Notes on the Mitotic Behavior of Long Chromosomes

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Received March 28, 1935

A few years ago I pointed out that the mitotic spindle is a complex structure and that morphologically at least the composing elements are easily distinguishable. In older publications this is a well recognized fact and it was emphasized for instance in such papers as those of Miss Bonnevie ('10 and earlier) and Wilson.

In elucidation of this statement, I have tried to show that the half-spindle component, extending between the chromosome and the pole, is in the Hemiptera not a single fibre but is composed of a number of very fine fibrillae. These are rather well defined and easily seen near the chromosome, but tend to become finer and more vague in the vicinity of the pole. In cross sections, this structure of the half-spindle component is even more striking, and there is, in such sections, strong indication that the fine fibrillae are arranged in a circle. Since the fibrillae come closer together as they approach the pole and disappear altogether before they reach it, the whole may be compared to a hollow, truncated cone. It is probably in conformity to the decreasing diameter of this cone that chromosomes frequently become narrower as they go to the pole (for instance, Fig. 9) and a consequence of passing beyond the truncated end that they round up again near the pole.

It may be observed here that in the Hemiptera and in several other forms with compact or very short chromosomes, the entire pole-ward surface of the chromosome serves as a base for the half-spindle component. But in other forms, where the chromosomes are longer, the half-spindle component is connected with the chromosome in a rather sharply delimited region of the latter (Figs. 1 & 2). This has long been known, as for instance in certain plants and urodele Amphibia, and has been shown in the drawings of many authors even when not specifically commented on. In the Amphibia the number of fibrillae involved in each component seems smaller than in the case of the Hemiptera, but so far as the essentials are concerned the same structural principle is observed in both.

In short the difference between the half-spindle components

of short and of long chromosomes lies mainly in the way in which the component is attached to the chromosome.¹⁾

Fig. 1. Spermatocyte chromosomes in spermatocyte metaphase of *Amphiuma*. 2 mm. oil imm. objective and 15× ocular. Fig. 2. Anaphase in spermatocyte division in *Amphiuma*. 2 mm. oil imm. objective and 10× ocular.

It is possible that this difference may have a simple explanation, as for instance that the half-spindle component arises independently of the chromosome and is relatively more uniform in size and shape than are the chromosomes of the species here involved. Thus a short chromosome might be provided with a component just as large as a much larger chromosome and hence a proportionately greater part of its surface is involved. But in my previous paper I have brought forward evidence for the view that it is the chromosome that is responsible for the formation of the half-spindle component, and it takes no large step in reasoning to suggest that it may play a further role in determining its exact shape. The difference in the components here at issue may then rest on some specific activity of the different chromosomes.

The X-chromosome of *Protenor* in the First Spermatocyte Division

A test is provided by those hemipteran chromosomes that are

1) It is possible that the Acrididae among the Orthoptera represent exceptions, but a cursory survey suggests that there the half-spindle component, although simpler than in Hemiptera, is nevertheless composed of 2 or at any rate very few fibrillae. However, I hesitate to commit myself to such a finding, especially since such keen observers as Bělař and various members of the McClung school have never described them as anything but single fibres.

exceptionally long, even during meiosis. An outstanding example is the unpaired X chromosome of the male of *Protenor belfragei*. Here is a chromosome at least as long as some of the amphibian chromosomes that show a restricted half-spindle component (Fig. 4).

Examination of many metaphases of the first spermatocyte division convincingly demonstrates that with the long axis of this chromosome always at right angles to the spindle axis, the entire poleward surface serves as a basis for the half-spindle component (Fig. 4, and also Wilson '11). The evidence thus bears out the view that we are dealing with a real difference in the influence exerted in the formation of half-spindle components by the chromosomes of Hemiptera and those of certain other forms with long chromosomes.

The case for this difference is strengthened by a study of the further mitotic movements of this sex chromosome. (The first division is equational for the X.) It will be remembered that in the anaphase movement of amphibian chromosomes, the region where the half-spindle component is attached seems to represent the active portion of the chromosome. Thus if it is median, the middle of the chromosome advances toward the poles while the two extremities trail more or less inertly behind, the whole forming a V with the apex toward the pole (Fig. 2). (Interesting exceptions to this rule are provided in the case of *Sciara* as reported by Metz and in *Micromalthus* by A. C. Scott.) But the X chromosome of *Protenor* shows its daughter halves proceeding toward the poles broadside on, with little or no bending of the body of the chromosome (Figs. 3-7). The only marked alteration in shape lies in the fact that it becomes somewhat shorter as it approaches the pole, but there is no change in its position with respect to the axis of the spindle. (There is indeed a tendency for the outline of the chromosome to become rough or diffuse, but this behavior, so reminiscent of the X of the male Orthoptera, has no direct bearing on the question.) Montgomery's Fig. 138 ('01) indicates that this mitotic movement of the X of *Protenor* was observed by him almost thirty-five years ago. There is thus a definite correlation between the form of the half-spindle component and the position of the moving chromosome.

In this connection reference may now be made to an element whose importance has been appreciated only within the last few years—the attachment body or insertion point (the achromite of McClung and the kinetochore of Sharp). That the presence of this special region or body in the chromosome has a considerable influence on crossing over is already well known to geneticists, but in addition there is now little doubt that a very close correlation exists between it and the half-spindle component. Since the latter

arises primarily under the influence of the chromosome, it is not unnatural to conclude that it is this attachment body rather than the chromosome as a whole that is directly involved in the formation of the half-spindle component.

So far as the X chromosome of *Protenor* or any hemipteran chromosome is concerned, this conclusion must lead to the suggestion

Fig. 3. Polar view of first spermatocyte in *Protenor*. Figs. 4-7. Anaphases of first spermatocyte in *Protenor*. Figs. 8-11. Anaphases and telophase of second spermatocyte in *Protenor*. 2 mm. objective and 15 $\times$ ocular.

that there we are concerned with an attachment body that is either diffuse or else is so large as to extend over the entire poleward

surface, whereas in the Amphibia and certain plants it is definitely restricted and occupies only a small region in the chromosome (as direct examination shows quite clearly). Support for this is gained from our knowledge of the chromosomes in *Ascaris megalocephala* cells prior to the diminution process. The peculiarities of the *Ascaris* case are clearly given in a figure published by Bonnevie ('13) and reproduced in my Fig. 12. This long chromosome shows spindle fibres attached to more than half of its length, only the extremities being devoid of such connections with the poles. During the anaphase movement, these ends of the chromosomes seem to be passive and

Fig. 12. Figure of *Ascaris* chromosome as given by Bonnevie ('10). 2 mm. objective and 15 $\times$ ocular. Fig. 13. Anaphase in division of undiminished chromosome of *Ascaris*. 2 mm. objective and 10 $\times$ ocular.

are simply carried along by the middle or spindle fibre section which proceeds to the pole broad-side on. This part of the *Ascaris* chromosome thus parallels the X of *Protenor* in the way it approaches the pole. (My Fig. 13 and also several cleavage figures by earlier authors.)

A further analysis of the case of *Ascaris* also may bear on the conditions in Hemiptera. The large chromosomes of *Ascaris* are of course multiple in character, being composed of 30 or more individual chromosomes. At the time of diminution all these small chromosomes become independent and in the descendant cells each one is provided with its own half-spindle component. The extremities of the large and multiple chromosomes, however, degenerate and the lack of spindle fibres to these portions thus serves as an indication that they are not equivalent to the middle section. The many fibres connecting the latter with the poles can be interpreted in no other way than that the small component chromosomes of the multiple do not lose their ability individually to form half-spindle components even though joined with many other small chromosomes. In effect the multiple chromosome therefore is a long chromosome with many small attachments bodies and these bring about a spindle structure which simulates that of hemipteran chromosomes. I use the word "simulates" advisedly, for there is no definite reason for believing that the ordinary hemipteran chromosomes are multiple in character. Nevertheless, there is inherent in these observations support for the claim that in Hemiptera the attachment body is more extensive than in such forms as the Amphibia.

Second Spermatocyte of *Protenor*

If now the second spermatocyte division be examined, it will become apparent at once that the behavior of the X is there radically different from that in the first spermatocyte. As is well known, it passes undivided to one of the two poles. But in its anaphase movement its long axis is parallel to the axis of the spindle instead of at right angles. In general this parallel position is maintained until it reaches the pole—as shown in my Figs. 8–11 and indeed also in figures given by Wilson ('12). In place of the broad half-spindle component of the first division, the X is now connected with the poles through a delicate fibre, terminally attached, and often difficult to see. Its anaphase movements are somewhat irregular, for although usually in or close to the general metaphase plate, it commonly lags behind the autosomes in their progress to the pole. In a few cases it may precede them, although toward the end of the anaphase such instances are rather rare.

In some respects this behavior thus parallels that of the X in the orthopteran *Gryllus* (Brunelli, '09), for there also the behavior is strictly regulated in an equational division whereas in the reduction the passage to one pole seems quite irregular. In general this evidence would seem to lend support to the contention of Bleier ('33) that univalent chromosomes are distributed to the poles as passive bodies, and in the case of *Gryllus* this view can only be strengthened by the fact that the axis of the X may shift and take almost any position with respect to the axis of the spindle. But, as I have already remarked, this latter irregularity is not shared by the X of *Protenor*, whose long axis has a definite position. This suggests that the *Protenor* X is subject to some regulatory force and further indication of this may be adduced from the presence of the two fibres which in the reduction division connect the X with opposite poles. I would be content to ascribe to these fibres a nonfunctional nature, were it not that there is often a slight attenuation at the points of the X where they attach (Figs. 9 & 10). In stages where the X is already markedly closer to one pole, the connection with this pole often appears thicker, a condition that I have been unable to demonstrate in the half-spindle components of the autosomes. Very similar conditions throughout are to be observed in the reduction division of *Pyrrhocoris apterus*. If therefore these fibres are not equivalent to the half-spindle component of the X in the equational division, they may nevertheless have some influence, if only on the position of the chromosome with respect to the spindle axis. In brief, the X of *Protenor* and *Pyrrhocoris* does not strictly follow the rule for

univalents postulated by Bleier.²⁾

It should also be noted that the presence of a partner, that is, a Y chromosome, does not in itself cause the X to alter its mitotic behavior. This is true even when the XY pair forms some kind of a union prior to the reduction division. In *Nezara* (Wilson '11), where the Y may be almost indistinguishable from the X and the two join just prior to reduction, the resemblance to the autosomes goes further in that their distribution is not entirely at random in the literal sense but is always toward opposite poles. Nevertheless they take their reductional position with their long axis parallel to the spindle axis, just like the unpaired X of *Protenor*. In *Ascaris felis* (Edwards, '11) conditions are even more striking, for the XY pair forms a tetrad that in general conformation is like the autosomal tetrad. But again, the reduction division finds the long axis of the sex chromosomes parallel to the spindle axis, although it indubitably is at right angles in the equational division. In short, so far as one can tell, the presence of a partner does not influence the behavior of the X in these respects. It would appear that in order to parallel the autosomal behavior, the sex chromosomes would have to be homologous. I am free to admit that I have no explanatory suggestion for these peculiarities. The fact remains that the spindle apparatus for such an unpaired chromosome as the X of *Protenor* is distinctly different in the two maturation divisions, whereas for the autosomes it is essentially the same. But whereas it may be conceded that in some other cases a univalent goes to either pole perfectly passively, there are instances in the Hemiptera in which the unpaired sex chromosomes are not mere inert bodies during the reduction division, but are in some way more directly involved in the mitotic mechanism.

Notes on Interzonal Connections

The tubular form of interzonal connections, that is, of the spindle element that connects separating sister chromosomes, has been discussed earlier (Hughes-Schrader '31, Schrader '31 and '32). In all the species considered at that time the chromosomes are short, and it has been suggested that entirely different conditions might prevail when chromosomes are long and provided with median attachments.

Thus in the large V-shaped chromosomes of *Amphibia* the conception of an outer layer forming an interzonal tube at anaphase

2) It may not be amiss to point out that Bleier's skepticism concerning the formation of unipolar spindles in conjunction with the univalents of certain species is hardly justified. The cases of *Sciara* and *Gossyparia* can hardly be dismissed on the basis of faulty technique and observation, and if this be not enough, the findings in *Micromalthus* by one of my students (A. C. Scott) establish the point beyond reasonable doubt (paper in press).

(Fig. 14) might meet with difficulty, especially when the extremities of each chromosome are spread apart. That the conditions are not identical with those met in the Hemiptera must appear from my Fig. 2. Here there is plainly not a single tube connecting the sister chromosomes, but there are interconnections between all the ends that are last to separate. Nor is there any indication that these connections are tubular. But, if in such cases the sheath is in a less viscous and perhaps less elastic state than in the Hemiptera, such figures might well be expected, and the fact that no tube is formed does not controvert the interpretations made for the Hemiptera. The conditions in the Amphibia would then be analogous to those observed when two appressed finger tips are dipped into mucilage and then separated slowly.

This does not necessarily dispose of the hypothesis that I suggested in 1931, that the half-spindle component and the interzonal connection are primarily parts of one and the same structure—a tube extending from each chromosome body to the two poles (Fig. 15). Admitting that in many instances this suggestion does not fit

Fig. 14. Diagram to show relation of interzonal connection to a chromosome sheath.
 Fig. 15. Diagram to show a condition in which half-spindle component and interzonal connection constitute a continuous tube.

the available observations, I must point out that it is singularly applicable to the case of *Protortonia*, for which it was first postulated (in *Protortonia* the chromosomes frequently appear like drops in a capillary tube, narrowing and widening with the diameter of this tube as they go to the pole). But if the half-spindle component in Amphibia represents such a tube, it is quite evident that it can not encompass the relatively huge body of the chromosome as it moves to the pole. It would then be necessary to postulate that the walls of the tube are ruptured, but that their material would tend to adhere to the chromosome and thus form the interzonal connections as seen in Fig. 2. It is perhaps not irrelevant to point out that the interzonal

connections of the long chromosomes so far studied are usually not so persistent or definite as in the case of hemipteran chromosomes. This in itself may well bespeak a difference in viscosity and elasticity in the two groups.

In thus discussing the possible nature of the interzonal connection, I make no claim to a final solution. It is for instance quite evident that further questions would arise from the consideration that in certain species the interzonal connections (which would be included in Bělar's Stemmkörper) have the power to expand or exert a thrust, as for instance in *Llaveia* (Hughes-Schrader '31) and certain hybrids of *Drosophila* (Dobzhansky '34). The difficulties in explaining this peculiarity have to my knowledge not been successfully surmounted in any of the recent hypotheses.

Summary

1) The type of mitotic movement as seen in the X of *Protenor* is radically different in the two spermatocyte divisions, and differs from that of correspondingly long chromosomes of Amphibia.

2) In the equational division, the entire broadside of the X serves as a base for the half-spindle component.

3) In the reduction division the X is connected with both poles through a single delicate fibre.

4) The nature of the interzonal connections in the case of long chromosomes is discussed.

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