A STUDY OF PARENTAL MODIFICATION OF VARIEGATED POSITION EFFECTS[1, 2]

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THE study of genetic modification of the interruptions in homogeneous development of pigment which characterize white-variegated position effects in *Drosophila melanogaster* produces a variety of information. The compound eyes, testis sheath, and malpighian tubules in the mutant white (w, 1–1.5) are colorless, whereas the eyes are red and the sheath and tubules uniformly yellow in wild-type flies. White variegated flies possess at least one w^+ allele which, through a chromosomal rearrangement R(w^+), is associated with heterochromatin (see review on position effect by LEWIS 1950) and accompanies the development of the variegated pigmentation. A R(w^+)/w fly will develop eyes wherein only part of the pigment cells form the products associated with visible pigment; some cells do not, and appear colorless. The other two tissues have patches of yellow or colorless cells (DEMEREC and SLYZINSKA 1937). The w allele completely blocks production of both brown pigment components (ommochromes) and red pigment components (drosopterins) of the wild-type eye. In the testis sheath of wild-type *D. melanogaster* certain pteridines develop, whereas the ommochromes and drosopterins do not. As the yellow pigment of the testis sheath is thought to be mainly sepia pteridine (GRAF and HADORN 1959) which is regarded as a substance produced in the synthetic chain leading to formation of drosopterins in eyes (FORREST and MITCHELL 1955), a comparison of the pteridines in these two tissues in white-variegated flies with their characteristically variable pigmentation might indicate the extent of correlation of pigment development in eyes and testis sheath.

The cause of the cell-to-cell somatic variation is unknown, although certain environmental and genetic factors can modify the variegation toward either a preponderance of unpigmented tissue or of pigmented tissue. For example, by adding heterochromatin to the genome in the form of fragments or whole Y chromosomes, genetic modification is realized which usually results in more pigmentation (GOWEN and GAY 1933; SCHULTZ 1936; BAKER and SPOFFORD 1959). This phenomenon was utilized here in analyzing the development of eye and testis sheath pigment simultaneously in males with one or two Y chromosomes.

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BAKER and SPOFFORD (1959) and SPOFFORD (1959) in their studies of white variegation found unusual parental effects on the amount of pigmentation in eyes of variegated offspring. The studies being reported in particular amplify, quantitate, and extend the unusual findings.

MATERIALS AND METHODS

Stocks: The w^+ allele employed in this study is the same one used by BAKER and SPOFFORD (1959), Dp(w^m)264–58a. This duplication is a 20-band segment of the X chromosome containing w^+ (close to one end) which has been relocated in inverted order within the proximal heterochromatin of 3L (SUTTON 1940). Although originally carried in a free X stock by DR. EILEEN S. GERSH, the duplication was introduced by DRS. BAKER and SPOFFORD in 1956 into two lines which have been kept separate since that time—one had free X chromosomes, the other attached-XY, attached-X chromosomes. The duplication in the former line is denoted by Dp^f; in the latter line, by Dp^a. The distinction implied by these symbols represents certain highly significant differences in behavior or state between Dp^f and Dp^a which were discovered during the course of this investigation.

In the experiments detailed below the Dp^a and Dp^f were contributed initially by one $\underline{yw}$/Y; Dp^a/Dp^a female and one yw/yw; Dp^f/Dp^f female respectively. The inbred white stock which were employed for testcrosses were (1) attached-X, attached-XY stock; $\underline{yw}$/Y females, $\widehat{XY}w$/Y males, and (2) a free-X yw stock (y = yellow body, 1–0.0; w = white eye, 1–1.5; $\underline{yw}$ = attached-X; $\widehat{XY}w$ = $Y^sX{\cdot}Y^L$,*In(1)EN* = $Y^sw\ y{\cdot}Y^Ly^+$, see LINDSLEY and NOVITSKI 1959).

Flies were reared at 23±1°C on a culture medium modified slightly from that described by CARPENTER (1950). Pair matings of genotypes being compared were made up simultaneously on medium from the same batch. One or two replicates of each set of matings were made, each replication in sufficient numbers to yield at least ten fertile sets of parents. Two sets of smaller size, five and seven pairs, were obligatory in two cases because of infertility. The males used for chromatography were obtained by sampling the progeny of each pair in each replication at least once in the course of making daily collections. Sampling was random in the sense that the flies collected were simply those which eclosed in a given two-hour period after clearing of culture vials. The number of progeny produced by each pair mating was 50–60 regardless of the combination of parents used except MP-13 as noted later.

Chromatographic techniques: The eye pteridines which were measured are the drosopterins (DP), sepia pteridine (SP), and 2-amino-4-hydroxypteridine plus biopterin (HB_1 and HB_2). In the testis sheath SP, $HB_1 + HB_2$, and *iso*xanthopterin (IX) were measured (abbreviations as in ZIEGLER-GÜNDER and HADORN 1958). The drosopterins refer to a complex of at least three compounds which comprise the red pigment (VISCONTINI, LOESER and KARRER 1958). Sepia pteridine is probably responsible for yellow pigment of the testis sheath; at least it is the major constituent (GRAF and HADORN 1959). In my experiments HB_1 and

HB_2 were inseparable on one-way chromatograms. Their identity and relative abundance in eyes or testis were determined by running two-way descending chromatograms of samples of either ten heads or 12 testes (first solvent: propanol-7 percent NH_4OH, 2:1; second solvent: butanol-glacial acetic acid-water, 4:1:5). Comparisons were then made between the location of known 2-amino-4-hydroxypteridine added as a pure substance and the location of biopterin as accumulated in the eyes of the mutant sepia. It was found that the major part of the HB fluorescence in eyes and testis sheath was contributed by HB_2—biopterin. In variegated eyes HB_2 was distinctly in execess of the normal wild type amount (BAKER and SPOFFORD 1959), whereas 2-amino-4-hydroxypteridine was somewhat reduced. Sepia pteridine is present in excessive amounts, and drosopterin and *iso*xanthopterin are present in general in lower amounts in variegated eyes as compared with wild type. (For studies on the pteridines of Drosophila, see HADORN and MITCHELL 1951; FORREST and MITCHELL 1954a, 1954b, 1955; FORREST, GLASSMAN and MITCHELL 1956; FORREST, HATFIELD and VAN BAALEN 1959; VISCONTINI, LOESER and EGELHAAF 1956; VISCONTINI, LOESER and KARRER 1958; VISCONTINI and RASCHIG 1958; VISCONTINI 1958. For more general papers see Ciba Foundation Symposium on Chemistry and Biology of Pteridines, 1954; ALBERT 1954; ZIEGLER-GÜNDER 1956; HADORN 1959).

The males chromatographed were aged four to five days, dissected in Drosophila saline solution, and the samples—heads and testes—placed on Whatman No. 1 filter paper (46 cm × 57 cm) prepared for descending chromatography. Fifteen to 20 heads were crushed individually 1 cm apart, and the testes corresponding to the heads were placed in the same order on the same sheet of paper. Paper blanks were also run on each sheet. The solvent was normal propanol:7% NH_4OH (2:1). The paper was equilibrated with this solvent mixture for two to three hours prior to the chromatographic run of 16 hours in the dark which achieved adequate separation of the fluorescent components. The paper was air-dried in a laboratory hood and observed under UV light emitted by a Shannon lamp. The appropriate spots were marked, cut out, and eluted separately in 1 ml of dilute ammonia (1%) in individual stoppered vials for two hours; then the papers were removed.

Fluorescent measurements were made with a Farrand (Type A) photofluorometer using as an arbitrary fluorescent standard 1 μgm of anthranilic acid per 1 ml elution solvent. The galvanometer was adjusted to give a reading of two (on the 1–10 scale) for the 1 ml of standard before each set of readings. Thus all measurements are in terms of fluorescent units of this arbitrarily chosen standard. A primary filter, Corning No. 9863, was inserted between the UV source and sample, while secondary filters were placed between phototube and sample. The combination of No. 3385 and No. 3389 secondary filters was used to measure drosopterin and sepia pteridine, and the combination of No. 3385 and No. 4308 was used to measure *iso*xanthopterin and the HB complex. All readings were corrected by subtraction of the fluorescent value of a paper blank which had been cut from the chromatography paper at the same level as the sample.

RESULTS

Mating plan: A detailed diagram of the mating scheme is presented in Figure 1. In order to distinguish the immediate parental source of the Dp(w^m), the convention was employed of placing a maternally-inherited duplication to the left of the diagonal and a paternally-inherited duplication to the right, e.g. $\underline{yw}$/Y; Dp^a/+ or $\underline{yw}$/Y; +/Dp^a.

The expression of white-variegated position effects can be modified in many ways. The mating plan outlined in Figure 1 was designed to provide an organized framework for observing the operation of three particular modifying effects—the parental source effect, the effect of extra heterochromatin, and the effect of homozygous *vs.* heterozygous mothers. The plan was designed to produce information which might clarify the relationship of one modifying effect to another. In order to follow readily the various sets of comparisons being made, the chart has been labeled in this manner: the P and M series refer to patrilineal and matrilineal stocks where the Dp is maintained in either males or females, respectively, by crossing each generation to appropriate white stocks. The numerals designating series 1 and series 2 divide the M and P series of $\widehat{XY}w$/Y (series 1) from the M and P series employing yw/Y males (series 2), thus distinguishing the effect of extra heterochromatin. Finally, MP-1 and MP-2 identify lines established by certain deliberate combinations of the M and P lines to produce females homozygous for the duplication. The reasons for this plan will now be explained.

It is known that white-variegated progeny of the same genotype may have drastically different phenotypic expression depending upon whether the Dp was contributed by the mother or by the father. This effect of parental source of Dp is illustrated by the variegated progeny of $\underline{yw}$/Y; Dp^a/+ ♀♀ and $\widehat{XY}w$/Y; +/+ ♂♂, which develop far less pigment in their eyes than do the progeny of $\underline{yw}$/Y; +/+ ♀♀ × $\widehat{XY}w$/Y; +/Dp^a ♂♂ (SPOFFORD 1959). In addition, daughters of these two crosses show significant differences in penetrance of Dp^a. Chromatographic assays of pteridines in eye and testis tissues of males from these reciprocal crosses were made. Are the quantitative differences exhibited in the eyes of the two kinds of sons paralleled by differences in testis sheath pteridines? It is of interest from a developmental standpoint to find whether both tissues are being similarly affected by the parental source of the duplication.

The mating plan as designed permits study simultaneously of a second effect —that of extra Y heterochromatin. As it was unknown whether the parental source effect of Dp obtains in crosses using attached-X females but free X males, the matings were arranged so as to provide separate matrilineal and patrilineal lines of the duplication in both $\widehat{XY}w$/Y males (series 1) and free X males (series 2). By examining the parental source crosses in terms also of additional heterochromatin, one might expect to resolve certain regularities in the effects on pigment formation. It was hoped that observations on penetrance and analysis by chromatography might establish the action of additional heterochromatin of the $\widehat{XY}w$ chromosome in enhancing pigment production in testes as well as in eyes.

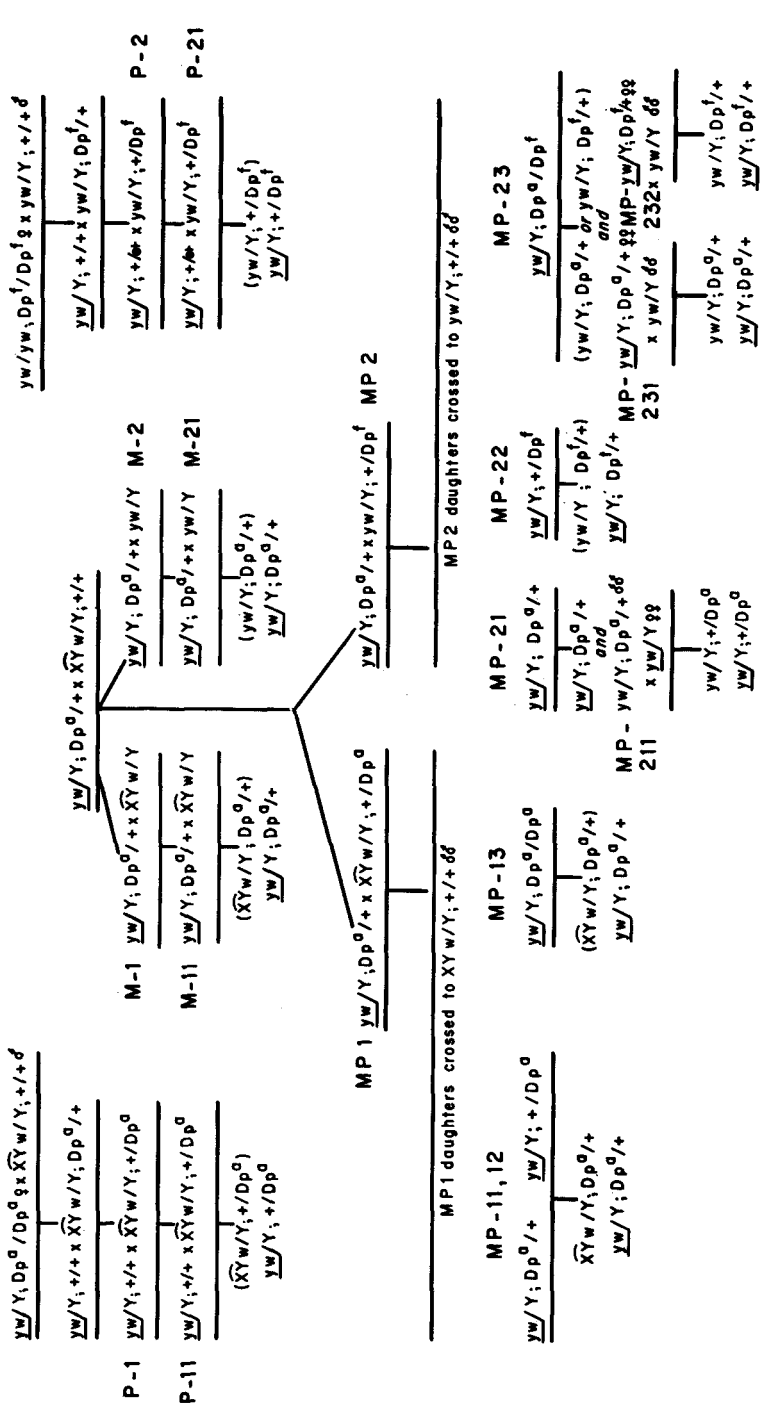

FIGURE 1.—An outline of the mating plan employed in producing the various parental stocks and progeny. For details on nomenclature, see Materials and Methods. The mottled males used in matings MP-1 and MP-2 were siblings of the males used in P-1 and P-2, respectively. Parentheses enclose certain groups of males that were chromatographed.

The mating plan provides for testing of a third modifying effect—that of augmented pigment production in progeny (heterozygous for Dp) of a $\underline{yw}$/Y; Dp/Dp mother compared with the progeny of a $\underline{yw}$/Y; Dp/+ mother. Series MP-1 and MP-2 in Figure 1 denote the deliberate recombination of matrilineal and patrilineal stocks to produce homozygous Dp females whose progeny could be compared with those of heterozygous Dp females. As in the case of other modifiers, inspection of progeny for penetrance of the duplication is an important criterion; and employment of chromatography of males proved especially useful, since phenotypic differences otherwise difficult to characterize without a large number of progeny could be easily detected and more subtly analyzed.

In summary, the mating plan was to provide simultaneous samples of all the kinds of flies to be compared. However, the experimental results also disclosed what appear to be real and significant differences between the way Dp^a and Dp^f act. Thus, the plan did not accomplish all that was intended. Sufficient data were accumulated to check the parental source effect completely in the $\widehat{XY}w$/Y series, but not in the yw/Y series. Comparative effectiveness of extra heterochromatin could be examined in both $\widehat{XY}w$/Y and yw/Y matrilineal series (M-11 and M-21, respectively), but it could not be examined in corresponding patrilineal matings (P-11 and P-21), where the difference between Dp^a and Dp^f invalidates such comparison. The desired comparisons of effects of homozygous Dp mothers *versus* mothers heterozygous for Dp are valid only in the Dp^a series (MP-1). Although the data collected from the MP-2 series are not directly comparable, they provide strong evidence that the original duplication now exists in two states, Dp^a and Dp^f.

Modifying effects associated with Dp^a: The parental source effect of Dp^a may be best described in two ways: the difference in penetrance of Dp^a in daughters and the difference in degree of expression in sons as determined chromatographically. As shown in Table 1, the mottled daughters of P-11 ($\underline{yw}$/Y; +/Dp^a) comprise a far larger percentage (47.7%) of the total female progeny (white plus mottled) than do $\underline{yw}$/Y; Dp^a/+ daughters of M-11 (29.4%). In addition, more pigment developed in P-11 than in M-11 daughters. Mottled sons from both crosses occurred in numbers insignificantly different from the expected 50 percent, but, as in their female siblings, M-11 sons ($\widehat{XY}w$/Y; Dp^a/+) exhibit markedly reduced pigment development compared to the $\widehat{XY}w$/Y; +/Dp^a P-11 sons. (This will be elaborated on later in the paper.)

When the MP-1 matings were made, a number of variegated females were produced which may have received Dp^a from either a mother or a father. (Note also that this group differs from the M-11 and P-11 ones in that the egg or sperm bearing the wild-type third chromosome is contributed by a Dp^a-bearing parent rather than by a homozygous testcross stock of $\underline{yw}$/Y; +/+, $\widehat{XY}w$/Y; +/+.) Since the mottled daughters ($\underline{yw}$/Y; Dp^a/+ and $\underline{yw}$/Y; +/Dp^a) of MP-1 showed a range of mottled phenotypes which could not be placed into distinct sets, it was impossible to designate the parental source of Dp^a. In addition, a number of Dp^a/Dp^a homozygous females were expected to survive, and their identification is inconclusive except by progeny test, although they tended to be somewhat

TABLE 1

Summary of genetic characteristics of Dp^a and Dp^f white-variegated progeny of mating plan with pteridine analysis of some males

Mating	Daughters: Genotype	Daughters: No.	Daughters: Percent w^m	Sons: Genotype	Sons: Tissue*	Sons: No.	Sons: Percent w^m	Pteridine analysis: No.	Drosopterin Mean	Drosopterin S.E.	Sepia Pteridine Mean	Sepia Pteridine S.E.	Biopterin + 2-amino-4-hydrox. Mean	Biopterin + 2-amino-4-hydrox. S.E.	*Isoxanthopterin* Mean	*Isoxanthopterin* S.E.
M-11	*yw*/Y;Dp^a/+	921	29.4	$\widehat{XY}$*w*/Y;Dp^a/+	E	880	48.7	57	0.247	±.002	1.121	±.106	1.757	±.155	.....	
					T	880	50.1	58	.....		0.814	±.076	1.010	±.085	4.286	±.480
MP-11, 12	*yw*/Y;Dp^a/+	1067	29.1	$\widehat{XY}$*w*/Y;Dp^a/+	E	1206	48.1									
P-11	*yw*/Y;+/Dp^a	815	47.7	$\widehat{XY}$*w*/Y;+/Dp^a	E	868	50.8	59	2.954	±.169	3.732	±.100	4.949	±.149	.....	
					T			59	.....		2.309	±.089	2.581	±.118	17.237	±.542
M-21	*yw*/Y;Dp^a/+	1018	25.3	*yw*/Y;Dp^a/+	E	959	0	12	0.000		trace		trace		.....	
					T	433	15.5	12	.....			0.350	±.357		0.367	±.171
MP-21	*yw*/Y;Dp^a/+	185	29.7	*yw*/Y;Dp^a/+	E	196	0									
					T	96	0									
MP-211	*yw*/Y;+/Dp^a	301	48.2	*yw*/Y;+/Dp^a	E	298	4.0									
P-21	*yw*/Y;+/Dp^f	1746	50.3	*yw*/Y;+/Dp^f	E	1629	23.2	17	0.153	±.030	0.557	±.137	1.041	±.247	.....	
					T	1629	48.0	51	.....		1.567	±.117	2.061	±.149	6.400	±.491
MP-22	*yw*/Y;Dp^f/+	286	59.8	*yw*/Y;Dp^f/+	E	295	45.1	15	0.285	±.052	0.600	±.178	1.600	±.452	.....	
					T	125	58.4	15	.....		.....		.....		7.857	±.504
MP-13 Dp^a/Dp^a mother	*yw*/Y;Dp^a/+	54	100.0	$\widehat{XY}$*w*/Y;Dp^a/+	E	53	100.0	21	0.771	±.154	1.605	±.206	2.829	±.367	.....	
					T			12	.....			2.450	±.310		7.133	±1.297
MP-23 Dp^a/Dp^f mother	*yw*/Y;Dp^a/+	168	100.0	*yw*/Y;Dp^a/+	E	242	65.8	23	0.496	±.138	0.600	±.143	1.481	±.375	.....	
	yw/Y;Dp^f/+			*yw*/Y;Dp^f/+	T	242	100.0	30	.....			2.980	±.271		5.963	±.664
MP-231 light mother	*yw*/Y;Dp^a/+	929	15.6	*yw*/Y;Dp^a/+	E	915	0									
MP-232 dark mother	*yw*/Y;Dp^f/+	377	54.4	*yw*/Y;Dp^f/+	E	398	42.5									

* E = eyes; T = testes.

more fully pigmented as a group than were the heterozygotes. As noted in Table 1, the daughters of the females which turned out to be heterozygous (MP-11, 12) resemble those of M-11 in that the penetrance of the duplication is essentially identical. Also, the expression of pigment in general among the progeny of MP-11, 12 was more similar to that of M-11 progeny than to P-11 progeny, with only a few exceptions showing much pigment. Thus, there is no evidence of a grandparental source effect.

Unlike similar tests on the M-11 and P-11 progeny, a chi-square test for heterogeneity within the 41 pairs comprising MP-11, 12 reveals very significant heterogeneity as to percentages of variegated flies from one mating to another, which could be largely accounted for by four exceptional pairs. Out of a total of 90 female progeny produced by these four pairs 55 were mottled, whereas 95 of the 99 sons were mottled. These numbers yield percentages (61% female, 96% male which are significantly different from the percentage of mottled progeny of the other 37 pairs (26.2% female, 43.8% male). The progeny of the exceptional pairs also developed more pigment. It is not known if these four exceptional pairs represent matings of mothers homozygous for Dp^a(MP-13) wherein a portion of the daughters and four of the sons are actually heterozygous for Dp^a but unpigmented or whether the exceptional progeny are actually from heterozygous mothers and the white-eyed progeny are not being recovered as adults in the usual numbers. Excluding these four exceptions, it may be said that most of the heterozygous daughters of the MP-1 mating seem to produce progeny which are indistinguishable from matrilineal (M-11) crosses and thus show no grandparental source effect attributable to having a $\underline{yw}$/Y; Dp^a/+ rather than an $\widehat{XY}w$/Y; +/Dp^a grandparent.

Characterization of the quantitative differences in pteridines between sons of M-11 and those of P-11 was deemed of possible value in determining whether both eye and testis sheath, which are quite different from one another in developmental origin (POULSON 1950), are affected similarly by the parental source effect. Highly significant quantitative differences in amounts of drosopterin (DP), sepia pteridine (SP) and the HB complex (biopterin and 2-amino-4-hydroxypteridine) in eyes distinguish the two types of sons, although no qualitative differences were observed (see Table 1). The amount of SP and HB (which is mainly biopterin) in the eyes bore a positive correlation, and there was less of both substances in M-11 than in P-11 sons (See Figure 2). The essentially straight line of Figure 2 leads one to infer that the parental source effect is acting on one process. However, the relation of both SP and HB to drosopterin differs in that M-11 sons show a positive correlation of both SP and HB to DP, whereas P-11 sons show negative correlation (Figures 3, 4). This may mean that at least two processes are being affected. Perhaps the M-11 sons characteristically never form enough precursors of drosopterin to make more than a small amount of visible pigment, whereas the P-11 sons accumulate excesses of the drosopterin precursors which are not converted into DP at a normal rate. The general shape of the curves in Figures 3 and 4 is reminiscent of the observation

of BAKER and SPOFFORD (1959) that the sepia and HB pteridines accumulate at intermediate values of visible pigmentation.

Highly significant differences in amounts of SP and the HB complex, as well as *iso*xanthopterin (IX), were observed also in the testis sheath, depending upon the parental source of Dp^a. Again, no qualitative differences distinguished the two kinds of sons. The relation of SP to HB was a direct positive one (Figure 5). *Iso*xanthopterin likewise showed a range of low values in M-11 sons, high values in P-11 sons (Table 1).

Within neither the $\widehat{XY}w/Y$; $Dp^a/+$ sons nor the $\widehat{XY}w/Y$; $+/Dp^a$ sons was there a correlation observed between the amount of SP of the eyes and in testis sheaths (Figure 6) nor between the amount of HB in eyes and sheaths (Figure 7). Moreover, the values for M-11 sons as a group were for both tissues lower than the values for P-11 sons. The relative lack of overlap between the two groups of points representing the two kinds of sons gives an indication of the genetic homogeneity within each group.

Direct examination of the source effect in free X males is impossible since no yw/Y; $+/Dp^a$ males from a patrilineal line were produced at the same time the M-21 matings were made. However, daughters of M-21 ($\underline{yw}/Y$; $Dp^a/+$) showed nearly identical penetrance and expression of Dp^a to that of daughters of M-11 and also daughters of MP-11, 12 and MP-21. Thus the expression of variegation in daughters appears to depend only on the source of Dp^a and not on the structure of their father's X chromosome. It seems legitimate, therefore, to compare the progeny of MP-211 ($\underline{yw}/Y$; $+/Dp^a$ ♀♀, yw/Y; $+/Dp^a$ ♂♂) with M-21 and MP-21. When 59 white-eyed sons of MP-21 were individually crossed to $\underline{yw}/Y$ females, 50 percent produced mottled progeny, establishing the fact that the fathers actually carried the duplication. The daughters of MP-211 appeared as the daughters of P-11 paternal source matings, 48.2 percent being pigmented, and the character of pigmentation was similar to the enhanced amount which develops in daughters of the patrilineal line. More sons (four percent) had pigmented eyes than ever occurred among sons of M-21 and MP-21 (zero percent). Thus it is suggested that the parental source effect of Dp^a is maintained in free X males.

The effect of added heterochromatin on pigment development of males in the Dp^a framework is drastic. The penetrance in yw/Y; $Dp^a/+$ males is at best about 30 percent of the expected 100 percent as determined by examining testes and malpighian tubules for pigment. Only progeny testing, such as was done in MP-211, reveals that Dp^a is actually present in about half of the white-eyed sons. In addition to poor penetrance of Dp^a in yw/Y males, the amount of pigment developed in such males showing variegation is markedly less than that developed in M-11 sons (see pteridine analysis, Table 1). Both kinds of sons had exactly the same kind of mother. This leads to the conclusion that additional Y heterochromatin in the genome is associated with development of far more variegated flies with more pigment in both the tissues inspected.

Modifying effects associated with Dp^f: One primary reason for distinguishing

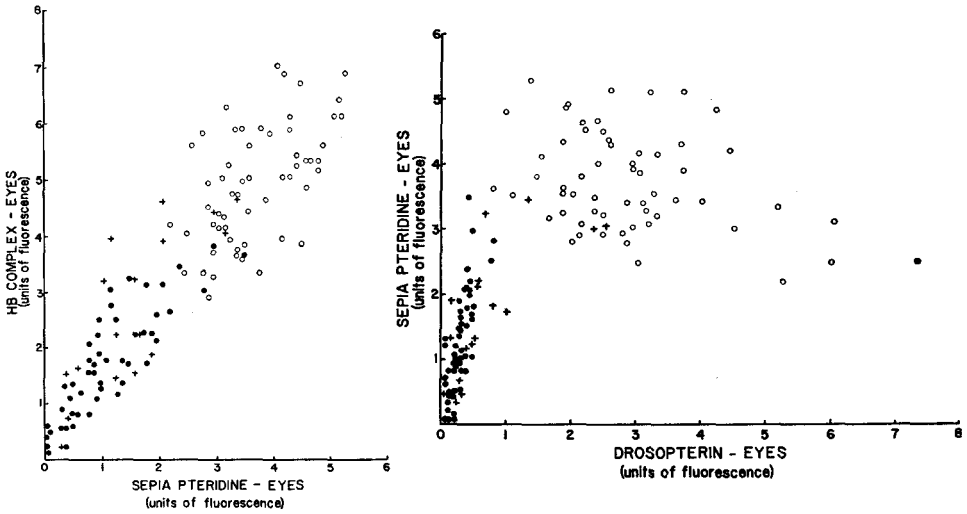

FIGURE 2.—Positive correlation of SP and $HB_1 + HB_2$ in eyes of M-11 sons ($\widehat{XY}w/Y;Dp^a/+$, black circles), P-11 sons ($\widehat{XY}w/Y; +/Dp^a$, open circles), and MP-13 sons ($\widehat{XY}w/Y; Dp^a/+$, plus signs). The units of measure are arbitrary units of fluorescence as explained in Materials and Methods. These units are employed in all the figures which follow.

FIGURE 3.—Positive correlation of DP and SP in the eyes of M-11 sons ($\widehat{XY}w/Y; Dp^a/+$, black circles); negative correlation in eyes of P-11 sons ($\widehat{XY}w/Y; +/Dp^a$, open circles). Note intermediate values for variegated sons of $\underline{yw}/Y; Dp^a/Dp^a$, MP-13 (plus signs).

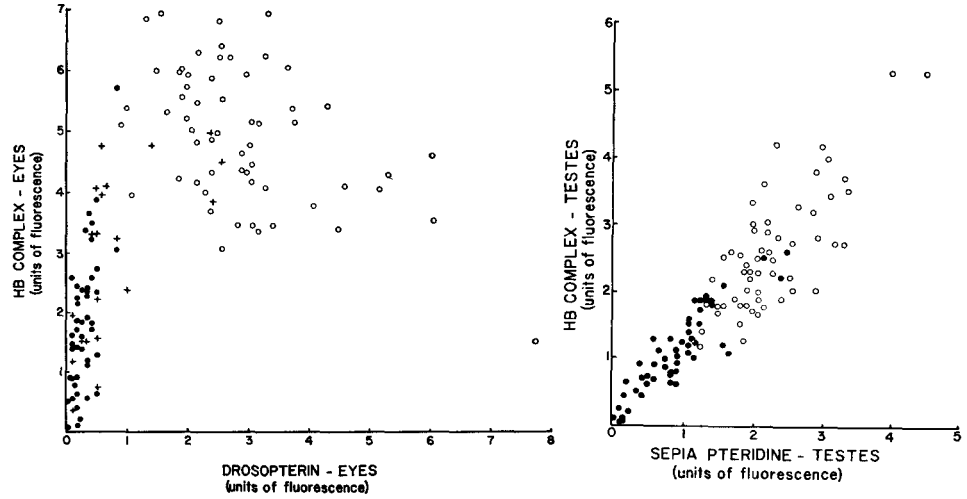

FIGURE 4.—Relation of DP to the HB complex. Symbols are the same as in Figure 3.

FIGURE 5.—Positive correlation of SP to HB complex in the testes. (Black circles = M-11 sons, $\widehat{XY}w/Y; Dp^a/+$; open circles = P-11 sons, $\widehat{XY}w/Y; +/Dp^a$.)

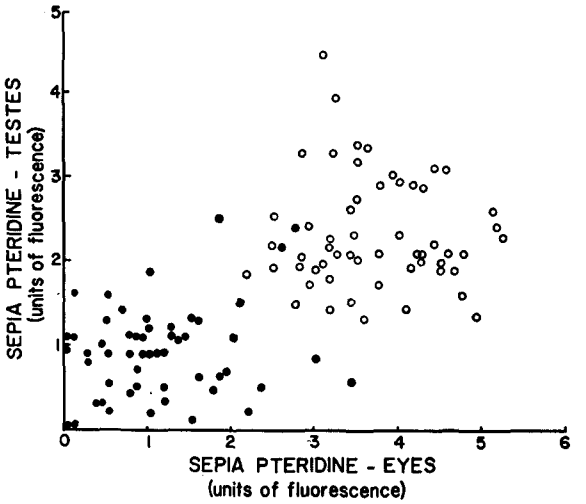

FIGURE 6.—Lack of correlation between eye and testis values of SP of M-11 sons ($\widehat{XY}w/Y$; $Dp^a/+$, black circles) as well as P-11 sons ($\widehat{XY}w/Y$; $+/Dp^a$, open circles).

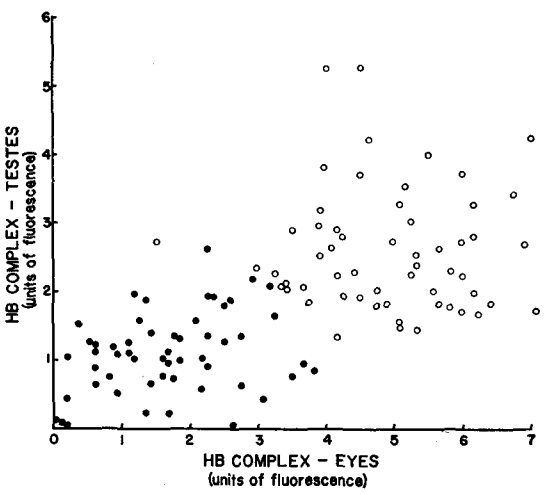

FIGURE 7.—Lack of correlation between eye and testis values of the HB complex of M-11 sons ($\widehat{XY}w/Y$; $Dp^a/+$, black circles) as well as P-11 sons ($\widehat{XY}w/Y$; $+/Dp^a$, open circles).

a Dp^a and a Dp^f state is that the parental source effect with Dp^f is either absent or, more likely, the reverse of that seen in Dp^a as described above. The main evidence for this is supplied by observations on penetrance of Dp^f in males and to some extent by chromatographic analysis. If the Dp^f parental source effect were similar to Dp^a, one would expect sons of a Dp^f mother to show equal or lower penetrance of the duplication than would sons of a Dp^f father, and the expression, in terms of pigment formation, would be significantly reduced. Also, the daughters of a Dp^f mother would be expected to show consistently lower

penetrance and expression of the duplication than the daughters of a Dp^f father.

Actually, however, mottled-eyed sons (yw/Y; Dp^f/+) of a Dp^f mother (sons of MP-22) numbered 45 percent of the total male progeny, whereas only 23 percent of the sons of a Dp^f father (mating P-21) had pigmented eyes. Although dissection of white-eyed flies for evidence of pigmented testis sheaths showed that the duplication is present in 48 percent of the sons in the latter case, similar dissection of the sons of a maternal source revealed that 58 percent had pigmented testes. Table 1 shows that the amount of drosopterin is significantly higher in MP-22 than in P-21 sons, but there is no significant difference in the amounts of SP or the HB complex, although they are slightly higher in MP-22 sons.

The variegated daughters (yw/Y; Dp^f/+) of the maternally contributed Dp^f (MP-22) number about 60 percent of the total female progeny, whereas mottled daughters of P-21 comprise 50 percent of the total female progeny. The abnormally high recovery of the Dp^f-bearing sons and daughters when the mother carries Dp^f (MP-22 and MP-232) is also unique to the Dp^f state and may be suggestive of a maternal influence of a Dp^f mother on her nonduplication bearing eggs. At any rate, it provides additional evidence supporting a distinction between Dp^a and Dp^f.

Maternal homozygosity effect: Let us first consider the matings using the Dp^a state of the duplication. The seven MP-13 matings (yw/Y; Dp^a/Dp^a ♀♀ × XYw/Y; +/+ ♂♂) were discernible because no white offspring were recovered. Homozygous females were as a rule darker than heterozygous females of the M-11 kind but not surely distinguishable from MP-11, 12 females. Progeny of MP-13 had distinctly more pigment than progeny of MP-11, 12. A chromatographic analysis of pteridines in MP-13 (XYw/Y; Dp^a/+) sons reveals higher values for all eye and testis pteridines than found in M-11 sons. The difference in drosopterin values is highly significant; the difference in HB complex values for eyes is barely significant at the five percent level; the differences between the means for all the other substances are not significant at this level. It would appear that in variegated sons, heterozygous for the duplication, from yw/Y; Dp^a/Dp^a mothers, the development of pigments is improved over that of identical sons from yw/Y; Dp^a/+ mothers.

Female progeny produced as a result of the MP-2 series of matings (which will include yw/Y; Dp^a/Dp^f females) could be of three genotypes—yw/Y; Dp^a/+, yw/Y; +/Dp^f, and yw/Y; Dp^a/Dp^f. There were in fact only two phenotypes recovered, lightly mottled females and females with a great deal more pigment, with essentially no intermediate phenotypes. A sample of 15 of the lightly mottled females and 75 darkly mottled ones were individually mated to yw/Y males; five of the 15 were fertile, while about one half of the 75 were fertile. Samples of the fertile females were studied. Ten of the darker females were identified as being Dp^a/Dp^f because they produced no white female offspring, although some of the male progeny were white-eyed; 12 dark-eyed females were heterozygous and identified tentatively as yw/Y; +/Dp^f. Five lightly mottled

females were heterozygous and identified as supposedly $\underline{yw}$/Y; Dp^a/+. Thus, unlike the MP-1 matings, heterozygous daughters of MP-2 were capable of being distinguished phenotypically. The testcross series MP-21, MP-22, and MP-23 thus are of unusual interest since these crosses could confirm the differences between Dp^a and Dp^f and provide evidence of the stability of these differences.

MP-21, a mating between supposed $\underline{yw}$/Y; Dp^a/+ and yw/Y; +/+ males produced progeny showing the penetrance and pigmentation characteristic of a maternal duplication of the Dp^a state (M-21). About 30 percent of the females were pigmented, but only slightly; all the males were white-eyed. Dissection of 96 of the males failed to reveal any with pigmented testes, but progeny testing of 59 (mating MP-221) revealed the presence of Dp^a in one half of the white-eyed males. The lack of pigment in any of the testes points to a possible difference between these sons and those of M-21.

Progeny of MP-22 were entirely different from those of MP-21. Nearly 60 percent of the females were well pigmented, a highly significant difference from the daughters of MP-21 (30 percent). Also, the male progeny (yw/Y; Dp^f/+) of MP-22 differed significantly from MP-21 sons (yw/Y; Dp^a/+) in that 45 percent of the former had pigmented eyes. A sample of the yw/Y; Dp^f/+ sons selected for dissection showed that 58 percent had well-pigmented testes and that considerable amounts of *iso*xanthopterin had formed.

The progeny of MP-23 individual matings of $\underline{yw}$/Y: $Dp^a/Dp^f \times yw$/Y; +/+ males showed a striking dichotomy in appearance of females, one half being lightly pigmented and one half more fully pigmented. Of the 168 female progeny of these ten Dp^a/Dp^f testcrosses, 90 were pair-mated to yw/Y; +/+ males, to check whether or not genetic evidence supported phenotypic evidence of persistence of a Dp^a:Dp^f dichotomy.

Of the 90 pair matings, 38 were fertile—26 mothers were lightly mottled, and 12 darkly mottled, thus giving an indication that the latter class was less fertile. However, since this particular set of matings was not repeated, it is not possible to say whether this difference is significant. All 38 mothers produced variegated daughters, but only the darkly mottled ones had variegated sons. Considering first the suspected Dp^a mothers, the lightly mottled ones, it is seen from the results of mating MP-231 ($\underline{yw}$/Y; Dp^a/+ ♀♀ × yw/Y; +/+ ♂♂) that no variegated sons were produced, only white-eyed ones, and only 15.6 percent of the daughters were variegated. Although these results closely resemble those of other series of $\underline{yw}$/Y; Dp^a/+ × yw/Y; +/+ matings, the penetrance of Dp^a in daughters of MP-231 is significantly lower (15.6 percent *vs.* 29 percent). The meaning and significance of this difference is unknown as yet, but the MP-231 results are regarded as providing strong support for the identification of these lightly mottled females as Dp^a.

The progeny of MP-232 ($\underline{yw}$/Y; Dp^f/+ ♀♀ × yw/Y;+/+ ♂♂) resembled phenotypically those of MP-22. There is a similarity in the penetrance of Dp^f also which, although not so drastic, is consistently in the same direction of recovery of more variegated than white females (Table 1). None of the males was dissected, so whether or not more than 50 percent of the sons actually carried

Dp^f is unknown. Nevertheless, the penetrance is significantly higher than was observed in sons of P-21 where the source of Dp^f was paternal. This further supports the conclusion that the parental source effect with Dp^f does act in the direction opposite to that with Dp^a.

Among the sons of MP-23, there would be expected a *yw*/Y; Dp^a/+ and *yw*/Y; Dp^f/+ class because there is evidence of Dp^a and Dp^f daughters. However, the genotype of the white and mottled sons cannot be deduced from the phenotypic percentages (34 percent white, 66 percent mottled) since the white-eyed males which have mottled testes might represent either Dp^a or Dp^f. The fact that all the testes are variegated does, however, give confirmation of the identity of the MP-23 mothers as homozygous. Note that the expression in these sons is even higher than in the *yw*/Y; Dp^f/+ males from MP-22, indicating an enhancement of pigmentation in the sons from a mother that carried two doses of the duplication, albeit in different states.

It is stressed that the maternal effect whereby progeny of a homozygous mother show better penetrance and expression than do progeny of a heterozygous mother is a one-generation effect. Progeny testing in series MP-231 and MP-232 re-establishes the differences as observed in the MP-21 and MP-22 progeny of heterozygous mothers.

It may be of interest to introduce data on the results of crossing other daughters of MP-2 to $\widehat{XY}w$/Y males. MP-2 daughters of the two phenotypes—lightly mottled and darkly mottled—were crossed individually to $\widehat{XY}w$/Y males, and progeny of 24 fertile pairs established the existence of three groups of mothers: (1) 12 darkly mottled and homozygous for the duplication, (2) eight darkly mottled and heterozygous, and (3) four lightly mottled and heterozygous. The figures given (Table 2) are the total progeny of each of the three groups, whereas all other sets of progeny discussed in this paper were first tested for within-group heterogeneity and then pooled, if appropriate. It is of interest to compare these results with both the MP-11, 12, 13 series of crosses and the MP-21, 22, 23 series.

Let us first compare the phenotypic data in Table 2 with the results of MP-11, 12, 13. The heterozygous daughters of the MP-1 matings could not be distinguished phenotypically, whereas the heterozygous daughters of the MP-2 mat-

TABLE 2

Progeny of $\widehat{XY}$w/*Y;* +/+ ♂♂ × ♀♀ *of three possible genotypes resulting from MP-2 cross*

	Daughters			Sons		
		White-variegated			White-variegated	
Genotype of mother	White	Red	Brown-red	White	Red	Brown-red
(1) *yw*/Y;Dp^a/Dp^f darkly mottled	0	97	92	0	160	0
(2) *yw*/Y;Dp^f?/+ darkly mottled	81	74	0	103	86	0
(3) *yw*/Y;Dp^a?/+ lightly mottled	46	0	32	32	0	53

ings ($\underline{yw}$/Y; Dp^a/+ ♀♀ × yw/Y;+/Dp^f ♂♂) can be distinguished and these distinctions are supported by progeny tests to $\widehat{XY}w$/Y males. Also, the daughters of MP-13, where the mother was homozygous for Dp^a, although showing some variation as to pigmentation did not show the dichotomy of phenotypes that daughters of Dp^a/Dp^f do (Table 2). Although no measurements were made, chromatographs of some white-mottled "red" and some white-mottled "brown-red" daughters of the homozygous females (Table 2) showed much more drosopterin in the "red" group than in the "brown-red", with the latter accumulating distinctively more HB and SP. It is believed that these two phenotypic classes correspond to the darkly mottled and lightly mottled daughters, respectively, of $\underline{yw}$/Y; Dp^a/Dp^f ♀× yw/Y; +/+♂. The distinction here made between the two kinds of mottled progeny must be tentatively assigned to the contributions of the $\widehat{XY}w$/Y paternal genome *versus* that of yw/Y males, a point which was not resolved in this study. Also, the supposed $\underline{yw}$/Y; Dp^f/+ mothers recorded in Table 2 did not produce a preponderance of mottled daughters, as did the MP-22 females with which they are being compared, another difference which may be attributable to the paternal genomes. The essentially wild-type pigmentation of sons of Dp^a/Dp^f mothers (when fathers are $\widehat{XY}w$/Y) is considered very different from the measured values of MP-13 sons, which were only partly pigmented. The most important characteristic brought out by the data in Table 2 is that the daughters of MP-2 matings when crossed to $\widehat{XY}w$/Y males produce offspring which fall into three genotypic groups comparable to MP-21, 22, 23, but distinctly unlike MP-11, 12, 13.

In $\widehat{XY}w$/Y and yw/Y sons of Dp^a/Dp^f mothers there cannot be distinguished two phenotypic classes which can be said to correspond to Dp^a or Dp^f, although the female siblings in both cases do display this dichotomy, which seems to correspond genetically to Dp^a and Dp^f (MP-231, MP-232). Therefore, one must assume in the case of presumed $\widehat{XY}w$/Y; Dp^a/+ sons of Dp^a/Dp^f females that such males have their pigmentation raised to a level higher than that observed when genetically identical sons come from mothers which were homozygous for Dp^a.

Development of pigment and precursors: In order to evaluate the generality of the conclusions reached concerning pigmentation in variegated eyes and testes, a comparative study was made of the pteridines in eyes and testes of $\widehat{XY}w$/Y; +/Dp^a males and Oregon-R wild type males at a series of different times in development from two days before eclosion to 12 days after eclosion. The measurements presented in Table 3 are in terms of the arbitrary fluorescent units described earlier in the paper. These developmental studies were done on flies reared at 25±.5°C after the completion of the studies discussed previously. The relatively larger standard errors registered for the mottled males is a reflection of the more heterogeneous nature of their pigmentation compared to more uniformly pigmented wild-type males.

The amount of drosopterin in the mottled males was as a rule less than in wild type (Figure 8) whereas the amounts of sepia pteridine and the HB com-

TABLE 3

Comparison of development of eye and testis pteridines in Oregon-R and $\widehat{XY}w/Y; +/Dp^a$ males. Values are mean ± standard error in arbitrary fluorescent units

	Pteridine	Genotype	Pupal stages: Age No.	−2 days 6	−1 day 6	Eclosion 12	18±1 hr 12	Imaginal stages: 2 days 16	4 days 16	9 days 17	12 days 16
Eyes	DP	Oregon-R		.27±.01	0.74±.23	1.080±.161	4.022±.193	5.800±.153	4.756±.105	5.735±.558	4.915±.100
		$\widehat{XY}w/Y;+/Dp^a$		.29±.16	1.57±.43	0.814±.131	.....	4.111±.255	2.489±.260	3.518±.202	3.875±.192
	SP	Oregon-R		.30±.06	0.58±.07	0.783±.078	1.667±.076	2.133±.058	1.894±.106	1.947±.084	1.925±.051
		$\widehat{XY}w/Y;+/Dp^a$		.40±.06	1.00±.11	1.107±.128	.....	2.933±.136	2.561±.136	2.606±.263	2.594±.201
	HB_1 + HB_2	Oregon-R		.72±.07	0.82±.22	1.050±.083	2.289±.145	3.178±.143	3.167±.095	4.329±.181	3.885±.118
		$\widehat{XY}w/Y;+/Dp^a$		.81±.09	1.73±.22	1.536±.075	.....	3.433±.179	4.339±.155	4.969±.196	5.200±.197
Testes	SP	Oregon-R		0 or trace	0 or trace	0.288±.002	0.389±.066	0.944±.050	1.422±.075	1.059±.123	1.520±.059
		$\widehat{XY}w/Y;+/Dp^a$				0.232±.002	0.867±.100	1.478±.176	2.211±.095	1.765±.074	1.850±.020
	HB_1 + HB_2	Oregon-R		0 or trace	0 or trace	0.444±.002	0.767±.101	1.389±.059	1.806±.059	1.735±.050	1.660±.075
		$\widehat{XY}w/Y;+/Dp^a$				0.585±.284	1.466±.186	2.056±.143	2.039±.123	2.512±.122	2.167±.106
	IX	Oregon-R		0-trace	0-trace	2.422±.200	5.467±.664	14.900±.788	15.825±.476	15.040±.170	14.453±.460
		$\widehat{XY}w/Y;+/Dp^a$		.33±.16	1.00±.46	2.800±.117	7.466±.917	13.600±1.335	15.210±.738	15.410±.684	14.989±.548

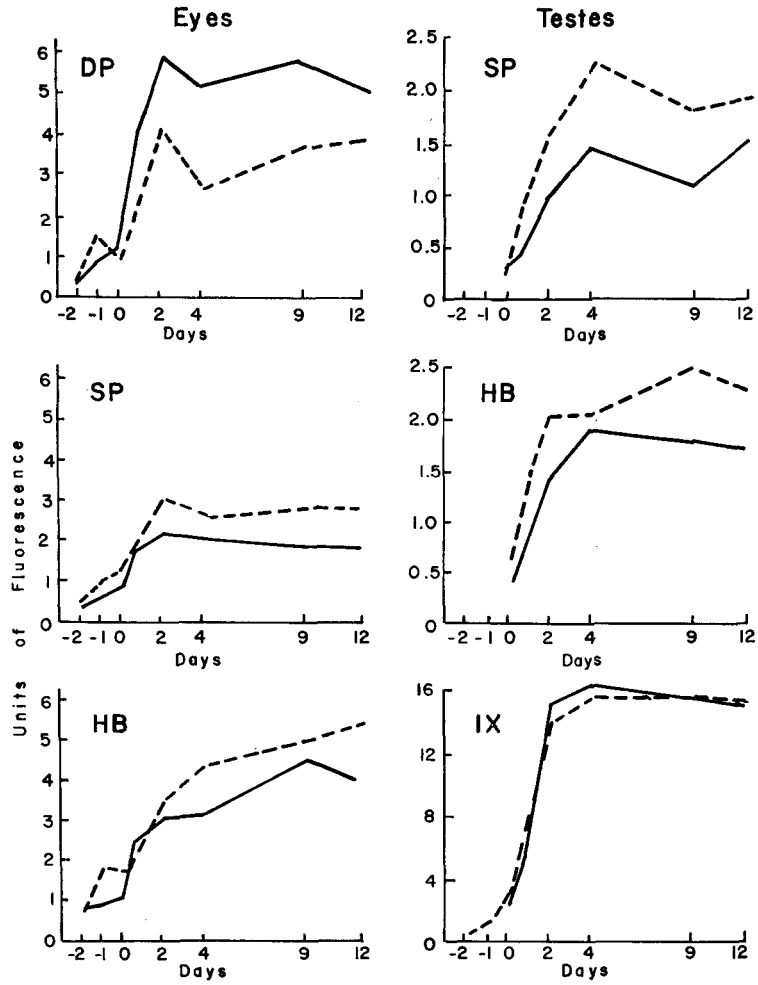

FIGURE 8.—Comparison of eye and testis pteridines developed in Oregon-R males (—) and $\widehat{XY}w/Y$; +/Dp^a males (---) which were reared concurrently. Day 0 is the time of eclosion. DP = drosopterin; SP =sepia pteridine; HB = biopterin + 2-amino-4-hydroxypteridine; IX = *iso*xanthopterin.

plex were usually higher in mottled than in wild-type males in both testes and eye tissue. If sepia pteridine is the main constituent of visible pigment in the testis sheath, it is of interest that the pigmented sheath cells of variegated testes accumulate more sepia pteridine than do the sheath cells of wild-type flies, which are completely pigmented. The *iso*xanthopterin content of the testes of the two kinds of males is essentially identical, except that the $\widehat{XY}w/Y$ males exhibit somewhat more variability. Since the synthetic pathways relating the various pteridines are not as yet known, the significance of these differences remains obscure. Apparently in males showing white-variegation, the partial blockage of red pigment formation in the eyes is accompanied by the accumulation of precursors (SP and the HB complex) in testis sheath as well as in eyes.

The values represented by the means, plotted in Figure 8, for imagoes fit very well the curves established in previous figures for 5-day-old flies. The extensive measurements made on 5-day-old flies reflect, at least in the case of $\widehat{XY}w/Y$; $+/Dp^a$ males, a difference from the wild-type condition which is characteristic throughout most of the pupal period and up to 12 days after eclosion.

DISCUSSION

Certain of the parental effects which have been described can most economically be interpreted as one-generation maternal effects. In the case of Dp^a, the progeny of a female homozygous for the duplication quantitatively express the pigment-forming action of Dp^a more strongly than do progeny of a heterozygous female. The pteridine analyses of the two sets of heterozygous sons, as well as the penetrance of Dp^a, support this statement. Implicit in such a conclusion is the assumption that one dose of Dp^a produces less pigment forming action in the egg than do two doses. Also it is possible that mutant white alleles and Dp^a are interacting such that two white alleles and one Dp^a in a heterozygous mother result in production of quantitatively less maternal pigment-forming substances than does the interaction of two white alleles with two Dp^a's in a homozygous mother. The most obvious pathway available in females for conveying maternally formed products into eggs is the transfer of nurse cell products into the developing oocyte. The egg is a closed metabolic system for the period from its fertilization until the hatching of the first larval instar. During this time (about 24 hours) differentiation is extremely rapid, and even the anlagen of imaginal gonads and eyes are laid down (POULSON 1950). Thus, there is a mechanism for the incorporation of maternal products into the cells of the imaginal anlagen. We do not know how these maternal products are maintained in the developing embryo and how they influence, eight to ten days later, the differentiation of pigment in an imaginal organ.

The other case in which a maternal effect may be tentatively invoked as an explanation of the results is the behavior of Dp^f in progeny of a maternal compared with a paternal source. It will be recalled that the progeny, heterozygous for Dp^f, produced by a Dp^f mother showed better penetrance and expression of pigmentation than progeny of a Dp^f father. This difference can be explained by assuming that the heterozygous Dp^f female adds pigment-forming substances to her eggs which are not added by white females to their eggs. Therefore, eggs ready for fertilization in a Dp^f mother would be, in a sense, conditioned for pigment development, whereas eggs of a mutant white mother would not.

Other cases of maternal influence on eggs have been noted from time to time (see CASPARI 1948). For example, in Ephestia kynurenine was demonstrated to be present in the homozygous recessive eggs (a/a) of a cross between an a^+/a♀ $\times$ a/a♂, whereas this substance is lacking in such eggs from an a/a♀ $\times$ a^+/a♂ cross. We have no knowledge of the chemical nature of the postulated substances accounting for the supposed maternal effects on white variegation in *D. melanogaster*.

In the Dp^a series, the progeny with a paternally contributed duplication exhibit

far more pigment development than do progeny of a maternal source. This is not in accord with expectations based on the postulated maternal action of the duplication. From the viewpoint of finding a consistent explanation accounting for all the phenomena observed, this is an exception which cannot be reconciled. Data are lacking which will permit critical judgment of this exception, until, for example, crosses between $\underline{yw}$/Y; Dp^a/+ and $\widehat{XY}w$/Y; Dp^a/+ are made, with a marked third chromosome in either the male or the female. Thereby male progeny coming from the same type of mother and carrying either a maternally or paternally contributed Dp^a can be studied simultaneously. This study has been postponed because the addition of a newly marked third chromosome would tend to destroy the isogenicity of the stocks.

The Dp^a/Dp^f females, when crossed to either $\widehat{XY}w$/Y or yw/Y males produced offspring with more pigment than offspring from mothers heterozygous for either Dp^a or Dp^f. This is consistent with an explanation in terms of maternal effect. This observation implies that there is no complementation between the function of Dp^a and Dp^f insofar as the maternal homozygous effect is concerned. In other words, although these two states of the duplication may be distinguished by the particular criteria previously enumerated, they behave as functionally the same state in the maternal homozygous effect.

Since all the $\widehat{XY}w$/Y sons of both Dp^a/Dp^a and Dp^a/Dp^f mothers are pigmented, it is of interest that the sons of Dp^a/Dp^f developed nearly wild-type pigmentation whereas sons of Dp^a/Dp^a developed only partially pigmented eyes. Since the Dp^a/Dp^f female is expected to yield both Dp^a and Dp^f sons, it would appear that the Dp^a sons look like Dp^f ones. Since any egg of Dp^a/Dp^f mothers has an equal probability of developing into a male or female, potential daughters and sons would be expected to receive the same kinds and amounts of maternally contributed substances prior to fertilization. Yet the daughters of Dp^a/Dp^f show divergent phenotypes, and the sons do not. The most obvious difference between the daughters and sons is, of course, the $\underline{yw}$ versus the XYw condition; both sexes have a free Y in addition. Perhaps the association of the extra heterochromatin of XYw in the sons results in the passing of some crucial threshold in pigment formation which is not surpassable in the $\underline{yw}$ daughters. While both sons and daughters have one duplication-bearing chromosome, the sons have only one white allele, whereas the attached-X daughters have two white alleles. Whether either of these factors influences the differences observed between sons and daughters of Dp^a/Dp^f females is not known.

The fact that phenotypically distinguishable and genetically distinct Dp^a and Dp^f categories could be established certainly supports the notion that the difference in state is a stable one. The difference reported is substantiated by an independent study by DR. JANICE B. SPOFFORD (unpublished). The characteristics of the two states must represent changes in the properties of the duplication and/or surrounding heterochromatin rather than alterations caused by modifying genes (unless very tightly linked). The states could be interpreted as representing two systems of modifiers of the duplication, one enhancing (Dp^a) and the other

suppressing (Dp^f) pigment development, with crossing over in females and lack of crossing over in males to account for the parental effects described. However, since the duplication was maintained in matrilineal lines, there is always the possibility that such modifiers would eventually cross out, resulting in progressive change in the character of mottling. Such progressive alteration was not observed. The most compelling argument against two systems of modifier genes is the observation that Dp^a and Dp^f as extracted from Dp^a/Dp^f females were unchanged in their respective actions.

SUMMARY

The study disclosed the existence of two states, Dp^a and Dp^f, of the duplication Dp(*w*^{m})264–58a in *Drosophila melanogaster* which produces position-effect variegation of the pigments in the eyes and testis sheaths. Dp^a flies as a rule develop less pigment than Dp^f flies when white-variegated stocks are compared. The variegated progeny heterozygous for Dp^a were more heavily pigmented if their mottled mothers were homozygous for Dp^a than if they were heterozygous for Dp^a. Variegated progeny of heterozygous Dp^a males, however, have significantly more pigmentation than do progeny of Dp^a females. Dp^f females, on the contrary, produce more heavily pigmented progeny than do Dp^f males. Also, the number of mottled progeny recovered from a $Dp^f/+$ female may be significantly higher than the number of white progeny recovered. This distorted ratio is not observed with $Dp^a/+$ mothers. The differences in penetrance and expression accompanying the parental source effects and maternal homozygosity effect in Dp^a or Dp^f are considered to be one-generation effects.

The homozygous *versus* heterozygous maternal effect is observed by comparing progeny of Dp^a/Dp^f females with progeny of either $Dp^a/+$ or $Dp^f/+$ females; thus there is no evidence for distinguishing a difference in action of Dp^a from Dp^f in terms of the homozygous maternal effect.

Chromatographic analysis of eye and testis sheath pteridines has demonstrated no correlation between sepia pteridine or $HB_1 + HB_2$ in these two tissues of $X\widehat{Y}w/Y$ males bearing Dp^a. A comparative developmental study showed that $X\widehat{Y}w/Y$; $+/Dp^a$ males as a rule developed less drosopterin in their eyes but more sepia pteridine and $HB_1 + HB_2$ in both eyes and testis sheaths than did Oregon-R wild-type males.

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