THE STRUCTURE OF THE Y CHROMOSOME IN THE SALIVARY GLANDS OF DROSOPHILA MELANOGASTER

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INTRODUCTION

THE genetic composition of the Y chromosome has been somewhat elucidated in recent years, thanks to the work on crossing over between the X and Y chromosomes (STERN 1927, 1929, STERN and OGURA 1931, KAUFMANN 1933, PHILIP 1934, NEUHAUS 1935).

If we add here the theoretical conclusions regarding the structure of the Y chromosome reached by some authors in their study of the structure of the inert region of the X chromosome (MULLER and PROKOFYEVA 1935; MULLER and GERSHENSON 1935), we may express the main body of information concerning the cytogenetic structure of the Y chromosome of *Drosophila melanogaster* as follows:

1. In metaphase plates (in nerve ganglia and in oogonia) the Y has the appearance of a J-shaped chromosome with two arms of different lengths, its larger arm being approximately equal to the whole X chromosome, the shorter arm being only half as long. At this stage the Y chromosome is completely heterochromatic (HEITZ 1933).

2. The Y chromosome has at least some of its parts homologous to the inert region of the X, which is proved by the occurrence of crossing over between the X and the Y chromosomes. It seems that both the short and the long arm of the Y can participate in such crossing over.

3. The Y chromosome contains a normal allele of the bobbed gene and two fertility complexes, k_1 and k_2. According to STERN (1927, 1929), the normal allele of bobbed and the fertility complex k_1 lie in the long arm of the Y. The short arm contains the fertility factor k_2. According to NEUHAUS, the normal allele of bobbed lies in the short arm and the long arm contains its very extreme mutant allele (NEUHAUS 1935).

4. According to MULLER and PAINTER (1932) the inert regions of the X and the Y chromosomes have a common origin.

5. Deficiencies for the Y chromosome do not result in the death of individuals carrying them.

6. The Y chromosome exerts an influence on the degree of mosaicism of flies of eversporting lines. (GOWEN and GAY 1933, DUBININ and HEPTNER 1934, NOUJDIN 1935).

7. The frequency of breakages of the Y chromosome as measured by the frequency of its participation in translocations seems to be roughly proportional to the length of this chromosome, as the latter is seen on metaphase plates, or even higher than this. Therefore, we may consider the chromonema of the Y chromosome (as well as that of the inert region of the X) as having essentially the same nature in respect to its length, degree of coiling and breakability as that of the active regions of chromosomes. But unlike the chromonema of the active regions, that of the Y chromosome is built up of a series of genes which are inactive in respect to viability and the majority of the external characters of the organism, but which are nevertheless connected with a usual amount of accessory chromatin, accompanying genes of the metaphase chromosomes (MULLER 1918; MULLER and ALTENBURG 1930; OLIVER 1932; MULLER and PAINTER 1932).

8. Breaks occurring in the inert region of the X chromosome are limited to some definite points. Considering this finding, MULLER and GERSHENSON (1935) conclude that the main difference between this region and the Y chromosome to which it is homologous on one hand, and the active regions on the other, consists in the X and the Y being built up of a small number of active genes which produce, at some stages of the life cycle of a cell, a large amount of non-genic substances forming several indivisible blocks. In metaphase the Y chromosome and the inert regions of the other chromosomes are composed chiefly of these blocks.

9. The relative size of the Y chromosome in salivary gland cell nuclei is not comparable with its relative size in metaphase plates. According to PAINTER (1933, 1934), the Y chromosome cannot be found in salivary gland nuclei at all. According to PROKOFYEVA (1935) and BAUER (1936), it is represented in salivary glands by a short segment lying in the region of the chromocenter. One can therefore assume that only the genetically active part of the Y chromosome is represented in the salivary glands. The relatively large size of the Y in metaphase can be explained either by supposing that its short chromonemata do not coil at that stage, or by the assumption made by MULLER and GERHENSON (1935).

It will be seen from the above survey that our information concerning the nature of the Y chromosome is very uncertain, and our conclusions are contradictory in many respects. Therefore the data mentioned in the last paragraph of our survey—the absence of the Y chromosome in salivary gland nuclei claimed by PAINTER and shown to be spurious by PROKOFYEVA and by BAUER—are of special interest. The present paper is devoted to a more extensive and exact study of this question, as well as to an attempt to interpret some facts described in the first eight paragraphs of the survey on the basis of the cytological analysis of the behavior of the Y chromosome in the nuclei of the salivary gland cells.

MATERIAL AND METHODS

For the present study the author used larvae of normal males and of attached-X females. The short and the long arms of the Y were studied separately in lines obtained by NEUHAUS (1935). He very kindly put these lines at the disposal of the author, who wishes to express her thanks. PAINTER'S acetocarmine method of treatment was used, with some modifications proposed by BRIDGES.

The structure of the inert region of the X chromosome (figures 1 and 2)

The inert region of the X is represented by a short segment lying to the right of the disc 20A of BRIDGES' map. This segment consists of eight discs

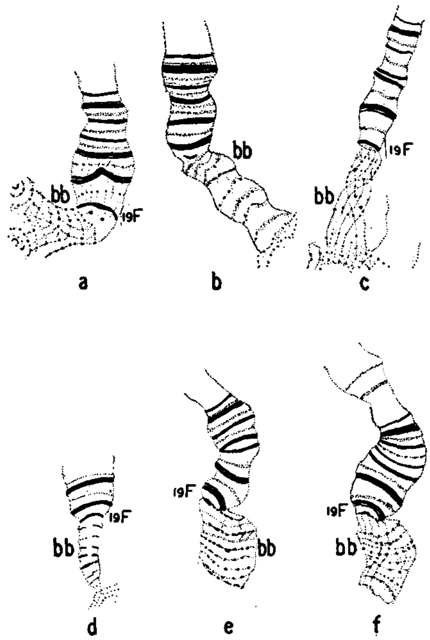

FIGURE 1.—(For 19F read 20A.) The structure of the chromocentral region of the X chromosome as seen in normal females.

Due to a large variability in the morphology of the whole region, it is difficult to identify exactly these discs with those shown on BRIDGES' map. The inert region of the X is often represented by several separate bundles of chromonemata with chromioles (fig. 1 a, c), and it is impossible to identify such structures with the landmarks shown on BRIDGES' map. But in some cases, thanks to an intimate conjugation of chromonemata, the structure of the inert region is clearly seen (fig. 1 d, e). In such cases the chromioles form typical discs characteristic for the euchromatic part of the X chromosome, and this condition makes it possible to give some outline of the morphology of the inert region of the X chromosome.

The first disc. Close to the 20A disc there lies a faint disc composed of very fine chromioles, which stains very badly, and therefore, in many cases, this disc is seemingly absent (20B).

The second disc has, in most cases, the same structure as the first disc, but sometimes it is more deeply staining and then seems to be composed of large chromioles (20B).

The third, fourth and fifth discs constitute the middle part of the inert region, and in most cases are better stained than other discs. Among them the fourth disc can always be identified as the most conspicuous one. It is composed of large, deeply staining chromioles and is the most conspicuous disc of the whole inert region of the X. The third and fifth discs are sometimes stained much less than the fourth (20CD1).

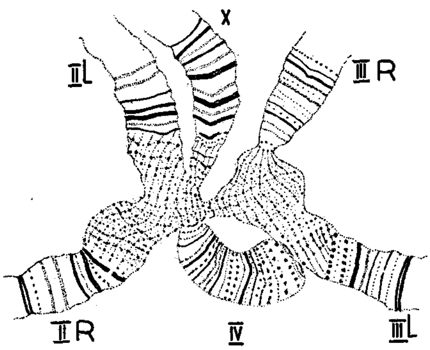

FIGURE 2.—Association of the chromocentral region of the X chromosome with that of autosomes.

The sixth disc. Its morphology varies very much. In cases when it is composed of chromioles lying very close to one another, it appears as a well stained sharp disc. But in some cases, when the sixth disc enters into the chromocenter as a part of it, it is represented by small chromioles lying on the disjoined chromonemata (20D2).

The seventh, eighth and ninth discs constitute the chromocentral part of the X chromosome and are mostly represented by separate, well stained chromioles lying far from one another, 20D3, 4 (fig. 2).

The general morphology of the inert region of the X can in some cases be profoundly modified due to the proximity of the chromocenter. Sometimes all the discs beginning with the first one are composed of separate chromioles lying on the widely disjoined chromonemata. Sometimes all the inert region constitutes a part of the chromocenter, being associated with the inert regions of other chromosomes.

The Y chromosome of normal males (figure 3)

In the nuclei of the salivary gland cells of *D. melanogaster* males, the inert region of the X conjugates with a small element, which the author has identified as the Y (PROKOFYEVA 1935).[1] In all the cases investigated, we observed but a single body without any indications of the presence of two arms. In many nuclei the conjugation of the inert region of the X with the Y is complete. In such cases the inert region of the X in a male does not differ in any respect from the inert region of the X in a female, and

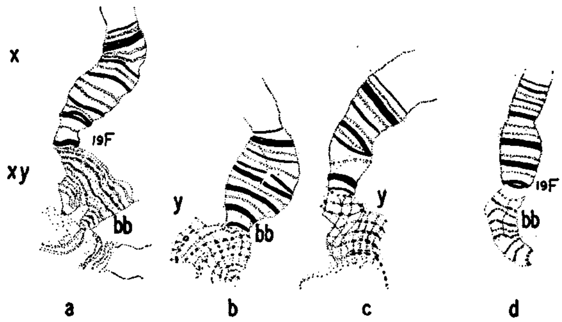

FIGURE 3.—(For 19F read 20A.) The structure of the chromocentral region of the X-Y compound as seen in normal males.

it is then impossible to observe the Y as a separate element (fig. 3d). By means of a study of the salivary glands of young larvae in which the somatic conjugation is not yet completed, we can find some nuclei with a Y incompletely conjugated with the inert region of the X, and here we can study the structure of the Y more easily (fig. 3 a, b). Like the inert region of the X, the Y is formed of 8–10 discs, the morphological appearance of which is similar to that of the discs of the inert region of the X. The third,

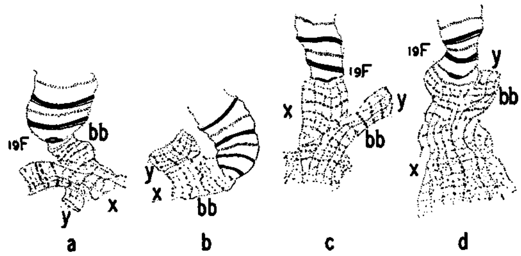

FIGURE 4.—(For 19F read 20A.) The structure of the chromocentral region of the X-Y compound as seen in attached-X females.

fourth and fifth discs of the Y are generally the best stained, as are the corresponding discs of the X. Like all the discs of the inert regions of chromosomes, the discs of the Y are formed of separate chromomeres which are stained with different intensities. In some cases one can see that while the middle parts of the inert region of the X and of the Y chromosomes (discs three, four and five) are already completely conjugated, their most proximal (fig. 3a) or distal parts still remain free.

[1] In this paper, the last disc of the X of Painter's map was wrongly identified as $19F_1$ of Bridges' map.

One can often observe a deeper staining of the euchromatic part of the proximal end of the X of males as compared with that of females, and in males the discs in the region 19EF, 20A are disproportionally enlarged (fig. 3d). Whether this condition is a result of a conjugation of some part of the Y with the region of the X in question (as it is in the case of the inert region), or is caused by some other kind of influence of the Y (SCHULTZ 1936), is as yet unknown.

The Y chromosome of $\widehat{XX}$ Y females (figure 4)

The salivary glands of the larvae of $\widehat{XX}$ Y females offer very favorable material for the study of the structure of the Y, because the latter often shows an incomplete conjugation with the inert regions of the X. In some

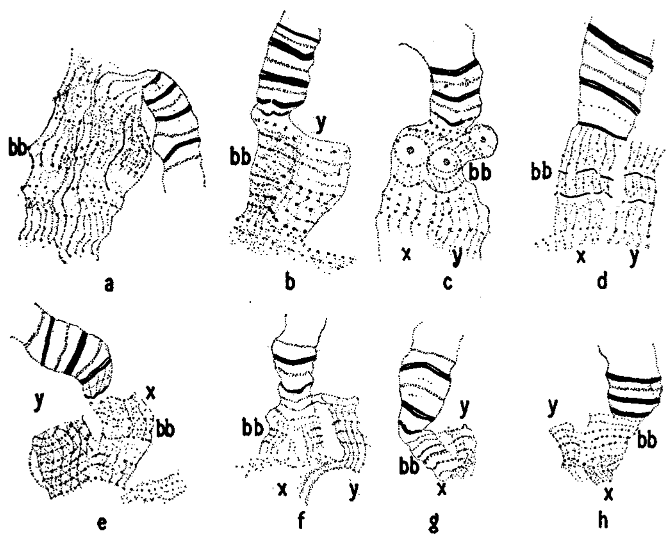

FIGURE 5.—The structure of the chromocentral region of the X-Y compound as seen in attached-X females having only a long arm of the Y chromosome.

nuclei one can clearly distinguish the more intensely staining third, fourth and fifth discs of the Y (fig. 4a), but in others, both the Y and the inert region of the X are composed of uniform chromioles (fig. 4 c, d).

The most interesting picture of the behavior of the Y in the $\widehat{XX}$ Y females is the frequently observed conjugation of its middle part (discs five, six and seven) with the corresponding part of the inert region of the X, the distal part of the Y as well as its very proximal end remaining free occasionally (fig. 4 a, c).

The long arm of the Y chromosome (figure 5)

The $\widehat{XX}$ Y females received from NEUHAUS were used as material for the present study. These females contained only the long arm of the Y which condition originated as a result of crossing over between the X and Y chromosomes. The study of the structure and behavior of the Y in this line as compared with the ordinary $\widehat{XX}$ Y females and in the males has not shown any sharp difference between them. Cytologically, the long arm of the Y corresponds closely to the inert region of the X. The most intensely staining discs of the Y and of the inert region of the X could have been observed also in the long arm of the Y. Other discs of the long arm also proved to be quite similar to the corresponding discs of the inert region of the X, and they underwent a complete conjugation with the latter. Occasionally only some distal discs of the long arm of the Y are conjugat-

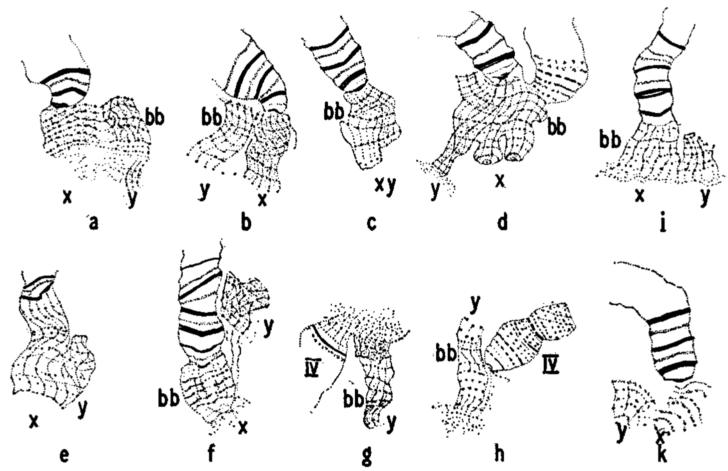

FIGURE 6.—The structure of the chromocentral region of the X-Y compound as seen in attached-X females having (a–h) only a short arm of the Y chromosome; (i–k) two short arms of the Y chromosome attached to one another.

ing while the remaining part of the Y remains free and has an independent connection with the chromocenter from the inert region of the X (fig. 5f). Such configurations could be observed only in cases of an incomplete conjugation. Some structures of the long arm of the Y vary greatly in different nuclei. Sometimes this arm looks like a comparatively small segment, having 7–9 discs, situated on the periphery of the chromocenter (fig. 5, e–h). But sometimes the chromonemata of the long arm of the Y, as well as those of the inert region of the X, are well developed and constitute altogether a considerable segment on the periphery of the chromocenter (fig. 5 a–d). The number of discs, however, (or the number of chromioles on a single chromonema) in the inert region of the X chromosome and the long arm of the Y, remains the same both in the cases when the Y and the inert region of the X appear as a large segment, and when they seem to be small, namely, from 8 to 10.

The short arm of the Y chromosome (figure 6)

For the study of the short arm of the Y the author used two kinds of $\widehat{XX}$ Y females obtained from NEUHAUS. The females of the first kind contained two short arms of the Y attached to one another, and those of the second kind had only one short arm. Cytologically they looked alike and showed no differences from the $\widehat{XX}$ Y females described above which had only the long arm of the Y. The short arm of the Y has the same morphology as that of the long arm of the Y, and of the inert region of the X. It is represented by a small segment having from 8 to 10 discs, the most intensely staining discs being situated in its middle part (fig. 6a). In most cases the short arm of the Y conjugates intimately with the inert region of the X, showing a quite identical structure with the latter. But occasionally one can observe an independent attachment of the short arm of the Y to the chromocenter. In such cases this arm looks like a very small element, the chromocentral region of which is associated, in most cases, with the chromocentral region of the IV chromosome (fig. 6 g, h). Sometimes one can see coiled bundles of chromonemata in the short arm of the Y.

SUMMARY AND CONCLUSIONS

The investigation presented here allows us to make some suggestions concerning the cytogenetic structure of the Y chromosome.

1. The chromonema of the Y chromosome and that of the inert region of the X chromosome have essentially the same structure as the chromonema of euchromatic parts of the chromosome. The distance between the chromioles of the Y chromosome is the same as that between the discs of the active parts of chromosomes. In other words, the number of chromomeres per unit of length of the chromonema of the Y chromosome is the same as in the euchromatic parts of chromosomes.

2. As shown by the study of breaks in the inert region of the X chromosome in sc^8 and sc^4 lines (MULLER and PROKOFYEVA 1935), the locus of bobbed lies somewhere near the third and fourth discs of this region, that is, just in the region of the most deeply staining discs. More detailed study indicates that the bobbed locus is connected with the fourth disc. It will be seen from the data presented here that a region corresponding to this part of the chromocentral region of the X can be identified in both the short and the long arm of the Y when they are studied separately as well as in the whole Y chromosome of $\widehat{XX}$ Y females and normal males. In other words, our cytological data substantiates the conclusion reached by NEUHAUS, according to which some allele of bobbed (either normal or mutant) is present in either the long or short arm of the Y.

3. Both arms of the Y chromosome show homology to the X chromosome not only in the region of bobbed, but in their other parts as well. As far as our technique allows us to make any conclusions, the short and the long arms of the Y chromosome and the inert region of the X have in the nuclei of the salivary gland cells an almost identical cytological structure. There is apparently some difference in the mode of conjugation of the two arms of the Y chromosome with the inert region of the X. Our next paper will be devoted to this question.

4. If the finest chromomeres of the Y correspond to the loci of single functional genes in metaphase, each arm of the Y must consist, according to MULLER'S and GERSHENSON'S assumption, of at least eight to ten separate blocks. MULLER'S and GERSHENSON'S study showed that some genes of the inert region of the X produce blocks of very different sizes ("block A" and "block B"). If this is true it is possible that the difference between the length of the two arms of the Y observed in metaphase can be explained by their being non-homologous in a very small number of genes, or even only in one gene.

5. The present investigation indicates that the Y chromosome has a tendency to become associated with the chromocenter by both its ends, similar to what is observed in the case of the IV chromosome. Whether this condition depends, as BAUER is inclined to believe, upon a nonspecific mutual attraction of all the terminal chromomeres which forces all the short chromosomes to bend, or upon some other cause, could not be decided.

6. The chief difficulty in a cytological investigation of the Y chromosome in the nuclei of the salivary gland cells is caused by an extremely high fragility of the chromonemata of this chromosome. It seems probable that this increased fragility of the chromonemata of the Y chromosome observed in the acetocarmine preparations and the high frequency of breaks in the Y and in the inert regions of the X and of the autosomes caused by X-raying, are based on some common condition.

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