

POSITION-EFFECT VARIATION AT SEVERAL CLOSELY LINKED LOCI IN *DROSOPHILA MELANOGASTER*^{1,2}

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THE phenomenon of position-effect variegation has been extensively studied, many inquiries having been made into its cause and differences in expression (for a general review, see LEWIS 1950). It has been established that variegation is in some way the result of chromosomal rearrangements which often relocate euchromatic sections in heterochromatic (GOWEN and GAY 1933; DEMEREC 1941; SCHULTZ 1941) or, on occasion, place heterochromatic segments in a euchromatic environment (SCHULTZ 1936; BAKER 1953). The genes affected are those located near the break points of the rearrangement. Direct proof of position-effect variegation has been obtained by the recovery of normal activity of those genes when they are transferred by crossing-over from the rearrangement to a normal chromosome (DUBININ and SIDOROV 1935; JUDD 1955).

Many investigators have noted and described the considerable phenotypic variations that exist between individuals exhibiting variegation. Factors which may contribute to differences in expression of variegation include temperature (GOWEN and GAY 1933), other chromosomal rearrangements (SCHULTZ 1941), extra heterochromatin (SCHULTZ 1936; GOWEN and GAY 1934; BAKER and SPOFFORD 1959), as well as genes which act as modifiers (DEMEREC and SLIZYNSKA 1937). In addition, differences in the phenotypic expression of variegation have been shown to result from residual effects of the genotype of the parents. SPOFFORD (1959) and HESSLER (1961) studying variegation at the white locus of *Dp(w^m)264.58a* have observed one-generation effects which include differences resulting from the sex of the parent contributing the duplication (parental-source effect), homozygosity *versus* heterozygosity of the mother for the duplication, and the Y chromosomal constitution of the mother between two white-mottled stocks of the above-mentioned duplication.

Differences between stocks have been observed both in amounts of pigmentation in general and in the parental-source effect. It was noted that one stock consistently produced more fully pigmented flies. This stock, referred to as *Dp^f*, showed more pigment if the duplication was inherited from the mother, while the stock showing more extreme variegation, *Dp^a*, produced more pigment when the rearrangement was passed through the father. Another stock, *Dp^g*, derived

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from Dp^f , also showed increased pigmentation but little or no parental effects (W. K. BAKER, personal communication).

The distinction noted between the latter two stocks relate to variegation at the white locus only. It was the purpose of the work being reported to discover whether the differences extend as well to other loci of the duplicated region and in this way to determine whether the mechanism causing these differences was specifically acting on the white locus alone or affecting a more considerable portion of the rearranged chromosome.

MATERIALS AND METHODS

The genes studied were included in a 20-band segment of the X chromosome relocated in reversed order in the proximal heterochromatin of 3L (DEMEREK 1940; SUTTON 1940). This is the same duplication, $Dp(w^m)264.58a$, used by Drs. HESSLER and SPOFFORD. The duplication was originally kept in a free X stock by DR. EILEEN S. GERSH and was then introduced into an attached-X stock and a free X stock by Drs. BAKER and SPOFFORD. These stocks were then kept separately. The attached-X stock was designated Dp^a , the free X stock, Dp^f . Another state of the duplication, Dp^e , was isolated from Dp^f by Drs. BAKER and HUBBY. These flies were more heavily pigmented than those normally obtained from a Dp^f line.

In the studies being reported, Dp^a and Dp^e were compared as to expression of variegation and response to parental genotype. Both lines of the duplication were carried with attached-X, attached-XY chromosomes, the duplication being passed each generation through attached-X females. Dp^a and Dp^e were carried in stocks consisting of attached-X $\gamma w/sc^s \cdot Y$; $Dp/+$ females and $\gamma w f Y^L \cdot Y^S/sc^s \cdot Y$; $Dp/+$ males. Mottled females of the two lines were crossed to attached-XY males carrying the recessive alleles of the loci to be studied. The genes used were apricot (w^a , at $1.5 \pm$, yellow-orange eyes); white (w , at $1.5 \pm$, between .005 and .02 units to the right of w^a , white eyes); facet (fa , at $3.0 \pm$, rough eyes); facet-notch (fa^n , at $3.0 \pm$, apical nicks in wings); facet-notchoid (fa^{no} , at $3.0 \pm$, .05 units to the right of fa , wings notched, more extreme than fa^n); split (spl , at $3.0 \pm$, .03 units to the right of fa^{no} , rough eye, slightly reduced in size, split bristles); notchoid (nd , at $3.0+$, .03 units to the right of spl , similar to fa^{no} , slightly more extreme); roughest² (rst^2 , deficiency of 3C4 to 3C6 inclusive, very rough eye); and roughest³ (rst^3 , inseparable from $In(1)rst^3$, slightly roughened eye). Cross-over data used to map fa , fa^{no} , spl and nd are from W. J. WELSHONS (personal communication). These genes were studied in several combinations to gain some information on a possible spreading effect of the heterochromatic influence on the euchromatin (DEMEREK and SLIZYNSKA 1937; SCHULTZ 1941).

The genes listed were carried in the following combinations:

1. Attached-X $\gamma w/sc^s \cdot Y$ females \times $w^a fa spl f Y^L \cdot Y^S/sc^s \cdot Y$ males
2. Attached-X $\gamma w/sc^s \cdot Y$ females \times $\gamma fa^n f Y^L \cdot Y^S/sc^s \cdot Y$ males
3. Attached-X $\gamma w/sc^s \cdot Y$ females \times $w^a fa^{no} f Y^L \cdot Y^S/sc^s \cdot Y$ males
4. Attached-X $\gamma w/sc^s \cdot Y$ females \times $nd f Y^L \cdot Y^S/sc^s \cdot Y$ males

5. Attached-X $\gamma w/sc^s \cdot Y$ females $\times rst^2 f Y^L \cdot Y^S/sc^s \cdot Y$ males
6. Attached-X $\gamma w/sc^s \cdot Y$ females $\times w^{60929} rst^3/sc^s \cdot Y$ males (it was not possible to add the fertility factors of the Y to the X chromosome because of the rst^3 inversion).

Each male was mated first to one female carrying Dp^s , and then to a female carrying Dp^a . Male offspring from these two pair matings were counted and classified. Eye characters were scored assigning one of six grades to the observed eyes: 0 for an eye with no wild-type areas (red or smooth areas, depending on the character under consideration), 1 for an eye with one fourth or less of the eye wild type, 2 for an eye with one fourth to one half of the eye wild type, 3 for an eye one half to three fourths wild type, 4 for an eye three fourths to completely wild type, and 5 for an eye completely wild type for the character being studied. Wings were differentiated into three classes: complete expression of the wild-type gene (nd^+ , fa^{n+} , or $fa^{n^{o+}}$), intermediate expression of the dominant allele (slight notching), or complete inactivity of the wild-type gene with consequent full expression of the recessive allele.

To test for the parental-source effect as observed by Drs. SPOFFORD and HESSLER at the white locus, males carrying the duplication and a marked attached-XY chromosome, stocks 1-6, were crossed to females with the duplication and a dominantly marked "normal" third chromosome. Employing this scheme, it was possible to tell whether the duplication was inherited from the mother or the father. Offspring showing the dominant marker inherited the rearranged chromosome from their father. However, offspring without the dominant marker may have inherited the rearrangement from the mother or from both the mother and the father. Progeny tests were made to distinguish between flies that were heterozygous and those that were homozygous for the duplication. The dominant markers used were Wrinkled (W , at 46.0; Wrinkled wings) giving approximately one percent crossing-over with the duplicated region, and Dicheate (D , at 40.4 to $41 \pm$). A CxD chromosome was used to insure a low percentage of crossing-over with the duplication which in this study was less than one percent.

RESULTS

Results of crosses to study expression of variegation of the neighboring loci to white with the two states of the duplication are given in Table 1. In the first cross, 1, notchoid flies were scored for wing phenotype. It was not possible to tell by observation of eye-mottling whether or not the duplication was present in any fly, as no recessive allele such as white or apricot was carried in the X chromosome. Therefore, all male offspring from the pair matings were scored. If the gene nd^+ in the duplication possessed full activity, it would be expected that 50 percent of the male offspring would have normal wings and 50 percent would show the notchoid phenotype (notchoid normally has complete penetrance). Whereas over 50 percent of the offspring of Dp^s crosses showed normal wings, only slightly over one percent of the offspring of Dp^a crosses had normal wings. Thus, the nd^+ gene seems to be completely active in the Dp^s stock whereas

TABLE 1

Comparison of expression of variegation in Dp^6 and Dp^4 males

Genotype of father	Dp contributed by mother	No. of flies scored	Percent nd^+	Percent int. nd^+	Percent nd				
(1) $nd f YL_1 YS/sc^8 Y$	g	695	53.96	7.77	38.27				
	a	569	1.23	33.74	65.03				
(2) $\gamma fa^n f YL_1 YS/sc^8 Y$	g	No. of flies scored 401	Percent fa^{n+} 38.90	Percent int. fa^{n+} 12.42	Percent fa^n 48.68				
	a	1249	11.45	25.38	63.17				
(3) $w^a fa^{n0} spl f YL_1 YS/sc^8 Y$	g	No. of eyes (wings) scored 492	Percent fa^{n0+} 87.60	Percent int. fa^{n0+} 4.07	Percent fa^{n0} 8.33	Avg. grade w^a 4.24±.18	Avg. grade spl 2.68±.66	No. of eyes (w^a fa^{n0} spl) 546	
	a	372	34.14	22.04	43.82	2.74±.25	1.12±.23	400	
(4) $w^a fa spl f YL_1 YS/sc^8 Y$	g	No. of eyes scored 702				Avg. grade w^a 4.33±.17	Avg. grade $fa spl$ 3.33±.23	No. of eyes w^a fa spl 401	
	a	504				1.77±1.06	.89±.54	1249	
(5) $rst^2 f YL_1 YS/sc^8 Y$	g	No. of eyes scored 496				Avg. grade rst^2 4.33±.18		No. of eyes rst^2 180	
	a	546				1.97±.17		1002	
(6) $w rst^2/sc^8 Y$	g	No. of eyes scored 1162	With pigment w^{m} 1.88	With pigment rst^2 1.70±.25	Without pigment w^{m} 0.0	Without pigment rst^2 1.40±.26	Lumped w^{m} 1.68±.16	No. of eyes w rst^2 1040	
	a	180	0.50	0.32±.25	0.0	0.94±.25	0.09±.17	2250	

activity is much reduced in Dp^a flies. However, it is more likely that the nd^+ gene in Dp^s does not have complete activity as evidenced by the 7.77 percent of flies with slightly notched wings. The very high percentage (over 50 percent) of normal-winged flies might be explained as due to a difference in viability between flies with Dp^s and flies without the duplication occurring as the result of some detrimental effect of the recessive notchoid gene, or as the result of a possible as yet undetermined advantage sometimes possessed by Dp^s flies and observed elsewhere with Dp^t (HESSLER 1961; SPOFFORD 1961).

Data from each pair mating were recorded separately and the values obtained from the offspring of each pair were averaged individually. Comparison of these values indicated that considerable variation in expression of variegation existed within each of the lines of the duplication. Consequently, analyses of variance were run on the wing characters, after arc sine transformations had been performed on the averaged percentages of the individual pair matings, in order to determine whether the differences between the states of the duplication exceeded those within each line and to determine whether the former differences were indeed significant. There was a highly significant difference between the two duplication states in the percentage of nd^+ flies, intermediate flies, and nd flies ($F = 53.4, 14.3, \text{ and } 11.8$, respectively, $df = 1 \text{ and } 58$, $P \ll .01$).

In the second cross, 2, the same scoring procedure was used. Again, the flies possessing the duplicated chromosome could not be distinguished from those without the rearrangement. The penetrance of facet-notch is 90 percent in males and considerably less in females. Consequently, instead of the 50 percent notched flies expected in cross 1, only 45 percent of the male offspring would be expected to have notched wings if the fa^{n+} gene present in the rearranged chromosome had full activity. Thus, 55 percent of the male offspring would be expected to have normal wings. Dp^s produced only 38.9 percent normal-winged flies, five percent of which presumably did not carry the duplication, showing that the fa^{n+} gene is not always active in its foreign environment. Dp^a showed an even more reduced activity of the dominant allele, producing only about one third as many normal-winged flies as Dp^s , showing the activity to be approximately one sixth, having subtracted five percent from each of the percentages recorded. The difference between the two duplication states is firmly established by an analysis of variance ($F = 227.6, 27.3, 1.0$ for fa^{n+} , intermediate fa^{n+} , and fa^n , respectively, $df = 1 \text{ and } 42$, $P \ll .01$ for the first two values).

In cross 3, it was possible to grade three characters at once and to compare directly the phenotypic variegation at three loci. The wing character fa^{no} was scored as the other wing characters except that each wing was scored separately instead of scoring both wings of the fly together. This was done to observe better a correlation between wing and eye variegation if such existed. As the apricot gene was present in the X chromosome, it was possible to recognize flies carrying the duplication by observation of w^a mottling; therefore, only flies showing the duplication were scored. In this case, 100 percent of the flies scored should have normal wings if the fa^{no+} gene is acting with its usual efficiency. It can be seen

that the activity of the dominant allele in Dp^s is somewhat reduced, but in Dp^a is reduced to less than half.

Apricot and split variegation were graded, as mentioned earlier, by putting flies into classes according to the amount of normal area within the eye. Only flies possessing the rearranged chromosome were scored. If the genes w^{a+} and spl^+ maintained normal activity in their changed location, all eyes would be given the grade of 5 for apricot and for split and would be completely wild type in appearance. If the genes showed no activity, all eyes would be rated 0 and would be completely apricot and completely rough. Observation of Table 1 shows that Dp^s crosses produced flies which had, on the average, well over three fourths (4.24) of the area of the eyes normal for pigmentation while Dp^a crosses produced flies with an average of less than three fourths (2.74) of the eye showing normal pigmentation. For split variegation, Dp^s gave, on the average, flies with between one half and three fourths of the eye area smooth (2.68), whereas Dp^a crosses resulted in flies with an average of about one fourth of the eye smooth (1.12). These differences between states are highly significant ($F = 27.5, 16.6,$ and 14.7 for fa^{no+} , intermediate, and fa^{no} flies, respectively. $F = 13.45$ for apricot and 14.17 for split variegation. 1 and 27 df, $P \ll .01$).

In cross 4 as in cross 3, three genes were scored simultaneously for phenotypic expression of variegation. However, the combination $fa spl$ is indistinguishable in phenotype from spl alone (WELSHONS 1958). It was thus necessary to grade the $fa spl$ combination as a single unit. Mottling for w^a and for $fa spl$ was graded as in the previous cross, with similar but even more obvious differences appearing between the two duplications. Comparison of flies for $w^a fa spl$ crosses gave F values of 189.41 for apricot mottling and 247.86 for facet-split variegation (1 and 44 df, $P \ll .01$).

The same problem occurred in cross 5 as in the first two crosses. As neither the white nor the apricot gene was present, duplication-bearing flies were not detectable. All male offspring of the pair matings were therefore observed for possible roughest variegation, following the procedure used in grading the other eye characters. Differences were observed between Dp^s and Dp^a offspring similar to those in previous crosses. In this cross, the differences are undoubtedly greater than can be seen by noting only the average eye grade. Fifty percent of the flies would be expected to carry the rearranged chromosome and therefore show a mottled or wild-type phenotype. Dp^s crosses showed over twice as many flies with the duplication, that is, variegated for the roughest character, as flies with entirely rough eyes. This can be explained as the result of the lower viability of roughest² flies, approximately 0.5 times that of wild type. But in Dp^a crosses, over twice as many flies with roughest eyes as with variegated eyes were found. It is evident that the differences between Dp^a and Dp^s would be greater if it were possible to have included only offspring from Dp^a crosses which actually carried the rearrangement. Differences in roughest² variegation resulted in an F value of 87.73 (1 and 49 df, $P \ll .01$).

Because the Y chromosome arms were not attached to the X chromosome in cross 6, many flies which would have shown mottling or a wild-type phenotype

in the presence of this additional heterochromatin, appeared as completely recessive. Additional complications arose because the roughest³ phenotype is itself the result of a position effect brought about by a long inversion of the X chromosome which causes the roughest allele to be placed next to proximal heterochromatin (GRÜNEBERG 1937). Roughest³ flies as kept in stock sometimes show a mottled phenotype. Hence, a position effect superimposed on a position effect was studied. In order to take these factors into account, the data were arranged in three ways. In the first two columns, only flies showing some pigment were averaged. In the next two columns, only flies showing smooth areas but no pigment were averaged, and in the next two columns, data on these groups of flies were combined. Eyes were graded as in the other crosses. Both Dp^g and Dp^a stocks produced flies with a much reduced activity of the *w*⁺ and *rst*⁺ genes in the rearranged chromosome, but Dp^g maintained over three times the activity of Dp^a for the *w*⁺ gene and over five times the activity for the *rst*⁺ gene. A comparison of the numbers of roughest flies and the numbers of variegated flies for each duplication serves to heighten the differences between the two lines. Whereas Dp^g produced approximately the same number of flies with the rearranged chromosome as without, Dp^a produced over 20 times as many flies which did not show the presence of the duplication as flies showing a variegated phenotype. Analysis of the data from *rst*^g crosses, using only flies with some pigment, gave an F value of 9.85 for white-mottling and an F value of 45.42 for mottling of the *rst*^g gene (1 and 41 df, P ≪ .01).

Results of the crosses to test the parental-source effect in Dp^g are given in Table 2. Cross 1 showed a slightly higher percentage of flies with normal expression of *nd*⁺ when the flies received the duplication from the male parent and a correspondingly lower percentage of flies with intermediate or recessive expression of this wing character. These differences were not significant, internal differences within each group of flies being greater than the differences between the two groups according to an analysis of variance of arc sine values of the recorded percentages. Similarly, in the second cross, 2, slightly higher percentages of *fa*ⁿ⁺ flies and slightly lower percentages of recessive flies were found when the rearrangement was inherited from the father. Again, the differences in percentages were not significant. In cross 3, slight differences were again found, this time in the expression of *fa*^{no+}, but these differences were not significant. Flies carrying the rearranged chromosome inherited from their fathers showed more normal expression of the *w*ⁿ⁺ and *spl*⁺ genes, that is, had eyes with more wild-type areas; however, the averaged differences in expression of *w*ⁿ mottling and *spl* variegation were not great enough to be significant. The F values obtained were 2.29 for *w*ⁿ and 4.30 for *spl* variegation (1 and 18 df, P > .05). Cross 4 showed differences in expression of *w*ⁿ and *fa spl* variegation between flies carrying the maternally inherited rearranged chromosome and those carrying the paternally inherited rearrangement. As in previous crosses, flies carrying the rearrangement inherited from the male parent showed, on the average, more nearly normal expression of the wild-type alleles. Analyses of variance for these characters gave F values of 7.31 for *w*ⁿ mottling and 3.20 for *fa spl* variegation (1 and 24 df, P ≪ .01).

TABLE 2
Study of parental-source effect in Dpp^g males

Genotype	No. of flies scored	Percent <i>nd⁺</i>	Percent int. <i>nd⁺</i>	Percent <i>nd</i>	Avg. grade <i>spl</i>	Avg. grade <i>fa spl</i>
(1) <i>nd f Y^L.Ys/sc^s.Y; Dp^g/+</i> <i>nd f Y^L.Ys/sc^s.Y; CxD/Dp^g</i>	139	72.66	23.74	3.60		
	89	73.03	23.60	3.37		
(2) <i>γ faⁿ f Y^L.Ys/sc^s.Y; Dp^g/+</i> <i>γ faⁿ f Y^L.Ys/sc^s.Y; CxD/Dp^g</i>	No. of flies scored 297	Percent <i>faⁿ⁺</i> 93.94	Percent int. <i>faⁿ⁺</i> 2.69	Percent <i>faⁿ</i> 3.37		
	199	95.48	2.51	2.01		
(3) <i>w^a faⁿ⁰ spl f Y^L.Ys/sc^s.Y; Dp^g/+</i> <i>w^a faⁿ⁰ spl f Y^L.Ys/sc^s.Y; CxD/Dp^g</i>	No. of eyes (wings) scored 226	Percent <i>faⁿ⁰⁺</i> 82.74	Percent int. <i>faⁿ⁰⁺</i> 7.52	Percent <i>faⁿ⁰</i> 9.74	Avg. grade <i>faⁿ⁰</i> 4.08 ± .23	Avg. grade <i>spl</i> 2.66 ± .25
	132	93.18	4.55	2.27	4.62 ± .12	3.47 ± .21
(4) <i>w^a fa spl f Y^L.Ys/sc^s.Y; Dp^g/+</i> <i>w^a fa spl f Y^L.Ys/sc^s.Y; W/Dp^g</i>	No. of eyes scored 228				Avg. grade <i>faⁿ⁰</i> 4.42 ± .14	Avg. grade <i>fa spl</i> 3.25 ± .15
	238				4.87 ± .04	3.67 ± .10
(5) <i>w rst³/sc^s.Y; Dp^g/+</i> <i>w rst³/sc^s.Y; W/Dp^g</i>	No. of eyes scored 262				Avg. grade <i>faⁿ⁰</i> 2.39 ± .19	Avg. grade <i>rst³</i> 2.49 ± .19
	230				2.65 ± .24	3.05 ± .55

for the first value, second value not significant at the five percent level). Cross 5 showed slight differences occurring for white-mottling and for roughest variegation. Internal differences were greater than between-group differences for white variegation while analysis of variance of *rst³* differences gave an F value of 1.98 (1 and 19 df, not significant at five percent level).

The results with Dp^s may be summarized by stating that there probably is a slight parental-source effect that favors more normal gene expression if the duplication is inherited from the father. Although this difference is not significant with respect to any of the genes studied, the direction of the difference is identical for all six genes. Thus it appears most likely that all of the genes investigated were being similarly affected by the parental source of the duplication.

Results from the crosses testing for a parental-source effect in Dp^a are given in Table 3. In cross 1 higher percentages of *nd⁺* and correspondingly lower percentages of recessive flies were obtained when the duplication was inherited from the father. These differences in percentages were not significant. In cross 2 slight differences were noted but were not significant. In cross 3 considerable differences were observed in expression of all three of the genes studied. Flies carrying the paternally-inherited rearrangement were significantly closer to wild type in expression of *w^{a+}*, *fa^{no+}*, and *spl⁺*. (F = 7.8 for *fa^{no+}*, 2.45 for percentages of intermediate *fa^{no+}*, and 11.60 for *fa^{no}* percentages. F = 11.4 and 38.3 for *w^a* and for *spl* variegation respectively. 1 and 24 df, P < .01 for all values). In cross 4, significant differences were observed between expression of *w^a* and *fa spl* variegation, flies carrying the paternally-inherited rearrangement being closer to wild-type in phenotypic expression (F = 11.86 for differences in expression of *fa spl* variegation). In cross 5 no differences were evident in expression of white variegation and the slight differences observed in expression of *rst³* variegation were not significant.

Therefore, the effect of the parental source of Dp^a on expression of these genes may be summarized by stating that Dp^a flies show more normal expression of the genes when the rearrangement is inherited from the father. Flies receiving the duplicated region from the mother are nearer to the recessive in phenotypic expression of the genes.

DISCUSSION

The results of the analyses show significant differences existing between the two states of the duplication, Dp^s and Dp^a, in spite of the variations that sometimes occur within each duplication stock; that is, differences between the two stocks far exceed their internal differences. It can easily be seen that the distinctions between the two stocks for mottling at the white locus indeed exist for other loci of the duplicated region. These differences are similar no matter whether the genes in question affect eye pigmentation, facet arrangement, or wing structure. It is therefore necessary to look for a general metabolic effect to explain the difference between Dp^s and Dp^a.

The fact that it must be a general metabolic effect causing the distinction between the states of the duplication would seem to point to an effect on the original

TABLE 3
Study of parental-source effect in Dp^a males

Genotype	No. of flies scored	Percent <i>nd⁺</i>	Percent int. <i>nd⁺</i>	Percent <i>nd</i>	Percent <i>fg^a</i>	Percent <i>fg^{no}</i>	Avg. grade <i>sp^a</i>	Avg. grade <i>fa spl</i>
(1) <i>nd f Y^L.Y^S/sc^a.Y; Dp^a/+</i> <i>nd f Y^L.Y^S/sc^a.Y; CxD/Dp^a</i>	176 90	17.61 34.45	59.09 51.11	23.30 14.44				
(2) <i>γ fa^a f Y^L.Y^S/sc^a.Y; Dp^a/+</i> <i>γ fa^a f Y^L.Y^S/sc^a.Y; CxD/Dp^a</i>	155 142	Percent <i>fg^{a+}</i> 72.26 63.38	Percent int. <i>fg^{a+}</i> 11.61 32.39	Percent <i>fg^a</i> 16.13 4.23				
(3) <i>w^a fa^{no} spl f Y^L.Y^S/sc^a.Y; Dp^a/+</i> <i>w^a fa^{no} spl f Y^L.Y^S/sc^a.Y; CxD/Dp^a</i>	No. of eyes (wings) scored 166 78	Percent <i>fg^{no+}</i> 65.66 74.36	Percent int. <i>fg^{no+}</i> 15.67 25.64	Percent <i>fg^{no}</i> 18.67 0.00			Avg. grade <i>sp^a</i> 1.83±.24 3.58±.18	
(4) <i>w^a fa spl f Y^L.Y^S/sc^a.Y; Dp^a/+</i> <i>w^a fa spl f Y^L.Y^S/sc^a.Y; W/Dp^a</i>	No. of eyes scored 526 402			Avg. grade <i>fa spl</i> 2.44±.26 3.12±.14	
(5) <i>w rst^β/sc^a.Y; Dp^a/+</i> <i>w rst^β/sc^a.Y; W/Dp^a</i>	No. of eyes scored 518 370			Avg. grade <i>rst^β</i> 0.23±.06 0.44±.11	

action of the heterochromatin in inducing a position effect on the genes within the duplicated region. As flies of the Dp^s line are closer to normal in the expression of all the genes previously affected, it would seem likely that in some way Dp^s is counteracting the suppressive effect of the heterochromatin on the action of neighboring genes. There are many possible mechanisms: one is that Dp^s produces a substance which acts as an inhibitor of the heterochromatic effect; another is that it acts by altering the time at which cells become determined in the imaginal discs; still another possibility is that the difference lies outside the duplication *per se*, perhaps in the heterochromatin.

A gene has been discovered by DR. SPOFFORD (personal communication) which produces striking changes in expression of variegation at the white locus. Flies possessing this modifier show a phenotype much closer to wild type. Preliminary experiments with an $w^a fa^{no} spl f Y^L \cdot Y^S/Y$; $Dp^a/+$ stock show that this modifier affects the other genes of the rearrangement as well as the white gene. Whether or not this modifier, which is linked to the duplication, constitutes the main differences between the states of the duplication, the fact that it does affect several loci shows that the differences noted between Dp^a and Dp^s at these several loci may possibly be brought about by the action of a single gene.

The results of the crosses testing for parental-source effects of Dp^s and Dp^a indicate that the effects noted for variegation at the white locus occur also at other loci of the rearrangement. It is not possible at this time to provide an explanation of the mechanism behind this parentally-caused difference in expression of variegation except to say that since all genes were affected similarly, an action on the original position effect is implied.

The simultaneous observation of variegation at two or three loci made it possible to test for the existence of a spreading effect such as was observed for w^{258-18} and the associated roughest gene by DEMEREC and SLIZYNSKA (1937) and for w^{258-21} and the neighboring split gene by SCHULTZ (1941). In both of these examples, the gene located closer to the heterochromatin and to the centromere was more often caused to lose activity and the gene beyond was never affected without the closer gene being affected as well. In other words, the influence of the heterochromatin or centromere moved along the chromosome and did not bypass one gene to affect another gene further away. In these two cases, it was not clear whether it was the location of the heterochromatin or the centromere that was responsible for the spreading since both were present on the same side of the euchromatic segment. In the rearrangement studied here, heterochromatin is present on both sides of the euchromatin whereas the centromere is, of course, at one side. Therefore, the spreading of the heterochromatic influence would be expected to occur from both sides of the duplicated region if it were the responsible agent. If the centromere were responsible, a polarized action would be observed. In the $w^a fa^{no} spl$ cross, flies were found which had eyes with apricot regions that were smooth, apricot regions that were split in phenotype, red regions that were smooth, and red regions that were split. Evidently the heterochromatin from each side is capable of reducing the activity of the genes located in the euchromatic region; there is no evidence of a centromere influence.

SUMMARY

In *Drosophila melanogaster*, two states of the duplication $Dp(w^m)264.58a$, designated Dp^a and Dp^s , were determined in previous studies to differ from one another in general phenotypic expression of variegation at the white locus and in phenotypic response to parental genotypes. The studies reported were made in order to determine whether these differences existed at other loci within the duplicated region: namely, facet, facet-notch, facet-notchoid, split, notchoid, roughest² and roughest³. The difference in expression of variegation of these loci with Dp^a and Dp^s was found to parallel the difference found in white-variegation. Therefore, the mechanism responsible for differentiating these two states of the duplication insofar as white-variegation is concerned acts in a similar manner on other loci over a considerable segment of the chromosome. Parental effects on these loci were observed in both states of the duplication to be similar to those noted for mottled expression of the white gene. It seems likely that the differences exhibited by the states of the duplication and the parental source are caused by modification of the original suppression of gene action responsible for the position effect.

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