PHENOTYPIC EFFECTS OF Y CHROMOSOME HYPERPLOIDY IN DROSOPHILA MELANOGASTER, AND THEIR RELATION TO VARIEGATION¹

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WHEN the history of the cytogenetic knowledge of the Y chromosome of *Drosophila melanogaster* is reviewed, the impression is gained that in part at least the Y has proven so refractory to genetic analysis because, *a priori*, the cytologist and geneticist alike have always expected so little activity from it, and have tended to emphasize its relative dispensability from the chromosome set and its heterochromatic nature. Needless to say such an expectation was at one time entirely reasonable, for neither the XXY female nor XO male (aside from infertility) show any evidence that the Y chromosome possesses a phenotypic action. But it is now known to be a cytogenetic system of very considerable complexity, although this complexity has not been worked out in even fair genetic detail. Not only does it possess a series of fertility factors (K_1 and K_2) in each arm, at least the bobbed locus which is homologous with that in the chromocentral region of X, a nucleolus organiser, some dozen or so bands when in the polytene state, and at least two specialized pairing organelles (similar to those of X, COOPER 1952), but, just as with the chromocentral regions of the other chromosomes, it has a pronounced interaction with variegating position effects.

The chromocentral regions of all of the chromosomes in *Drosophila melanogaster* are commonly held to be genetically depauperate elements, together sharing ill-defined but wide-ranging and important cytologic and genetic properties. These properties are said by most to be conferred upon these regions by heterochromatin, or more properly, are said to be owing to their heterochromatic nature. Now heterochromatin, unlike all other genetic entities, is accepted as pluripotent to an almost fabulous degree. Heterochromatin is said to affect the entire dimensions of all chromosomes of a set, to play a leading role in nucleic acid metabolism, to affect rates of development, of mutation, of chromosome pairing, of crossing over, of chromosome breakability and rejoinability, to determine localization of chiasmata, to bring about conjunction at meiosis, to affect gene penetrance and expressivity, and so on. There is very little doubt that each of these effects has been shown or made reasonable as a property within one or another heterochromatic element, but it does not seem reasonable to lump them together as attributes simply to be *expected* from heterochromatic portions of chromosomes or of entirely heterochromatic chromosomes (if such exist). Can a manifold of properties engendered by an allegedly little differentiated system, such as heterochromatin is supposed to be, be willingly accepted

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with so little proof? Not until the heterochromatic regions are genetically analysed can it be known what the actual basis for these attributes may be. At the present, however, any hypothesis which importantly involves heterochromatin is, by that very token, likely to be so elastic as to be untestable.

The Y chromosome is also heterochromatic, or heteropycnotic in the cytologist's sense, like the other members of the chromocentral set. For this reason a good number of experimenters find in it a convenient means for "adding heterochromatin" to a genotype. Regrettably there is a tendency to lose sight of the fact that a Y chromosome, not simply "heterochromatin," has been added to the genotype, and that the deductions may more properly relate to the genetic constitution of the Y chromosome than to that yet to be demonstrated catch-all, "heterochromatin." In comparison with what is known for the euchromatic lengths, there seems no question that the polytene bands of the Y are somehow related to its genic constitution, whether the bands contain genes, or simply act as spacers separating genes that carry on unrelated or mutually conflicting activities. Until more is known of the cytogenetics of the Y chromosome and of the chromocentral regions, it will probably be more profitable to endeavor to dissect their genetic systems with respect to their activities, than to add to the already impressive total of attributes assigned to "heterochromatin."

The substance of the paper which follows is partially the outcome of segregation studies in male and female complex hyperploidies in *Drosophila melanogaster*, all of which were carried out at $25^{\circ} \pm 0.5^{\circ}\text{C}$. As will become clear, certain Y chromosome hyperploidies and fractional hyperploidies can be recognized by the visible phenotypic effects of the Y chromosomes alone. These are of a sort which brings forcibly to mind the variegating position effects, and so the problems of dosage effects by Y chromosomes and their possible relation to the V-type position effects are discussed. They are not discussed as involving attributes of heterochromatin, but as the outcome of the actions of supposed genic systems in the chromocentral elements which are subject to test and possible disproof. Whatever one's view of heterochromatin, those who consider these problems must find themselves especially indebted to the work and reflections of JACK SCHULTZ, as well as to E. B. LEWIS for his very helpful review of position effects (LEWIS 1950).

ANALYSIS OF AN APPARENTLY SPONTANEOUS MOTTLE

Among the offspring from a pair mating in a series of crosses: *Ins (1) dl-49, Bm¹, sc v/ y² apr cv v f⁺ Y^S × Canton-S (= CS) male*, there occurred a single exceptional *v* female with blotched eyes, having pale washed-out areas on approximately the posterior fifths of both eyes. Bred by a CS male, the exceptional female produced 108 offspring: 11 ♀ and 15 ♂ exceptions, and 48 ♀ and 34 ♂ regular offspring. Five of the exceptional females resembled their mother, having washes of color removed from posterior portions of the eyes. In addition, two of the regular females had wild type eyes in which color was also conspicuously reduced or diluted in a more or less triangular blotch at the posterior marginal field. All other offspring seemed phenotypically normal.

These mottled-eyed *F*₁ females, bred by CS males, again produced some mottled

female offspring, and the irregular ratios in their progeny of mottled to non-mottled classes, as well as the absence of mottled-eyed males, suggested a genetic system involving factors of more than one chromosome. Systematic replacement of autosomes, and of the X chromosomes, in fact showed that the mottling occurred only in the presumably $\gamma^2 \text{ apr cv v } \widehat{Y^S}/X/Y$ females, for XXV daughters without the $X\widehat{Y^S}$ chromosome, and $X\widehat{Y^S}/X$ females without a Y, had unblotched eyes of normal phenotype.

Test crosses of mottled-eyed $[Mo] \gamma^2 \text{ apr cv v } \widehat{Y^S}/CS/Y$ females by $sc, dl-49 v, B^{M1}$ regular sons of $[Mo] sc, dl-49 v, B^{M1}/\gamma^2 \text{ apr cv v } \widehat{Y^S}/Y$ females also gave numbers of mottled-eyed female offspring. Oddly, some of these $[Mo]$ females proved to be $sc, dl-49 v, B^{M1}/CS$ in X-chromosomal constitution. It was clear, then, that the $X\widehat{Y^S}$ -chromosome is not essential for the production of a mottled eye. Since these phenotypically wild type but mottled-eyed females gave less than 40% nondisjunction, whereas $dl-49, B^{M1}/+/Y$ females regularly give nearly 80% secondary nondisjunction of the X chromosomes (COOPER 1948), it was suspected that they carried two Y chromosomes. This was reasonable, for half of the fathers should have been X2Y males. In each cytologically analysed case the mottled-eyed $sc, dl-49 v, B^{M1}/CS$ females did in fact prove to be XX2Y, whereas their not mottled sisters were XXY. Two possibilities seemed most promising for test: (1) the vermilion alleles present in the $dl-49, B^{M1}$ and $X\widehat{Y^S}$ chromosomes variegate in the presence of two Y-chromosomes, or their near equivalent (as in the $\overline{X}Y^S/X/Y \text{ } \varnothing$); (2) two Y chromosomes (or their equivalent) will *by themselves* cause mottling of the female eye.

The first possibility was easily eliminated. Among the regular males of $[Mo] sc, dl-49 v, B^{M1}/CS/2Y$ by $f B/Y$ males, CS/2Y males should be frequent. Crosses of such mottled-eyed females by their regular CS brothers produced some CS/CS females which also had mottled eyes. These mottled homozygous wild type females were cytologically shown to be XX2Y; clearly, unless an unusual maternal effect was involved, or an unusual Y chromosome, the mottling must be related primarily to the number of Y chromosomes present, and not to a peculiar vermilion allele.

PROOF THAT SUPERNUMERARY Y CHROMOSOMES GENERALLY HAVE A PHENOTYPIC EFFECT

If a maternal influence is not necessary for the mottling of the eyes of a $+/+$, $v/+$, or v/v female carrying two Y chromosomes, nor any special genes or special Y chromosomes, then (1) it should be possible to produce mottling in a standard wild type stock simply by providing the females with two Y chromosomes; (2) on the average, half of the crosses of regular males from secondary nondisjunctive stocks by their exceptional XXY sisters should give mottled female offspring, and these mottled-eyed females should be XX2Y; (3) such mottling ought to be brought about by Y chromosomes of unrelated stock origins; (4) if the effect also occurs in males, then it is to be expected that the highly hyperploid male (X3Y or X4Y) will, like its XX2Y sister, have mottled eyes; and, finally, (5) females having a genotype equivalent to XX2Y, whether carrying free normal Y chromosomes or not, ought to have mottled eyes. Each of these expectations has been tested and, as shown below, each in fact is realized in practice. The normal Y chromosome, therefore, has a characteristic phenotypic effect when hyperploid.

TABLE 1

Crosses of XXY or attached-X/Y females by regular sons of XXY females. These males are equally likely to be XY or X2Y. If two Y chromosomes cause the eye of a female to mottle, then one half of these crosses should produce mottled-eyed daughters

(= [Mo] ♀ ♀) (see text, p. 245)

Constitution of ♀	Total no. of crosses	Number giving [Mo] ♀ ♀
CS/CS/ Y ^{CS}	11	2
y v f car y v f car/ Y ^{CL}	8	4
sc, dl-49 v, B ^{M1} / y ² apr v/ Y	32	16
sc, dl-49 v, B ^{M1} / CS/ Y	17	5
sc, dl-49 v, B ^{M1} / Swed-b/ Y	27	12
dl-49 v ^o , f/ y ² apr v/ Y	33	16
Totals	128	55

$$\chi^2 = 2.53, P_1 = 0.1$$

Production of mottled-eyed females in the wild type Canton-S stock

A wild type stock of Canton-S, inbred for 120 consecutive generations by brother-sister pair-matings, was the source of the female parents. A *f B* stock, carrying the Y chromosome and all its autosomes from the Canton-S stock, provided the male parents. Pair-matings: CS ♀ × *f B*/ Y^{CS} gave but 1 CS/ CS/ Y^{CS} female in approximately 12,000 female offspring.² By crossing the exceptional female by a *f B*/ Y^{CS} male, and continuing to do so with her exceptional female descendants thereafter, the secondary nondisjunctional stock was multiplied. Eleven pair-matings were made of CS/ CS/ Y^{CS} females by regular brothers (having an equal likelihood of being CS/ Y^{CS} or CS/ 2Y^{CS}). Of these, two produced a number of mottled-eyed Canton-S females. Thereafter a mottled-eyed wild type Canton-S line was maintained through some 20 generations by pair-matings of mottled-eyed Canton-S females by their Canton-S brothers. The mottling of the eyes was attributable only to the presence of two Y chromosomes in the mottled-eyed females, or to some peculiarity of the Canton-S stock that results in variegation when a female carries two Y chromosomes. As will become clear, the former interpretation is the correct one.

Do half the crosses of exceptional females by regular brothers produce mottled-eyed daughters?

Table 1 summarizes the results of 128 pair-matings of regular males (equally likely to be XY or X2Y) from secondary nondisjunctional stocks by XXY or attached-X/Y females. If a female carrying two Y chromosomes generally has mottled eyes, then roughly half of these pair-matings should give mottled-eyed females among their offspring. In all, 55 of the 128 matings produced some mottled daughters; $\chi^2 = 2.53$, and $P \approx 0.1$, a result consistent with the prediction.

² There was also but 1 *f B*/0 exceptional male among a like number of male offspring. Such a low frequency of primary nondisjunction (0.016%) is usual when isosequential stocks are employed.

Aceto-orcein squashes were made of the ovaries of 60 of the mottled-eyed daughters from these crosses, and of 21 of their not mottled-eyed sisters. Of the mottled-eyed females (19 of which were v/v , and 41 were $v/+$ or $+/+$), 56 were in fact XX2Y. Very likely the other 4 were also, but the preparations did not reveal their karyotypes. All 21 of the not-mottled sisters were XXV (of which 17 were v/v , and 4 were $v/+$). As anticipated, then, the mottled-eyed females produced by these crosses have 2 Y chromosomes.

Mottling produced by the Swedish-b Y chromosome

It is not the case that the Canton-S Y chromosome is peculiar, or the mottling the consequence of a unique genetic quality, for the unrelated Y chromosome of the Swedish-b stock is also effective. Two secondary nondisjunctional stocks, maintained by selection, of $sc, dl-49 v, B^{M1}/y^2 apr v/Y^* \text{ ♀♀}$ and X (wild type)/ $Y^* \text{ ♂♂}$, provided regular females and males for pair-matings of the type: $sc, dl-49 v, B^{M1}/X^*/Y^* \text{ ♀} \times sc, dl-49 v, B^{M1}/2Y^* \text{ ♂}$. In the one case the stock had the wild type X^* and Y^* of Swedish-b origin, in the other these chromosomes were from Canton-S. In both cases the autosomes were isosequential and all derived from the Canton-S stock. The matings were made in all combinations, and the relevant data are summarized in table 2.

Each of the crosses produced mottled-eyed females regardless of the chromosomal background, or of the origin of the X or Y chromosome. Mottling by supernumerary Y chromosomes, then, is not a peculiarity of the Canton-S Y chromosome.

Mottling in the X3Y male

Although mottled-eyed females are usual in crosses of XXV females by X2Y males, their frequency bearing an inverse relation to the rate of secondary non-disjunction, only relatively rarely do mottled-eyed males occur (e.g., from 1 to 3% of the males from $\text{♀ } dl-49, B^{M1}/X/Y \times \text{♂ } X/2Y$.) In progenies from such crosses X2Y males must be the preponderant class of males, XY next most frequent, and X3Y by far fewest of all; X4Y males, on the other hand, should not normally occur in this cross. Both by their low frequency and the fact that two extra Y chromosomes mottle the female's eye, it seemed very likely that the occasional mottled-eyed males are X3Y in constitution. This is indeed the case.

TABLE 2

Crosses of the type: $sc, dl-49 v, B^{M1}/X^/Y^* \text{ ♀} \times X^*/2Y^* \text{ ♂}$. Percent of secondary nondisjunction (XX-Y), total female offspring (N ♀ ♀), and the number (n) and percent of mottled [Mo] daughters produced*

Cross and origin of X^* and Y^*		Percent (XX-Y)	N ♀ ♀	[Mo] daughters	
n ♀	in ♂			n	Percent
Swed-b	× Swed-b	79.5	388	87	22.4
CS	× Swed-b	76.9	144	29	20.1
CS	× CS	83.1	159	27	17.0
Swed-b	× CS	82.3	293	36	12.3

Six males having a wild type X chromosome (CS or Swed-b) and blotched eyes, and 12 males with a *sc, dl-49 v, B^{M1}* X chromosome and mottled vermilion eyes, all from matings of *sc, dl-49 v, B^{M1}/ X/ Y* ♀♀ × *X/ 2Y* ♂♂, were cytologically proven to be X3Y. An additional male with very slightly variegated eyes proved to be X2Y. In a second, similar series of 57 mottled males, *both* parents of which carried two Y chromosomes, 55 were X3Y and two, with only slightly variegated eyes, were X2Y (COOPER 1949). The not mottled-eyed brothers were almost all X2Y, few being XY. In no case was a not-mottled X3Y male encountered. As a general rule, then, the addition of two Y chromosomes to an otherwise normal genotype may produce a mottling of the eyes whether or not the fly is female (XX2Y) or male (X3Y).

Mottling in genotypes equivalent to XX2Y

Finally, it can readily be shown that mottling may be produced in a female whose constitution is roughly equivalent to XX2Y; for example, in a $Y^S X \widehat{Y^L} / Y^S X \widehat{Y^L}$ female. " $Y^S X \widehat{Y^L}$ " symbolizes a remarkable X chromosome that carries portions of both arms of Y, including all of the fertility factors, as well as all of the essential genetic material of a nearly totally inverted X chromosome (LINDSLEY and NOVITSKI 1950). Four pair-matings of: Muller-5/ *v cv y y⁺* (= $Y^S X \widehat{Y^L}$) ♀♀ × *v cv y y⁺* / 0 ♂♂ gave a total of 279 *v cv* females (*viz.*, $Y^S X \widehat{Y^L}$ homozygotes), of which 237 (85%) had mottled-eyes of varying degrees of variegation.

All five lines of evidence, then, confirm the view that two supernumerary normal Y chromosomes can cause the eye of an XX2Y female, or of an X3Y male, to become blotched or mottled. In itself this is a very surprising matter, not merely because the Y chromosome seems genetically to possess few phenotypic effects of its own, but especially because of the rather general interaction of supernumerary Y chromosomes with variegating rearrangements. As is well known, extra Y chromosomes tend to suppress the mottling brought about by rearrangements, and to restore the phenotype to normal. Thus, for example, in each case the mottling of the eyes that is induced by *T(1;3)w^{m1}* and *In(1)w^{m4}* is suppressed by extra Y chromosomes (GOWEN and GAY 1934; SCHULTZ 1936). It is an odd fact, then, that although extra Y chromosomes may suppress a variegation brought about by structural rearrangements, supernumerary Y chromosomes themselves can produce a variegation of the eye in the absence of such rearrangements.

THE SOMATIC EFFECTS OF SUPERNUMERARY Y CHROMOSOMES

Most conspicuous among the phenotypic effects of supernumerary Y chromosomes, in strains not carrying variegating rearrangements, are those affecting the uniformity of the coloration of the eye, but there are also effects of hyperploid normal Y chromosomes on overall morphology, and on fertility, as well. Both male and female fly seem well adjusted to the presence of a single extra Y chromosome (BRIDGES 1916), and, unless carrying variegating rearrangements, they are ordinarily of expected phenotype. No mottled-eyed XXY females have been detected by me, and only rarely has a mottled-eyed male proven X2Y rather than X3Y. Such exceptional mottled males are quite possibly mosaics of X3Y and X<3Y tissues.

Ordinarily the mottling of the eye is not sufficiently striking to draw the attention of one not examining the eyes minutely, nor is it generally easy to detect. A moderate or strong overall coloration of the eye, as well as approximately 30 $\times$ magnification is necessary for the accurate classification of mottling. The following genetic backgrounds give phenotypes that, with practice, generally permit good recognition of mottling in XX2Y females: $+/+$, $apr/+$, $v/+$, $car/+$, apr/w^e , apr/v , v/car , apr/apr , v/v , car/car , bw/bw , etc. In the X3Y male, $+$, apr , v , car , bw/bw are all favorable phenotypes. Very pale-colored eyes, such as hemi- or homozygous apr , w^e , etc., can at times be classified reasonably well, but are not ordinarily reliable.

The $apr\ v/apr^+v$ constitution of the first female fly in which Y-mottling was discovered provides an especially favorable background for its detection. The eye color of a young strongly mottled fly of this makeup may have an overall orange cast, and in ordinary illumination the mottling blotches appear as lemon yellow washes of paler color at the posterior margin of each eye. At times, and perhaps typically, the blotch in each eye is roughly triangular, with the broad base of the triangle along the posterior margin of the eye, the apex near the eye's center. At other times the wash of color may be in the form of a narrow, light streak from the eye's center to the mid posterior margin. The two eyes are generally not symmetrically affected, although they do tend to be mottled to similar extents. Only infrequently does the mottling extend to the anterior margin of the eye. When it does so, most of the posterior half of the eye is generally washed of color. Mottling, if slight, may be restricted to one eye only. It may be reduced to a patchy wash of color near the mid-point of the posterior rim of the eye, or involve only a few ommatidia, sometimes but one. As the fly ages, darkening of the eye may emphasize the color contrast of the washed-out area and normal field of the eye. For some genotypes, however, ease of detection decreases with advancing age. This is especially so in the case of wild type eyes in which the mottling grade is a mere freckling of the posterior field of the eye.

If Y-mottling is extreme, there is no problem in detecting it with ordinary illumination at 30 $\times$ magnification, even in homozygous wild type flies. Commonly, and especially with weaker grades of mottling, however, the variegation of the eye is difficult to recognize unless the eye is obliquely shadowed across its surface, but otherwise brightly illuminated from the sides and below, as though in dark field (COOPER 1949). In practice this can be done conveniently by casting, from a few millimeters' distance, the shadow of the tip of a "pusher" or forceps obliquely across the eye. If, in addition, a very bright light source is used, and the flies are examined on a white porcelain tile, there is ordinarily no difficulty in observing whatever mottling may be present.

Three sorts of mottling are recognized in shadowed eyes: (1) a pale blotch or wash of color that contrasts with the overall eye-field; (2) a darker freckling of the eye (especially of the wild type eye), which may be extensive, or restricted to an ommatidium; and, rarely (3) a bright colorless point where an ommatidium is evidently devoid of pigment. Whether the seemingly darker freckles possess more pigment, or less (like the colorless ommatidia), is not known. For all types of this mottling by Y chromosomes, however, one rule concerning the region of the eye that is mottled seems to hold. Mottling commonly involves the mid-portion of the eye nearest the

posterior rim; i.e., near the 3 o'clock position for the left eye, the 9 o'clock position for the right eye. If slight, it is generally restricted to this site, and only one eye may be affected. Increasingly strong mottling extends anteriorly from this region and along the rim of the eye, widening the field of involvement. With the stronger grades of mottling both eyes are generally involved. In some stocks, furthermore, a seam of irregular facets may also be present in the dorsocaudal portion of the eye of some mottled flies.

Certain subsidiary somatic effects of hyperploidy for Y chromosomes are often present in XX2Y females and X3Y males. Where mottling can be detected, these somatic effects are generally correlated with the more extreme grades of mottling. The posterior and middle legs (infrequently the anterior legs), of one side or both, symmetrically or not, may be markedly shortened, thickened, bowed, laterally flattened and deformed, with tarsi that are twisted and curled inwards. The end of the tibia may be spatulate and coarsely bristled in an unusual manner. In very crippled flies the membrane of the wing is frequently coarse and less transparent, and in females the appearance of the abdomen may resemble that of a super-female. Such females are weakened and often of reduced viability and fertility, or even sterile. It is not known whether this reduction of fertility in females is brought about by a primary effect of Y chromosomes on the ovaries, or is a secondary consequence of the generally low viability and weakness of these extremely affected females. Whether or not the "crippling" is a primary effect of supernumerary Y chromosomes is also not known; if it is a direct interaction with certain necessary genes or factors elsewhere in the genome, these elements must be very commonly distributed through laboratory stocks in much the fashion as the apparently ubiquitous podoptera factors (GOLDSCHMIDT, HANNAH, and PITERNICK 1951).

Although colorless eyes, or nearly colorless, such as those of *w*, *apr v*, *apr v car*, etc., are not generally classifiable as to mottling, if there are strong somatic effects of the type just described there need be no great handicap to recognition of at least some XX2Y females, and X3Y males, from crosses in which hyperploid offspring are expected. While the expression of crippling is correlated with mottling of the eye, the eye need not be visibly mottled for a fly to be of crippled phenotype. Thus crippled flies occur equally frequently among the white and wild type classes of females from crosses of *w*/+ / Y ♀♀ × *y w*/2Y ♂♂, even though mottling of the eyes can be recognized only in the *w*/+ / 2Y daughters. Regrettably penetrance for these gross somatic effects of Y chromosomes seems lower than that for eye mottling, at least at 25°C, so no certain judgement can be made from phenotype alone as to the Y chromosome constitution of the pale-eyed sibs that are not crippled.

THE EFFECT OF EXCESS Y CHROMOSOMES ON FERTILITY

It is a familiar fact that males which are X0 (BRIDGES 1916; SAFIR 1920), $\bar{X}Y^s/0$, or $\bar{X}Y^L/0$ (STERN 1929b) are sterile, even though otherwise they are phenotypically normal. As STERN showed, the sterility of such males is brought about by deficiencies for fertility factors present in the Y chromosome, a set from each arm of Y being necessary for male fertility. As $\bar{X}Y^s/Y$, $\bar{X}Y^L/Y$ and X2Y males are of normal fertility, or very nearly so, the conclusion seemed reasonable that overdoses of Y

TABLE 3

Fertility tests of hyperploid males having mottled eyes, hence presumably X3Y in constitution. The figures in brackets indicate the number of males in each group that was successfully analysed to chromosome constitution. Except for 3 of the 4 fertile sc, dl-49 v, B^{M1} mottled males, all males analysed were X3Y as expected. The 44 males of established constitution are included in the total sample of 76 cytologically analysed males discussed on page 250

Source of ♂ X-chromosome	Number of ♂♂ mated	Number fertile	95% confidence limits
Canton-S	165 [20]	1 [1]	0.06- 3.4%
sc, dl-49 v, B ^{M1}	41 [17]	5 [4]*	3.9 -28.5%
car	17 [0]	0	
Swedish-b	12 [2]	0	

* 1 ♂ was X3Y, 3 ♂♂ were X2Y.

chromosomes would not have a sterilizing effect. Nevertheless, as surprising as it may seem, three Y chromosomes are sufficient to sterilize a male, as SCHULTZ first found (MORGAN, BRIDGES and SCHULTZ 1934).

The sterility of males having three Y chromosomes is demonstrated in table 3. All of the males tested for fertility had mottled eyes which were ascribable only to the effects of Y hyperploidy. Each male was mated with successive females for a period of not less than seven days, and in many cases extending to three or more weeks. An attempt was made to determine the cytological constitution of more than half of the males tested, but regrettably in only a fraction of them were division figures found and a determination possible. It is clear that the series of sc, d-49, v, B^{M1} males is out of line with the others. Not only was 12% of this group fertile, but all three of the variegated males that proved to be cytologically X2Y (p. 247) possessed the sc, dl-49 v, B^{M1} X chromosome. Excluding this discrepant series, then, only one fertile male occurred among the remaining 195 tested males. The 95 % confidence interval for fertility among X3Y males is, therefore, approximately from 0.05 % to 2.9 %.

The sterility of X3Y males resembles in many respects that of X0 males and other males which are sterile owing to the absence of one or more fertility factors of the Y chromosome (SAFIR 1920; SHEN 1932; COOPER 1949). The testes and allied genital structures of X3Y males are all normal in gross appearance, spermatogenesis is regular, and normal appearing bundles of spermatozoa are produced. The spermatozoa do not become motile, however, and customarily the bundles degenerate at the proximal end of the testis. Only occasionally do the paragonia and testicular ducts of the X3Y male contain free spermatozoa, but even in these instances the sperm have always proven immotile. On dissection, females that have copulated with X3Y males prove to have empty ventral receptacles and spermathecae. No doubt the rare, slight, and evanescent fertility of an X3Y male is due to a mitotic loss of one or more Y chromosomes. Such a loss might occur in a secondary gonial cell, giving rise to a cyst of X2Y (or XY) spermatocytes capable of giving motile spermatozoa. Very likely, however, the losses that confer limited fertility on these males must also involve cells destined to become nutritive cells of the testis, for motility and fertility of the spermatozoan appear to be conferred upon it by extra-spermatocytic factors, rather than by intrinsic ones (STERN 1936; STERN and HADORN 1938).

Whether or not the X4Y male is viable has not been determined. In studies of hyperploid segregations, to be reported elsewhere, a large number of crosses of XX2Y ♀♀ × X2Y ♂♂ have been made. If viable, X4Y males (from fertilizations of X2Y-eggs by 2Y-sperm) should form small fractions of the progenies. Reasoning from what has so far been found about the effects of supernumerary Y chromosomes, these hyperploids should be very strongly mottled forms (the $X/X^s/sc^s Y^L/Y$ female is an extreme mottle), and the male at least would be expected to be sterile. Up to 4% of the mottled males from such crosses may have diminutive testes, sometimes sac-like, which in gross appearance seem empty of cells. Furthermore a small fraction of the extremely mottled females also have atrophied ovaries and are sterile. Whether these are the otherwise unidentified X4Y males and XX3Y females is not known. According to STERN's (1929a) findings, at least the XX3Y female is a viable type. Although it is an attractive thought that three Y chromosomes may also sterilize a female, it is entirely possible that these extremely mottled flies, bearing gross somatic effects as they do, are sterile not because Y chromosomes directly affect the female reproductive system, but wholly owing to their general phenotypic debility.

STURTEVANT (1945) discovered the remarkable recessive transformer gene (*tra*; 3: 44.0-45.3) which he demonstrated to convert genetic females into extreme female intersexes which are wholly male in morphology and behavior, but sterile. The sexually "transformed" flies, however, remain female in their developmental rate and in their expression of the sexually dimorphic mutants scute-3 (lethal in female), eosin, and facet. The *tra/tra* intersexes have normal male external genitalia, and a complete set of internal male reproductive organs. The testes, however, are under-sized, and the addition of a single Y chromosome is apparently without significant effect for the intersexes remain sterile, and the testes small. It seemed of interest to discover whether the transformed "male" would mottle with two Y chromosomes, as its female genotype should dictate, or, like a true male, would require three Y chromosomes for mottling of the eye.

In crosses of mottled $[Mo] \widehat{X}X/2Y, tra/+ \text{ } \times \text{ } X/Y, tra/+ \text{ } \times \text{ } \sigma\sigma$, the total transformed $\widehat{X}X \text{ } \sigma\sigma$ should constitute approximately one eighth of the whole progeny. If two Y chromosomes are sufficient to mottle the intersexual form, then mottled transformed "males" should be numerous and the ratio of $[Mo] \widehat{X}X \text{ } \sigma\sigma$ to transformed $[Mo] \widehat{X}X \text{ } \sigma\sigma$ close to 3:1. These expectations are borne out, as the following cross shows.³

♀♀ $[Mo] y^v y^{v/2Y}, tra/+ \text{ } \times \text{ } \sigma\sigma y^2 apr v/Y, tra/+$
 351 ♀ $[Mo] y^v$, 320 ♀ y^v , 108 "♂" $[Mo] y^v$,
 142 "♂" y^v , and 916 ♂ $y^2 apr v$; total offspring 1837.

The extra Y chromosome did not affect the sterility of the female intersexes, nor were the internal male genitalia normal in size. The testes of the four mottled "males"

³ Regrettably two of the male parents in the 7 pair matings may have been X2Y. However, this does not affect the conclusions drawn. If three Y chromosomes were necessary to mottle the intersex, rather than two, and even if all the male parents were X2Y, at most no more than one sixth of the transformed "males" would have mottled eyes, and mottled females should be many times as frequent as mottled "males" (namely, in a ratio of approximately 12:1).

that were dissected had no more than half a coil, were lumpy along their lengths, and contained no spermatocytes, no spermatozoa, and no dividing cells. As STURTEVANT (1945) and GOLDSCHMIDT (1953) have emphasized, the genetic response of the transformed "male" is that of a female fly.

FRACTIONAL HYPERPLOIDS

Experiments on sex chromosome segregation that make use of the mottling and sterility brought about by Y chromosomes have provided interesting information on the penetrance of these effects in hyperploid females and males carrying fractional Y chromosomes. The results, summarized in table 4, permit the following conclusions to be drawn.

In both male and female, a hyperploidy of something less than $+2Y$ chromosomes above normal for the genotype produces mottling in at least some individuals, and sterility among some males. However, the penetrance for mottling (i.e., the fraction of the genotype that is mottled) is not in a direct correspondence with that for the sterilizing action of Y chromosomes in males. Thus $\widehat{X}Y^s/2Y$ males are many-fold more fertile than $\widehat{X}Y^L/2Y$ males, although quite probably they are equally frequently mottled.

The degree of hyperploidy for sets of fertility factors of the Y chromosome (set K_1 in Y^L , set K_2 in Y^s) is certainly not the sole agent that determines male sterility and mottling of the eye in either sex. Since the $Y^s\widehat{X}Y^L/0$ male is fertile (LINDSLEY and NOVITSKI 1950), it must carry sufficient, and presumably complete, sets of K_1 and K_2 . If the K -factors were decisive, then the $Y^s\widehat{X}Y^L/2Y$ male and $X3Y$ male should correspond in both degree of mottling and sterility, since each carries $3K_1 +$

TABLE 4

The effect of varying degrees of Y chromosome hyperploidy on the fertility of males, and on the mottling of the eyes of males and females of favorable genotypes (see p. 252)

Constitution of male	Approximate percentage of class that is:		Constitution of female	Approximate percentage mottled
	Mottled	Fertile		
$\widehat{X}Y^s/Y$	0	100	XXY	0
$\widehat{X}Y^L/Y$	0	100	$XXXXY$	0
$Y^s\widehat{X}Y^L/Y$	0	100	$\widehat{X}\widehat{X}/Y^{st}/Y$	42†
$X/2Y$	<1	100	$X/\widehat{X}Y^s/Y$	78
$\widehat{X}Y^L/Y^{st}/Y$	8	*	$X/\widehat{X}Y^L/Y$	‡
$\widehat{X}Y^s/2Y$	76	40	$Y^s\widehat{X}Y^L/Y^s\widehat{X}Y^L$	85
$\widehat{X}Y^L/2Y$	80	4	$XX/2Y$	100?
$Y^s\widehat{X}Y^L/2Y$	68	11	$XX/scY^L/Y$	100?
$X/3Y$	100?	<1	$XXX/2Y$	‡
			$X/\widehat{X}Y^s/scY^L/Y$	100

* Percentage not determined.

† Y^{st} is a fragment that includes the chromocentral region (XC) of X from XR through block-A, a nucleolus organizer (from either Y^s or XC), and the tip of Y^s with factors K_2 . Data from MORGAN, STURTEVANT, and BRIDGES (1926), COOPER 1948 (= " Y^s " of figures and text), and unpublished.

‡ It has been determined only that at least some of this class have mottled eyes.

$3K_2$. Nevertheless the $Y^S\widehat{X}Y^L/2Y$ male is both less frequently mottled and more fertile than the $X3Y$ male. This is borne out also by the proportionately lower frequency of mottling in $Y^S\widehat{X}Y^L/Y^S\widehat{X}Y^L$ females than in $XX2Y$ females, where again the two genotypes correspond with respect to fertility factors, both having $2K_1$ and $2K_2$. Some material other than K_1 and K_2 , then, from either the chromocentral portion (XC) of X, or from Y, has a strong action on mottling and sterility, and is evidently absent from the $Y^S\widehat{X}Y^L$ chromosome. DUBININ and HEPTNER (1935) have likewise given evidence for the existence in Y of a non-distal factor or factors, M_y , importantly affecting variegation.

In like fashion it is evident from table 4 that mottling and male sterility do not bear a direct correspondence with hyperploid frequencies of sex chromosomal spindle attachments, bobbed loci, or nucleolus organizers. Very likely, then, as others have contended, the materials that have genetic activity in the chromocentral region of X, and in the Y chromosome, are complex and of a nature not yet ascertained.

Although not included in the above table, attached-X stocks bear importantly on this question, for different stocks may respond in their own fashion to extra Y chromosomes. Some give strong and regular mottling in their $\widehat{X}\widehat{X}/2Y$ daughters, whereas others may give negligible frequencies and only a slight expression of mottling with two Y chromosomes. Sons that are $X3Y$, regardless of their $\widehat{X}\widehat{X}/2Y$ sisters' reaction to extra Y chromosomes, are regularly mottled and sterile in any case. Such results strongly suggest that the proximal regions of a normal X and Y, at least in part, are not alike.

As L. V. MORGAN (1938) pointed out, nearly all (if not all) attached-X stocks arose from oblique exchanges between the arms of either $\widehat{X}Y^S$ or $\widehat{X}Y^L$ chromosomes, or by some sequence of exchanges between X and Y chromosomes. As neither Y^S nor Y^L cytologically corresponds with XC in its basal portion (cf. figures in HEITZ 1933 and KAUFMANN 1933, 1934, or figures and summary in COOPER 1950), and as evidently there exists a number of potential exchange points between X and Y (LINDSLEY 1955), it follows that if the basal portions of Y may replace those of X, a whole family of $\widehat{X}\widehat{X}$ chromosomes can exist in which each member differs from the other in the composition of its chromocentral region. Such differences are adequate to account for the dependency on stock origins of the penetrance and expressivity of mottling in $\widehat{X}\widehat{X}/2Y$ females. For such reasons also, similar differences may be expected among different stocks of $\widehat{X}Y^L$ and $\widehat{X}Y^S$ chromosomes, and great care must be taken in judging the relative genetic effects of the separate arms of the Y chromosomes from experiments involving $\widehat{X}Y^{Arm}$.

ARE V-TYPE POSITION EFFECTS AND Y CHROMOSOMES COMPLEMENTARY IN ACTION?

Since the characteristic phenotypes of dominant and recessive V-type position effects which mottle the eyes are commonly suppressed or profoundly modified by supernumerary Y chromosomes (summarized in LEWIS 1950), it may be wondered whether in turn they are directly and equally antagonistic to the action of the Y chromosomes. If so, it might be expected that the workings of the position effect and the supernumerary Y chromosomes could effectively complement one another, the result of their interaction being the simultaneous eradication of most, perhaps all,

of the phenotypic expression of each on the eye. It is also conceivable that the sterilizing effect of hyperploid Y chromosomes on males could be ameliorated for, as shown above, sterilization very likely involves the action of some elements that are not themselves K_1 - or K_2 -fertility factors. Two tests of these possibilities were made.

Mottled-K (KRIVSHENKO 1954) was used as a dominant V-type mottle. Mo^K is associated with an inversion in the right limb of the second autosome, one break of which lies within the chromocentral region. It is lethal when homozygous, but of good viability when heterozygous, producing a sharply classifiable mottling of the eyes in all XX females and XY males carrying the rearrangement. In XXY females and X2Y males, however, the eyes of Mo^K heterozygotes range in gross appearance from wholly wild type to faintly and sparsely freckled eyes. The normal Y chromosome, then, suppressed the phenotype of Mo^K . The eyes of XX2Y and X3Y Mo^K heterozygotes, however, are blotched or washed of color in the very manner characteristic of this degree of Y-hyperploidy. The dominant V-type position effect Mo^K , therefore, does not markedly counteract or suppress the action of the Y chromosomal elements that variegate the eye. The same is true for the recessive V-type position effect of $In(1)w^{m4}$.

SCHULTZ (1936) has shown that an extra Y chromosome strongly suppresses variegation of the eyes of w^{m4} hemi- and homozygotes, and this I can confirm. Nevertheless $w^{m4}/w^{m4}/2Y$ females and $w^{m4}/3Y$ males have mottled eyes as dictated by the hyperploid sets of Y chromosomes. As with Mo^K , increasing the number of Y chromosomes suppresses the position effect of the rearrangement, but the position effect itself does not appear to have an equivalent antagonistic action on the variegation of the eye that is brought about by the supernumerary Y chromosomes. Nor does w^{m4} make the X3Y male fertile. Sixteen $w^{m4}/3Y$ males were repeatedly mated with fresh females (of XX, XXY, and XX2Y genotypes); in every case the cross was sterile.

COMMENT

The effects of the Y chromosome which have been described above are almost certainly general for ordinary stocks of *Drosophila melanogaster*, and not due to some interaction between supernumerary Y chromosomes and a particular mutant gene or rearrangement peculiar and common only to the stocks used in these experiments. SCHULTZ (1941a, and in: MORGAN, BRIDGES and SCHULTZ 1935), for example, has already noted that the X3Y males are a sterile class. It is not possible to say for certain whether the variegating effects of supernumerary Y chromosomes have been observed before, although many have mentioned crosses or occurrences of XX2Y females, and of X3Y males. While it may be that the seemingly anomalous effects of supernumerary Y chromosomes on variegation of the light locus (SCHULTZ 1936, and in: MORGAN, BRIDGES and SCHULTZ 1934; MORGAN and SCHULTZ 1942), or on that of bw^A (DUBININ and HEPTNER 1935), are actually instances of the Y chromosome effect on the eye that I have just described, the presence of light or any other mutant locus is not normally required. In any event, the phenotypic responses of both light and bw^A are explicable in terms of the general hypothesis offered in the discussion below.

Although it is true that the flies in which a Y-induced variegation of the eye was first observed were actually of a very complex genetic nature (see p. 243), it has been shown that flies of pure Canton-S wild type lines variegate when suitably hyperploid for Y chromosomes, as do strains derived from Swedish-b and many other diverse stocks, presumably little related, that were obtained from the California Institute of Technology, Indiana University, the University of Missouri, Cold Spring Harbor, and elsewhere. Not only is the effect shown within stocks having autosomes and X chromosomes of different origins, but the Y chromosomes too need not be of any particular strain or sort. Y^{CS} , Y^{Sw-b} , $sc^8 \cdot Y$, $Y:bw^+$, $sc \cdot Y^L$, and so on, are all at least very similar in this respect, for when they are members of a set of Y chromosomes in an XX2Y female, or in an X3Y male, the phenotypic effects on the eye and on male fertility are alike.

The fact that supernumerary Y chromosomes mottle wild type or self-colored eyes makes it possible to carry out some segregation studies not otherwise feasible. Nevertheless considerable care is necessary, if mottling alone is to indicate the Y chromosome constitution, for both penetrance and expressivity fluctuate within and between stocks. Although flies with variegated eyes that occur in progenies from XX2Y females by XY males, or from XXY females by X2Y males, may generally be classified with confidence as XX2Y females and X3Y males, non-mottled flies of these constitutions may also occur. Furthermore it is a surprising fact that both hyperploid males and females may scale down the Y-hyperploidy within the gonad, even though retaining a higher somatic hyperploidy. This is most convincingly observed in flies with suitable Y chromosome markers; e.g., in $y v = /sc^8 \cdot Y/Y:bw^+$; bw/bw females and $y v/Y^{CS}/sc^8 \cdot Y/Y:bw^+$; bw/bw males. In each instance the occurrence of a vermilion eye (i.e., not $v bw$) and a wild type body color (owing to the y^+ allele of $sc^8 \cdot Y$) indicates the presence of both marked Y chromosomes in the soma, and the eyes of such flies may be strongly mottled. Yet some of these males may be fertile to a normal degree, and in each such case they have bred as $y v/Y^{CS}/sc^8 \cdot Y$; bw/bw males. In other words, $Y:bw^+$ has been lost in at least a large sector of one or both gonads of such males. In similar manner an occasional female transmits only one of the two Y chromosomes to her offspring. The reverse cases, in which somatically $y v$ not-mottled females and males (or $v bw$ females and males) have transmitted both $sc^8 \cdot Y$ and $Y:bw^+$ to their offspring, have not been observed. Whether the seemingly high frequency of fertile sc , $dl-49 v$, $B^{M1}/3Y$ males (see p. 250) is a consequence of such a gonadic scaling down of Y chromosomes is not known.

DISCUSSION

As earlier remarked, the mottling of self-colored eyes which occurs in the presence of supernumerary Y chromosomes is perplexing for, as is well known, Y chromosomes commonly suppress the mottling brought about by variegating (or V-type) position effects (GOWEN and GAY 1934; SCHULTZ 1936, and in: MORGAN, BRIDGES and SCHULTZ 1935, reviews in: SCHULTZ 1941a, b, 1948, 1952; LEWIS 1950; HANNAH 1951). As a general rule, striking V-type position effects in *Drosophila melanogaster* involve displacements of chromosomal elements (MULLER 1930) to and from chromo-

central regions (SCHULTZ 1936), with the formation of new euchromatic-chromocentric associations. In *D. virilis*, at least, V-type position effects may also arise in the case of purely chromocentral displacements (BAKER 1953), and this may prove to be the case in *D. melanogaster* as well (STERN and KODANI 1955). In striking contrast, wholly euchromatic rearrangements give rise preponderantly to stable, S-type position effects (LEWIS 1950). Chromocentral change, therefore, is a cardinal element in variegating position effects, although not all displacements involving chromocentral elements result in detectable variegation.

The euchromatic genes (and, in *D. virilis*, chromocentric) involved in the production of variegated phenotypes are of no particular sort, but as a group are necessarily characterized by the production of terminal, autonomous, cellular differentiations (MULLER 1930; LEWIS 1950). Both the stability of phenotype and the normal action of such genes may be restored when the variegating euchromatic locus is removed from its chromocentral involvement, in whole or in part, as in the cases of some induced reversions of *w^{m11}* (PANSIN 1938), of *rst³* (KAUFMANN 1942), and presumably of variegating *In(2LR)40D* (HINTON 1950). In these cases, the position effects largely or entirely disappear. Nevertheless, the obvious fact must be kept in mind that the original change responsible for the V-type position effect may have conjointly involved position effects on chromocentric loci, as well as those on the euchromatic loci to which attention has chiefly focussed. Whatever the case may prove to be, as LEWIS (1950) so aptly comments, the common attributes of the V-type position effects suggest a division of the genetic material into two major components, one cytologically corresponding with the euchromatic lengths of the polytene chromosomes, the other with the chromocentral regions.

It is by no means a simple matter to account for variegation in terms of what is known. None of the older interpretations that require mutable loci, unstable rearrangements, excessive reduplication of affected loci, duplications undergoing somatic crossing over, abnormal reproduction of genes in twisted genomata, or in lateral attachments, mechanical asymmetries and torsions imposed on loci by somatic pairing of heterozygous rearrangements, modes of conformation of the chromocenter more or less persisting in groups of related cells, and so on, are reconcilable with the facts as they now stand. Nor do such explanations, or conceivable ones involving the simple introduction of euchromatic elements into the chromocentral environment normally foreign to them, make understandable the suppression of mottling in otherwise variegating genotypes to which chromocentral elements, or of duplications of chromocentral elements, have been added in the form of Y chromosomes, or the occurrence of mottling in normal genotypes hyperploid for Y chromosomes.

The chemical or "kinetic" interpretations of position effects which stress interference with immediate or early gene products (OFFERMANN 1935, and others), or changes in competitive combining powers and reaction efficiencies of genes (STERN and HEIDENTHAL 1944), or accumulations of precursors (LEWIS 1950), or interactions of substrates (BAKER 1953) or of gene products (BELGOVSKY 1946), and so on, can in principle explain the occurrence of a phenotypic change, or apparent inactivation of a gene, consequent to rearrangement, but none bears an implicit relation that would automatically explain variegation of phenotype. Something in addition is required.

More than any other, SCHULTZ (1936-1952)⁴ has emphasized the role of the chromocentral elements in variegation. They are viewed by him as nullifiers of euchromatic loci newly conjoined to them, and in this kind of interpretation he has been followed and supported by NOUJDIN (1938, 1939, 1946a, b), PROKOFYEVA-BELGOVSKAYA (1939, 1946, 1947), and others. Variegation is envisaged as a consequence of "heterochromatization," or effective somatic inactivation of euchromatic loci brought into close continuity with chromocentral material. The inactivation might be in a literal sense, or the displaced euchromatic loci, inefficiently competing with those of the chromocenter for nucleic acids, might fail to be reproduced in certain somatic cells and thus be lost in a sort of intrachromosomal diminution. Or again, acquiring properties inferred from the allometric growth of the chromocentral elements in nurse cells (SCHULTZ 1948, 1952; and in: MORGAN, SCHULTZ and CURRY 1941), they are viewed as retarded in their multiplication when compared with that of the normal euchromatic complement. Since "heterochromatization", a process which seems to encompass all of these effects, and still others, is variable from cell to cell, variegation of phenotype would characterize the action of rearranged euchromatic loci that have autonomous effects.

SCHULTZ explains the suppression of variegation when Y chromosomes are added to the genotype (presumably also when other chromocentral elements are added), by holding that the general pool of nucleic acids is adequately heightened by their presence. Displaced euchromatic loci thus would become capable of maintaining normal rates of reproduction and action, and would not heterochromatize, for they would no longer need to be starved by their more or less ineffective competition with adjacent chromocentral elements for nucleic acids at threshold levels. This has been a fruitful view, even though containing what seem to be contradictions and being subject to dispute regarding important facts (PROKOFYEVA-BELGOVSKAYA 1939; COLE and SUTTON 1941; CALLAN 1948; GERSCH 1949; SCHULTZ and CASPERSSON 1949). Nevertheless, even within the wide limits which such an interpretation grants, it is difficult to account for the mottling of the eye that accompanies Y chromosome hyperploidy in otherwise normal, non-variegating genotypes.

NOUJDIN (1938), DEMEREC (1940, 1941), RAFFEL and MULLER (1940), KAUFMANN (1942), LEWIS (1950), and HANNAH (1951), have all emphasized that just as with the euchromatic break, the locations of breaks in the chromocentral elements which are associated with V-type position effects are important, and that the chromocentral regions are by no means of a linearly uniform property with respect to causation of variegation. It is not merely necessary that the affected euchromatic locus be continuous with, or adjacent or subadjacent to a chromocentral element for variegation to occur, but the chromocentral break must include or neighbor a sensitive site. Such responsive sites might be imagined as more or less repeated loci of special function occurring in the chromocentral regions much as PONTECORVO (1943) has portrayed heteropycnotic regions adjacent to kinetochores. Just as position effects may "spread" along a euchromatic length (MULLER 1930; MULLER and PROKOFYEVA 1935; DEMEREC

⁴ SCHULTZ has contributed most widely to the subject of variegation, and those interested in the full range of his discoveries and views should refer also to his contributions in: MORGAN, BRIDGES and SCHULTZ 1933-38; MORGAN and SCHULTZ 1942; MORGAN, SCHULTZ, BRIDGES and CURRY 1939; and MORGAN, SCHULTZ and CURRY 1940-41, and in SCHULTZ and CASPERSSON 1939; 1949.

1940, 1942), BAKER (1954) has shown that they may "spread" along chromocentral ones in *D. virilis*. It is quite possible, then, that chromocentral "spreading" may also occur, and this could account for disbalances arising from a simple rearrangement even were the numbers of relevant loci within a chromocentral element quite high.

As earlier commented by SCHULTZ (1936-1952, and in: MORGAN, BRIDGES and SCHULTZ 1934, 1935), chromocentral loci may be viewed as together forming a system which is in balance with loci elsewhere in the euchromatic lengths. As a purely formal way of looking at it, we may suppose that normal autonomous differentiations within cells require a state which is a consequence of euchromatic-chromocentric interaction, and that this state is of some range in latitude or time. Changes in the euchromatic-chromocentric balance which may occur through ordinary fluctuations in genotype (for example, by nondisjunction of sex chromosomes) would, in the long run, act selectively to affect the adjustments of those genes sensitive to the results of balance and unbalance. Were this so, thresholds of individual loci primarily concerned with autonomous cellular differentiations might differ, provided all fall within the narrowest limits of the normal range in state. Thus the actions of genes need not be impaired by displacements not including or affecting them, provided that any changes in balance which may occur do not fall below their threshold requirements. But the displaced loci, if themselves subject to position effect in much the way STURTEVANT (1925), or OFFERMANN (1935), or LEWIS (1954) conceive, might now operate outside, or marginal to, their present needs and variegation result, for then differentiation would not be uniform from cell to cell. The addition of one extra Y chromosome would suppress the V-type position effect if the state supposedly brought about by euchromatic-chromocentric balance were shifted to a point that the displaced, variegating gene no longer acted under marginal or limiting conditions. Were unbalance extreme, even genes that were not displaced might variegate, and it is assumed that some such gene or genes are responsible for the variegation in hyperploids having two Y chromosomes above normal. Just as maleness or femaleness in *Drosophila* is evidently determined by an unbalance of autosomal male-trending genes with X-chromosomal female-trending genes, and intersexuality by varying degrees of balance (BRIDGES 1932), in this case variegation is also interpreted as a consequence of unbalance.

A balance interpretation of variegation has much to commend it, for V-type position effects on euchromatic loci may be enhanced by heterozygosity for amorphic alleles or deficiencies at the locus (LEWIS 1950), by homozygosity for the rearrangement (DEMEREK and SLIZYNSKA 1937; SCHULTZ 1941a, etc.; LEWIS 1950), and especially by deficiencies for chromocentral elements as in the X0 male (SCHULTZ 1936; NOUJDIN 1936) and in individuals deficient for the chromocentral region of 2R (SCHULTZ 1941a, and in: MORGAN, SCHULTZ, *et al.* 1940, 1941). Conversely they are suppressed by the addition of Y chromosomes, and by duplications of chromocentral material from 2R, and from the 4th and X chromosomes (SCHULTZ 1941a, and in: MORGAN, SCHULTZ and CURRY 1940; NOUJDIN 1938, 1939, 1946a, b). Furthermore, as SCHULTZ (1941a) has found, V-type position effects are nevertheless expressed in intersexes even in the presence of an extra Y chromosome. Finally such an interpretation can encompass the production of variegation in Y-hyperploids with

otherwise normal genotypes, for in principle a point of unbalance ought to be attainable at which even some normal alleles would operate outside the threshold limit at which their full phenotypic expression is regularly reached.

It is true that I have not observed variegation other than that of eye coloration (and sometimes corneal defects) in wild type Y-hyperploids. It is also the case that V-type position effects may involve not only eye coloration and corneal irregularities, but also integumentary coloration, presence and absence of hairs, bristle form, and so on, and all seem suppressable by the simple addition of Y chromosomes. How is it then that the wild type Y-hyperploid is not conspicuously variegated for these characters? It may be that sufficiently high levels of disbalance simply are not achieved or, if achieved, their effect may involve so many elements of differentiation that it proves lethal. Certainly the frequent somatic deformation in highly hyperploid flies (see p. 249) bespeaks the possibility of more general effects than those on the eye alone.

In the same fashion, but probably involving other genes, a balance interpretation can adequately account for the sterilizing action of supernumerary Y chromosomes on males, as SCHULTZ (1941a; and in: MORGAN, BRIDGES and SCHULTZ 1934, 1935) has already noted. A male of *Drosophila melanogaster* requires the two different fertility groups of the Y chromosome for sperm motility and fertility. But these, and very likely other factors in the Y chromosome (see p. 252), may be viewed as opposing the actions of genes elsewhere in the chromosome set. When too many Y chromosomes are present, the effect of the genes of Y would exceed the balance and the male be rendered infertile. It remains to be shown what effect, if any, such disbalance may have in the female.

The cytologist is familiar with numerous instances, as in coccids (HUGHES-SCHRADER 1948), cecidomyids, and other insects (WHITE 1954), in which balances may be maintained, in at least certain tissues, between a differentiated class of chromosomes and the remainder of the set. On the whole, balanced or redundant systems have many attractive aspects for the interpretation of development and the control of phenotype, no less for the explanation of gradual yet extensive evolutionary changes in the genotype despite an overall retention of the original basic genetic system. They are furthermore very susceptible to direct genetic test, and it is precisely this aspect of the interpretation of V-type position effects just outlined which makes it seem worthwhile.

Although the unification of the effects of simple Y-hyperploidy with that of the V-type position effects is easily and naturally encompassed in this admittedly broad view, it may still prove to be the case that the variegation of wild type (or non-mottled) eyes by supernumerary Y chromosomes is an epiphenomenon, parallel to, but not immediately involved with V-type position effects. It is odd that $w^{m4}/3Y$ and $X/3Y, Mo^{\kappa}/+$ males both show the characteristic dorsocaudal blotching of the eye characteristic of variegation by Y chromosome hyperploidy alone, whereas $w^{m4}/2Y$ and $X/2Y, Mo^{\kappa}/+$ males are wild type or nearly so (p. 254). After all, *two* additional Y chromosomes are required to variegate the wild type eye, while in the above cases only *one* additional Y chromosome seems to give variegation. At first glance it would seem that in these cases the Y chromosome is carrying out two

independent actions, rather than merely recreating, and then superseding in the usual way, a balance with opposing, non-chromocentral elements. Nevertheless SCHULTZ (1943) has discovered a w^{m4} derivative that requires not one, but two Y chromosomes for suppression of its variegation. It remains to be seen whether study of loci such as this one, as well as accumulations of unrelated V-type position effects within one genotype, will provide evidence consistent with the general balance interpretation and with the view that Y-induced variegation is a consequence of upset of this balance.

Finally, it must have been noted that I have deliberately employed "chromocentral region," rather than "heterochromatin," throughout my discussion. "Heterochromatin" today is what an investigator chooses to make it or imagine it, and I agree wholeheartedly with the views on this score expressed by BAKER and CALLAN (1950). "Heterochromatin" occurs in fully 17 of the 20 numbered divisions of the polytene X chromosome (PROKOFYEVA-BELGOVSKAYA 1939; KAUFMANN 1944, 1946; SLIZYNSKI 1945), if by "heterochromatin" we mean only sites of ectopic pairing or of unusual breakability or rejoinability. "Heterochromatin" also occurs in the Y chromosome and all chromocentral regions. But these latter are differentiable genetic systems, and it would seem best to demonstrate first the propriety and usefulness of referring their actions to allegedly common heterochromatic properties, or to different kinds of "heterochromatin," rather than directly to their genetic constitutions. Conversely, there is little logic to the transfer of the genetic properties of Y chromosomes and chromocentral regions to the "interstitial heterochromatin," of which few facts are known but to which much is imputed. Analysis of the genetic organization and action of the chromocentral elements has barely begun, and quite possibly it holds keys to many of our genetic puzzles, as, for example, position effect.

SUMMARY

1) Y chromosomes have a characteristic pair of phenotypic effects in *Drosophila melanogaster* when there are two above normal for the genotype, namely in XX2Y females and X3Y males: they cause a mottling of the self-colored eye, and they sterilize the male. These effects are shown to occur in a number of unrelated strains, and with a number of different Y chromosomes. They are probably general within the species.

2) There are frequently other somatic irregularities observable in flies hyperploid for Y chromosomes: eye facets may be irregular, the legs may be shortened and deformed, the membrane of the wing abnormal, and so on. These effects have not been studied, and may depend on interactions with certain genes that are common in laboratory strains.

3) XX2Y, *tra/tra* females that are transformed to extreme male-type intersexes variegate just as their female genotypic constitution dictates; they remain female in their response to the supernumerary Y chromosomes.

4) Fractional hyperploids demonstrate that something less than two supernumerary Y chromosomes is sufficient to cause mottling, and that the factors concerned with sterilization of hyperploid males are at least in part independent of

those causing variegation of the eye. Furthermore, sterilization of males involves the action of some elements that are not themselves members of the K_1 - and K_2 -fertility groups.

5) The proximal regions of the X chromosome and Y are, at least in part, not alike cytologically, or in their direct genetic contributions to variegation. In principle, a family of attached-X chromosomes must exist, the members of which differ in their chromocentral components. In this way the variable response among different attached-X stocks to Y chromosome hyperploidy may be explained.

6) The V-type position effects of Mo^K and w^{m4} are suppressed, in whole or in part, by one extra Y chromosome; nevertheless they themselves evidently do not prevent expression of the characteristic variegation brought about by two extra Y chromosomes.

7) XX2Y females and X3Y males occasionally become mosaic for the Y chromosomes so that the original constitution is retained in the soma, but a Y chromosome is lost in the germ tract. Such mosaic males are fertile.

8) V-type position effects are discussed, and a purely formal explanation involving a euchromatic-chromocentric balance is proposed, of a directly testable nature, which can account for both the suppression and production of variegation by supernumerary Y chromosomes, as well as for the V-type position effects themselves. Despite the suggestion of such an all-encompassing view, it remains possible that the variegation caused by Y chromosomes is an independent phenomenon.

9) The sterilization of 3Y males may also be explained in terms of a genotypic balance.

LITERATURE CITED

- BAKER, J. R., and H. G. CALLAN, 1950 'Heterochromatin'. *Nature* **166**: 227-228.
- BAKER, W. K., 1953 V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin. *Genetics* **38**: 328-344.
- 1954 The anatomy of the heterochromatin. *J. Heredity* **45**: 65-68.
- BELGOVSKY, M. L., 1946 On the causes of mosaicism associated with heterochromatic chromosome regions. *Am. Naturalist* **80**: 180-185.
- BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. *Genetics* **1**: 1-52, 107-163.
- 1932 The genetics of sex in *Drosophila*. *Sex and Internal Secretions*, Chap. iii: 55-93. Baltimore, Williams & Wilkins.
- CALLAN, H. G., 1948 Ribose nucleic acid in the *Drosophila* egg. *Nature* **161**: 440.
- COLE, P. A., and E. SUTTON, 1941 The absorption of ultraviolet radiation by bands of the salivary gland chromosomes of *Drosophila melanogaster*. *Cold Spring Harbor Symposia Quant. Biol.* **9**: 66-70.
- COOPER, K. W., 1948a A new theory of secondary non-disjunction in female *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. U.S.* **34**: 179-187.
- 1948b The evidence for long range specific attractive forces during the somatic pairing of dip-teran chromosomes. *J. Exp. Zool.* **108**: 327-335.
- 1949 The cytogenetics of meiosis in *Drosophila*. Mitotic and meiotic autosomal chiasmata without crossing over in the male. *J. Morphol.* **84**: 81-122.
- 1950 Normal spermatogenesis in *Drosophila*. *Biology of Drosophila*, Chap. 1: 1-61. New York, John Wiley.
- 1952 Studies on spermatogenesis in *Drosophila*. *Am. Phil. Soc. Ybk.* 1951, pp. 146-147.

- DEMEREK, M., 1940 Genetic behavior of euchromatic segments inserted into heterochromatin. *Genetics* **25**: 618-627.
- 1941 The nature of changes in the white-Notch region of the X chromosome of *Drosophila melanogaster*. *Proc. 7th Inter. Congr. Genet., J. Genet. (Suppl. Vol.)*: 99-103.
- DEMEREK, M., and H. SLIZYNSKA, 1937 Mottled white 258-18 of *Drosophila melanogaster*. *Genetics* **22**: 641-649.
- DUBININ, N. P., and M. A. HEPTNER, 1935 A new phenotypic effect of the Y chromosome in *Drosophila melanogaster*. *J. Genet.* **30**: 423-446.
- GERSH, E. S., 1949 Influence of temperature on the expression of position effects in the scute-8 stock of *Drosophila melanogaster* and its relation to heterochromatization. *Genetics* **34**: 701-707.
- 1952 Pigmentation in a mottled white eye due to position effect in *Drosophila melanogaster*. *Genetics* **37**: 322-338.
- GOLDSCHMIDT, R., 1953 Heredity within a sex-controlled structure of *Drosophila*. *J. Exp. Zool.* **122**: 53-95.
- GOLDSCHMIDT, R., ALOHA HANNAH, and L. K. PITERNICK, 1951 The podoptera effect in *Drosophila melanogaster*. *Univ. Calif. (Berkeley) Publ. Zoöl.* **55**: 67-294.
- GOWEN, J. W., and E. H. GAY, 1934 Chromosome constitution and behavior in eversporting and mottling in *Drosophila melanogaster*. *Genetics* **19**: 189-208.
- HANNAH, ALOHA, 1951 Localization and function of heterochromatin in *Drosophila melanogaster*. *Advances in Genet.* **4**: 87-125.
- HEITZ, E., 1933 Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. *Z. Zellforsch.* **20**: 237-287.
- HINTON, T., 1950 A correlation of phenotypic changes and chromosomal rearrangements at the two ends of an inversion. *Genetics* **35**: 188-205.
- HUGHES-SCHRADER, S., 1948 Cytology of Coccids (Coccoidea-Homoptera). *Advances in Genet.* **2**: 127-203.
- KAUFMANN, B. P., 1933 Interchange between X- and Y-chromosomes in attached X females of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. U. S.* **19**: 830-838.
- 1934 Somatic mitoses of *Drosophila melanogaster*. *J. Morphol.* **56**: 125-155.
- 1942 Reversion from roughest to wild type in *Drosophila melanogaster*. *Genetics* **27**: 537-549.
- 1944 Cytology: Organization of the chromosome. *Carnegie Inst. Wash. Ybk.* **43**: 115-120.
- 1946 Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. *J. Exp. Zool.* **102**: 293-320.
- KRIVSHENKO, J., 1955 *Drosophila melanogaster*: New mutants. *Drosophila Inf. Ser.* **28**: 74-75.
- LEWIS, E. B., 1950 The phenomenon of position effect. *Advances in Genet.* **3**: 73-115.
- 1954 The theory and application of a new method of detecting chromosomal rearrangements in *Drosophila melanogaster*. *Am. Naturalist* **88**: 225-239.
- LINDSLEY, D. L., 1955 Spermatogonial exchange between the X and Y chromosomes of *Drosophila melanogaster*. *Genetics* **40**: 24-44.
- LINDSLEY, D. L., and E. NOVITSKI, 1950 The synthesis of an attached XY chromosome. *Drosophila Inf. Ser.* **24**: 84-85.
- MORGAN, L. V., 1938 Origin of attached-X chromosomes in *Drosophila melanogaster* and the occurrence of nondisjunction of X's in the male. *Am. Naturalist* **72**: 434-446.
- MORGAN, T. H., C. B. BRIDGES, and J. SCHULTZ, 1933 Constitution of the germinal material in relation to heredity. *Carnegie Inst. Wash. Ybk.* **32**: 298-302.
- 1934 *ibidem*, **33**: 274-280.
- 1935 *ibidem*, **34**: 284-291.
- 1936 *ibidem*, **35**: 289-297.
- 1937 *ibidem*, **36**: 298-305.
- 1938 *ibidem*, **37**: 304-309.
- MORGAN, T. H., and J. SCHULTZ, 1942 Investigations on the constitution of the germinal material in relation to heredity. *Carnegie Inst. Wash. Ybk.* **41**: 242-245.
- MORGAN, T. H., J. SCHULTZ, C. B. BRIDGES, and V. CURRY, 1939 Investigations on the constitution of the germinal material in relation to heredity. *Carnegie Inst. Wash. Ybk.* **38**: 273-277.

- MORGAN, T. H., J. SCHULTZ, and V. CURRY, 1940 Investigations on the constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **39**: 251-255.
- 1941 *ibidem*, **40**: 282-287.
- MORGAN, T. H., A. H. STURTEVANT, and C. B. BRIDGES, 1926 The constitution of the germ material in relation to heredity. Carnegie Inst. Wash. Ybk. **25**: 308-312.
- MULLER, H. J., 1930 Types of visible variations induced by X-rays in *Drosophila*. J. Genet. **22**: 299-334.
- MULLER, H. J., and A. A. PROKOFYEVA, 1935 The individual gene in relation to the chromomere and the chromosome. Proc. Nat. Acad. Sci. U. S. **21**: 16-26.
- NOUJIN, N. I., 1936 Influence of the Y chromosome and of the homologous region of the X on mosaicism in *Drosophila*. Nature **137**: 319-320.
- 1938 A study of mosaicism of the eversporting displacement type in *Drosophila melanogaster*. Bull Biol. Med. Exp. U.R.S.S. **5**: 548-551.
- 1939 Effect of the 'inert' regions of chromosomes on the expression of mosaic characters. Compt. Rend. Acad. Sci. U.R.S.S. **22**: 602-605.
- 1946a Multiple ratios of mosaic frequencies. Compt. Rend. Acad. Sci. U.R.S.S. **53**: 835-837.
- 1946b Heterochromatin, rate of embryonic development, and mosaicism. Compt. Rend. Acad. Sci. U.R.S.S. **54**: 61-63.
- OFFERMANN, C. A., 1935 The position effect and its bearing on genetics. Bull. Acad. Sci. U.R.S.S. 1935(1): 129-140.
- PANSHIN, I. B., 1938 [The cytogenetic nature of the position effect of the genes white (mottled) and cubitus interruptus.] Biol. Zhur. **7**: 837-865. (in Russian; cited from LEWIS 1950, and others.)
- PONTECORVO, G., 1943 Meiosis in the striped hamster (*Cricetulus griseus* Milne-Edw.) and the problem of heterochromatin in mammalian sex chromosomes. Proc. Roy. Soc. Edinburgh B **62**: 32-42.
- PROKOFYEVA-BELGOVSKAYA, A. A., 1939 Cytological mechanism of mosaicism and chromosome rearrangement. Compt. Rend. Acad. Sci. U.R.S.S. **22**: 270-273.
- 1946 Heterocyclicity of the system of cell nucleus. Compt. Rend. Acad. Sci. U.R.S.S. **53**: 741-745.
- 1947 Heterochromatization as a change of chromosome cycle. J. Genet. **48**: 80-98.
- PROKOFYEVA-BELGOVSKAYA, A. A., and V. V. KHVOSTOVA, 1939 Distribution of breaks in the X chromosome of *Drosophila melanogaster*. Comp. Rend. Acad. Sci. U.R.S.S. **23**: 270-272.
- RAFFEL, D., and H. J. MULLER, 1940 Position effect and gene divisibility considered in connection with three strikingly similar scute mutations. Genetics **25**: 541-583.
- SAFIR, S. R., 1920 Genetic and cytological examination of the phenomenon of primary non-disjunction in *Drosophila melanogaster*. Genetics **5**: 459-487.
- SCHULTZ, J., 1936 Variegation in *Drosophila* and the inert chromosome regions. Proc. Nat. Acad. Sci. U. S. **22**: 27-33.
- 1941 The function of heterochromatin. Proc. 7th Inter. Congr. Genet., J. Genet. (Suppl. Vol.): 257-262.
- 1942 The evidence of the nucleoprotein nature of the gene. Cold Spring Harbor Symposia Quant. Biol. **9**: 55-65.
- 1943 A derivative of *In(1)w^{m4}* showing a different reaction to heterochromatin. *Drosophila Inf. Ser.* **17**: 64-65.
- 1948 The nature of heterochromatin. Cold Spring Harbor Symposia Quant. Biol. **12**: 179-191.
- 1952 Interrelations between nucleus and cytoplasm: problems at the biological level. Exp. Cell Research, Suppl. **2**: 17-42.
- SCHULTZ, J., and T. CASPERSSON, 1939 Heterochromatic regions and the nucleic acid metabolism of the chromosomes. Arch. Exp. Zellforsch. Gewebezücht. **22**: 650-654.
- 1949 Nucleic acids in *Drosophila* eggs, and Y chromosome effects. Nature **163**: 66-67.
- SHEN, T. H. 1932 Zytologische Untersuchungen über Sterilität bei Männchen von *Drosophila melanogaster* und bei F₁-Männchen der Kreuzung zwischen *D. simulans*-Weibchen und *D. melanogaster*-Männchen. Z. Zellforsch. **15**: 547-580.

- SLIZYNSKI, H., 1945 'Ectopic' pairing and the distribution of heterochromatin in the X chromosome of salivary gland nuclei of *Drosophila melanogaster*. Proc. Roy. Soc. Edinburgh B **62**: 114-119.
- STERN, C., 1929a Über die additive Wirkung multipler Allele. Biol. Zentr. **49**: 261-290.
- 1929b Untersuchungen über Aberrationen des Y-Chromosoms von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **51**: 253-353.
- 1936 Interspecific sterility. Am. Naturalist **70**: 123-142.
- STERN, C., and E. HADORN, 1938 The determination of sterility in *Drosophila* males without a complete Y chromosome. Am. Naturalist **72**: 42-52.
- STERN, C., and G. HEIDENTHAL, 1944 Materials for the study of the position effect of normal and mutant genes. Proc. Nat. Acad. Sci. U. S. **30**: 197-205.
- STERN, C., and M. KODANI, 1955 Studies on the position effect at the cubitus interruptus locus of *Drosophila melanogaster*. Genetics **40**: 343-373.
- STURTEVANT, A. H., 1925 The effects of unequal crossing over at the Bar locus in *Drosophila*. Genetics **10**: 117-147.
- 1945 A gene in *Drosophila melanogaster* that transforms females into males. Genetics **30**: 297-299.
- WHITE, M. J. D., 1954 *Animal Cytology and Evolution*. xiv + 454 pp. Cambridge.