8, 309 (1959)). The tritium is on the 4 (thymidine) or 4 and 5 (cytidine) carbon atoms of the pyrimidine nucleus.

[20] Mitchell, H. K., personal communication.

[21] The transformation of the soft, segmented larval cuticle into the hard, unsegmented cuticle of the prepupa, called the puparium, takes only a few minutes at 25°C. The time at which the transformation occurred was known to the nearest half hour.

[22] Fine-grain Autoradiographic Stripping Plates, AR-10, Kodak, Ltd., London, Eng.

[23] Taylor, J. H., in *Physical Techniques in Biological Research*, G. Oster, and A. W. Pollister, ed., (New York: Academic Press, 1956).

[24] Bergeron, J. A., *Stain Technology*, **33**, 221 (1958).

[25] Bridges, C. B., *J. Heredity*, **26**, 60 (1935).

[26] Rudkin, G. T., and J. A. Aronson, unpublished data.

[27] Taylor, J. H., personal communication.

[28] Experiments in progress in which one of the glands of a larva was treated with ribonuclease before autoradiographic exposure, have revealed that tritium introduced in cytidine is removed by the enzyme from the puff regions, as well as from the rest of the chromosomes. A very few nuclei contain RNAase resistant label in their chromosomes. Presumably they correspond to the nuclei that incorporated H^3-thymidine and have utilized the pyrimidine moiety of the injected cytidine in DNA synthesis. In parallel experiments it was found that deoxyribonuclease does not affect the observed frequency (100 per cent) of nuclei labeled by H^3-cytidine.

[29] Breuer, M., and C. Pavan, *Chromosoma*, **7**, 371 (1955).

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*PARENTAL CONTROL OF POSITION-EFFECT VARIEGATION: I. PARENTAL HETEROCHROMATIN AND EXPRESSION OF THE WHITE LOCUS IN COMPOUND-X DROSOPHILA MELANOGASTER**

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Communicated by Sewall Wright, May 11, 1959

In studies of the role of extra heterochromatin in position-effect variegation at the white locus,[1, 2] discrepancies between the offspring of reciprocal crosses stimulated a search for possible genetic factors controlling gene expression in the succeeding generation. Two genotypes of mothers have already been found to enhance pigmentation in the eyes of genetically identical offspring—a recessive modifier and homozygosity as compared with heterozygosity for a w^m rearrangement.[3, 4]

It is well known that the direct effect of extra Y-heterochromatin in the genome of a fly is partial restoration of the normal phenotype disturbed when a chromosomal rearrangement juxtaposes a normally euchromatic locus to interrupted heterochromatin. The experiments reported here indicate that extra Y-heterochromatin in the parental genotype also "residually" affects offspring phenotype.

Exploratory.—Variegation was due to a 15-band insertion including w^+, Dp(w^m)-264.58a, into the proximal heterochromatin of 3L. The heterochromatin content of the genotype of the test flies was augmented by a normal Y, sc^{S1}. Y^L#2 or Y^S. Y^S#2 or Y^S:y^+ bb^+ −5 (described elsewhere[2]). Males used in the experimental crosses had an attached-XY chromosome, Y^S w y·Y^L y^+; females had a y w attached-X chromosome from a recent single ancestor. In order to reduce extraneous genetic variation, the long autosomes of all stocks were rendered initially as co-isogenic as

possible by the Cy; Ubx/Xa method[2] and crosses between stocks to yield the experimental parents were such as to randomize or eliminate background genetic differences between parents being compared. Even the distal parts of the normal and Dp-bearing third chromosomes were probably nearly identical.

All test crosses were made reciprocally for the duplication. Residual effects of the paternal extra heterochromatin (Y^F) could be assessed in XY/Y sons of XY/Y^F fathers; residual effects of the maternal Y^F, in XX/Y daughters of XX/Y^F mothers. In the other progeny, the direct effect of the Y^F is confounded with the residual effect. There were thus 18 crosses (including $Y^F = Y$) distributed into four categories, indicated in Table 1. The experiment was seriated, each of the five series including for each cross at least 5 simultaneous pair matings in vials containing aliquots of a single batch of medium. Parents were transferred to fresh medium at least once.

TABLE I

PARENTAL EFFECTS OF FIVE Y-CHROMOSOMES AND SOURCE OF Dp(w^m) ON OFFSPRING EYE PIGMENTATION[a]

Parent Contributing Dp	Y^F	Y	Genotype of Offspring	"Y^F" of Indicated Cross: Y Mean	Y No.[b]	$sc^{S1}.Y^L$ Mean	$sc^{S1}.Y^L$ No.	$Y^S.Y^S$ Mean	$Y^S.Y^S$ No.	$Y^S:y^+bb^+5$ Mean	$Y^S:y^+bb^+5$ No.	O Mean	O No.
♀	♀	♂	XX/Y; Dp/+	0.116	156	0.150	132	0.309	251	0.250	238	0.038	43
♂	♀	♂	XX/Y; Dp/+	0.130	148	0.205	278	0.521	431	0.561	324	0.114	157
♀	♂	♀	XX/Y^F; Dp/+	0.116	156	0.082	179	0.063	213	0.125	335	0.000	0
♂	♂	♀	XX/Y^F; Dp/+	0.130	148	0.078	98	0.051	110	0.108	92	0.000	0
♀	♀	♂	XY/Y^F; Dp/+	0.388	156	0.392	95	0.463	227	0.558	159	0.000	0
♂	♀	♂	XY/Y^F; Dp/+	0.847	132	0.771	191	1.025	352	1.395	215	0.001	3
♀	♂	♀	XY/Y; Dp/+	0.388	156	0.427	231	0.360	351	0.445	346	0.281	299
♂	♂	♀	XY/Y; Dp/+	0.847	132	1.013	84	0.981	140	0.930	97	1.017	132

ORTHOGONAL ANALYSIS OF VARIANCE

Offspring Genotype	Source of Variance	D. F.	Mean Square[c]	Variance Estimate Total	Variance Estimate Per Cent
XY/Y^F; Dp/+ (excluding $Y^F = 0$)	Parental source of Dp	1	3.1291*	0.1537	65.4%
	Y^F in genotype	3	0.3190*	0.0264	11.2%
	Between series	4	0.1096	...	...
	Residual[d]	35	0.0550	0.0550	23.4%
XY/Y; Dp/+	Parental source of Dp	1	4.1660*	0.1658	82.3%
	Father's Y^F	4	0.0151	...	...
	Between series	4	0.0904*	0.0071	3.7%
	Residual	42	0.0193	0.0193	10.0%
XX/Y^F; Dp/+ (excluding $Y^F = 0$)	Parental source of Dp	1	0.0003	...	...
	Y^F in genotype	3	0.0097†	0.0007	21.8%
	Between series	4	0.0016	...	...
	Residual	35	0.0026	0.0026	78.2%
XX/Y; Dp/+	Parental source of Dp	1	0.2240	...	...
	Mother's Y^F	4	0.2559†	0.0218	60.7%
	Interaction between Dp-source and Y^F	4	0.0382*	0.0064	17.8%
	Between series	4	0.0231†	0.0017	4.8%
	Residual	35	0.0060	0.0060	16.7%

[a] Sum of the visually estimated amounts of pigment in both eyes.
[b] Number of flies showing any pigmentation.
[c] Asterisk indicates significance at the 1 per cent level; dagger at the 5 per cent level.
[d] The three possible two-way interactions were tested against the three-way interaction and except in the one instance noted were not significant. Their sums of squares and degrees of freedom were pooled to give the "residual" mean square.

For all offspring, the amount of pigment in each eye was estimated visually and recorded, the scale ranging from 0 for white to 1.0 for a wild-type eye. The estimates correlate highly with photofluorometric measurements after chromatography.[2]

Each mean in Table 1 is an unweighted average over the 5 series. The value for any one series was the weighted mean of all pigmented offspring when preliminary

analyses of variance indicated homogeneity between replicate pairs; otherwise, it was the unweighted mean of sib means. No transformation of scale was attempted since, though within-sib σ was correlated with m for means below 0.25 (one-eighth of the amount of pigment in a wild-type fly), σ was practically constant for larger m's. Orthogonal analyses of variance were performed on the series means.

The greatest source of phenotypic variation in sons (86 per cent in one case, 65 per cent in the other) was the parental source of Dp (w^m), there being 2.5 times as much pigment when the father rather than the mother contributed it to XY/Y sons. The paternal Y^F had no residual effect on sons. The absence of interaction between Dp-source and father's Y^F confirms the lack of divergence at possible modifying loci on the third chromosome in the Y^F stocks.

Most of the variation in XX/Y daughters (62 per cent) is attributable to the residual effect of the maternal Y^F. Thus at least part of the differences between sons with different Y^F's has the same cause. Whether sex of the parent transmitting the duplication affected average grade of pigmented daughters depended on the maternal Y^F—the increase with paternal source being least (1.1-fold) when the mother had a normal Y, greatest (3.0-fold) when she had no Y.

Resolution of Direct and Parental Effects.–The second experiment assessed direct as well as indirect effects of $Y^S{:}y^+\ bb^+ - 5$(abbreviated as Y^5) and the normal Y, since the residual effects of mother's Y^F were observed in both XX/Y and XX/Y^5 daughters. Ten pair matings of each cross (Table 2) were made simultaneously and transferred to fresh medium three more times.

Table 2 gives both the mean grade of offspring which were pigmented and the percentage of all offspring which they constituted, theoretically 50 per cent if full penetrance and no viability differential. Pairs of mean grades were compared by t tests based on within-sib σ's according to the Cochrane and Cox method described by Snedecor.[5] When the replicate pairs were heterogeneous, the unweighted mean was used with an error computed from the excess of total variance over that attributable to within-sib variation.

The maternal heterochromatin has the same phenotypic effect on both Y- and Y^5-bearing daughters (cf. lines 1, 4, 7, 9 versus 2, 5, 8, and 10, respectively). A maternal Y^5 rather than Y more than doubles the proportion of pigmented daughters (though never over 50 per cent), all comparisons being significant at the 0.01 per cent level. The amount of pigment when present is similarly increased, no comparison failing significance at the 5 per cent level. Most interestingly, lack of any maternal Y more drastically reduces penetrance and expression when the mother carries rather than lacks Dp(w^m) (cf. lines 3 and 6).

In contrast to its residual effect as compared to the Y, the direct effect of Y^5 reduces both penetrance and pigmentation to about 65 per cent (cf. lines 1, 2, 4, and 5 versus 7, 8, 9, and 10). All these comparisons are consistent though not all statistically significant. Background stock differences between this and the preliminary experiment may account for the lesser direct pigment enhancement by the Y^5 here.

Independence of the direct and maternal effects of the Y^5 on penetrance when incomplete is shown by a low interaction $\chi^2 = 0.999$ $(0.30 < P < 0.35)$.

A paternal rather than maternal source of Dp(w^m) more than doubles the proportion of pigmented daughters (e.g., cf. lines 1 and 4), although as before the *amount*

TABLE 2

RESULTS OF FURTHER CROSSES EMPLOYING Y, Y^s:y^+ bb^+ − 5 AND NO FREE Y CENTROMERE, VARYING PARENTAL SOURCE OF Dp(w^m)

		Genotype of:[a] Father	Mother	Total	Per Cent with Pigment[b]	Mean Pigment ± Error	Number of Sib-ships
		Daughters (XX/Y^F; Dp/+)					
Y	(1)	Y; +/+	Y; Dp/+	332	13.9	0.070 ± 0.017	4
	(2)	Y; +/+	Y^5; Dp/+	230	34.8	0.123 ± 0.019	2
	(3)	Y; +/+	O; Dp/+	159	1.3	0.030 ± 0.010	2[c]
	(4)	Y; Dp/+	Y; +/+	458	34.7*	0.073 ± 0.009	8
	(5)	Y; Dp/+	Y^5; +/+	794	49.5	0.219 ± 0.037	10
	(6)	Y; Dp/+	O; +/+	446	36.6*	0.051 ± 0.005	8
Y^5	(7)	Y^5; +/+	Y; Dp/+	494	8.7	0.039 ± 0.008	5
	(8)	Y^5; +/+	Y^5; Dp/+	1,034	18.8	0.118 ± 0.020	9
	(9)	Y^5; Dp/+	Y; +/+	516	22.3†	0.049 ± 0.005	9
	(10)	Y^5; Dp/+	Y^5; +/+	661	44.9	0.140 ± 0.042	7
				159	27.0	0.074 ± 0.017	2
	(11)	Y^5; Dp/+	O; +/+	633	25.6*	0.038 ± 0.003	10
O	(12)	O; +/+	Y; Dp/+	678	0.0	...	8
	(13)	O; +/+	Y^5; Dp/+	493	0.0	...	6
	(14)	O; Dp/+	Y; +/+	261	0.0	...	4
		Sons (XY/Y^F; Dp/+)					
Y	(15)	Y; +/+	Y; Dp/+	325	28.6	0.166 ± 0.025	4
	(16)	Y^5; +/+	Y; Dp/+	438	30.8	0.135 ± 0.022	5
	(17)	O; +/+	Y; Dp/+	609	23.6*	0.077 ± 0.010	8
	(18)	Y; Dp/+	Y; +/+	378	49.2	0.520 ± 0.192	6
	(19)	Y^5; Dp/+	Y; +/+	445	54.4	0.535 ± 0.093	8
	(20)	O; Dp/+	Y; +/+	258	48.1	0.568 ± 0.196	4
Y^5	(21)	Y; +/+	Y^5; Dp/+	181	39.8	0.241 ± 0.104	2
	(22)	Y^5; +/+	Y^5; Dp/+	893	40.3	0.248 ± 0.128	9
	(23)	O; +/+	Y^5; Dp/+	491	32.4*	0.198 ± 0.028	6
	(24)	Y; Dp/+	Y^5; +/+	687	56.9	0.960 ± 0.114	10
	(25)	Y^5; Dp/+	Y^5; +/+	712	48.7*	1.000 ± 0.236	9
O	(26)	Y; +/+	O; Dp/+	146	0.0	...	2[c]
	(27)	Y; Dp/+	O; +/+	483	0.0	...	8
	(28)	Y^5; Dp/+	O; +/+	664	0.4	0.005 ± 0.002	10

[a] The crosses from which these parents came all contain the XY and XX derived initially from one stock used in the experiments recorded in Table 1, the XY/O/XX stock. Several different compound-X chromosomes had been used in males of stocks crossed to yield the parents of Table 1.

[b] Asterisk indicates significant inhomogeneity of frequency of white versus variegated flies between sibships at the 5 per cent level; dagger at the 1 per cent level.

[c] Since the Dp-bearing mothers cannot be distinguished phenotypically from their +-bearing sisters, 18 such were used. Only if w^m daughters appear can the mother's genotype be established. Since these two progenies had 1 w^m daughter apiece, it is likely that other Dp-bearing mothers escaped detection.

of pigment, when present, is greater only in Y-bearing daughters of Y^5 mothers (cf. lines 2 and 5).

Examination of the phenotypes of the sons indicates that the Dp-source effects and the direct and residual maternal effects of extra Y-heterochromatin are of the same magnitude as in daughters, making due allowance for the 50 per cent upper limit on proportion pigmented. With a maternal Dp(w^m), XY/Y^5 sons include 1.3 times as many pigmented flies as XY/Y sons (cf. lines 15 and 21), a ratio not unexpected, given the direct (0.65-fold) and residual (2-fold) effects inferred from their sisters. The amount of pigment when present is roughly quadrupled when the Dp(w^m) was paternal rather than maternal (cf. lines 15 versus 18, 16 versus 19, 21 versus 24, and 22 versus 25), and is increased 1.8-fold by Y^5 as compared to Y (cf. lines 15 versus 21, 16 versus 22, 18 versus 24, and 19 versus 25). Although there appears to be a significant residual effect of the paternal Y or Y^5, it occurs only when the Dp(w^m) was maternal and was not established by the earlier experiment.

Testcrosses of the above progeny have indicated no persistent (i.e., "grand-

parental") effect of the various Y^F's. They do suggest a phenotypic influence of the sex of the grandparent from which the Dp(w^m) was inherited, an influence not attributable to paramutation such as occurs at the R locus in maize.[6] Further investigation is required to clarify the mechanism of this influence.

Any factor which increases pigment in Dp-bearing flies may operate either (1) locally to palliate the inhibition of normal gene action in the vicinity of disturbed proximal heterochromatin or (2) more efficiently to employ in pigment synthesis whatever the w^+ gene still manages to produce. The direct phenotypic effect of any kind of Y as compared to its total absence may be either or both of these. Another such factor must be a normal product of the Y^5 fragment which persists in the egg after the maturation divisions, since the maternal effect of the Y^5 as compared to the Y is as great when the mother lacks Dp(w^m) as when she possesses it. In the presence of Dp(w^m), there is an additional residual maternal effect of both the normal Y and the Y^5 in contrast to total lack of the Y. One of many possible explanations might be that the impaired activity of the abnormally situated w^+ gene evokes substances inhibitory to pigment formation which accumulate in the cytoplasm of the maturing oöcyte. Since both the Y and Y^5 partially restore normal w^+ activity, they would lessen this accumulation. This hypothesis has the merit of explaining also the greater efficacy of the w^+ gene in the rearrangement when introduced by sperm, but requires test. In any case, the earliest detectable phenotypic effect of these oöplasmic substances occurs at the time of pigment synthesis in the pupa.

Summary.—The amount of heterochromatin in the genome has long been known to influence directly the extent of somatic variegation induced by a position-effect chromosomal rearrangement in *Drosophila*. It has now been shown that the extent of pigmentation in white-variegated eyes does not depend solely on this direct effect of heterochromatin but also is clearly enhanced if: (1) the maternal genome contains certain Y-chromosome fragments, and (2) if the rearrangement responsible for the position-effect variegation is paternal, as compared with maternal, in origin. From the standpoint of the genetic control of pigment differentiation, the novel feature displayed here is that the parental genotype can influence an ontogenetic process in the offspring that takes place rather late in the time sequence leading to an imago.

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

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