 0.68	12.14 ± 0.33	1.10 ± .06	S
white-apricot^2	46.51 ± 3.98	53.51 ± 3.96	1.16 ± .13	S
white-eosin	20.72 ± 2.42	10.75 ± 1.90	0.52 ± .09	S

pensated strains while measurements on white-eosin, the non-dosage compensated strain, represent the control situation. Inspection of the male/female ratios indicates that wild-type and dosage compensated males have more pigment per unit area than females. *t*-tests performed on these data indicate that in two cases this difference is significant. In the remaining two cases, it can be seen that the results are consistent but not significant at the 95% level. Similar measurements on the partially coisogenic strains are shown in Table 3 and it is again apparent, by inspection of the male/female ratio, that wild-type and dosage compensated males exhibit more pigment per unit area than females.

Comparisons of pigment values of females *versus* 2*X* intersexes and males *versus* 1*X* intersexes are shown in Tables 4 and 5, respectively. Examination of these data for females reveals that the effect of intersexuality on dosage compensated mutants is opposite to that on the non-dosage compensated control. Intersexuality reduces the amount of pigment in prune and white-apricot^2, while it increases the pigment in white-eosin. Comparisons of pigment values for males

TABLE 4

Pigment determinations: X-*linked females* versus 2X *intersexes*

Strain*	Female	2*X* intersex	Ratio 2*X* intersex/female	Significance at 95%
prune	151.43 ± 8.49	141.68 ± 8.45	0.94 ± .06	S
white-apricot^2	42.12 ± 3.22	39.49 ± 2.02	0.94 ± .05	S
white-eosin	20.75 ± 1.61	22.48 ± 1.75	1.09 ± .11	S

* White-apricot comparisons could not be made due to the extreme inviability of 2X intersexes.

TABLE 5

Pigment determinations: X-*linked males* versus 1X *intersexes*

Strain	1*X* intersex	Male	Ratio 1*X* intersex/male	Significance at 95%
prune	152.08 ± 11.36	179.52 ± 7.96	0.85 ± .05	S
white-apricot	11.05 ± 0.59	10.26 ± 1.01	1.10 ± .11	NS
white-apricot^2	40.27 ± 3.53	44.53 ± 3.14	0.91 ± .10	S
white-eosin	7.71 ± 0.84	7.13 ± 0.81	1.09 ± .10	S

versus 1*X* intersexes show that, for the dosage compensated mutants prune and white-apricot^2, there is a reduction in pigment in the intersexes. For the dosage compensated mutant white-apricot and the non dosage compensated control white-eosin the reverse is true, intersexuality bringing about an increase in pigment content. *t*-tests demonstrate that all but one of these changes are significant at the 95% level. These changes in pigment content under the influence of intersexuality will be discussed in terms of presently available models of sex physiology which attempt to explain the regulatory phenomenon.

Autosomal mutants: An investigation of the effect of changing the sex physiology in which an autosomal, sexually dimorphic, pigment mutation acts was undertaken in conjunction with the study of the *X*-linked dosage and non dosage compensated pigment mutations. Pigment determinations for the non-isogenic strains are presented in Table 6. These data provide the basis for the classification of the glass alleles into three groups:

(i) Group I has as its single representative the glass^3 allele which differs from the other 7 alleles in two ways: a) it has a much higher pigment content such that its eye color appears nearly wild-type; and, b) the entire surface of the eye is pigmented. (ii) Group II is represented by the P, BG, 2, 63a14, and 63f6 alleles and is characterized by: a) a low level of pigmentation approximating 5% of wild type; b) a pigment distribution pattern in which the central portion of the eye is pigmented while the marginal area is not; and, c) a noticeably greater amount of pigment in the males leading to a sexual dimorphism for pigment content which is reflected in the male/female ratios (notice that the P and BG alleles appear to be more extremely dimorphic than the others). (iii) This group, represented by the 60j and 60j9 alleles, differs from Group II with respect to the third characteristic. For these two alleles there is no apparant sexual dimorphism (see male/female ratio).

After having been made partially coisogenic, these alleles were again assayed for pigment content. These data, presented in Table 7, exhibit certain differences from the non-isogenic data previously shown. The alleles may now be subdivided

TABLE 6

Pigment determinations: non-isogenic glass strains

Strain	Female	Male	Ratio male/female	Significance at 95 %
glass (P)	26.32 ± 5.15	75.05 ± 21.69	2.87 ± .02	S
glass (BG)	16.88 ± 3.17	57.58 ± 9.38	3.51 ± .78	S
glass^2	35.90 ± 3.18	73.75 ± 7.38	2.06 ± .20	S
glass ^{3}	815.05 ± 62.84	910.26 ± 79.77	1.12 ± .05	S
glass ^{60j}	7.56 ± 1.56	8.08 ± 1.78	1.12 ± .38	NS
glass^{60j9}	8.73 ± 1.73	8.65 ± 1.44	1.01 ± .16	NS
glass^{63a14}	24.68 ± 3.26	49.02 ± 7.21	2.00 ± .32	S
glass^{63f6}	25.57 ± 2.31	47.58 ± 3.97	1.87 ± .22	S

glass (P): Pasadena strain
glass (BG): Bowling Green strain

TABLE 7

Pigment determinations: partially coisogenic glass strains

Strain	Female	Male	Ratio male/female	Significance at 95 %
glass (P)	21.04 ± 2.04	62.85 ± 7.71	2.99 ± .33	S
glass (BG)	47.76 ± 6.87	156.65 ± 17.41	3.33 ± .55	S
glass^{2}	95.60 ± 14.85	173.14 ± 12.44	1.83 ± .22	S
glass^{3}	932.35 ± 66.97	977.76 ± 65.43	1.05 ± .06	S
glass^{60j}	18.28 ± 3.94	68.42 ± 17.55	3.80 ± .95	S
glass^{60j9}	16.86 ± 3.49	60.83 ± 11.57	3.63 ± .35	S
glass^{63a14}	138.15 ± 17.93	238.28 ± 26.25	1.74 ± .22	S
glass^{63f6}	127.21 ± 20.18	244.70 ± 32.60	1.94 ± .23	S

into two groups: (I) The glass^{3} allele exhibits a similar expression in males and females, as above, with pigment production increased almost to wild-type levels. (II) This group contains all the other alleles and exhibits both the non-random pigment distribution and the sexual dimorphism. The alleles may be classed into those showing mild dimorphism (2, 63a,14, 63f6) and those showing extreme dimorphism (P, BG, 60j, 60j9).

It should be noted that the 60j and 60j9 alleles, which earlier showed no dimorphism, now exhibit a striking one. This change could have one of the following explanations: 1) the non-isogenic strain contained a modifier gene (or genes) which reduced pigment production in males and which was subsequently eliminated when the strains were made partially coisogenic; or, 2) there existed a pigment threshold level such that mutants which contained extremely low levels of pigment were unable to express their intrinsic dimorphism. In this case isogenization would bring about increased biochemical efficiency, allowing for increased pigment synthesis and consequent expression of the dimorphism. By raising the 60j and 60j9 non-isogenic strains at 18°C to increase pigment production and subsequently examining these strains, evidence was obtained which was consistent with the latter hypothesis (see Table 10). The glass locus which was believed to contain two types of mutant pigment alleles, those which exhibit sexual dimorphism and those which behave as "normal" autosomal mutations, appears, in fact, to contain only one type of pigment mutation, namely, that which shows different expression between the sexes. The glass^{3} allele may be considered to be a mutation affecting only eye shape since pigment production is very nearly at wild-type levels in the coisogenic strain.

The comparison between glass females and $2X$ intersexes is shown in Table 8. The general reduction in pigment content from the coisogenic levels is due to the presence of a reversed metacentric compound X chromosome bearing the mutant alleles for yellow body color (y) and for forked bristles (f) both in the females and the $2X$ intersexes. These data demonstrate that pigment production is significantly increased in $2X$ intersexes such that they have approximately twice the pigment of females. It should be noted that the glass^{3} allele, which does not exhibit sexual dimorphism under normal circumstances, nonetheless exhibits a

TABLE 8

Pigment determinations: glass females versus 2X *intersexes**

Strain	Female	2X intersex	Ratio 2 X intersex/female	Significance at 95%
glass (P)	5.79 ± 1.27	18.41 ± 3.24	3.28 ± .77	S
glass (BG)	7.77 ± 1.81	15.81 ± 2.84	2.14 ± .70	S
glass^{2}	10.55 ± 1.82	22.74 ± 4.56	2.18 ± .40	S
glass^{3}	481.94 ± 47.69	742.45 ± 74.94	1.56 ± .26	S
glass^{60j}	6.42 ± 1.86	8.15 ± 0.92	1.41 ± .60	S
glsas^{60j9}	5.22 ± 1.25	7.36 ± 0.91	1.49 ± .43	S
glass^{63a14}	23.34 ± 3.53	38.40 ± 2.16	1.67 ± .21	S
glass^{63f6}	26.29 ± 5.10	49.15 ± 4.28	1.92 ± .32	S

* Both glass females and 2*X* intersexes carry reversed metacentric compound *X* chromosomes marked by yellow body *(y)* and forked bristles *(f)* to allow distinction of 2*X* intersexes from 1*X* intersexes. This accounts for the reduction in pigment content from isogenic females.

response to intersexuality identical to that of the sexually dimorphic alleles. *t*-test values for these comparisons indicate significant differences at the 95% level in every case.

Glass males and 1*X* intersexes are compared in Table 9. Of the 8 alleles, 4 respond to intersexuality by significantly increasing pigment production. The remaining 4 show a similar trend though the difference is not statistically significant. In general, there appears to be an increase in pigment content of intersexes over normal adults, as in the non dosage compensated controls. It should be observed that 2*X* intersexes exhibit a much greater increase over females than do 1*X* intersexes over males. This asymmetry of change due to intersexuality will be discussed below.

DISCUSSION

Two main theories have been proposed to explain dosage compensation in Drosophila. The first, developed mainly by MULLER (1950), postulated the existence of modifier genes on the *X* chromosome called dosage compensators, the

TABLE 9

Pigment determinations: glass males versus 1X *intersexes*

Strain	1X intersex	Male	Ratio 1X intersex/male	Significance at 95%
glass (P)	160.01 ± 29.30	62.85 ± 7.71	2.56 ± .44	S
glass (BG)	159.95 ± 23.30	156.65 ± 17.41	1.02 ± .10	NS
glass^{2}	202.45 ± 25.42	173.14 ± 12.44	1.17 ± .15	S
glass^{3}	1048.52 ± 87.09	977.76 ± 65.43	1.08 ± .12	NS
glass^{60j}	71.28 ± 18.66	68.64 ± 17.63	1.14 ± .48	NS
glass^{60j9}	62.39 ± 6.84	60.83 ± 11.57	1.06 ± .26	NS
glass^{63a14}	265.07 ± 14.91	238.28 ± 26.25	1.12 ± .13	S
glass^{63f6}	293.46 ± 26.63	244.70 ± 32.60	1.22 ± .22	S

TABLE 10

*Pigment determinations: temperature experiments**

Non-isogenic strain	25°C Female	25°C Male	25°C Ratio male/female	18°C Female	18°C Male	18°C Ratio male/female
glass (P)	.089	.333	3.74	.227	1.050	4.62
glass (BG)	.085	.253	2.98	.192	0.855	4.45
glass^{60j}	.033	.029	0.88	.053	0.092	1.74
glass^{60j9}	.038	.035	0.92	.079	0.112	1.42

* These spectrophotometer readings are uncorrected for eye size.

net effect of which was the inhibition of genetic activity in the female such that it was equivalent to that of the male.

MULLER observed that a white-apricot male, carrying a duplication for this mutant allele, was significantly darker than a white-apricot female despite the equivalence of dose. He interpreted this to mean that the *X* chromosome contains genes at other loci which can act to reduce the eye color of the normal two dose female to that of the one dose male. MULLER, of course, realized that the addition of an *X* chromosome duplication to a male genome might drastically influence its "sex physiology".

In order to eliminate the sex determinative process as a causative factor in the dosage regulation, MULLER employed a sex transformation mutant recently discovered by STURTEVANT (1945), transformer (*tra*), which converts chromosomal female zygotes into male-like adults ("pseudomales"). MULLER theorized that if sex physiology played a determinative role in compensation, white-apricot pseudomales should resemble the two dose duplication males which he had constructed. If, on the other hand, it did not, they should resemble regular white-apricot females. Finding the latter to be the case, MULLER ruled out sex as a controlling factor in the phenomenon. Since the dosage compensators were present in both the male and the female genome and in the same dose relations as the genes which they were to regulate, MULLER proposed a method of operation for the dosage compensators in which each of the two doses of modifiers in the female had twice the effect that a single dose had in the male.

GOLDSCHMIDT countered MULLER's experiments with transformer on the basis that pseudomales had an essentially female physiology and, from a developmental point of view, should be regarded not as "male-like" but as extreme intersexes. He noted that, when transformer was introduced into the non dosage compensated strain, white-eosin, the pseudomales were identical to the females and suggested this as evidence against MULLER's conclusions on sex. More recent studies on transformer by BROWN and KING (1961) have tended to substantiate GOLDSCHMIDT's contention. In particular, they observed that transformed females are intermediate in mass between males and females when young, resemble females rather than males in terms of wing size and contain cells that sometimes undergo processes akin to oogenesis. To demonstrate that the sexes are basically different developmental systems, GOLDSCHMIDT (1955) noted that developmental

rates in the sexes differ, that phases of growth are not synchronous, that times of determination of particular tissues vary between the sexes and that "the rhythm of differentiation of individual organs" differs. Moreover, GOLDSCHMIDT contended that frequently autosomal loci exhibit a sexual dimorphism for the expression of a character, which should not be expected if dosage of gene is the only factor to be accounted for. Thus, GOLDSCHMIDT believed that a physiological interpretation based on the fact that males and females differ developmentally is sufficient to account for both of these types of gene expression and that a complexly interacting modifier system like MULLER's need not be invoked to explain dosage compensation.

Detailed new evidence suggesting a physiological interpretation has been gathered by KOMMA (1966) through his studies with the sex transformation genes transformer and doublesex. KOMMA hypothesized that if sex physiology was the regulatory agent involved, then a change in physiology should bring about a change in gene action. By assuming that there is a gradient of physiological milieu from "femaleness" to "maleness" and that the relative "femaleness" or "maleness" of the milieu acts to decrease or increase, respectively, gene action, KOMMA predicted that $2X$ intersexes and pseudomales should have a higher gene activity than their normal sisters (since they would be more male-like) while $1X$ intersexes should have a lower activity than their normal brothers (since they would be more female-like). By measuring the activity of the X-linked enzyme, glucose-6-phosphate dehydrogenase, in normal and intersexual flies, KOMMA verified the former expectation for $2X$ intersexes and pseudomales but failed to substantiate the latter for $1X$ intersexes and concluded that sex physiology plays a role in the regulation of this enzyme.

If one examines the effects of doublesex on pigment content of the dosage compensated mutants, prune, white-apricot and white-apircot^2, in the present study, it is apparent that these data are not consistent with the model endorsed by KOMMA. Although for two of the three mutants (prune and white-apricot^2) $1X$ intersexes have significantly lower levels than their normal brothers, there is an insignificant increase in pigment content of white-apricot $1X$ intersexes over their normal brothers. In addition, dosage compensated $2X$ intersexes exhibit a significant decrease in pigment content over their normal sisters, a result in direct contrast to that predicted by GOLDSCHMIDT's model.

The effects of intersexuality on pigment levels in the X-linked mutants are, nevertheless, significant at the 95% level (with the exception of the white-apricot male *versus* $1X$ intersex comparison) and must be explained. A possible explanation may be found in the data for the non dosage compensated mutant, white-eosin. It is expected that this mutant should not respond to changes in the sex physiology and yet white-eosin intersexes also exhibit significant changes in pigment content over normal adults. It seems logical to conclude from this observation that significant changes in pigment content are being brought about by changes in physiology which are not strictly related to dosage compensation but which may be due to generalized effects on metabolism by the doublesex mutation. Evidence to substantiate this contention may be found in the study by

HILDRETH (1965) of various characteristics of the basitarsi of doublesex-produced intersexes. Effects of doublesex on the number of transverse rows of bristles, on the average number of bristles in longitudinal rows, and on the average length of the basitarsi themselves indicate that the intersexes respond in differing ways and suggest that closely related characteristics of the organism may show dissimilar responses to intersexuality. It must be concluded that the effects of *dsx* on eye pigment are not necessarily connected with sex physiology or the regulation of sex-linked dosage compensated genes.

Because GOLDSCHMIDT's theory postulated that a developmental mechanism could explain not only dosage compensation but also cases of autosomal dimorphism, concurrent studies with the third chromosome pigment mutation, glass (*gl*), were undertaken. Doublesex was introduced into eight partially coisogenic glass strains and female, male, and $1X$ and $2X$ intersex pigment levels were compared. With each of the eight alleles, pigment levels of both $2X$ and $1X$ intersexes were increased over females and males, respectively. It should be noted that the increase in $2X$ intersexes over females is much greater than in $1X$ intersexes over males. One might be led to conclude that this difference in effect is a reflection of a regulatory role of sex physiology on this locus. It could be conjectured, for example, that female pigment levels are depressed from male levels because of some effect of "female physiology" which is not present in $2X$ intersexes and that this is the explanation for the dimorphism. Yet it should be pointed out that: 1) the females and $2X$ intersexes carry a compound X chromosome, employed to distinguish $2X$ from $1X$ intersexes, which appears to decrease pigment synthesis such that the difference between the females and the $2X$ intersexes may be exaggerated by the presence of this chromosome; and, 2) the glass^3 allele, which never exhibits sexual dimorphism, nevertheless responds to intersexuality in a manner identical to the dimorphic alleles. Thus, until further investigation of this point, it must be concluded that the increase in pigment content in glass intersexes is unrelated to sex physiology, *sensu strictu,* and is again a result of generalized effects of the sex transformation mutant on metabolism.

If one assumes from the foregoing considerations that sex physiology, *per se,* plays no direct role in the regulation of the activity of X-linked hypomorphic eye pigment mutations and that KOMMA's results with glucose-6-phosphate dehydrogenase can be attributed to a generalized effect on the metabolism by the sex transformation mutants, how can one explain dosage compensation?

STERN (1960) predicted that if dosage compensating genes act in the kinetic fashion hypothesized by MULLER, then metafemales ($3X2A$), homozygous for the mutant white-apricot, should have an eye color lighter than the regular white-apricot mutant females. When STERN obtained such metafemales and found that they were not lighter but exhibited the same shade of pigmentation, he suggested a more complex functional relationship than that originally envisioned.

Two noteworthy cytological observations bearing on the problem of dosage compensation in Drosophila are those of DOBZHANSKY (1957) and MUKHERJEE (1966). By calculating the size of X chromosomes in salivary gland preparations, DOBZHANSKY demonstrated that the single male X chromosome is approximately

the same size as the two synapsed female *X* chromosomes, despite a DNA ratio of 1 to 2 (ARONSON, RUDKIN and SCHULTZ, 1954). This led him to conclude that this is a cytological expression of dosage compensation whereby the male *X* "works twice as hard" as a single female *X*. Similarly, MUKHERJEE, measuring RNA synthesis on salivary gland chromosomes by uptake of tritiated uridine, demonstrated that there were the same number of grains over the single *X* chromosome in the male as over the two synapsed *X* chromosomes in the female, evidence tending to confirm DOBZHANSKY's earlier conclusions. Although later experiments by MULLER and KAPLAN (1966) questioned DOBZHANSKY's conclusion that the single male *X* was equal in size to the synapsed female *X* chromosomes, their findings tended to confirm the belief that a single male *X* chromosome was, in fact, larger than a single female *X* chromosome. These observations, which suggest that dosage compensation is accomplished at the level of gene transcription by an enhancement of genetic activity in the male rather than a depression in the female, also suggest that MULLER's model is untenable.

The problem of dosage compensation suffers significantly from the fact that no molecular models have been proposed to explain the phenomenon. If one assumes that MUKHERJEE's experiments are a true reflection of the nature of messenger RNA synthesis, then one is led to the conclusion that dosage compensation occurs at the level of gene transcription. One hypothesis which could be proposed to account for this observation, and which is presently favored as a working hypothesis by the authors, is that regulation occurs by feedback control. One would predict that dosage compensated mutants are the result of lesions which affect only the catalytic ability of the enzyme product of the gene while non dosage compensated mutants represent impairments which occur in that portion of the gene involved in the controlling mechanism. Secondary effects of these latter lesions would result in reduced catalytic ability.

The observation of gene dosage effects, for example, MULLER's experiments with white-apricot duplications and deficiencies, seem to preclude mechanisms involving simple feedback. Yet it would seem premature to generalize from this observation how regulation at various steps in a complex biosynthetic pathway would be affected under normal diploid conditions. If one compares homozygous v^+ females to v^+/v females, a gene dosage effect is observed at the immediate enzyme level yet there is no dosage effect observable at the phenotypic level (KAUFMAN 1962). The true test of mechanisms of feedback control to explain dosage compensation will be forthcoming as more biochemically oriented experiments are performed.

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SUMMARY

The sex transformation gene doublesex, which converts chromosomal male and female zygotes into 1*X* and 2*X* diploid intersexual adults, was introduced into various *X*-linked and sexually dimorphic autosomal eye mutant strains under partially coisogenic conditions. Measurements of red pigment content in normal

and intersexual adults of these strains were performed in order to test the hypothesis that physiological differences between the sexes can account not only for dosage compensation of *X*-linked loci but also for dimorphic expression of autosomal mutations. The present studies do not support this physiological model and suggest that sex differences, *per se*, cannot explain regulation in these cases. A model based on feedback control to explain the regulation of *X*-linked genes is discussed.

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