

PARENTAL CONTROL OF POSITION-EFFECT VARIATION.
II. EFFECT OF SEX OF PARENT CONTRIBUTING WHITE-MOTTLED
REARRANGEMENT IN *DROSOPHILA MELANOGASTER*¹

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Received March 16, 1961

THE extensive phenotypic variation among individuals exhibiting position-effect variegation has long hampered investigations of the mechanism of position effect. The variegated phenotype itself is associated with loci near the breakage points of chromosomal rearrangements. The genes subject to position effect are not mutant in the strict sense, at least in the germ line, since they can be recovered with full activity by any recombination which restores them to a structurally normal chromosome region (JUDD 1955; DUBININ and SIDOROV 1935). Most rearrangements which cause variegation include breaks in both heterochromatic and euchromatic regions of chromosomes, although some position effects have been demonstrated for rearrangements involving only breaks in heterochromatin (BAKER 1953).

Among the factors which have been identified as affecting position-effect variegation, and which thus contribute to individual variation in phenotype, are temperature, other heterochromatin-euchromatin rearrangements, modifying genes, amount of heterochromatin, especially in the form of Y chromosomes (for detailed references, see LEWIS 1950) and in particular, various more restricted regions within the Y heterochromatin (BAKER and SPOFFORD 1959). In addition to these foregoing factors, which operate on the individual whose phenotype is examined, there are several factors whose action is initiated in the generation prior to the individual whose phenotype is affected. These factors give rise to "parental effects." Those already established, for certain genotypes incorporating a specific rearrangement of the white (*w*) locus of *Drosophila melanogaster*, are: homozygosity rather than heterozygosity of the mother for the rearrangement (SPOFFORD 1958; HESSLER 1961), the mother's Y chromosomal constitution (SPOFFORD 1959), and the sex of parent contributing the rearrangement to the offspring. This last has been shown only in crosses employing attached-X mothers (SPOFFORD 1959; HESSLER 1961). Inasmuch as it was the only parental effect not easily explicable by the accumulation of maternal gene products in the oöplasm during maturation of the egg, it seemed desirable to discover whether the crucial aspect of the reciprocal crosses in influencing phenotype of the offspring was the X chromosomal constitution of one or both parents or simply the fact of transmission via egg or via sperm.

Hence, the present set of experiments was undertaken to discover what interaction there might be between the parental-source effect and X chromosomal

¹ Work performed under contract No. AT (11-1)-431 for the Atomic Energy Commission.

constitution of parents and offspring. Both these experiments and concurrent experiments reported by HESSLER (1961) revealed differences in the behavior of the position effect between two of the stocks. With the first stock used, the variation was more extreme when the rearrangement was transmitted to an individual through the egg rather than through the sperm. On the other hand, the rearrangement from the other stock gave more extreme variation when it was transmitted through the sperm than through the egg. Both of these distinct parental-source effects are demonstrated in the present experiments in strictly comparable genotypes and regardless of the parents' X chromosomal constitution.

MATERIALS AND METHODS

The gene whose phenotypic variegation was studied was the w^+ allele included in a 20-band section, 3B3-4 to 3D5-6, from the X chromosome, inserted in inverted sequence into the proximal heterochromatin of the left arm of the third chromosome. The resulting deficient X chromosome has been described as $N^{264\cdot58}$ (SUTTON 1940; DEMEREC 1940). The duplication in the third chromosome, $Dp(w^m)264\cdot58a$, was carried in genomes with complete X chromosomes, usually with the recessive allele white (w) in a structurally normal euchromatin.

Three X chromosomes have been employed: a normal X with the recessive markers yellow (γ) and white, an attached-X (reversed metacentric) chromosome homozygous for γ and w , and a compound \widehat{XY} chromosome, $Y^s w \gamma \cdot Y^L \gamma^+$. Four stocks were rendered as coisogenic as possible by the Muller-5; Cy ; Ubx^{150}/Xa technique (BAKER and SPOFFORD 1959):

Stock 1, γw ; $+/+$, has structurally normal free X chromosomes and wild-type third chromosomes. After its initial derivation as an isogenic stock, it has been maintained in mass culture.

Stock 2, γw ; $Dp(w^m)$, has one or both third chromosomes carrying the duplication. To prevent the accumulation of modifiers systematically differentiating this stock from 1, it has been kept by pairing a variegated female with a male from stock 1 and, in the next generation, mass mating their variegated offspring. The females whose eyes are most heavily pigmented are then selected from the progeny of the mass mating for pairing again in repetition of the cycle. The stock was continued when possible from the single females all of whose progeny had eye pigment and who were therefore homozygous for the duplication third chromosome. As will be indicated later, free X flies heterozygous for the duplication often develop no pigment in their eyes. On the other hand, females homozygous for the duplication (comparable males are at least sterile and are probably inviable) and their heterozygous offspring almost invariably have some eye pigment (SPOFFORD 1958). After the initial coisogenization, two distinct $Dp(w^m)$ free X lines were maintained. After the eighth crossing back to stock 1 one of these lines lost its ability to produce fertile females homozygous for the duplication, while at the time of writing, the other line still produces homozygous fertile females.

In stock 3, $Y^s w \gamma \cdot Y^L \gamma^+ / Y / \underline{\gamma w}$; $+/+$, males have the compound \widehat{XY} in addition to the normal free Y and females have the attached-X plus a Y, whereas both

sexes carry the wild-type third chromosome. The relative coisogenicity of this stock has been maintained originally by frequent and now by routine crossing of $\gamma w/Y$; $+/+$ stock 1 males with $\gamma w/Y$; $+/+$ stock 3 females, followed by crosses of their $\gamma w/Y$; $+/+$ daughters to stock 3 $Y^s w \gamma Y^L \gamma^+/Y$; $+/+$ males.

Stock 4, $Y^s w \gamma \cdot \bar{Y}^L \gamma^+/Y/\gamma w$; $Dp(w^m)$, has one or both third chromosomes carrying the duplication. After derivation as a stock coisogenic with the others, it was maintained in mass culture without further intercrossing for a period of two years. The systematic differences in the behavior of the duplication carried in stocks 2 and 4 arose during this interval. (In all of the crosses employed, 50 percent of the offspring were expected to be heterozygous for the duplication. The white-eyed offspring in progenies with low percentages of pigmentation reported here were not tested further for presence or absence of the duplication. However, many previous tests of offspring exhibiting similar ratios have failed to disclose a significant deviation from a 1:1 genotypic ratio.) The duplication in the free X stock 2 will be designated Dp^f and that in the attached-X stock 4 Dp^a (HESSLER 1961). The Dp^f line employed here is the one with only eight passages through homozygous females; the experimental crosses were initiated, however, with the immediate offspring of the last homozygous Dp^f mother in the line.

Flies were reared at $23 \pm 1^\circ C$ on a culture medium similar to that described by CARPENTER (1950). Pair matings were made in vials containing aliquots of the same batch of medium and subsequently transferred to fresh vials of another single batch of medium; these produced the flies which were compared directly. All offspring were scored as to sex and presence or absence of any pigmentation. The amount of pigment when present was estimated for each eye. When there were many pigmented offspring in a sibship, the amount of pigment was estimated for a random sample of limited size. Scores on such estimates range from 0 for an eye with no trace of brown or red pigment to 1.0 for an eye apparently wild type in pigmentation. The values for the two eyes of a fly were summed to provide the "grade" for the fly. When pigment was present, it was a mixture of brown, or ommochrome, sepia, and red, or drosopeterin, in varying proportions. There is considerable subjective error possible in the scoring of eyes; however, the total numbers scored per category tended to reduce the error of the mean grade for each category and those categories compared directly were scored in the same time interval. A fair linear correlation has been established between this estimated grade and the amount of drosopeterin measured photofluorometrically after chromatography (BAKER and SPOFFORD 1959). The relationship between grade and ommochrome, or between ommochrome and drosopeterin, has yet to be established.

EXPERIMENTAL RESULTS

Parental-source effect in crosses between and within free X and compound X stocks

In designing crosses to test the possibility that the X chromosomal constitution of either or both parents was relevant to the parental-source effect on variegation, it was not anticipated that a significant difference had arisen between the two

Dp-bearing stocks 2 and 4. Consequently, not all the crosses were made which would completely distinguish between Dp^f and Dp^a . Furthermore, in view of the distinctness of Dp^f and Dp^a , some crosses were missing which would have been desirable to establish completely that the X chromosomal constitution of either parent or offspring is irrelevant to the parental-source effect. However, the crosses performed permitted enough comparisons to support an interpretation which was eventually substantiated by the series of crosses reported in the next section.

Five replicate pair matings of three-day-old flies were made and transferred four times, according to the following scheme:

- (1,2)—stock 1 $y w/y w$; $+/+$ ♀ × stock 2 $y w/Y$; $Dp^f/+$ ♂
 (2,1)—stock 2 $y w/y w$; $Dp^f/+$ ♀ × stock 1 $y w/Y$; $+/+$ ♂
 (3,4)—stock 3 $y w/Y$; $+/+$ ♀ × stock 4 $Y^s w \gamma \cdot Y^L \gamma^+/Y$; $Dp^a/+$ ♂
 (4,3)—stock 4 $y w/Y$; $Dp^a/+$ ♀ × stock 3 $Y^s w \gamma \cdot Y^L \gamma^+/Y$; $+/+$ ♂
 (3,2)—stock 3 $y w/Y$; $+/+$ ♀ × stock 2 $y w/Y$; $Dp^f/+$ ♂
 (4,1)—stock 4 $y w/Y$; $Dp^a/+$ ♀ × stock 1 $y w/Y$; $+/+$ ♂
 (1,4)—stock 1 $y w/y w$; $+/+$ ♀ × stock 4 $Y^s w \gamma \cdot Y^L \gamma^+/Y$; $Dp^a/+$ ♂
 (2,3)—stock 2 $y w/y w$; $Dp^f/+$ ♀ × stock 3 $Y^s w \gamma \cdot Y^L \gamma^+/Y$; $+/+$ ♂

It will be noted that the crosses group into pairs reciprocal for the Dp-bearing chromosome, if the distinction between Dp^a and Dp^f is ignored. Both early and late-eclosing offspring from this first round of crosses were mated individually to flies from stocks 1 and 3 as corroboration of first-generation results and to exclude the existence of other factors, not noted in the above genotypic descriptions of the crosses, which might differentiate the stocks and modify this variegation system.

The results of the primary interstock crosses are summarized in Table 1. In the genotypic description of the offspring, $Dp/+$ symbolizes maternal transmission of the duplication; $+/Dp$, paternal.

The first pair of crosses was essentially a repetition of a pilot experiment three months earlier, in which the cross of $+/+$ ♀ × $Dp^f/+$ ♂ yielded three pigmented sons out of 948 (0.32%) and three pigmented daughters out of 1008 (0.30%), in contrast to the cross $Dp^f/+$ ♀ × $+/+$ ♂, which yielded 55 pigmented sons out of 2915 (1.89%) and 18 pigmented daughters out of 3244 (0.55%). No statistical heterogeneity had been demonstrable between replicate sibships in that experiment, so that at least the difference in frequency of pigmentation in sons between the reciprocal crosses was statistically significant ($P < .001$). The same kind of difference between reciprocal crosses—more pigmentation when the duplication was inherited from the mother—persisted in crosses (1,2) and (2,1). For sons, the χ^2 of the difference is 15.4, $P = .0001$, although, as before, for daughters the significance of the difference is debatable.

The second pair of crosses was essentially a repetition of those published earlier, with which the present results are in agreement: either percent pigmented or amount of pigment was higher when the duplication was inherited from the father. However, the significance of the discrepant results of the last two pairs

TABLE 1
Results of reciprocal crosses between stocks with Dp-bearing and with normal third chromosomes, with free and with compound X chromosomes

Cross† fertil	No. of pairs	Daughters			Sons						
		Genotype	No.	Percent w ^m	No. graded	Average grade	Genotype	No.	Percent w ^m	No. graded	Average grade
(1,2)	4	$y w/\gamma w; +/Dp^{\dagger}$	380	0.5	2	.002±	$y w/Y; +/Dp^{\dagger}$	302	2.0	5	.002±
(2,1)	4	$y w/\gamma w; Dp^{\dagger}/+$	444	2.7	12	.011±.004	$y w/Y; Dp^{\dagger}/+$	370	9.2	34	.016±.004
(3,4)	4	$y w/Y; +/Dp^{\ddagger}$	129	51.2	65	.170±.039*	$\bar{X}Yw/Y; +/Dp^{\ddagger}$	96	62.5	56	.860±.205**
(4,3)	2‡	$y w/Y; Dp^{\ddagger}/+$	108	31.5	34	.146±.030	$\bar{X}Yw/Y; Dp^{\ddagger}/+$	76	46.0	33	.380±.073
(3,2)	8§	$y w/Y; +/Dp^{\dagger}$	105	57.1	60	.391±.072**	$y w/Y; +/Dp^{\dagger}$	109	6.4	7	.011±.004
(4,1)	2	$y w/Y; Dp^{\ddagger}/+$	234	26.1	54	.043±.007	$y w/Y; Dp^{\ddagger}/+$	225	0.4	1	.002±
(1,4)	5	$\bar{X}Yw/\gamma w; +/Dp^{\ddagger}$	638	48.6	216	.199±.033**	$y w/Y; +/Dp^{\ddagger}$	628	0		
(2,3)	3	$\bar{X}Yw/\gamma w; Dp^{\ddagger}/+$	259	49.4*	86	.980±.206*	$y w/Y; Dp^{\ddagger}/+$	224	8.5	19	.009±.003

* Indicates heterogeneity between pairs or between transfers significant at the 5% level; **, at the 1% level, in this and the subsequent tables.
 † See text for identification of crosses.
 ‡ Although four pairs were fertile, only two were $Dp^{\ddagger}/+$. The other two mothers proved to be $Dp^{\ddagger}/Dp^{\ddagger}$ and their progeny will not be discussed here.
 § Eight pairs of this cross were made, rather than five.

of crosses was not appreciated in time to reschedule the second generation crosses appropriately. In particular, although a single genotype of father, $\gamma w/Y; Dp^f/+$, had $\gamma w/Y; +/Dp$ sons more often pigmented when the mother had attached-X's (cross 3,2) than when she had free X's (cross 1,2), the $\gamma w/Y; Dp/+$ sons of attached-X mothers in cross (4,1) were very much less frequently pigmented than those of free X mothers in cross (2,1). Furthermore, the $\gamma w/Y; +/Dp$ sons of cross (1,4) were completely white-eyed, although in the companion cross (2,3) the proportion of pigmented $\gamma w/Y; Dp/+$ sons did not differ significantly from that in cross (2,1).

Of the various conceivable hypotheses which could be invoked to resolve these apparently conflicting results, the single feasible one finally suggested by the data from the testcrosses and by independent concurrent work by DR. ANITA HESSLER has already been published: namely, that the duplications in stocks 2 and 4 had differentiated systematically to the extent that more pigment in general is produced in the presence of Dp^f than of Dp^a , while Dp^f is most effective in pigment production when transmitted via the egg; Dp^a , via the sperm.

The progeny of the testcrosses are recorded in Tables 2, 3, 4 and 5. Although all types of crosses were made twice employing early-eclosing flies as parents for the one and late-eclosing flies as parents for the other group, statistical tests in no case indicated significant heterogeneity between these two groups so that the data have been pooled in the tables.

$\gamma w/Y$ sons (Table 2): Sons with structurally normal sex chromosomes were produced by a greater variety of crosses than any other class of offspring. These consequently provide the greatest number of comparisons of the phenotypic effects of diverse parental constitutions. The results were relatively unambiguous since in only one instance was heterogeneity demonstrable among the replicate sibships of a single type of cross. The phenotypes of these sons depended on the two conditions already noted, *viz.* the type of duplication and its parental source, and on a third condition: whether the mother had had free or attached-X chromosomes. Types of crosses which differed in other respects but not in these produced statistically indistinguishable $\gamma w/Y$ sons. Thus, (1) the father's X chromosome, whether normal or compound with the Y, had no effect on the phenotype of his sons by $\gamma w/\gamma w; Dp^f/+$ mothers. Also, (2) there was no "grandparental" source effect— $+/Dp^f$ sons of either X chromosomal class of mother are not separable statistically by whether their fathers had been $+/Dp^f$ or $Dp^f/+$, nor are the $Dp^f/+$ sons of $\gamma w/\gamma w$ mothers separable statistically by whether their mothers had been $+/Dp^f$ or $Dp^f/+$. Furthermore, (3) whether the Y chromosome came directly from stock 1 or stock 3 had no effect on the phenotype of $Dp^f/+$ sons of $\gamma w/\gamma w$ mothers and probably had no effect on the phenotype of $+/Dp^f$ sons of $\gamma w/\gamma w$ mothers. Lastly, (4) the results of the first generation crosses yielding $\gamma w/Y$ sons agree so well with the results of the comparable testcrosses that it seems highly probable that the γw -bearing normal X chromosome introduced from stock 2 or from stock 1 is not influenced by prior association with Dp^f in a parental or grandparental genome.

TABLE 2

γ w/Y sons produced by test crosses of mottled progeny of reciprocal crosses with stocks carrying Dp-bearing and normal third chromosomes, free and compound X chromosomes

Cross producing parent carrying Dp	No. tested	Maternal genotype	Paternal genotype	Dp in sons	No.	Percent w^m	No. graded	Average grade
(1,2)	6	$\gamma w/\gamma w; +/+$	$\gamma w/Y; +/Dp^f$	$+/Dp^f$	200	0
(2,1)	12	$\gamma w/\gamma w; +/+$	$\gamma w/Y; Dp^f/+$	$+/Dp^f$	306	2.9	9	.019±.008
(3,2)	2	$\gamma w/\gamma w; +/+$	$\gamma w/Y; +/Dp^f$	$+/Dp^f$	147	1.4	2	.006±...
(1,2)	6	$\gamma w/Y; +/+$	$\gamma w/Y; +/Dp^f$	$+/Dp^f$	101	12.9	10	.023±.009
(2,1)	5	$\gamma w/Y; +/+$	$\gamma w/Y; Dp^f/+$	$+/Dp^f$	139	11.5	15	.007±.002
(3,2)	9	$\gamma w/Y; +/+$	$\gamma w/Y; +/Dp^f$	$+/Dp^f$	197	9.6	17	.017±.005
(2,3)	12	$\gamma w/Y; +/+$	$\gamma w/Y; Dp^f/+$	$+/Dp^f$	261	11.1	27	.030±.008
(1,2)	4	$\gamma w/\gamma w; +/Dp^f$	$\gamma w/Y; +/+$	$Dp^f/+$	331	5.7	19	.012±.003
(2,1)	6	$\gamma w/\gamma w; Dp^f/+$	$\gamma w/Y; +/+$	$Dp^f/+$	425	6.8	29	.012±.002
(3,2)	5	$\gamma w/Y; +/Dp^f$	$\gamma w/Y; +/+$	$Dp^f/+$	242	38.8	84	.053±.009
(1,2)	8	$\gamma w/\gamma w; +/Dp^f$	$\widehat{X}Yw/Y; +/+$	$Dp^f/+$	465	8.6**	40	.034±.008
(2,1)	5	$\gamma w/\gamma w; Dp^f/+$	$\widehat{X}Yw/Y; +/+$	$Dp^f/+$	401	5.5	22	.015±.006
(4,1)	2	$\gamma w/\gamma w; +/+$	$\gamma w/Y; Dp^a/+$	$+/Dp^a$	106	0.9	1	.010±...
(4,1)	4	$\gamma w/Y; +/+$	$\gamma w/Y; Dp^a/+$	$+/Dp^a$	67	1.5	1	.002±...
(1,4)	7	$\gamma w/Y; +/+$	$\gamma w/Y; +/Dp^a$	$+/Dp^a$	114	0.9	1	.010±...
(4,1)	4	$\gamma w/Y; Dp^a/+$	$\gamma w/Y; +/+$	$Dp^a/+$	137	0.7	1	.010±...

The average values of the two indices of variegation in $\gamma w/Y$ sons for the net seven effectively different kinds of crosses are:

$Dp^f/+$ from $\gamma w/Y$ mothers: 38.8% pigmented, average grade .053±.004

$+/Dp^f$ from $\gamma w/Y$ mothers: 11.0% pigmented, average grade .021±.004

$Dp^f/+$ from $\gamma w/\gamma w$ mothers: 6.8% pigmented, average grade .020±.003

$+/Dp^f$ from $\gamma w/\gamma w$ mothers: 1.7% pigmented, average grade .017±.007

$Dp^a/+$ from $\gamma w/Y$ mothers: 0.7% pigmented, average grade .010±.....

$+/Dp^a$ from $\gamma w/Y$ mothers: 1.1% pigmented, average grade .006±.....

$+/Dp^a$ from $\gamma w/\gamma w$ mothers: 0.9% pigmented, average grade .010±.....

The last three categories include small numbers of offspring; they are in line with expectation, however.

$Y^{s_w}y.Y^L y^+/Y$ sons (Table 3): Again no "grandparental effect" introduced heterogeneity into the only three categories of these sons obtained. It was obviously impossible to test the effects of maternal sex chromosome constitution. $Dp^f/+$, from one cross only, was fully penetrant and had the highest average grade, 1.872±.018. No mating yielded $+/Dp^f$ sons, which would be expected to rank next in pigmentation. In crosses giving $+/Dp^a$ sons, 55.4 percent of the sons

TABLE 3

Y^Sw y-Y^Ly⁺/Y [$\widehat{X}Yw/Y$] sons produced by test crosses of mottled progeny of reciprocal crosses with stocks carrying Dp-bearing and normal third chromosomes, free and compound X chromosomes

Cross producing parent carrying Dp	No. tested	Maternal genotype	Paternal genotype	Dp in sons	No.	Percent w^m	No. graded	Average grade
(3,2)	7	$y w/Y; +/Dp^f$	$\widehat{X}Yw/Y; +/+$	$Dp^f/+$	285	54.0	154	$1.872 \pm .018$
(3,4)	15	$y w/Y; +/+$	$\widehat{X}Yw/Y; +/Dp^a$	$+/Dp^a$	235	55.3	130	$.822 \pm .045$
(4,3)	7	$y w/Y; +/+$	$\widehat{X}Yw/Y; Dp^a/+$	$+/Dp^a$	142	55.6	78	$.726 \pm .157^{**}$
(3,4)	18	$y w/Y; +/Dp^a$	$\widehat{X}Yw/Y; +/+$	$Dp^a/+$	654	46.8**	291	$.461 \pm .160^{**}$
(4,3)	16	$y w/Y; Dp^a/+$	$\widehat{X}Yw/Y; +/+$	$Dp^a/+$	605	44.6	267	$.511 \pm .155^{**}$
(4,1)	9	$y w/Y; Dp^a/+$	$\widehat{X}Yw/Y; +/+$	$Dp^a/+$	460	50.0	224	$.578 \pm .089^{**}$

were pigmented with an average grade of $.786 \pm .098$. In crosses giving $Dp^a/+$ sons, less than half of the total, or 46.9 percent, were pigmented with an average grade of $.512 \pm .112$, the lowest. The differences between these average grades are all highly significant.

y w/y w daughters (Table 4): Data taken on this genotype were merely corroborative of the foregoing interpretation because of the rarity of pigmentation regardless of mating plan. No differences appeared among the crosses yielding $+/Dp^f$ females in which four (0.6%) of the 711 daughters were pigmented, with an average grade of .018. Nor did any appear among the crosses yielding $Dp^f/+$ females, in which 20 (2.4%) of the 822 daughters were pigmented with an average grade of .014. The difference in frequency of pigmentation between those with a maternally derived Dp^f and those with a paternally derived Dp^f was, however, significant at the one percent level, with a χ^2 value of 7.48. In fact,

TABLE 4

y w/y w daughters produced by test crosses of mottled progeny of reciprocal crosses with stocks carrying Dp-bearing and normal third chromosomes, free and compound X chromosomes

Cross producing parent carrying Dp	No. tested	Maternal genotype	Paternal genotype	Dp in daughters	No.	Percent w^m	No. graded	Average grade
(1,2)	6	$y w/y w; +/+$	$y w/Y; +/Dp^f$	$+/Dp^f$	169	0.6	1	$.010 \pm \dots$
(2,1)	12	$y w/y w; +/+$	$y w/Y; Dp^f/+$	$+/Dp^f$	361	0.8	3	$.011 \pm \dots$
(3,2)	2	$y w/y w; +/+$	$y w/Y; +/Dp^f$	$+/Dp^f$	150	0.7	1	$.030 \pm \dots$
(1,2)	4	$y w/y w; +/Dp^f$	$y w/Y; +/+$	$Dp^f/+$	312	2.6	9	$.020 \pm .006$
(2,1)	6	$y w/y w; Dp^f/+$	$y w/Y; +/+$	$Dp^f/+$	510	2.4	12	$.010 \pm .003$
(4,1)	2	$y w/y w; +/+$	$y w/Y; Dp^a/+$	$+/Dp^a$	138	1.4	2	$.036 \pm \dots$

pooling these with the comparable females of the preceding generation, the chance of a random difference between $Dp^f/+$ and $+/Dp^f$ became less than one per 2000, with a χ^2 in excess of 12.

y w/Y daughters (Table 5): The four major categories could again be distinguished. Minor yet significant differences existed within several of the categories which were not, however, attributable to such conceivable factors as father's sex chromosome constitution and probably not to a "grandparental effect". Two categories of $Dp^f/+$ daughters, identical except for the father's genotype—free X from stock (1) or compound $\widehat{X}Y$ from stock (3)—were indistinguishable as to daughter phenotype; likewise, two categories of $Dp^a/+$ daughters differed as to father's X chromosome but not as to phenotype. As to a residual "grandparental effect", the four categories of $+/Dp^f$ daughters indeed show significantly more pigment when the duplication is inherited from the paternal grandfather than from the paternal grandmother; however, any such grandparental effect is restricted (a) to *y w/Y* daughters, since their *y w/Y* brothers did not exhibit a similar effect, and (b) to Dp^f since the picture with $+/Dp^a$ daughters is not consistent and there is no indication of such an effect with $Dp^a/+$ daughters.

In spite of this heterogeneity within the categories, the differences between them are striking and statistically significant. $Dp^f/+$ daughters were always pigmented, with an average grade of $1.400 \pm .143$. Pigmented flies constituted 61.6

TABLE 5

y w/Y daughters produced by test crosses of mottled progeny of reciprocal crosses with stocks carrying Dp-bearing and normal third chromosomes, free and compound X chromosomes

Cross producing parent carrying Dp	No. tested	Maternal genotype	Paternal genotype	Dp in daughters	No.	Percent w^m	No. graded	Average grade
(1,2)	6	<i>y w/Y; +/+</i>	<i>y w/Y; +/Dp^f</i>	$+/Dp^f$	83	47.0	38	.679±.078
(2,1)	5	<i>y w/Y; +/+</i>	<i>y w/Y; Dp^f/+</i>	$+/Dp^f$	108	63.0	68	.490±.119**
(3,2)	9	<i>y w/Y; +/+</i>	<i>y w/Y; +/Dp^f</i>	$+/Dp^f$	186	69.9	123	.677±.071**
(2,3)	12	<i>y w/Y; +/+</i>	<i>y w/Y; Dp^f/+</i>	$+/Dp^f$	194	59.3	97	.399±.033
(3,2)	5	<i>y w/Y; +/Dp^f</i>	<i>y w/Y; +/+</i>	$Dp^f/+$	203	54.2	100	1.293±.057
(3,2)	7	<i>y w/Y; +/Dp^f</i>	$\widehat{X}Yw/Y; +/+$	$Dp^f/+$	318	58.2	178	1.460±.199*
(4,1)	4	<i>y w/Y; +/+</i>	<i>y w/Y; Dp^a/+</i>	$+/Dp^a$	81	48.2	39	.223±.039*
(1,4)	7	<i>y w/Y; +/+</i>	<i>y w/Y; +/Dp^a</i>	$+/Dp^a$	107	65.4	70	.172±.021
(3,4)	15	<i>y w/Y; +/+</i>	$\widehat{X}Yw/Y; +/Dp^a$	$+/Dp^a$	221	48.0	102	.242±.032
(4,3)	7	<i>y w/Y; +/+</i>	$\widehat{X}Yw/Y; Dp^a/+$	$+/Dp^a$	108	53.7	55	.121±.016
(4,1)	4	<i>y w/Y; Dp^a/+</i>	<i>y w/Y; +/+</i>	$Dp^a/+$	144	35.4	51	.171±.062*
(4,1)	9	<i>y w/Y; Dp^a/+</i>	$\widehat{X}Yw/Y; +/+$	$Dp^a/+$	432	38.0	164	.184±.063**
(3,4)	18	<i>y w/Y; +/Dp^a</i>	$\widehat{X}Yw/Y; +/+$	$Dp^a/+$	569	35.3**	199	.129±.056*
(4,3)	16	<i>y w/Y; Dp^a/+</i>	$\widehat{X}Yw/Y; +/+$	$Dp^a/+$	603	27.4	164	.157±.069**

percent of the females, on the average, in the heterogeneous crosses yielding $+/Dp^f$ daughters, with an average grade of .556. Pigmentation was also fully penetrant in $+/Dp^a$ daughters, with an average grade of $.196 \pm .015$. In crosses producing $Dp^a/+$ daughters, only 33.2 percent of the females had any pigment, with an average grade of $.156 \pm .030$.

In summary, every class of testcross offspring supports one or more of the following generalizations: (1) The duplication-bearing third chromosome of the free X stock (Dp^f) leads to a far greater degree of eye pigment production than that of the compound X stock (Dp^a). (2) Both Dp^f and Dp^a exhibit parental-source effects, but of opposite direction. Whereas Dp^a , as earlier shown, is more effective in pigment production in a heterozygous fly when inherited from a heterozygous father than when inherited from a heterozygous mother, Dp^f is the more effective when inherited from the mother. (3) The X chromosomal constitution of the father is immaterial to the expression of w^m in a fly whose X chromosome(s) come(s) from the mother only. (4) The parental-source effect endures for only one generation; there is probably no "grandparental effect". And lastly, (5) the free X sons of attached-X mothers develop pigment more often and in greater amount than do the free X sons of free X mothers. This may well be completely attributable to the residual maternal effect of the attached-X mother's Y chromosome, although no direct evidence is provided here to exclude other hypotheses, such as parental-source effect on w^m modifiers which quite possibly exist in the free X chromosome, or a residual maternal effect of w^m modifiers which might well differentiate the free and attached-X chromosomes—there may, for instance, be more heterochromatin in the attached-X than in the two free X chromosomes. However, the first of these seems the most likely.

Confirmation of Dp^f versus Dp^a distinction in compound X genotypes

If the foregoing distinction between the two forms of duplication-bearing chromosomes is assignable to the duplicated region itself or to the adjacent heterochromatic region of the third chromosome, the differentiating properties of Dp^f and Dp^a should persist after passage through many generations in a common genotypic background. The compound X rather than the free X background was chosen, largely because of anticipated difficulties in maintaining a $\gamma w/\gamma w$; Dp^a stock in the face of the exceedingly low penetrance of Dp^a in such genotypes. Consequently, a compound X stock was begun from the $Y^s w \gamma \cdot Y^L \gamma^+/Y$; $Dp^f/+$ and $\gamma w/Y$; $Dp^f/+$ flies which had been produced from the just-described testcrosses. In the seven months of its maintenance as a mass-cultured stock (hereafter referred to as Dp^f-1) no signs of homozygosity for the Dp chromosome were observed in either males or females, paralleling the experience with the parent line of the free X duplication stock.

At the end of the seven month period, eight w^m males taken from each of the three stocks— Dp^f-1 , stock 2 with Dp^f , and stock 4 with Dp^a —and 15 stock 3 $+/+$ males were each paired individually with stock 3 $\gamma w/Y$; $+/+$ females. Virgin w^m daughters of Dp fathers (and virgin daughters of $+/+$ fathers) were paired

individually with the sons of the $+/+$ fathers, and transferred twice to vials containing fresh medium. These latter matings served as the source of the parents for the experimental crosses. Thus the different duplication-bearing parents should differ minimally in respect to their compound X, Y, second and fourth chromosomes. Any euchromatic loci on the third chromosome which might be important modifiers of the variegation and which might have differentiated the Dp^1 from Dp^a chromosomes were thus given an opportunity to recombine and produce a discernible heterogeneity among individual experimental matings of ostensibly a single type.

The experimental pair matings, between four-to-five-day-old flies, were made on two occasions, the first using offspring collected from the first transfer, the other, from the second transfer, of the source matings just described. The number of pairs in each type of test cross made on the two occasions are indicated in parentheses:

- (10 + 12) $Dp^1-1/+$ ♀ × $+/+$ ♂
- (10 + 6) $Dp^1/+$ ♀ × $+/+$ ♂
- (10 + 12) $+/+$ ♀ × $Dp^1-1/+$ ♂
- (10 + 8) $+/+$ ♀ × $Dp^1/+$ ♂
- (20 + 22) white-eyed ($Dp^a/+$ or $+/+$) ♀ × $+/+$ ♂
- (10 + 13) $+/+$ ♀ × $Dp^a/+$ ♂

At the time the second group was mated, the first group of matings was transferred to fresh vials of the same batch used for the second group of matings. Parents were discarded a week later.

Flies eclosing from both original and transfer vials of the first group were counted; since χ^2 tests indicated no significant difference between transfers in ratios of mottled to white offspring, the counts for both vials representing a pair of parents were combined. However, the amount of pigment per eye was estimated only for those offspring eclosing from the one batch of vials used for both groups of matings, to minimize environmental differences. Neither χ^2 tests of ratios nor analyses of variance of phenotypic grades indicated differences between experimental pair matings attributable to date of mating. Hence, the results for the two groups have been pooled in Table 6.

Many of the pair matings were sterile, particularly those with Dp^1 from stock 2.

Of the 42 white-eyed daughters of $+/Dp^a$ mothers, 22 proved to be $Dp^a/+$, 12 $+/+$, and eight were sterile.

The variation in phenotype from fly to fly within a sibship, even when highly inbred, has always characterized position-effect variegation. The replicate sibships from the same class of mating for the most part varied no more in average grade than random samples of the same sizes drawn from a single population. Histograms of the relative frequencies with which sibship means fall in the ranges 0–0.09, 0.10–0.29, 0.30–0.49, . . . , 1.90–2.0 are given in Figure 1 for both sexes from each of the four major types of matings. Sibships with Dp^1-1 or Dp^a fathers, however, did differ very significantly among themselves. Recombination of any modifying loci in the third chromosome euchromatin in the heterozygous grand-

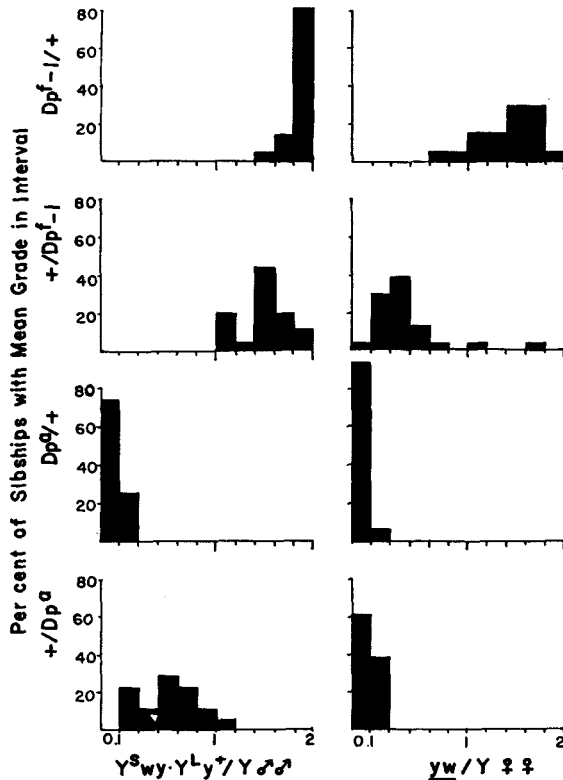


FIGURE 1.—Histograms of frequency with which sibships of the indicated genotype from the indicated cross have average grades falling in the intervals 0–0.09, 0.10–0.29, 0.30–0.49, . . . , 1.90–2.00. A chromosome symbol to the left of the slash indicates inheritance from the mother; to the right, from the father. Dp^f-1 = duplication, initially from the free X stock, carried in a compound X stock for seven months prior to assay. Dp^a = duplication carried for more than two years in compound X stock.

mothers could produce such between-sibship heterogeneity and would be masked in the progeny of heterozygous Dp^f-1 or Dp^a mothers by the larger within-sibship variability which would result from further recombination. However, the within-sibship variances were if anything smaller when the mother rather than the father was $Dp/+$, even after allowing for the tendency for variances to be smaller when means approach either extreme (0 or 2.0). Therefore, the + and Dp third chromosomes were effectively the same at potentially modifying euchromatic loci.

In spite of the observed sibship heterogeneity, the distributions of sibship means were clearly different for the different types of matings. The distinction between Dp^f and Dp^a is not lost when the two are put into the same genetic background and is therefore assignable to the duplicated region itself. The comparison between Dp^f-1 and Dp^a is shown graphically in Figure 2.

The remote possibility existed that Dp^f had arisen from Dp^a by unequal cross-

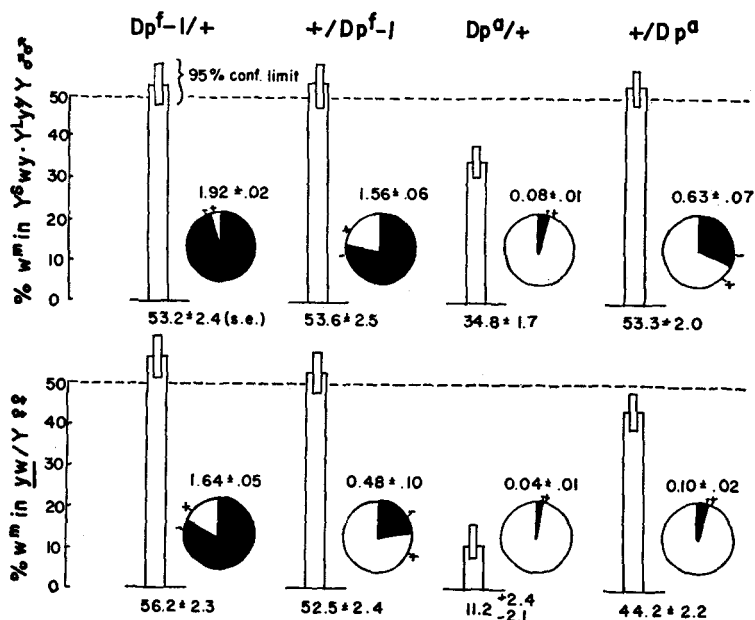


FIGURE 2.—Comparison of the four genotype-cross combinations in both sexes of offspring. Vertical bars signify the proportion of pigmented individuals in the daughters and sons of crosses of $Dp^f/+ \times +/+$, 95 percent confidence limits indicated by the inserted small bars. Pie diagrams signify the average amount of pigment in the category, with + and - signs indicating the 95 percent confidence limits.

ing over following imprecise pairing of the duplicated region in an originally $y w/y w$; Dp^a/Dp^a female in the free X line. DR. EILEEN SUTTON GERSH very kindly examined the salivary chromosomes of both the Dp^f and the Dp^a stocks. No differences could be found anywhere in the entire chromosome region. If any excess or deficiency of chromosomal material is involved in the difference between the two forms of the duplication, either it is restricted to adjacent heterochromatin, within which crossover recombination is virtually nonexistent and which is exceedingly difficult to study in salivary preparations, or else it amounts to considerably less than one band. Such a microdeficiency has been established by convincing noncytological tests in the case of certain "recombinational whites" arising during the elegant study of pseudoallelism at the white locus by GREEN (1959).

Six individual matings gave anomalous results and have been excluded from Table 6. Of these, one (with a Dp^f-1 mother) had sons with an average grade of 1.51 and daughters of 0.78, thus falling into the range characteristic of $+/Dp^f-1$ sibships. As a tentative explanation, it is conceivable that the greater pigment-promoting tendency of Dp^f is separable either recombinationally or mutationally from its parental-source effect. Two exceptional matings (with Dp^f mothers) were alike, having only three pigmented sons in 36, averaging .002 in grade, and three pigmented daughters in 28, averaging .015 in grade. Two with Dp^f-1 fathers and

TABLE 6
 Comparison of Dp^a with Dp^l after ten generations in compound X stock.
 $Y_{sw} y.Y_{Ly}^+ / Y$ males and $y w / Y$ females

Sex of $Dp/+$ parent	Kind of Dp	No. of fertile pairs	Daughters			Sons				
			No.	Percent w/m	No. graded	Average grade (of sib means)	No.	Percent w/m	No. graded	Average grade (of sib means)
♀	Dp^{l-1}	13	470	56.2	122	1.635 ± .048	417	53.2	108	1.922 ± .025
♀	Dp^l	9	224	46.9	60	1.295 ± .112	279	50.5	76	1.926 ± .022
	Total	22	(694)	(53.2)*	(182)	(1.464 ...)**	696	52.2	184	1.923 ± .018
♂	Dp^{l-1}	16	415	52.5	112	.477 ± .095**	403	53.6	127	1.565 ± .063**
♂	Dp^l	9	78	48.7	29	.360 ± .135	103	43.7	37	1.687 ± .074
	Total	25	493	51.9	141	.442 ± .077	506	51.6	164	1.609 ± .049
♀	Dp^a	22	731	11.2**	73	.042 ± .011	825	34.8**	250	.079 ± .014**
♂	Dp^a	18	529	44.2	160	.096 ± .020**	612	53.3	193	.631 ± .072**

Comparison of $Dp/+$ with $+/Dp$ offspring:

Kind of Dp	Daughters		Sons	
	χ^2 of ratio	F of graded	χ^2 of ratio	F of graded
Dp^{l-1}	.98	9.13†	.0008	22†
Dp^l	.025	28.8†	1.18	10†
Dp^a	176.†	5.13† (66.1† after arcsin transformation)	48.1†	71.† (19.† after arcsin transformation)

† Indicates the difference between $Dp/+$ and $+/Dp$ to be significant at the 5% level; ‡, at the 1% level.

one with a Dp^f father were alike, netting 22 pigmented sons in 48, averaging .436 in grade, and 33 pigmented daughters in 77, averaging .081 in grade. The progeny of these five matings suggests a change from Dp^f to a "state" resembling Dp^a both in over-all pigment production and in direction of the parental-source effect.

Furthermore, in matings employing $Dp/+$ mothers, possible alterations from Dp^f to Dp^a or the reverse can be detected in individual daughters, since the phenotypes of $Dp^f/+$ and $Dp^a/+$ females are so discrete in their statistical distributions. Sons of these same matings, or offspring of $Dp/+$ fathers, cannot be so unequivocally classed as Dp^f or Dp^a on the basis of their own individual phenotypes, and thus were not included in the search for evidence of change of state of the duplication in the progeny of the presently described crosses. Thus from the given evidence no conclusion about the conditions of such a change can be drawn. Eleven of the 122 daughters of Dp^f-1 mothers and two of the 60 daughters of Dp^f mothers had much less pigment than the remainder, averaging 0.155. These are, however, included in the data of Table 6, since no means of identifying their opposite numbers from the reciprocal crosses would permit a similar exclusion. Two of the 75 daughters of Dp^a mothers averaged 1.425, and were obviously excluded from Table 6. The numbers involved are too small to warrant estimates of the rates of change. Only a few of these "mutant" daughters were progeny-tested. The data on these indicated a change both in over-all pigment production and direction of parental-source effect. Obviously the phenomenon of change of state of the duplication merits much further study.

DISCUSSION

It is, first of all, worthy of note that the foregoing results include no exceptions to the rule that the more whole Y chromosomes in the genome, the more suppressed the variegation, or, in other words, the more normal the phenotype attributable to the euchromatic loci near broken heterochromatin. This is more obvious in the case of comparisons within sex—of free X with compound \overline{XY} males when both have whole free Y chromosomes, and of free X with Y-bearing attached-X females. Other conditions, such as greater heterochromatin in the attached-X than in two free X's, or even sex-linked modifying loci with incomplete dosage compensation, may also play a role in the over-all ranking of pigment-forming capacity in the increasing order $\gamma w/\gamma w < \gamma w/Y < \gamma w/Y < Y^s w \gamma \cdot Y^L \gamma^+ / Y$. The simplicity of this ranking is, however, deceptive, since earlier work has shown that neither of the compound X genotypes forms as much pigment as the $\gamma w/\gamma w$ unless some part of an additional Y chromosome is present.

HESSLER (1961) obtained an excess of pigmented offspring from heterozygous Dp^f mothers. A similar excess was found here in the first series of crosses with heterozygous Dp^f fathers but was not repeated in the last series of experiments. Other unpublished data further indicate that such excesses, though statistically significant in magnitude, are sporadic; the circumstances under which they may be repeatedly elicited have yet to be defined. Consequently, it is fruitless to specu-

late at this date on whether Dp-bearing flies are occasionally more viable than their non- w^m sibs on occasion, or whether more novel mechanisms, such as meiotic drive or segregation distortion, are involved.

Certainly there is a notable over-all difference in penetrance and expression of the w^m locus in experiments performed at widely different times. A subjective change of standards of grading in the interim would account for apparent differences in expression but not for differences in penetrance. The origin of the differences is probably environmental, although most identifiable environmental factors have been rigidly controlled. A systematic difference in stock genomes, which could arise and spread throughout the stocks in the interval between experiments, cannot be ruled out.

The hypothesis advanced by NOUJDIN (1944) to account for the various maternal or parental effects which he reported for scute variegation was that structural heterozygosity promotes a transiently inherited "heterochromatization" of the regions bearing the variegating + alleles, fathers differing usually from mothers in the degree of structural heterozygosity of heterochromatic regions. Such "heterochromatization" would not suffice to account for the difference in parental-source effect between Dp^f and Dp^a unless additional *ad hoc* hypotheses are framed, such as that there are undetectable structural differences in the 3L proximal heterochromatin adjacent to the euchromatic duplication.

Many features of this variegation system require a more systematic exploration before a mechanism accounting for the already established attributes of Dp^f and Dp^a can be sensibly postulated. For instance, it has yet to be determined whether flies bearing Dp^a transmitted through sperm, but of Dp^a -bearing mothers, are as intensely pigmented as $+/Dp^a$ offspring of $+/+$ mothers. This result would invalidate the earlier suggestion that Dp^a in females functions so imperfectly as to lead to the accumulation in eggs of materials inhibitory to the pigment-forming action of the w^m locus. Whether either or both of the distinctions between Dp^f and Dp^a will be found to hold for the other loci included in the duplications will determine whether the eventual mechanism will be couched in terms of the immediate relationships of neighboring parts of the chromosome or rather in terms of a specific action on the physiological system affected by the white locus. Furthermore, it is unlikely that this is the only variegation system in which parental effects of the sort described here can be found. This may indeed be a quite common attribute of position-effect variegation systems, reflecting aberrations in the basic normal premeiotic functions of heterochromatin in the over-all process of differentiation during ontogeny.

SUMMARY

Two states of activity of the white locus in the rearrangement $Dp(w^m)264\cdot58a$ have been identified and tested on a common genetic background: in Dp^f the white locus is much less suppressed than in Dp^a . Dp^f yields a more nearly wild-type phenotype when transmitted via egg than via sperm, while Dp^a yields a more nearly wild-type phenotype when transmitted via sperm than via egg. The

specific parental-source effect of Dp^f is independent of X and Y constitution of both parents and offspring. The parental-source effect of Dp^a , tested only in progeny of attached-X mothers, is independent of the X-Y constitution of the father and the offspring. The effects are limited to one generation; there is no "grandparental effect."

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the stimulation and encouragement provided by DR. WILLIAM K. BAKER, in whose laboratory this work was accomplished, and the enlightening and provocative discussions with DRs. EILEEN SUTTON GERSH, JOHN L. HUBBY and ANITA Y. HESSLER.

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