BRANCHED CHROMOSOMES AS SYMMETRICAL DUPLICATIONS

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(With Nine Text-figures)

The problem

Since the discovery of the linear arrangement of the genes, the possibility of the occasional existence of "branch" strands has become a disputable question. But this possibility would constitute a step towards a non-linear arrangement, for, if we allow the branches to close in on themselves, we obtain a network which would result in a three-dimensional arrangement of the genes. Only a one or a two-dimensional distribution of elements would allow a pairing of all partners which is a prerequisite for crossing-over—and therefore for evolution—where for mechanical reasons involved in the breaking and reuniting of the bonds between the more elementary parts, the space factor has to be overcome. If we discard the assumption concerning the refusion of branches which leads to a three-dimensional distribution and thus hinders free interchange, and conclude that such anastomosed conditions would have been eliminated because of their diminishing of the pace of evolution in the organisms carrying them, the question then arises: Is not the mechanics of crossing-over of such a nature (requiring for instance a rotation of the chromonema) as not to admit even a two-dimensional distribution of genes, such as would be involved in a branched chromonema? Another question concerning the point of branching itself is this: Can the genes, fundamentally of a bipolar structure and bearing the same relationship to one another as links in a chain, be sometimes multipolar? As pointed out by Kossikoff and Muller (1935), it is important, in order to find an answer to this question, to carry out a crucial examination of the instances of branched chromosomes reported in the literature.

If we examine the literature of *Drosophila*, we find one well-known case, the genetical side of which seemed to indicate that we were dealing with a branched chromonema. This is Bridges' "Pale translocation", in which a fragment of the second chromosome was cut out and transferred to the middle region of the third chromosome. The piece was too small

to be definitely recognisable in the condensed stage of the chromosome at metaphase, but an examination of the chromosomes in the salivary glands by Kossikoff and Muller (*ibid*) showed that we were dealing here with an insertion and not with a side attachment. Meanwhile, however, further research on salivary gland chromosomes did reveal some evidence of branched chromosomes. One case, reported by Heitz (1934), is that of an apparent branch normally present in one of the chromosomes (the *X*?) of *D. virilis*. Heitz interprets this as a real branch. Another example of such apparent "branching" has been recognised by us in the bulb normally present in the *X*-chromosome of *D. melanogaster*, as seen in the salivary glands. The present paper concerns itself with the interpretation of this case and with the method by which such structures can arise. After our present interpretation of this case had been arrived at, we met with references to Bridges' as yet unpublished work on the similar case of "Dominant eyeless", an aberration originally produced by X-rays in the fourth chromosome of *D. melanogaster*, in work of Muller. Bridges' interpretation of his case is in some respects similar to our own, although there are one or two important differences involved.

Observations on females

The bulb (referred to by Bridges as the "puff") appears as a thickened part of the left end of the *X*-chromosome that sets in at the level of the "tenth" (in the terminology of Muller and Prokofyeva) band. It comprises a distance approximately equivalent to the space that normally would be taken up by five bands of the average size and spacing for this general region. The bulb is not, as generally represented in the illustrations in the literature, a more or less spherical expansion provided with cross-striations which run perpendicularly to the long axis of the chromosome, like the rest of the bands. Close examination reveals a bilateral structure of the bulb with striations running in planes parallel to the general axis of the chromosome. The chromosome, in other words, is branched at this point, forming a sort of cross, each of the two conjugants contributing one branch (Fig. 1 *a* and *b*).

To corroborate this interpretation, dissociated *X*-chromosomes were examined which had happened to remain or to become dissociated in the female and which should therefore show an asymmetry of this structure. Such accidental dissociations of the ends of the chromosomes which might occur during the process of making the preparation are occasionally to be found. A case in point is represented in Fig. 2. Here the asymmetry of each chromosome is clearly to be seen.

The stock known by the designation "Notch 172 *b*" (Patterson), in which the left end of one of the *X*-chromosomes is broken off and leaves the corresponding part of the other in haploid condition, was then examined. (This fly is viable because it has a fragment of the left end bearing the so-called "viability gene" attached to the right end, but the attraction of this small piece, lying at the chromocentre, is not usually sufficient to result in synapsis with its homologues at the other end and

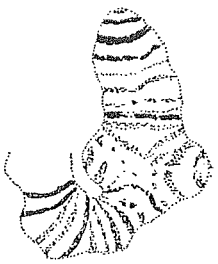

Fig. 1 *a*.

Fig. 1 *b*.

Fig. 2.

thus leaves the normal end free for observation.) The resulting haploid condition of the bulb in the salivary gland preparations of the female is shown in Fig. 3.

What is the nature of this branching? A close examination of the branch reveals: firstly, that its diameter (taken in the direction of its bands) is comparable to that of the diploid chromosome; secondly, that each branch in itself reveals a bilateral structure, the bands or rings (looking from the tip) being elongated with their long axis perpendicular to the main axis of the chromosome. This leads us to conclude that the

bulb represents a duplication of a special type which might be called symmetrical, in which the elements are arranged successively in both their direct and reversed order, *–c–d–e–e–d–c–*, forming by the pairing of these elements a branch-like structure, but one which consists not of a real branch but of a *single continuous chromonema* folded sharply at

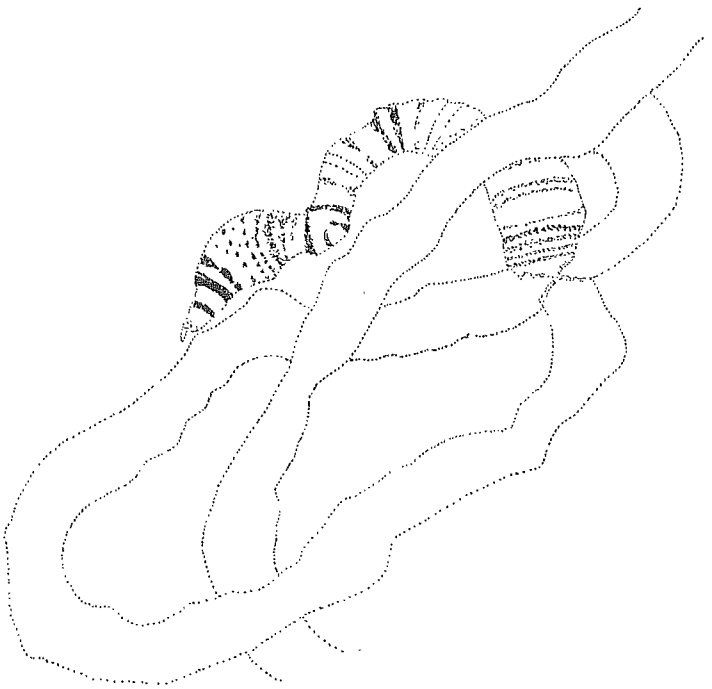

Fig. 3.

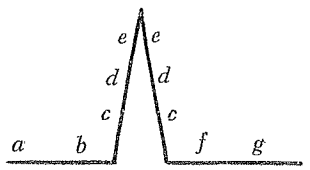

Fig. 4.

one point (*e–e*) and bent over against itself. It seems that in the case of the bulb there are three different chromomeres involved: one at the very tip, which forms a more or less flattened ellipse; a second one lying farther towards the base, forming an elliptical band; and very probably a third which does not quite succeed in conjugating in the salivary gland chromosome, but takes a diagonal position due to the action of the other

non-duplicated chromomeres which tend to conjugate in the other direction.

ORIGINATION OF "SYMMETRICAL DUPLICATIONS"

The probability of a wholly chance attachment of a duplication exactly at the end of the affected region and in inverted order is so small as to exclude this possibility. An explanation can be found, however, in the postulate of the rearrangement having occurred at a stage when the chromosomes are about to reduplicate or have reduplicated to form two sister strands that still have their homologous loci apposed. Supposing the latter to be the case, there are two possible conformations which could give rise to the duplication. These are shown in Figs. 5 and 6.

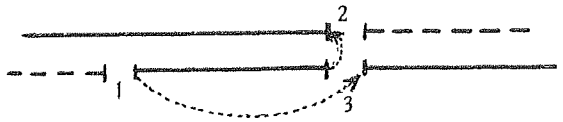

Fig. 5.

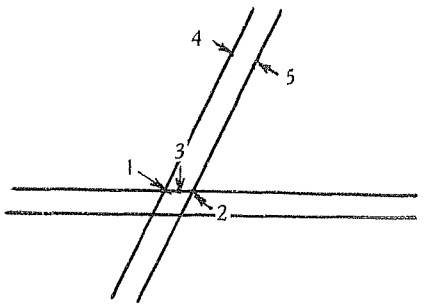

Fig. 6.

First, the duplication can be formed *in situ*, as it were: for this case the occurrence of three breaks (at points 1, 2, 3 in Fig. 5) and two reattachments (between $2L$ and $3L$, and between $1R$ and $3R$) are required. Secondly, it can be formed by transfer of a fragment of one chromosome to another (or from one region to another of the same chromosome): in this case five breaks and three reattachments are required as shown in Fig. 6 (the points of breakage are indicated by perpendicular arrows).

A remarkable prerequisite for the formation of such "symmetrical duplications" consists in the condition that both of the sister strands have to break at the same level. Such a fact cannot be interpreted as the result of independent events in the two sister strands, for too high a grade of coincidence would be required. The two breaks are, therefore,

to be referred to one and the same cause. This conclusion bears on our general interpretation of the mechanism of rearrangements.

It is unlikely that the union of sister strands came about through the failure of one chromomere or gene to divide in timely fashion, for we have no grounds for assuming such an effect of X-rays in addition to the quite different effect of breakage and rearrangement of different strands, and further very special assumptions would be required to explain how such an effect could really result in a permanent attachment of the divided components of both strands to the same undivided gene (presumably now at its opposite poles). It is therefore likely either that the two identical breaks in the sister strands were caused by a common agent, acting on both at once by virtue of their being in contact and crossing one another at the point in question, or else that they were caused by the fact that breakage preceded chromonemal division, and

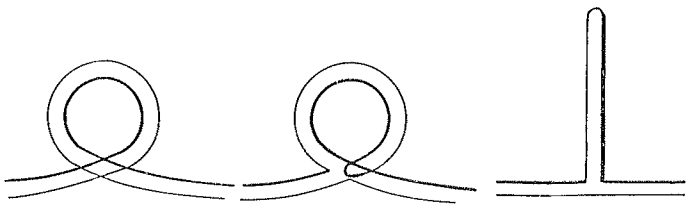

Fig. 7.

that chromonemal division in its turn preceded attachment between those broken ends that happened to lie near together.

Either of these methods can be postulated for either of the two possible conformations already mentioned. In the case of the first conformation, however, that of a rearrangement *in situ* (duplicating inversion), if the already split chromosome is bent to form a loop, as shown in Fig. 7, not only are all the three breaks referred to one origin but the difficulty of explaining a reattachment of points ordinarily separated by an appreciable distance is overcome. This does not, however, constitute critical evidence in favour of this conformation. In fact, dominant eyeless, as shown by Bridges, must have involved the second conformation. (We cannot, however, follow Bridges in regarding the fact of the attachment of the branches to one another as a matter for surprise or conjecture, for union of the arms would be expected to occur at the "hinge", in the same way as any of the other new attachments occurs, once breakage of both sister strands at that point is admitted.)

Do we have other examples that could be interpreted as changes in both sister strands?

Panshin's (1935) remarkable case of two simultaneous mutations at one locus, involving the appearance of the two mutant allelomorphs lozenge and spectacled in the two sister *X*-chromosomes (destined for the first two cleavage nuclei) derived from the same X-rayed spermatozoon, should probably be interpreted as two changes produced by the same cause (either two mutations, or two rearrangements with one of the breaks approximately or exactly at the same point in both sister strands). That the identity of the loci of the changes is a mere coincidence is too improbable an hypothesis to be worth consideration. The only alternative to considering this as a case of related mutation or breakage in two sister strands is to suppose that a preliminary effect ("premutation") was produced in the undivided chromosome, at the locus in question, and that it was followed by an after-effect that was different in the case of the two daughter chromosomes.

Patterson, in his work with induced mosaics (1933) produced, by raying mature sperm, some "aberrant" offspring, deficient for some chromosome region in all the cells of the body, and some (about a fourth as many) mosaic offspring, in which half or less than half of the fly bears a deficiency. His results can be interpreted, firstly, as a consequence of a mixture of two kinds of chromosomes in the mature treated sperm, some being in the single strand stage (these would give rise to the "aberrant" types) and some in the double strand stage, *i.e.* with the chromosome split into the two chromatids (these would produce the mosaics), or, secondly, on the assumption that all the chromosomes in the mature sperm are split, and that the two chromatids are not affected independently but simultaneously in the majority of cases, or thirdly, on the assumption that all the chromosomes are unsplit, but that either the breakage or the reattachment or both occur later, as an after-effect, after splitting has been completed.

SYNAPTIC ATTRACTION IN SYMMETRICAL DUPLICATIONS

Why do we have a pairing of the two arms of the duplication rather than either a pairing of the two duplications as a whole with one another (in which case the chromosome would not show the branching) or a random mixture of the two possibilities?

The two arms of the duplication in one chromosome are at the very start of the synaptic process nearer to one another than to the duplication in the homologous chromosome, in fact, they are touching at the point of union of the two arms. The pairing of the two arms with one another (causing the "branching") can therefore take place early in the

synaptic process, at a time when the sister strands formed by reduplication of the original chromosomes are conjugating to form the chromosome bundles, but before the bundles derived from the two original homologous chromosomes have come together. Synapsis no doubt starts at the points at which homologous elements happen to lie nearest together, and then extends from here farther along the chromosomes. Thus the pairing of the two arms of the duplication will be more or less accomplished before the two homologous chromosomes achieve their union. The double branching will therefore be a constant feature in the case of small symmetrical duplications. In the case of longer duplications there will be increasing tendency for the two branches to pair with each other as well; the strength of the tendencies involved could be tested by the inspection of longer duplications.

It might be of interest to consider here the relationship between certain structural features of the chromosomes of the salivary glands and the action of the forces of its elementary parts, the chromonemal threads. There are two chief forces acting in a chromonema: a transverse force, caused by the attraction of homologous parts (interthread), and a longitudinal force transmitted along the chromonema (intrathread), characterised by the coefficients of cohesion and elasticity. The threads tend to maintain a certain length at the same time that their elements (the genes or the chromomeres) tend to conjugate with one another to form rings. This is readily achieved in the case of a simple arrangement of genes, and a multiple chromosome of a simple cylindrical shape results. A conflict of forces comes in the case of rearrangements, especially at the points of breakage. Lines running lengthwise on the surface of the chromosomes are in that case not of the same length for a given section. These lines represent single chromonemas which form the sheath of the multiple chromosomes and in tending to maintain a given length they cause a bending of the chromosome. Therefore we find that generally a convex outline is followed by a concave one. The convex line of a symmetrical duplication, like the bulb of the haploid-*X* forms, is followed, as can be seen in the case of Notch 172*b* (Fig. 3), by a concave line, so that the whole describes an S-shaped curve. The same has been found to be the case in the *X*-chromosome of the male. In the normal female, which has a diploid *X*-chromosome, the internal forces of the two halves which make up the bulb and the left end, being equal and opposite, cancel each other and thus do not interfere with the straight conformation of the left end. Here the chromomeres not included in the duplication associate intimately and form distinct rings whereas the bulb itself

usually has a granulated structure. In the case of the haploid end in the female (Fig. 3), where the end has more freedom to bend, we find that a closer association of the chromomeres of the duplication results, giving well-defined rings. But here in turn we can observe the expected greater dissociation of the chromomeres to the left of the duplication. The appearance of the bulb in the male is different: we shall come back to it later.

Perhaps the structural changes described above might serve also as an indication of the forces acting in the four-strand chromosome complex at the time of crossing over. The structure of the bulb and its effect on distribution of chromonemal forces in its vicinity may also account, for instance, for the diminished crossing-over present in the left end of the *X*-chromosome, which was established by Muller by comparison of the crossing-over map with the map based on mutation frequency.

EVOLUTIONARY HISTORY OF THE BULB

Examination of the *X*-chromosome of *D. simulans* revealed a similar structure of the bulb, which was found to be perfectly homologous to the bulb of *D. melanogaster* in location and shape. The establishment of this duplication must therefore lie very remote in time, *i.e.* it antedates the evolutionary split of the common ancestral species. It has recently been pointed out by Bridges (1935) and by Muller (1935 *a*, *b*) independently, that duplications of small chromosome regions can become established in the course of evolution, and then serve to increase the number of genes available for evolutionary differentiation of the genetic complex.

Now genes included in any duplication are subject to much stronger changes than other genes in the course of evolution, as first pointed out by Muller (1918) in connection with polyploids and with duplications of whole chromosomes, since, being supernumerary, the duplicated genes are not indispensable for the life of the organism. We might, therefore, expect a duplication relatively soon to lose its synaptic affinity with the chromosome region that remained *in situ*, from which it was derived, and if the presence of this duplication is not in itself disadvantageous, it will thus become incorporated in the hereditary material and come to bear the same relationship to the latter as does any other group of genes to the rest of the genome, *i.e.* the traces of its having been a duplication will gradually be effaced. Why then should the genes in the two mirror-image sections of the bulb have kept their synaptic affinity for one another and have remained so similar in regard to the cytological

appearance of their region of the chromonema bundle, *i.e.* why should not these parts have undergone strong differentiation in respect to one another?

In seeking an answer to this question, we may observe that, in the case of symmetrical duplications, the genes have a special arrangement which allows those in the two arms to undergo interchange with one another. "Diagonal crossing-over" produced by an interchange between a point lying in one arm of the duplication in one chromosome and a corresponding point lying in the other arm of the duplication in the homologous chromosome, would lead to the formation of one double chromosome having two spindle fibres but no region corresponding to the original free end and one doubled free end without any spindle fibre; thus these cross-overs would be lost. On the other hand, crossing-over between the two arms of one duplication, lying in the same chromosome strand, merely inverts the order of the allelomorphs in relation to one another, those from the left passing into the right arm and *vice versa*. (That two points of crossing-over may occasionally lie so close together in a chromosome is proved by the demonstration by Kossikoff and Muller (*ibid*) that the cross-overs previously found by Bridges within the translocated section of "Pale translocation" must have been double cross-overs.) The same effect, inversion of the arms with respect to each other, could also be produced by double crossing-over between homologous chromosomes, if one point of crossing-over lay in one arm and the other in the other arm of the duplication and if the duplication figure of one chromosome made a loop that crossed, while that of the other was uncrossed (one arm in one chromosome conjugating with the other arm in the other). Now if a chromosome containing such a change of sequence of the arms undergoes, in later generations, occasional single crossing-over of an ordinary kind in the region of the duplication, with a chromosome having the original sequence, duplications will thereby be produced which have their two arms quite identical in the regions between the two points of crossing-over, *i.e.* in the regions distal (with respect to the hinge of the figure) to the later point of crossing-over, and proximal to the earlier point of crossing-over. In some cases both arms will be derived from the original left arm and in others from the original right arm. In this manner mutations which are detrimental when "tetrazygous" have a chance to be eliminated and those which are indifferent or advantageous have a chance to be accumulated, and the genes of the two arms would thus be able to retain their homology.

After we had arrived at our present interpretation of the bulb, the

excellent article of Bridges (1935), giving detailed drawings of all the chromosomes of normal *D. melanogaster* and depicting various duplications in them, came to hand. There seems little doubt that some of these duplications involve structures of a fundamentally similar type to that which we conceive the bulb to have ("symmetrical duplications"); none of them, however, are shown as apparent branches (nor is the bulb itself). We have found indications of apparent branching, similar to that of the bulb, in some other situations on the *D. melanogaster* chromatin; however, this condition seems on the whole to be rare. In this connection, it may be noted that smaller symmetrical duplications than the bulb—and these have apparently become established oftener—would have much less opportunity for the occurrence of the process of crossing-over, previously described, which is necessary for the maintenance of the homology and therefore of the synaptic affinity of the two arms; there would therefore be less tendency to that folding which gives rise to the apparent branched condition; in smaller duplications, moreover, there would also be more conflict of forces involved in such folding. Hence lack of folding does not prove that a given conformation is not duplicational. On the other hand, certain dubious features of some of the observations—such as the heavy lines shown bordering some of the presumed duplications above and below, and the depicting of typical transverse bands in the bulb—show the importance of further investigation before we can decide just which structures of the *Drosophila* chromatin are to be considered as duplications.

SEX DIFFERENCE IN CHROMOSOME MORPHOLOGY

During the course of these investigations a notable sex-limited difference in the morphology of the *X*-chromosome was found. While the diameter of the paired *X*-chromosomes in the salivary glands of the female is comparable to that of the autosomes, in the male, where the *X*-chromosome is haploid, the average diameter is not one-half that of the autosomes, as would be expected if the size of the sheath was determined directly by the number of chromonemal threads, *i.e.* if the distance between threads was constant, nor 0·7 ($\sqrt{\frac{1}{2}}$) of that of the autosomes as it would be if there was a constant ratio between the volume of the chromosome and the number of its threads. Our measurements showed that the diameter of the haploid piece in the female (in the above-mentioned case of Notch 172*b*) bears approximately the relation of 0·65 to that of the diploid chromosomes, while the *X*-chromosome in the male cell bears the relation of approximately 0·875. In other words, on

comparing haploid segments of the *X*-chromosome in males and females, we find a large sex difference, these segments being of larger diameter in the males. Comparison of the lengths of corresponding segments in both sexes with the lengths of given regions of the autosomes, shows that this transverse expansion of the *X*-chromosome in the male is not due to its lengthwise contraction.

In the male the chromioles within each ring are more widely spaced, giving to the chromosome as a whole the appearance of being more faintly stained. Evidently, the force of synaptic attraction that holds together homologous parts is less strongly expressed in the male than in the female, in correspondence, perhaps, with the difference in the tendency to crossing-over in male and female germ cells under natural

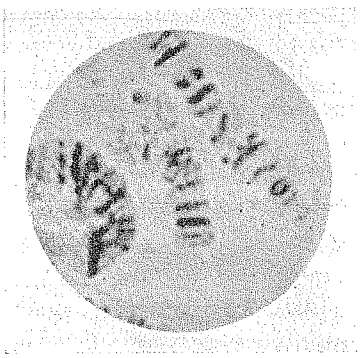

Fig. 8. Microphotograph of "bulb" in male.

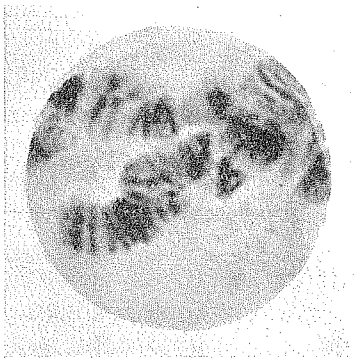

Fig. 9. Microphotograph of "bulb" in female.

conditions and somatic cells under X-raying. As Muller suggests, this difference in synaptic force is more evident in the less multiple ("haploid") chromosome bundles, than in the more multiple ("diploid") bundles, since in the former there is already a lesser amount of the common synaptic force present which tends to make all threads gravitate together, as it were.

The above sex difference expresses itself most strikingly in the bulb, which in the male is increased to a large scarcely staining body with a nearly imperceptible outline, probably altered by the process of preparation. Since in the bulb the forces of synapsis operating transversely from chromiole to corresponding chromiole are already disturbed, and weakened in their effect, by the attraction between the chromioles of the two arms, the two further causes of weak attraction—namely, the

less multiple condition of the chromosome (its origination from a haploid), and the influence of sex, together result in a structure in which the association of homologous chromioles is almost too weak to give any visible striations (compare figs. 8 and 9). A similar effect has been observed by Prokofyeva (1935) in the chromocentre, where the various counter-attractions of the several proximal chromosome regions result in a considerable obscuration of the striational structure.

SUMMARY

1. The bulb of the *X*-chromosome of *D. melanogaster* and branched chromosomes in general are interpreted to be symmetrical duplications formed by a continuous unbranched chromonema.

2. An explanation of the formation of symmetrical duplications is advanced, and evidence for a single event producing more than one break is presented.

3. The bulb antedates the evolutionary split of *D. melanogaster* and *D. simulans*, since it is present in both species. The homology of the two arms of the duplication and therefore of the duplicated genes themselves, could be preserved by the possibility of crossing-over which exists in this type of rearrangement.

4. Some of the observed structural features are referred to certain internal forces in the chromosomes, such as the attraction between homologous chromomeres and the tension within the chromonemas.

5. Sexual dimorphism in regard to the *X*-chromosome in general and to the bulb in particular has been described. This is interpreted as an expression of a difference in the force of synaptic attraction in the cells of male and female.

REFERENCES

BRIDGES, C. B. (1935). "Salivary chromosome maps." *J. Hered.* **26**, 60–4.

FRIESEN, H. (1932). "Vergleichende Untersuchung der Häufigkeit somatischer Chromosomenbrüche bei *Drosophila*—Weibchen und Männchen." *Biol. J.* **1**, 47–51.

HEITZ, E. (1934). "Über α- und β-Heterochromatin sowie Konstanz und Bau der Chromomeren bei *Drosophila*." *Biol. Z.* **54**, 588–609.

KOSSIKOFF, R. V. and MULLER, H. J. (1935). "Invalidation of the genetic evidence for branched chromonemas in the case of Pale translocation." *J. Hered.* (in press).

MULLER, H. J. (1918). "Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal factors." *Genetics*, **3**, 422–99.

—— (1935 *a*). "The origination of chromatin deficiencies as minute deletions subject to insertion elsewhere." *Genetica*, **17**, 237–52.

MULLER, H. J. (1935 *b*). "A viable two-gene deficiency phenotypically resembling the corresponding hypomorphic mutations." *J. Hered.*

PANSHIN, L. (1935). "New evidence for the position effect hypothesis." *C.R. Acad. Sci. U.S.S.R.* (in press).

PATTERSON, J. T. (1933). "The mechanism of mosaic formation in *Drosophila*." *Genetics*, **18**, 32–52.

PROKOFYEVA, A. A. (1935). "The structure of the chromocentre." *C.R. Acad. Sci. U.S.S.R.*