The Dosage compensation of *Drosophila* and mammals as showing the accuracy of the normal type*

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It has been established for over fifty years in *Oenothera* and for forty-five in *Datura* and *Drosophila* that the effect of aneuploidy of a whole chromosome is, in relation to the amount of chromatin change, far more damaging than alteration in the number of entire chromosome sets. This finding came as no surprise to those who at that time thought of the phenotype as resulting from chemical reactions. Yet the *Drosophila* X-chromosome, although known to carry many genes with functions unrelated to sex determination, varied in number in relation to the autosomes without causing more than the adaptive differences between the sexes.

1. DOSAGE COMPENSATION IN *DROSOPHILA*

The early course of development of the concept of dosage compensation was a zigzagging and rather confusing one, that has usually been misinterpreted. But the reader who prefers to omit the account of it given (in small type) in the next eight paragraphs is unlikely to find his understanding of the remainder of this article impaired.

The peculiarity of expression of genes in the *Drosophila* X, when realized to be one, tended to be interpreted on the assumption that the normal genes in the X, in consequence of the natural selection caused by the 1X:2X method of sex determination, had come to have, individually, an even more nearly equal effect in one and two doses than the autosomal genes were thought to have, in a situation in which the doses of all other genes were somehow held constant. Incidentally, the 'presence and absence' hypothesis of Bateson and Punnett had taken it for granted that, in general, one dose of a gene has as much effect as two. In the *Drosophila* group, however, Altenburg in his M.A. thesis (1912, unpublished) had shown the theoretical unsoundness of this position, on the ground that the concentration of a gene's products, determined by its dose, could be expected to have an influence on the phenotype.

The difficulty involved in the case of the genes in the X was early aggravated by the phenotypic intermediacy of females heterozygous for the dominant sex-linked mutant Bar (narrower) eye, in contrast to the very similar phenotypes of the Bar male and the homozygous Bar female. Muller, in considering this situation in a passage in *The Mechanism of Mendelian Heredity* (1915, p. 30) stated that this 'intermediate condition . . . is probably due to the competing influence of the other [the normal] allelomorph . . .' This preferred view, which we

may here term 'the first interpretation', is now known to be incorrect as applied specifically to Bar, although valid for a few other cases. However, the passage continues: 'it might be contended that since in the male there is a different chromosome complex (XABCD/YABCD) from that in the female (XABCD/XABCD) it is this difference in other factors that causes the heterozygous female to have a wider eye than the male ...' What was referred to here was of course the different doses of other genes in the X, since the autosomes remained constant and the Y was already known to be relatively inactive in *Drosophila*. The proposed relation was later (Muller, 1930) developed as 'intra-chromosomal genic balance.'

In regard to this 'second alternative', however, the passage of 1915 continues as follows: 'this argument is rendered improbable here, when we recall that in only one out of many cases of sex-linked inheritance in which the heterozygous female is intermediate [three other examples of which are given on pp. 30–32] is the male different from the homozygous female.' The unstated but implied conclusion here is that of the first alternative, i.e. that probably the mutant alleles in most heterozygotes compete with the normal ones, just as Bar was thought to do, although not so effectively as the latter, and that they tend to produce effects contrasting with those of normal alleles, but that one and two doses of a given allele, whether mutant or normal, usually produce nearly the same effect.

It is true that Mohr (1919 et seq.) surmised, on the ground of the 'exaggerating' effect of 'deficiencies' of a small part of any chromosome on the expression of most recessive mutant genes lying in the corresponding part of the homologous chromosome, that one dose of a gene resulted in a less normal phenotype than do two doses. But it was as yet quite unproved that a 'deficiency' represents an actual loss. It had also been conjectured that a deficiency is a kind of inactivating 'chain' or 'line' mutation of adjoining genes, whereby they became unable to produce the normal alleles' effects yet competed with the latter. Without salivary chromosome techniques, this question could not be resolved cytologically.

Moreover, three clear-cut cases were recorded by Bridges (in a chapter, 'Non-disjunction of the fourth chromosome', in Morgan, Bridges & Sturtevant, 1925, pp. 138–139), of fourth-chromosome mutants—shaven, eyeless, and bent-wings—that resulted in more extreme phenotypes in flies known to have only one fourth-chromosome than in those homozygous for these genes. But these 'exaggerations'—and also those caused by interstitial 'deficiencies'—were here interpreted as meaning that more 'plus' than 'minus' modifiers for the character in question had happened to be located within the given region. Primarily, the cases were presented in evidence that genes are in chromosomes.

Thus, when Stern (1929) reported a case of demonstrable dosage change—involving bobbed bristles, a mutant with a locus in both the X and the Y—it was widely thought to be the first such case. On the basis of the bobbed case, Stern (1929, 1930; Stern & Ogura, 1931) concluded that, in general, recessive mutant genes (at least) do have distinctly unequal effects when their dosage is varied. Since the other mutant genes in the X, taken as a whole, do not seem to behave in this way, he reached a conclusion quite coinciding with the 'second alternative interpretation', i.e., that the rest of the X interacts with any given gene in it to equalize that gene's expression in male and female. This relation had somehow been caused by natural selection for optimal and therefore similar phenotypes in the two sexes. But bobbed, being in both X and Y, was an exception that helped to prove the rule. However, on a diagram, he represented the normal allele of bobbed as having already attained, in one dose, the maximal and hence optimal effect—a result not to be expected if the principle applied to normal genes.

Nevertheless, it still remained possible that, through natural selection, the expression of the genes in the X that were *not* represented in the Y had become much less affected by individual changes in their dose, given an otherwise constant genome, than had that of other genes, while the genes in what we have termed chain mutations engage in competition, more evident when their alleles are mutant.

Definite evidence of the 'second alternative', and therefore against the view just expressed, was presented in the finding (Muller, League & Offermann, 1931; Muller, 1933)* that recessive

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* These findings, and their interpretation in terms of dosage compensation, made in 1930 and communicated by the senior author in that year to several geneticists elsewhere (see Muller, 1933, footnote 3), were withheld from publication for more than a year for the obtaining of confirmatory evidence.

mutant genes (other than bobbed) sampled in the X of *Drosophila* and found to act as 'hypo-morphs', in that their effect approached normal as their relative dose was raised, as well as Bar eye, which was termed a 'neomorph', usually express themselves distinctly more strongly when in *two* doses in the male than in two doses in the female. The method used was to get, by irradiation, a short piece of the X, containing the mutant gene in question, and then by appropriate crosses to get males with that fragment of X and an allele of the same kind in the unbroken X, for comparison with males having only the unbroken X. Muller's interpretation of these findings has been that they are manifestations of 'dosage compensation'. Other tests also proved that a dose of a hypomorph demonstrably below the normal dose caused 'exaggeration' of the mutant character. Not long afterwards the salivary-chromosome method showed deficiencies in general to be actually without the part in question (Mackensen, 1934).

Although the phenomenon of dosage compensation was thus disclosed by a study of *mutant* genes, the implication for normal genes in *Drosophila* was more important. This implication was that the normal genes of the X, except those engaged in sex differentiation, have by the natural selection of appropriate X-contained mutations become interadjusted, as of course implied although considered improbable when the second alternative was first mentioned. The interadjustment has been such that in the normal diploid type the otherwise expected effect of altering the dose of a given gene in the X is counteracted by the influence of the simultaneous and equal alterations of dose of one or more other genes in the X, the compensators of the given gene. Thus, within the limits of relative-dosage difference between male and female, the amount of final effect of these genes in the X remains about the same regardless of their dose-ratio to autosomal genes.

Nevertheless, the now normal genes in all the chromosomes have been subjected to a lengthy natural selection for phenotypic stabilization against diverse influences other than those of dosage or heterozygosis that might disturb their expression (Muller, 1918, 1933; Plunkett, 1933*a*, *b*). This selection must of course have included much selection throughout the genome of other genes, that interacted with the former and brought these up closer to a saturation level of expression, although because of biochemical hindrances not all the way up to it. An incidental result, evident in the usual 'dominance' of normal over mutant genes, has been that, as also indicated in 1915, even an uncompensated one dose–two dose difference in normal genes ordinarily causes too small a phenotypic difference to allow it to be observed by superficial means. Hypomorphic mutant genes, by contrast, having 'potencies' (effectiveness) further below the phenotypic saturation level, more readily disclose to the observer the effects of dosage differences, and hence of dosage compensation also. A similar situation exists for those neomorphs the action of which follows a pathway appropriately related to that of the original gene although in a new phenotypic direction. In a sense, then, the mutants sensitize the observer to these functionally advantageous interactions that actually occur among the normal genes as well.

This compensation theory and further developments of it (Muller, 1933, 1950) became strongly substantiated by evidence gathered from very diverse directions. It was concluded that, typically, a given gene in the *Drosophila* X has its own group of several compensators, distinctively scattered about that same chromosome. In fact, the compensators are sometimes different ones even for different phases of a

given complex gene's actions (work of Lieb, to be explained in section 6). Furthermore, it could be inferred that the selective steps by which the individual compensators had become established, being based on very slight, 'second-order' advantages, had frequently been rather coarse in relation to the adjustment to be reached. For the same reason, even anti-compensators had often become established also—doubtless by virtue of other, more important effects connected with them. Yet eventually, through a succession of slowly achieved corrections, a balance far too accurate to be detected by ordinary observation had been attained, and the over-all result from selection had therefore turned out to give a very delicate adjustment indeed. Of course, any given compensating gene may have, and probably it usually does have, other, more major, as well as other minor effects, while as a compensator to a given gene it contributes only a mite to the entire manifold of processes that result in the compensation of so many different genes at once.

2. DOSAGE COMPENSATION IN MAMMALS

More recently, beginning with the discovery by Ohno, Kaplan & Kinosita (1959) that in somatic cells of mammals only one of the female's X chromosomes is in extended condition in interphase, it has become evident that the genetic inactivity associated with chromosome condensation results in a type of dosage compensation in this large group of animals also (Mary F. Lyon, 1961). For the somatic cell of the female, despite its possession of two representatives of each of the genes of the X, has only one effective dose—at least throughout by far the major portion of the X—and hence is like the male with its one dose, in regard to the effect produced in the cell by such a gene.

In seeking an evolutionary, functional interpretation of this result one is led, from the very existence of it, to the inescapable inference that in mammals as in diptera, despite a normal gene's seeming dominance, two doses of it, without compensation, are not really equivalent in their effect to one dose, when the genome in general remains at the diploid dose. One must infer further that in the X-chromosome, again as in diptera, a compensation mechanism has been established by natural selection, whereby most of the normal genes of the X in diploid somatic cells have come to exert about the same effect when present in one dose, in the single X, as when in two doses, in two X's. Obviously, for mammals, though not necessarily for diptera, the optimum dose for these genes if somehow uncompensated, in diploids, is now the one dose of a single X.

The mammalian dosage-compensation mechanism works by the condensation of the X-chromosome as a whole, controlled by an influence apparently stemming from one or only a few regions or genes in the X and spreading from there. This type of control has been demonstrated by the observations of Cattanach (1961), Ohno & Cattanach (1962), and Liane B. Russell (1963) on translocations between the X and autosomes. The establishment of this mechanism, because it acted in a 'wholesale' way, must have been far simpler and have involved much stronger selective forces than the ones, depicted above for diptera, that acted piecemeal for the individual

genes of the X. This being the case, the existence of the mammalian mechanism would not carry nearly so strong an implication as does the dipteran one regarding the need for high precision of the effects of *individual* normal genes.

3. HOW DIFFERENT IN PRINCIPLE ARE THE FINDINGS IN THE TWO GROUPS?

The question has presented itself, however, whether in this respect the dipteran dosage-compensation mechanism may not be essentially like the mammalian one after all, in working by a process that involves virtually the whole of the X-chromosome at once. If so, of course, it would not be necessary to conclude that there is a significant advantage in having so delicate an adjustment of the effectiveness of each individual normal gene as is called for on the theory that had earlier been devised for diptera.

The source of this doubt goes back to observations reported by Offermann in 1936, although he did not question the compensation theory that he had helped to found. He reported that the polytene X present in the salivary nuclei of larval *Drosophila melanogaster* males is greater in its horizontal diameter than it would be if all the salivary chromosomes were approximately circular in cross section and if the cross-sectional area of the male's X were only half that of the conjugated, originally diploid X of females. This would also make the volume of the male's X correspondingly larger, since the lengths were found by Offermann to be alike.

According to his measurements of diameters, the cross-sectional area of the male's X would not be 0·5 but 0·75 that of a conjugated pair of chromosomes (autosomes) of a given specimen. At the same time, his measurements of a region of the female's X that had been left unconjugated, by reason of a small deletion (Notch 172b deficiency) of the homologue, indicated a cross-sectional area that was even less than 0·5—about 0·42—that of the pairs of conjugated autosomes of the same individual. Accepting, as all the investigators have, the essential likeness of diameter of conjugated X's and autosomes, this would seem to make the cross-sectional area of a male's X not far from double (1·8 times) that of an unconjugated part of a female's X, even though only 1·5 the area of half of a pair of conjugated X's.

It seems probable that the finding of so small a diameter for a short unconjugated portion of the female's X is to be understood as expressing (1) a deformation of that portion from its usual cross-sectional shape, occasioned by its being bent around in the plane parallel with the slide, and hence compressed vertically, in adjustment to the relatively short interstitial deletion of the homologous chromosome and, in addition (2), by the pressure of apposition between the two homologues, to approach a cylindrical shape for the whole, on both sides of this deletion. As for the unexpectedly large width of the male's X, this was conjectured to be a possible result of a difference in stresses between the X-chromosomes compared, in this case expressing a lesser synaptic force in the male's X, caused for instance by the smaller number of strands as compared with those in females. However, observations by ourselves and others, to be mentioned in sections 4 and 5, even if only qualitatively correct, would

not allow this to be the entire explanation. At any rate, as Offermann also noted, the unconjugated X of the male's salivaries is stained so much more lightly than are the pair of conjugated X's of the females as to indicate the presence of not more than half as much chromatin in the former as in the latter.

More recently, statements have been made by other investigators to the effect that 'the X in male larvae is not appreciably smaller in diameter than are the bipartite chromosomes in either sex.' However that may be, all these authors recognize the male's X to be distinctly paler, and in this respect as well as in its greater width unlike portions of doubly represented chromosomes in which there has for any reason been a failure of conjugation between the homologues. According to Dobzhansky (1957), the offspring of *D. insularis* crossed by *D. tropicalis* show this situation most clearly, inasmuch as here no salivary chromosomes undergo any conjugation at all, and all of them are alike in width and darkness in both sexes, except that the width of the single X of the male is 'about double' that of the rest and its staining is correspondingly lighter. Of course if this were true its cross-sectional area would really be four times as great as that of the others. This is contrary to the estimate given elsewhere in the papers referred to, that the volume of the X in the male is about equal to that of the double X's of females. For with a four-fold area, the male's X would be as much larger than a pair of conjugated X's of the female—twice as much larger— as they in turn were larger than their individually considered unconjugated portions. Other inconsistencies will be pointed out later.

In any case, however, such a situation would seem really to challenge the earlier dosage-compensation theory, especially since it is sometimes held that a chromosome's apparent width when it is in an extended form (as in the salivaries) indicates its activity. For then the mechanism of compensation in *Drosophila* might appear, at first sight at least, to be like that in mammals in principle, even though reverse in its details. For it might be thought to work by means of an influence emanating from one or a few genetic sources but exerted on all or a large part of an X chromosome at once. The mammal, however, would have the effectiveness of the female's two X's reduced to that of the male's single X, by the condensation of one of the two X's, whereas the dipteran had the size and effectiveness of the male's single X raised to that of both the female's X's together.

The here-postulated concentration of source of this influence, although not a necessary condition of such an assumed regulation of the size and activity of the X as a whole in diptera, would nevertheless appear, merely on the face of it, to be not unlikely, if such regulation did exist. For the situation would then be analogous to the mammalian one and it would be far simpler both in origin and workings than the mechanism earlier depicted. But, for these very reasons, it would not lead to nearly such far-reaching and important conclusions regarding the survival value of very high precision in the degree of effectiveness of individual normal genes. It was therefore decided that a cytological investigation of a special kind should be conducted by the junior author on the salivary chromosomes of *D. melanogaster* males, and that the subject in general should be restudied by the senior author. (See our preliminary abstract, 1964.)

4. CYTOLOGY

It was decided to use for examination cases of translocation between the X and the fourth chromosome, that had resulted from breakage in known positions in the X. Translocations allow a kind of dissection of the phenomena in question (as they did in mice), and more specifically a determination of whether they are subject to 'spreading'. Among the advantages of the given choice of translocations were the availability of four different 'TX4' (or 'TX-IV') stocks, involving five breaks of the X, the positions of which had already been determined, four of them being widely separated. This foreknowledge allowed the observer more readily to trace both (or all) portions of the X to their formerly broken ends. The use of the small distinctive fourth chromosome, lying as it does near the chromocenter, made the autosome concerned easier to find and to recognize. The diversity in position of the breakages of the X made it possible to obtain more light than otherwise on the degree of concentration of the genetic source of any effects on chromosome appearance to be found in either the X or the attached autosome parts.

The translocations used are listed below, arranged according to their breakage positions from left to right in the euchromatin of the X. The breakage positions are shown in parentheses according to Bridges' scheme. All the breakages in the X are in the left arm. This arm includes all the euchromatin as well as, near the centromere, most of the heterochromatin of the X. The distal portion, carrying the free end of the X, is designated by us as DX. In Figs. 1, 3, 4, and 5, that portion of the left arm that lies proximal to or at the right of the break is designated as PX, and is still connected to the centromere of the X. As will be explained, the special case in Fig. 2 has required a different designation.

The right arm of Chromosome IV is euchromatic; its left arm, heterochromatic. Thus when, as in all but the first of our figures of translocations, the break in IV is close to the distal end of the right arm, we designate all but the tip of that arm as P-IV, only the tip being D-IV. In the first case, however, since the break in IV is in its purely heterochromatic left arm (usually buried in the chromocenter), all of its right arm is designated R-IV and the distal portion of the X is attached to L-IV.

(1) Case 'e6' (so designated here because of its place in the Bloomington stock list, D.I.S. 37). In this case DX became attached at its broken end to the proximal broken end of the heterochromatic left arm of IV. Thus the whole right arm of IV is indirectly connected, via the centromere of IV and the chromocenter, to DX, while PX is capped by the tiny distal piece of the left arm of IV. (9A, 101.)

(2) 'W13' is a transposition, a case in which two breaks have occurred in one chromosome, the X. The piece embraced by these breaks has been inserted between the two portions formed by the one break of the other chromosome, the fourth, broken near the end of its right arm. The distal and proximal portions of the twice-broken X-chromosome have united at their broken ends, leaving the X deleted. The mid-portion of the X thus removed was a large one, comprising about half of its euchromatic part. In Fig. 2 it has been designated MX. It is inserted in an inverted position with respect to the centromere-carrying portion of the right arm of IV and

is capped by the small distal piece of IV. These pieces of the fourth chromosome are shown as P-IV and D-IV, respectively.

(3) 'TX-IV Sidky' (13B8 and 102F).

(4) 'TX-IV Bar-Stone' or B^S: (16 A1 and 102F).

Fixation and staining was by means of aceto-lacto-orcein according to the method outlined by Nicoletti (1959). In our case the glands remained in the stain-fixative for only 3 min. and were then viewed and photographed by means of phase-contrast optics.

In the clearer slides of all four of these cases it was apparent that the width of each portion of the X seen was distinctly less throughout its length—except for the usual variations arising from chance arrangement, stretching, compressing, staining, and natural regional differences of genetic and functional origin—than the width of either piece of the second or third chromosomes. Although, because of such variations, it was felt that actual measurements would give an impression of greater accuracy than was feasible, the width of the single X, relatively to that of a pair of major autosomes, was usually in the range of 0·7 (resulting in a cross-sectional area of 0·5) to somewhat less than 0·9 (area 0·8). An intermediate (0·8) width would of course give an 0·64 area in cross section, and therefore a 1·3 area in comparison with just one member of a conjugated pair. However, the X parts tended also to be noticeably lighter than autosomes—a result that seemed more marked than would have been expected as a sole consequence of their smaller diameter in the vertical direction, that of vision.

Most striking of all was the fact that at each junction between the unconjugated X portion and the conjugated (double) portion, R-IV, of the fourth chromosome there was a very sudden transition in width from the narrower X to the much wider fourth, even when the effect of the tiny unpaired portion of the fourth projecting beyond this junction point was allowed for. This difference in width between the X and the fourth (which could, of course, be compared more readily, because of the junction and the consequent subjection of them and the X to similar stresses, than could the X and other autosomes) often appeared great enough, in fact, to have resulted from an actual doubleness of volume of the double fourth, per unit of length, relatively to the single X.

Another important point is the fact that there is no consistent difference between the size and staining characteristics of DX and PX, other than those seen in unbroken X's. This is true no matter at which of the widely separated positions the X had been broken to form the four different translocations (including the transposition) used.

All these points are to be noted to some extent in the photographs of the representative specimens presented in Figs. 1 to 5, of cases 1 to 4, respectively, Fig. 5 being another view of case 4. As is always true in salivary chromosome work, a single figure of a case by no means shows all parts typically. It should perhaps be added that in males without translocations the normally structured X exhibits the same differences from the autosomes as seen in the present specimens.

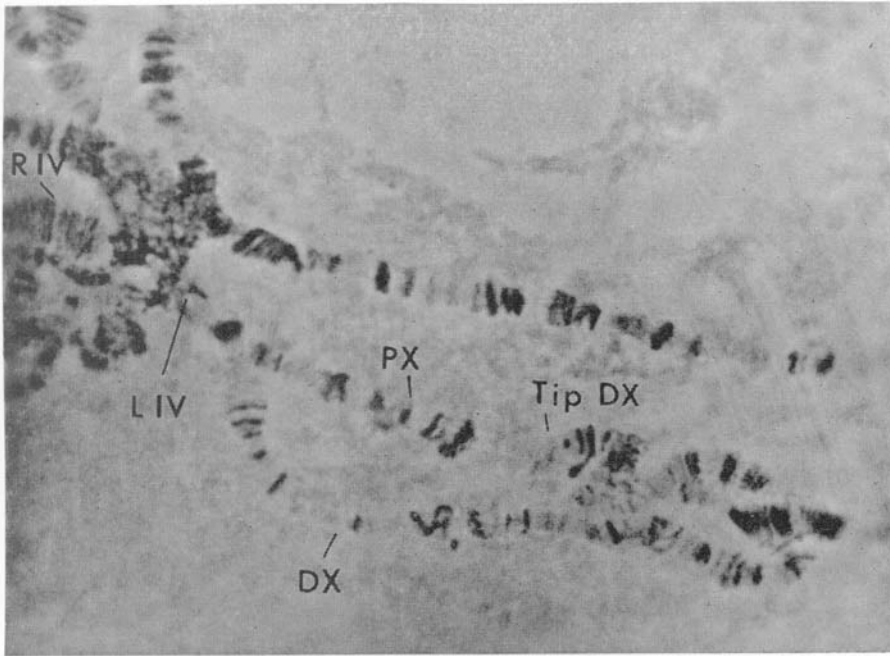These results, taken together, show that the male's one X has a distinctly smaller
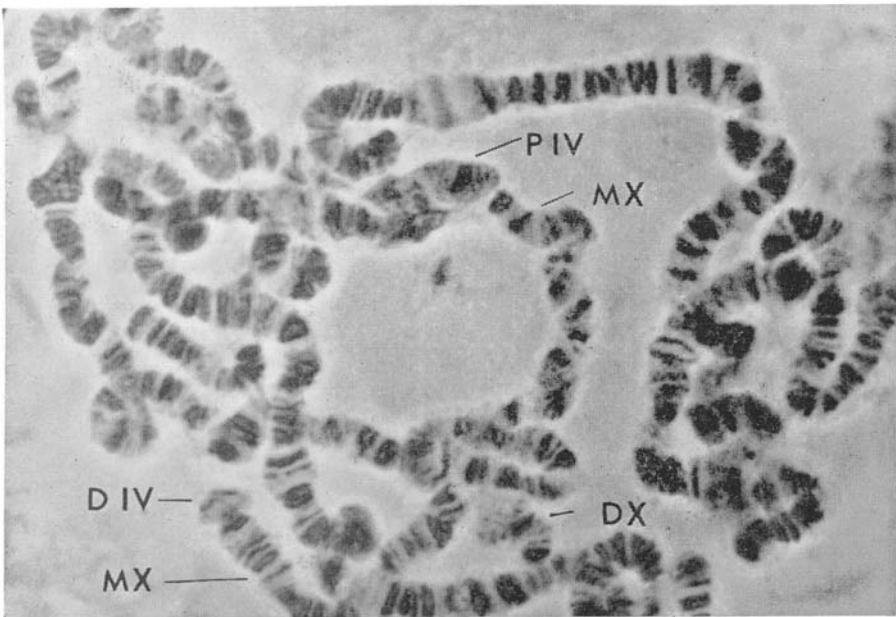
e 6

Fig. 1. Translocation, e6.

W 13

Fig. 2. Translocation, W13.

All photos taken by Zeiss phase optics at $10 \times$ *by* $100 \times$, *except for Fig.* 4 *which is* $10 \times$ *by* $40 \times$.
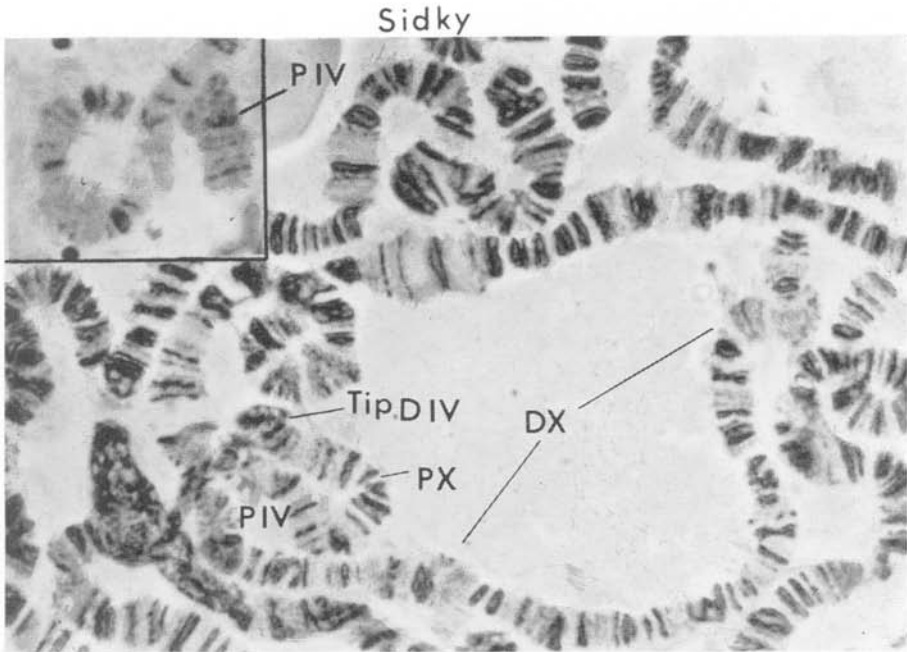
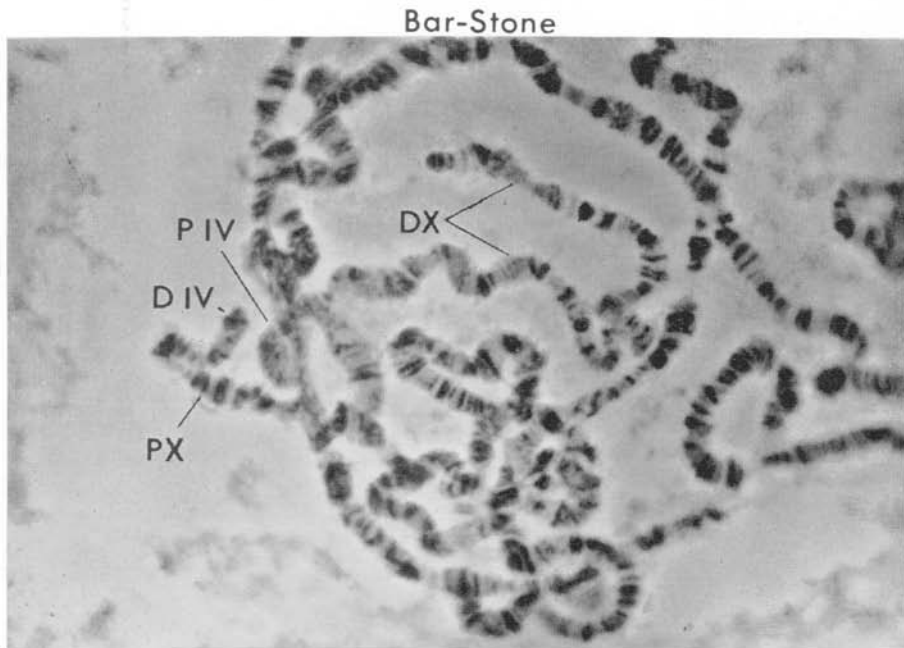H. J. MULLER AND W. D. KAPLAN

Fig. 3. Translocation, Sidky. (Inset in upper left corner shows DX and PIV junction.)

Figs. 4 and 5. Translocation, Bar-Stone. (Fig. 4 shows all components of the translocation; Fig. 5 shows the PIV and DX junction.)
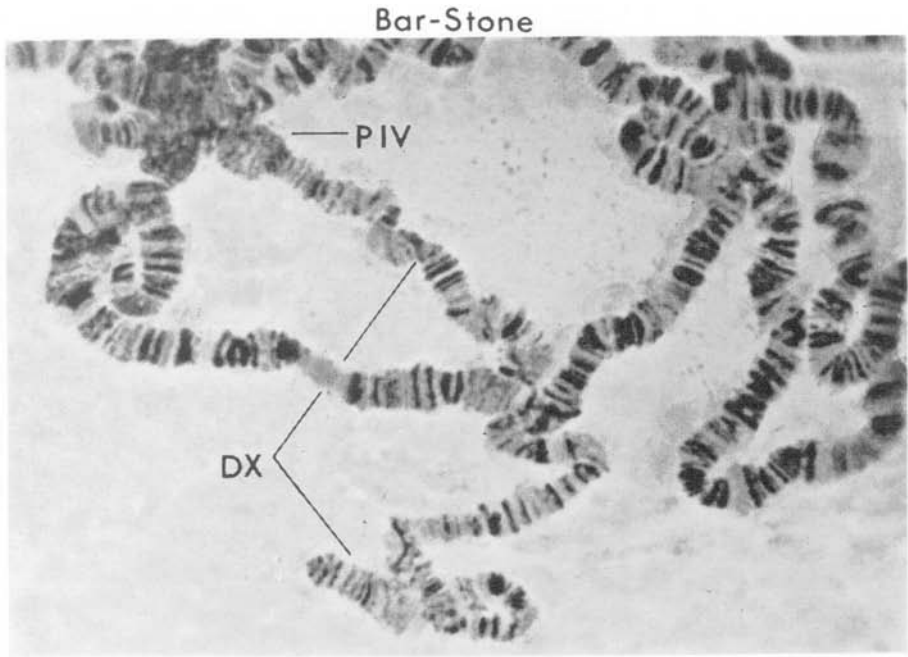
H. J. MULLER AND W. D. KAPLAN
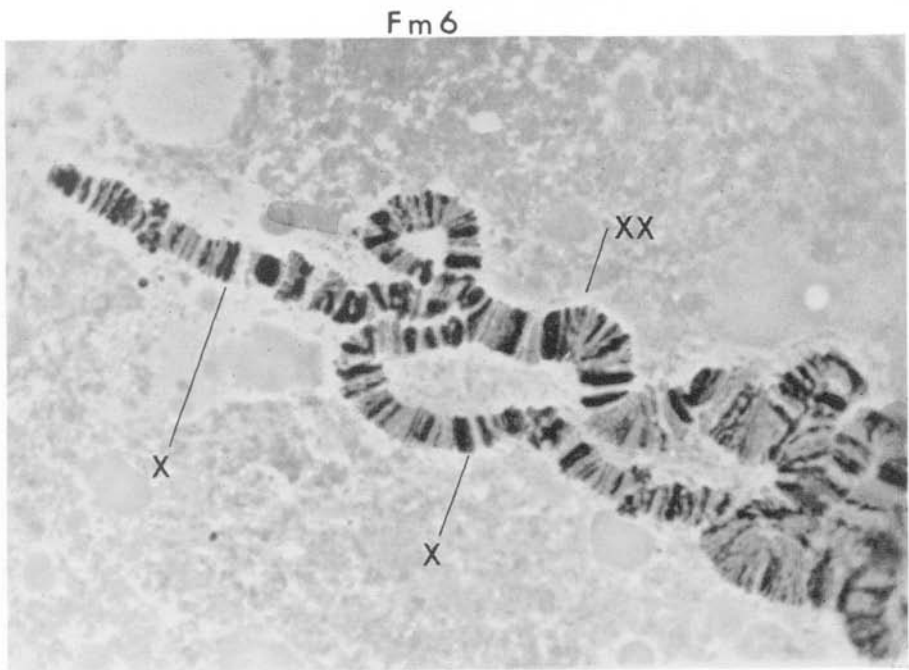
Bar-Stone

Fig. 5

Fm 6

Fig. 6. Inversion, Fm6.

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cross section, in relation to the autosomes, than has the female's pair of conjugated X's. Whether it is about equal to a member (one-half) of the female's pair or somewhere between that and 1·6—most likely about 1·3—times the latter's in cross section is not made certain by these results alone. That it is probably somewhat more than equal, however, is made more probable, among other things, by its distinctly lighter staining.

The above evidence is given greater weight by that presented in our Fig. 6. This is a photograph of a case in which we found that a rather long region of a female's double X had failed, because of heterozygous inversions caused by the inversion-complex termed 'FM6' (Grell & Lewis, 1956), to synapse with the normally structured X. It will be seen that the unsynapsed parts are distinctly narrower, in relation to the synapsed parts of the X or autosomes, than is the X of the male either when it is normally structured or involved in a translocation. Moreover, the unsynapsed parts appear to be about as heavily stained as the synapsed ones, and therefore more heavily stained than the male's X. This preparation was made and examined by the same techniques as used in our preparations of translocations. In this case, of course, the objection raised, in section 3, paragraph 4, regarding short unsynapsed regions do not apply. Thus it becomes very probable that the male's X is really somewhat larger in cross section, and less heavily stained, than half of the female's double X, or than an unpaired part of the double X.

The translocation results show, further, that whatever may be the cause of the various distinctive attributes of the male's single X, this cause is not concentrated in one main center in the X, from which the influence diffuses along the chromosome. For if it were of this nature, the phenomena would not be just as evident as before in both parts of the X after the X had been divided. Nor would they stop abruptly short at the small fourth chromosome—nor stop regardless of where (within the limits of our experiment) the autosomes had been attached to the X. This argument also holds against any idea of diffusion along the chromosome of influences that stemmed from several or many centers in the X, even if they originated in all parts of the X. Moreover, the typical narrowness and paleness of the portion MX of the 'W13' translocation shows more specifically that there cannot be main centers of the kind in question at either or both normal ends of the X chromosome, somehow capable of influencing only the X, since this MX piece lacks both those ends.

5. CRITIQUE OF THESE AND RELATED CYTOLOGICAL RESULTS

Thus it can be concluded that the morphological and biochemical properties above considered are determined autonomously in each region of the X, without diffusion, a situation quite unlike that in mammals. And this is what had earlier been concluded for the phenomena of dosage compensation themselves in *Drosophila*, on the basis of observations that will be reviewed in section 6.

It was only after our own work had been completed that we came across an article of 1964 by Rudkin, and through it an abstract by Aronson, Rudkin & Schultz published in 1954. The valuable data obtained by this group in 1954 were presented

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in the later article. However, early in that article, much as in that quoted from in our section 4, paragraph 5, the statement is still made, and not later withdrawn, that the salivary 'X chromosome of *D. melanogaster* . . . appears to have approximately the same diameter as the autosomes, regardless of the sex.' In spite of this, the 1954 measurements of chromosome width presented by Rudkin show a very significantly smaller cross section—about 0·6 as great—for the male's one X as for the female's double X, if with Rudkin one reckons the latter by doubling the result found by these investigators for a short unsynapsed portion of it.

We have seen, however, in section 3, that when, as in Offermann's study, the female's unpaired portion is a relatively short interstitial one, twice this size may be too small an estimate for the female's double X. In that case the size of the male's single X would be too great in relation to the double X when estimated by a comparison of the male's X with a short unpaired portion of the double X. In fact, the measurements might even be reconcilable with the male's X being only half the female's double one. Since in the 1954 study the measurements of the female's X were made only on a short terminal part (the bands involved not being stated) which had been left unpaired by reason of a translocation, it does not seem clear from this alone whether the male's single X is in cross section really 0·6 of the female's double one or even less. However that may be, the male's one X as measured does turn out to be very decidedly smaller than the female's *double* one, much as in our own studies but contrary to the above-quoted affirmations of their apparent equality.

That the male's one X is very probably smaller in diameter and content than the female's double X is a conclusion that has also been arrived at in quite another dipteran group, that of mosquitoes. The following statement was made by Simões *et al.*, in an abstract published in 1961 which Dr Frota-Pessoa kindly called to our attention and translated after seeing our own 1964 abstract. 'In *A.* [*Anopheles*] *darlingi* we noticed that the X chromosome in the salivary gland of some individuals has the same thickness and colorability as the other chromosomes, but in other individuals the X chromosome is paler and much thinner than the autosomes, this situation probably corresponding to the haploidy of the X in males.'

On the other hand, the measurements by Aronson *et al.* given in the above-cited paper by Rudkin indicate that, in the region they dealt with, the male's single X is some 9 % (± about $2\frac{1}{2}$ %) greater in horizontal diameter than the corresponding unpaired portion of the female's double X, thus being 1·09 times the latter. Hence, *if* the unpaired part of the latter has not been more compressed than the male's X in this region (for reasons discussed in section 3, paragraph 4), the male's X is in cross-sectional area some 19 % (± 5 %) larger than one member of the female's paired X's. It is on this reckoning that the statements about their relative areas made in the second and third paragraphs of this section have been based. Yet if there had been such compression the difference would be reduced and even its existence would be rendered uncertain. At any rate, even when taken at its face value, the increase of nearly 20 % would not be out of harmony with our own cytological observations (see section 4), and it would agree qualitatively although very far from quantitatively with those reported by Dobzhansky, cited earlier.

In addition, Rudkin (op. cit.) reported measurements of integrated light absorption made by Aronson *et al.* in their 1954 work. No significant difference was found, at the ultraviolet wave length used (257 mμ) for peak absorption by nucleic acids (both DNA and RNA), between the given portion of the above-mentioned terminal unpaired member of the female's double X and of the male's single X. Neither was there a significant difference when visible light absorption by DNA alone after Feulgen staining was measured at 546 mμ.

This result for DNA is paralleled by a finding reported in 1964 in an abstract by Pavan and Frota-Pessoa. They measured by radioautography after the incorporation of tritiated thymidine the relative amounts of DNA in salivary-gland chromosomes of *Rhynchosciara angelae*. In correspondence with the observation that in this species also the X is paler in the male than in the female, they found that the male X, but not the female double X, is less labeled than the autosomes.

At the wave lengths for peak absorption of ultraviolet by protein, Aronson *et al.* found no significant difference at 275 mμ, representing the more complex proteins. However, at 231 mμ, representing simpler proteins such as histones, having little or no tyrosine, tryptophane, or other cyclic amino acids, a 10 % ± 4·7 % greater total content was indicated in their homologous regions for the male's X than for one of the unpaired terminal parts of the female's X.

This reported difference may appear contrary to evidence that some histones tend to inhibit gene action if, as Rudkin and Dobzhansky believe likely, the genes in the male's X are more active. In our section 7 conceivable means of resolving this apparent discrepancy are suggested. However, the possibility should also be looked into whether there may not have been more places in the seemingly denser X of the female at which some of the light-absorbing materials were overlying others to such an extent as not to cause an appreciable increase in absorption. In that case the X of the male might not really contain more of the simpler proteins than did one of the female. Since the method of radioautography is practically free from this difficulty it will be of special interest to see the data obtained by its use in the work that was carried out by Pavan and Frota-Pessoa and in that which, according to a personal communication, is being carried out by them.

6. RELATED RESULTS FROM BREEDING TESTS

It is appropriate at this point to turn back to some of the long-standing evidence from breeding experiments showing the piecemeal nature of dosage compensation in *Drosophila*, unlike what is true in mammals. Such evidence had early been obtained by Offermann, by Patterson, and by the senior author (see Muller, 1933). We will cite here, however, only two representative groups of results, taken from the similar but more comprehensive tests later conducted by Lieb, also under Muller's direction (see Muller, 1950). As with Offermann's tests, these concerned the effects on sex-linked mutant-gene expression in females exerted by the presence of diverse extra pieces of the X chromosome. The pieces, which were obtained by using X-IV translocations, were taken, except in special cases, from regions other than those in which the mutant genes studied were themselves located.

The first group of cases to be cited is concerned with the effect of a piece of the X extending from cytological map region 10A to 13A, derived from a transposition (TX-IV A124) whereby this piece had become inserted within chromosome IV, as in 'W13'. With this piece as a supernumerary, in females homozygous for the hypomorphs forked bristles, apricot eyes, and scute bristles, the forked character was definitely made somewhat more extreme, as expected for a compensator. The effect on apricot eye, if any, was so small as to be doubtful. As for scute, although it is located not far from apricot, the effect of the piece on the scutellar bristles—those most conspicuously reduced in number in the scute mutant—was to make the reduction far more extreme, by about 80 % (a marked 'compensation'). At the same time, the counts of other bristles reduced in the scute mutant showed that the sternitals were reduced by about 40 %, hence much less so that the scutellars, but that the anterior and median orbitals remained unaffected and the posterior orbitals were even anti-compensated by this extra piece, showing about 10 % increased frequency.

It is evident from this example, firstly, that a given X-chromosome region (here 10A to 13A) exerts a very different compensatory influence on different sex-linked genes lying outside of its own limits. Secondly, its influence differs even for genes relatively close to one another. Thirdly, and still more striking, it differs even for different parts, or different phases of the operation, of the same general gene.

The same principle is found to hold when the effects of the addition of other regions are studied. This is shown by our second example, which deals with the effects, on the same genes as before, of the right-hand part of the Sidky translocation (14C-20). This piece could not readily be tested directly with forked, since that gene lies within it, but the absence of compensation by the right-hand part of the B^S translocation (16A-20), and the definite compensation exerted by the left-hand part of the Sidky translocation (0-14C), make it very likely that the right-hand part of TX-IV Sidky contains no compensator, nor anti-compensator. Incidentally, other evidence showed that there are at least three compensators of forked, in widely different positions, but that none is between 0 and 4C, and that there are probably no substantially anti-compensating genes. The right-hand part of the Sidky translocation also failed to influence apricot. As for scute, this piece of the X was found to act as a strong anti-compensator for the scutellar bristles, increasing their frequency by about 90 %, a slight compensator for the sternitals (-10%), a strong compensator for the anterior and median orbitals (-40%), and a non-influencer of the posterior orbitals.

7. GENERAL CONSIDERATIONS

It follows from the preceding and other examples that in *Drosophila* the individual genes, and sometimes even different operations traceable to the same general gene, have their own separate groups of compensators. These in each case tend to balance one another so as to attain, all taken together, an appropriate degree of compensation for the given gene. This very complicated type of compensation is quite

different from that caused, as in mammals, by a generalized influence originating in one or a few centers and spreading out to affect in the same way all the genes that it reaches.

Those who have regarded the reportedly greater volume of the male's single X than of either member of the female's double X in *Drosophila* as an expression of a kind of dosage compensation by which a gene in the single X of the male 'works apparently twice as hard' as one in either X of the female (Dobzhansky, op. cit.) seem to overlook the innumerable ways by which a given gene can exert a stronger effect without itself being more active. Moreover, just because molecular geneticists have made the very important discovery that the genes themselves can be repressed and de-repressed (or activated), and that these effects can sometimes be carried along through cell reproduction, by no means implies that this mode of action, on the first step of the biochemical processes traceable to a given gene, is the only or even the most likely one by which any portion of the phenotypic characteristics ultimately affected by that gene can be influenced by other genes or by environmental agencies.

To make such a supposition would be contrary to the weight of genetic evidence and interpretation, even from the standpoint of the genetics of a half century ago (see for instance Altenburg & Muller, 1920; Morgan, Sturtevant, Muller & Bridges, 1915; Muller, 1914). True, it was only when such work as that of Ephrussi, Beadle, Tatum, etc. mapped out steps in some of these ramified gene-to-character pathways, and then succeeded in representing them with chemical formulae, that many biochemists awoke to the existence of this situation, and then only in given cases. But many geneticists had long realized that, in general, an increase or diminution or a change in the quality of a phenotypic effect that was dependent in some measure upon a given gene could usually be caused by an influence acting upon any of numerous steps in the pathway between that gene and that effect, or acting upon any of the usually much more numerous pathways leading from other genes, that finally merged, in part, with the one that led from the particular gene in question to the given phenotypic character. Moreover, it is pertinent in this connection that in evolution any mutant arising that happens to work is selected for and can become established. This conclusion is arrived at on both theoretical and observational grounds.

It it nevertheless true that any mechanism of dosage compensation that does not operate by regulating the activity of the gene itself must be less economical and serviceable in its operation than one which does influence the primary agent, the gene concerned. For in the case of the less direct type of operation the first step or steps in the pathway from the given gene to the given 'character' would ordinarily be alike whether the gene was ultimately to be rendered more effective or less so. Hence enough material would have to be produced at these earlier steps for either contingency. That would mean, in the situation in which a sex-linked gene was to be less effective (i.e. in the female), using up more material, and also encumbering the cell with more, than if the gene itself had been less active in this situation.

For this reason, in a very long-term evolution, some type of compensation that operated by affecting gene activity might eventually become substituted for the less direct type, in the case of one compensated gene and then another, and in fact the

mammalian method would seem to be the most efficient of all, where still attainable without too much disturbance of already-existing arrangements. Perhaps some such substitution, involving however a relatively greater activity of *individual* genes in the male's X, has already taken place to some extent in *Drosophila*, and is somehow associated with the seemingly larger volume and higher content of histone-like protein of the male's X than of either X of the female.

True, it would seem paradoxical for greater gene activity to be connected with a larger histone content, even though it might be speculated that *repressors* in the male's X worked *less* in consequence of histone binding. Other qualifications here stem from a report by Littau *et al.* (1965) that (1) arginine-rich histone, unlike the lysine-rich kind, does not cause chromosome pycnosis, and (2) that localized functional extension and condensation, presumably associated with greater and lesser synthesis of RNA by DNA, proceeds without alteration of the amounts of these two histones bound in the given regions.

That even in diptera there are ways of influencing the structure and presumably the activity of the X as a whole differently from the autosomes is shown by Pavan and Basile's interesting finding of a differential swelling of the X in *Rhynchosciara angelae* in consequence of a protozoan infection. However, the effect is the same on the X's in both sexes. It does not seem too surprising that special influences can act upon and spread from special chromosome regions. If this were impossible the mammalian type of compensation could never have originated.

In the case of any given gene of the *Drosophila* X, it need not be supposed that one dose of it in the male has an actually raised biochemical effectiveness (nor, more specifically, a raised activity) in relation either to its effectiveness at an earlier evolutionary stage or to that which it would have had if its compensators could somehow have been removed. Vice versa, one dose of the gene in the female need not have an actually decreased effectiveness. Either one of these conditions or a combination of both must hold, but which is the case may differ from locus to locus, so long as the effects in the two sexes are equalized, to a nicety extending considerably beyond that occurring in 'dominance'. Concluding, however, that the compensators must work in this relative sense at least, by reducing and/or enhancing the effectiveness of the gene to be compensated, it can also be inferred that each individual compensator gene acting as a reducer must exert more influence, in relation to the genes it is compensating, when in two doses than in one. Contrariwise, as an enhancer, it must be more influential when in one dose. For otherwise there would be no equalization of effects.

In any case, the fact that two such different mechanisms as those in *Drosophila* and mammals, obviously serving the same function, should both have evolved, independently, attests to the importance of that function. It also illustrates the point that in evolution anything is accepted that works sufficiently well. The function in both kinds of compensation must be the exact regulation of the degree of effectiveness of the *normal* genes. This exactness is especially emphasized by the fact that, likewise to regulate normal gene effectiveness, 'dominance' has also been evolved, and polished to such a degree that, *incidentally*, it causes the *uncompen-*

sated effects of one and two doses of genes to be not readily distinguishable in most cases (see section 1, penultimate paragraph). Yet the small degree of phenotypic difference that would still result from the difference between one and two doses is demonstrated, by the very existence of dosage compensation, to be too great. That is, there is survival value in steps of even smaller grade, that regulate the amount of gene effectiveness with considerably more fineness than does the mechanism underlying 'dominance'.

Although the fact of the independent evolution of both the *Drosophila* and mammalian systems greatly strengthens this conclusion, the piecemeal type, when taken by itself alone, provides' far stronger evidence of it than does the wholesale type, when taken alone. For the piecemeal type required the selection and establishment of operations each of which conferred a very much smaller amount of advantage, and the mutations that underlay them must have been correspondingly less advantageous and more numerous.

8. CONCLUSIONS REGARDING 'NORMALITY'

Since even such tiny steps, so slightly and to the ordinary view imperceptibly altering the effectiveness of an individual gene, can affect fitness enough to become established, it follows further that in the great majority of cases it is after all valid to speak of a 'normal gene' and a 'normal type'. This gene or type can vary only within very narrow limits of effect without a significant reduction in the average over-all fitness of the organism. At least, this would be true of genes such as those sampled, that have a 'major' effect, but the distinction here implied between them and so-called 'polygenes' is a vague and dubious one. Moreover, the argument would at least apply to those mutants (of such genes) whose main effects, as compared with those of their normal alleles, were quantitative ones. This would hold for those termed hypomorphs, amorphs, antimorphs, and hypermorphs, which taken together include the great majority of mutants that have thus far been worked with.

The variation so usual within populations constitutes no evidence against this conclusion. Undoubtedly some 'polymorphisms' within them are useful, though a survey of the evidence (Morton, Krieger & Mi, 1966) makes this very questionable for the majority of suspected ones found in man. Other intrapopulational variations have been caused by relative recency of given selectional conditions and by prior mixture between populations adapted to unlike conditions of living and/or differentiated by drift. But such of these variations as are greater than the intrapopulational ones would subside in the ordinary course of natural selection. Greater variation that is partly functional persists, of course, in a composite group splintered by strong, very long-continued hindrances to inter-mixing, based on the geography or behavior of its parts. Moreover, any wider spread of selection means, in effect, its relaxation, and thus a blunter peak.

However, the greatest part of the continuing genetic variation observed within a genetically united population must usually have been caused by multiple genes each of which, mutating separately, has given rise to its own small mutational load before

the mutants are eliminated by selection. It is mainly by the integration, for any given character, of these small accumulated loads that the character comes to have its relatively high variation. Of course this variation is in evolution a very necessary 'evil', since it allows natural selection a grasp by which in time of changed needs or opportunities the constitution of the population may be altered adaptively.

Such integrated variation must commonly be much larger, however, from one member of the population to another, than the separate mutational steps which afford the basis of it, of dominance, or of dosage compensation. Nevertheless some of these small separate steps, being ultimately subjected to a natural selection of 'second-order' intensity and speed, do in time become established so as to afford the individual normal gene the very exactly stabilized and, between the sexes, equalized effectiveness that it has. It is of course necessary to except here such cases of differential effectiveness between the sexes as are useful. Finally, it follows from all these facts and considerations (as well as others) that, ordinarily, the gene normal to a population confers, under the conditions of living to which that population has long been adapted, a significantly higher fitness when homozygous than when heterozygous for a mutant allele, despite the seeming dominance of that normal gene.

9. SOME FUTURE STUDIES NEEDED

There is much possibility for more extended investigation of the *Drosophila* type of dosage compensation. A great deal remains to be done on the cytological front. One immediate need here is for actual measurements on *long* portions of the double X that have remained unsynapsed, for comparison with corresponding portions of both single and double X's. If it becomes firmly established that the male's single X is more swollen than a single member of the female's double X, various attacks involving cytology can be used in investigating the basis and the consequences of any such difference. In any case, various types of chromosome rearrangements, some involving breaks of the X in still different postions, should be studied.

On the genetic front, it would be of interest to seek, systematically, a series of heterozygously-viable small deletions ('deficiencies') extending, all told, over as much of the X as possible. Their effects as compensators, minus or plus, would then be tested out on a series of hypomorphic and other mutants involving specific loci, and also on 'visible deficiencies' such as Notch wing. As another line of attack, the phenotypic expression of hypomorphic and other mutant genes should be more exactly observed when they are within parts of the X that have undergone structural change, but for this purpose genes close enough to the breakage point to be subject to position effects should be avoided. As yet, however, no difference in expression attributable to the rearrangement has been observed in any such cases. Similar observations should be made on mutant genes, not *too* far from the breakage point, that lie within autosomes that have engaged in translocation with the X.

In tracing the mechanism of genetic action in dosage compensation, studies of repression and de-repression associated with such compensation will of course be practicable eventually. And so will the often more recondite studies of the biochemi-

cal pathways of compensation mechanisms that operate further than that from the gene level.

As one of various evolutionary approaches, more studies—both through cytology, breeding tests, and chemistry—are needed, going beyond those of Muller and Lamy (see Muller, 1950), of the compensation of genes in arms of the X that have until relatively recently, in terms of evolutionary time, been autosomal (or vice versa). And of course the prevalence and nature of piecemeal dosage compensation remains to be investigated among dioeceous organisms other than *Drosophila*.

SUMMARY

Examination, in the salivary gland nuclei of *D. melanogaster* males, of four cases of translocation between the X and fourth chromosome, involving breaks of the X in widely different positions, disclosed no influence of the rearrangement on the width, morphology or chromatin-staining of either of the separated parts of the X, or on the fourth chromosome. Both parts of the single X were distinctly narrower than were the double major autosomes, as is true for the X of normal males but not for the double X of females. At the junction between a portion of a translocated single X and the double fourth chromosomes the transition in width, morphology and staining was abrupt and striking. As in structurally normal males, however, the parts of a single X, here removed from one another by the translocation, did appear to be somewhat swollen, as compared with half of a double chromosome, but to be correspondingly paler in stain, so as to indicate an unaltered amount of chromatin (see also Offermann, 1936; Rudkin, 1964; Pavan & Frota-Pessoa, 1964).

The above evidence of the regional autonomy in characteristics of the different parts of the X studied by us, and also of the fourth chromosome, is in contrast to the lack of such autonomy found in translocation studies on mammals, where the X chromosome and that joined with it are subject to an influence diffusing along them and thus acting 'wholesale', rather than 'piecemeal'. Likewise, a re-examination of the earlier genetic evidence on dosage compensation in *Drosophila* leads back again to a decidedly 'piecemeal' interpretation of its operation and evolution, according to which most genes in the X, and sometimes even different phases of the action of the same gene, have their own system of separately evolved, scattered compensators, which are also located in the X.

The fact that two so differently working compensation mechanisms as those in *Drosophila* and mammals have evolved independently to serve the same function emphasizes the importance of that function. That is, it points up the survival value of having the effectiveness of normal genes regulated to a very exact level. For the compensation enables the single representative of the X in the male cell to become equivalent to the two representatives of the X in the female cell. Moreover, this equivalence is of a considerably finer grade than that already afforded by the phenomenon termed 'dominance', which has evolved to meet the same basic need (that of phenotypic stabilization), and which has, incidentally, made even the *uncompensated* effects of one and two doses of either sex-linked or autosomal normal genes not readily distinguishable in most cases.

Taken by itself, the 'piecemeal' mechanism of *Drosophila* provides far stronger evidence for this conclusion than does the 'wholesale' mechanism in mammals. For the former must have required the establishment of far more numerous mutational steps and, taken individually, each of these steps was of correspondingly lesser survival value. Since they nevertheless affected fitness enough to become established, it also follows that usually a normal gene—or at any rate one of the kinds whose mutants have usually been studied—confers a significantly higher fitness when not heterozygous for such a mutation in it, despite the seeming recessiveness of most mutations. Thus, the expression 'normal gene' continues to have a very high validity.

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