

**STUDIES IN SPERMATOGENESIS,
WITH ESPECIAL REFERENCE TO THE
"ACCESSORY CHROMOSOME".
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STUDIES IN SPERMATOGENESIS WITH ESPECIAL REFERENCE TO THE "ACCESSORY CHROMOSOME."

By N. M. STEVENS.

In connection with the problem of sex determination it has seemed necessary to investigate further the so-called "accessory chromosome," which, according to McClung ('02), may be a sex determinant. This view has been supported by Sutton ('02) in his work on *Brachystola magna*, but rejected by Miss Wallace ('05) for the spider.

The forms selected for study have been taken from several groups of insects, and are all species whose spermatogenesis has not been previously worked out. They are (1) a California termite, *Termopsis angusticollis*; (2) a California sand-cricket, *Stenopelmatus*; (3) the croton-bug, *Blattella germanica*; (4) the common meal-worm, *Tenebrio molitor*; and (5) one of the aphids, *Aphis anotheræ*.

A brief account of a chromatin element resembling the accessory chromosome in *Sagitta* has been added for comparison. The spermatogenesis of each form will be described in detail, and a general discussion of the results and their relation to the accessory chromosome and sex determination will follow. The spermatogenesis of the aphid has been included in another paper, but a summary of results and a few figures will be given here for reference in the general discussion.

METHODS.

The testes were fixed in various fluids—Flemming's strong solution, Hermann's platino-aceto-osmic, Gilson's mercurio-nitric, Lenhossek's alcoholic sublimate acetic, and corrosive acetic. Flemming's and Hermann's fluids followed by safranin gave good results in most cases. The mercurio-nitric solution and Lenhossek's fluid gave excellent fixation and were preferable to the osmic mixtures when it was desirable to stain the same material with iron-hæmatoxylin, and also with various anilin stains.

Heidenhain's iron-hæmatoxylin, either alone or with orange G or erythrosin, was used more than any other one stain. With osmic fixation safranin gave better results in some cases, because of the

abundance of spindle fibers and sphere substance which were stained by hæmatoxylin. The safranin-gentian combination used by Miss Wallace and others in the study of the accessory chromosome did not prove to be especially helpful with these forms. Thionin was found to be a very useful stain for distinguishing between the accessory chromosome and an ordinary nucleolus. Licht-grün was often used in combination with safranin.

RESULTS OF INVESTIGATIONS.

Termopsis angusticollis.

In the termite it was not found to be practicable to dissect out the testes. The tip of the abdomen was therefore fixed and sectioned, young males whose wings were just apparent being used. The cells are all small, and could not be studied to advantage with less than 1500 magnification (Zeiss oil immersion 2 mm., oc. 12).

In the spermatogonium there is a very large nucleolus (plate 1, fig. 1), which in the iron-hæmatoxylin preparations is very conspicuous, but does not stain like chromatin with thionin or other anilin stains, nor does it behave like an accessory chromosome during the maturation mitoses. Before each spermatogonial division it divides as in figures 2 and 3, and the same is true for each maturation mitosis. Figure 4 shows the 52 chromosomes of a spermatogonial division in metaphase. Figures 5 and 6 are young spermatocytes, showing the division of the nucleolus. Figures 8, 9, and 10 show a stage immediately following that shown in figure 6 and evidently persisting for some time. The spireme thread is very fine, stains deeply, and is wound into a dense ball, often concealing one (fig. 10) or both nucleoli (fig. 8). Figure 11 shows the next stage; the bivalent chromosomes are so disposed as to give the familiar "bouquet stage," with the loops directed away from the centrosome and sphere (*c*). Figures 12, 13, and 14 show the later development of the same stage, the chromatin loops becoming thicker by the concentration of the smaller granules to form the larger ones seen in figure 14. The loops now straighten out and extend in various directions across the nuclear space (figs. 15, 16, 17). In fig. 18 *a* a longitudinal split is seen in several chromosomes. Figures 18 *b*, 19, 20, and 21 show various stages in the contraction of these split bivalent chromosomes to form diamond-shaped tetrads, each side of which is a univalent daughter chromosome. The tetrads come into the spindle in this form (figs. 22, 23), and change to the form shown in figure 24 during the metaphase (figs. 22, 26, 28). Figures 25 and 27 show the 26 bivalent chromosomes, or tetrads, in

early and late metaphase, respectively, and figures 29, 30, and 31 in anaphase. This is certainly a reduction division, for the tetrads are always somewhat elongated and come into the spindle with their longer axes parallel with the axis of the spindle. The aberrant bodies in these figures are probably remains of the nucleoli; they are found only in iron-haematoxylin preparations. Figures 31 and 32 show exceptional cases where the cell has divided. Usually the two daughter nuclei are formed in an undivided cell. The resting-stage between the two divisions is only partial. The nucleolus appears and divides into two (figs. 33-36), and the chromosomes change into the dyad form (fig. 36), in which they come into the second maturation spindle (figs. 37, 38). The equatorial plate again shows 26 chromosomes (fig. 39). The formation of the spermatozoa is peculiar in that the original spermatocyte cell-body, as a rule, does not divide; but the four nuclei resulting from the two maturation divisions develop into sperm-heads in one cell. All have a nucleolus (fig. 41), and in a slightly later stage (fig. 42) the elongated nuclei have a distinct centrosome and sphere at the posterior end. Later stages are shown in figures 43, 44, and 45.

The points of greatest interest in the spermatogenesis of *Termopsis angusticollis* are, (1) the fact that no accessory chromosome is present; (2) that the method of tetrad formation and reduction are clear, despite the fact that the cells and the chromatin elements are quite small; and (3) the failure of the cell-bodies to divide and the consequent development of four spermatozoa in one cell.

Stenopelmatus.

The spermatogonium of *Stenopelmatus* contains from one to three large nucleoli, which stain much less with thionin than does the spireme (plate II, figs. 46, 47, 48). As the distinct chromosomes come into view in the prophase of mitosis, two are seen to be nearly twice as long as the others, but of equal length (figs. 48, 49, 50.) There are 46 chromosomes in the equatorial plate of a spermatogonial spindle (fig 50). Besides the nucleolus (*n*), there appears in the young spermatocyte a conspicuous element (*x*) which stains deeply with all chromatin stains (fig. 51). It is closely applied to the nuclear membrane and is connected with an end of the spireme (figs. 51-54). At first it is quite small, and it gradually increases in size during the spireme stage. There is no "bouquet stage" in this form. Figure 55 shows the spireme segmented and split longitudinally. The segments have begun to open out at the center to give the cross which is the typical tetrad form in *Stenopelmatus*. Figures 56, 58, 59, and 60 show various stages in the contraction of the split segments to form crosses and

diamond-shaped rings. The tetrads usually remain connected by delicate linin threads, as shown in figures 57 and 60, also in figures 62 and 63, the latter taken from the metaphase of the first maturation spindle. If these linin connections persist, as they appear to do, from the segmentation of the spireme to metakinesis, the first division of the contracted tetrads must be longitudinal, corresponding to the split in the segments of figures 55, 57, 58, etc. The chromosomes in the metaphase usually appear as dumbbells (fig. 66) or elongated crosses (fig. 67), but occasionally one can be found which still shows its tetrad nature (fig. 64), so clearly indicated in the quadrivalent crosses of figure 59. In the anaphase the chromosomes are often split as in figure 68, and occasionally the two components can be seen as plainly as in figure 65. Figure 61 shows the various shapes assumed by the element x during the tetrad-stage of the chromosomes. This element x almost invariably appears in a vesicle near one pole of the spindle (figs. 67, 68); in exceptional cases it is found nearer the equatorial plate, as in figure 66, or even in the same plane with the ordinary chromosomes, but always somewhat isolated from them. In position and form this element resembles the accessory chromosomes described by Baumgartner ('04) for *Gryllus domesticus*; in its mode of origin it seems to differ from the other accessory chromosomes yet described.

Figures 69 and 70 show the 23 bivalent chromosomes in metaphase; in figure 69 the element x is shown partly behind the large chromosome and at a different level. In figures 66 and 67 the one exceptionally large chromosome doubtless represents the two larger ones of the spermatogonia. In the anaphase the element x is sometimes as conspicuous as in figure 71; in other cases it is concealed either behind or within the polar mass of chromatin. In this form there is a distinct resting stage between the two maturation mitoses (figs. 72-75). The element x is conspicuous in one-half of the cells (figs. 72, 73); it may be included in the nucleus as in figure 72, or it may be partly or wholly outside, as in figures 74, 75, and 76. In the latter case, but not in the former, it is surrounded by its own membrane. As the chromatin begins to condense for the second mitosis, disintegration of the element x becomes apparent. This is most easily made out in cases where the element is isolated, as in figures 75 and 76; but there seems to be little doubt that it disappears before the metaphase of the second maturation mitosis. It is not possible to count the chromosomes in this stage, they are so crowded together, but it is not probable that such a conspicuous chromatin element as that seen in the first division could escape detection, even if it were in the equatorial plate among the chromosomes. No aberrant element is ever seen in these spindles;

and, moreover, all of the spindles and all of the spermatids appear to be exactly alike at the same stage. The chromosomes are double in the prophase (fig. 77) and always appear double in the equatorial plate (fig. 78), the paired elements corresponding to those of figure 65.

In figure 80, plate III, a pair of spermatids is shown with nuclear membrane formed and the spindle fibers twisted in a characteristic manner. Figure 81 is a slightly later stage with the spindle-remains massed against the nuclear membrane. Curiously enough there appears in the nucleus of every spermatid a body similar to the element x of the spermatocytes of the first order (figs. 82-86). This body is often applied to the nuclear membrane and connected with the spireme (figs. 84-86). It decreases in size and finally disappears (figs. 88-91). The spindle-remains divides (fig. 83), and a small part of it (a) goes to form the acrosome at the apex of the head (figs. 85-92). The larger part is probably utilized in the formation of the tail, for it gradually disappears as the tail develops.

The centrosome which, although small, is conspicuous in each mitosis, is seen in figure 83 between the two parts of the spindle-remains, applied to the outside of the nuclear membrane. In figures 85, 86, and 87 the relation of the tail (or its axial fiber) to the centrosome is shown. In figures 87 and 88, instead of the small spherical centrosome of figures 83 to 86, we have a much elongated body, at first (fig. 87) applied for its whole length to the nuclear membrane, but later lying along one side of a middle piece (m), as shown in figure 89, and in a later stage in figures 90 to 92. The mature spermatozoon with its forked anterior end appears in figure 93.

The points of especial interest in the spermatogenesis of *Stenopelmatus* are the development of the aberrant chromatin element x during the growth stage of the spermatocyte of the first order, its distribution to one-half of the spermatocytes of the first order, its disappearance during the rest stage between the two maturation divisions, and the development of a similar, though smaller, element in all of the spermatocytes.

Blattella germanica.

Unlike the spermatogonia of *Stenopelmatus*, those of *Blattella* have both a faintly-staining nucleolus and a deeply-staining chromatin element (x), and moreover the two are always closely associated (figs. 95, 96). The number of chromatin elements in the equatorial plate of late spermatogonial mitoses is 23 (fig. 97). Later events indicate that one of the 23 is the element x , but it is impossible to distinguish it here. Figure 98 is a very early stage of the spermatocyte of the first order, showing the element x as a U-shaped body. The centrosome

was also conspicuous in all of the cells of this group. The spireme here, as also in figure 99, is fine and closely interwound. In figure 99 and again in figure 100 the element x is joined to the spireme as it is throughout the spireme stage. In the "bouquet" or "polarized" stage the combined nucleolus and element x are always at one side of the group of loops and down very close to the base of the figure (figs. 101, 103). In figure 102 most of the loops are cut across. The stage shown in figures 104 and 105 is a later one than that just described. Here we have again a continuous spireme connected with the element x , making it seem improbable that the bivalent chromosomes are really separated in the bouquet stage. Figure 106 gives some of the variations in form of the combined nucleolus and element x . The last of the five figures was taken from a giant cell containing at least twice the usual amount of chromatin. In one giant cell four unusually large combinations of this kind were found, and a corresponding amount of chromatin in the spireme. In figure 107 one sees the spireme divided into segments still joined by linin bridges. In figure 108 similar segments may be seen, one of them showing a longitudinal split. The element x is present, but the nucleolus has disappeared. In many cases the split, if it appears at all, closes quickly and the chromosome bends in U-shape, as in figure 109, plate IV. This figure also shows two centrosomes (c). In other cases the split persists as in figure 110 and leads to the formation of crosses of a tetrad character (figs. 111, 112, 113), as in *Stenopelmatus* and many other insects. Figures 114 to 117 show later stages of the U-shaped chromosomes. Perfect rings are rare. All sorts of variations are seen, broad and narrow U-shapes, rings split at one point or the opposite points, a U split at the bottom (fig. 114), pairs of parallel rods (fig. 115), and occasionally rods constricted in the middle and showing a longitudinal split in each half, as in figure 116. Figure 117 shows different views of the split rings. Apparently all of these forms straighten out so that the two components of the bivalent chromosome stand end to end as dumbbells or compressed crosses in the metaphase of the first maturation spindle (figs. 123-125). The element x remains concentrated and more or less spherical in form. Figures 118-122 are equatorial plates, with x absent in figure 120, in the same plane as the 11 other chromosomes in figure 119, far to one side in figure 118, and near one pole of the forming spindle in figure 122. It is also shown in various positions with regard to the spindle in figures 123 to 126 and 128 to 132. In figure 125 it is apparently double, and again in figure 129. In figure 130 one lagging chromosome shows the dyad nature of the products