The Spermatocyte Divisions of the Lucustidae

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THE SPERMATOCYTE DIVISIONS OF THE LOCUSTIDÆ.

BY C. E. M'CLUNG.

With Plates VII, VIII, IX, and X.

I. INTRODUCTION.

UNDER the title, "A Peculiar Nuclear Element in the Male Reproductive Cells of Insects" (**16**), I published a preliminary account of the process characterizing the maturation divisions of the Locustidæ. This was of a general character and served merely as a basis for a description of the accessory chromosome in these cells. It is my present intention to give a detailed history of the spermatocyte divisions occurring in this family, after the manner followed previously in considering corresponding stages in the Acrididæ (**17**). Besides giving this account of processes, however, I shall be able to draw some comparisons between the two families. Eventually I hope to complete such a comparative study of all the Orthopteran families. Material for this larger investigation is now partially on hand, and is being added to as circumstances permit, so that it may be possible to carry through a study of the maturation stages in this order of insects within a few years.

The value of comparative cytological study was urged by Vom Rath (**25**), and its importance in relation to the accessory chromosome and the maturation mitoses received recognition in both my earlier papers (**16, 17**). Recently Montgomery (**15**) has added his influence to the movement.

The observations upon which the present paper are based were originally made upon *Xiphidium*. The cells in this genus are, unfortunately, small in size, and much difficulty was experienced during the early work in getting clear images. This embarrassment was further increased by the large number of chromosomes and their habit of compact arrangement. Later it was found that species of *Anabrus*, *Orchesticus*, *Microcentra* and *Scudderia* have cells much better adapted for study, and because of this they have been largely utilized. The account which follows is therefore based upon a study of all these genera, and is considered representative of the family. The accompanying figures are principally from *Orchesticus*, since the number of stages represented exceeded those in material derived from other genera. I am indebted to a friend and former student, Mr. W. S. Sutton, now of Columbia University, for a generous supply of *Orchesticus* and *Anabrus* testes from his collections.

II. TECHNICAL METHODS.

For the fixation of material used in these studies, it has been found that the osmic acid mixtures of Flemming and Hermann are the most generally applicable and are productive of the best results. In connection with these, however, Gilson's aceto-nitric-sublimate mixture has been tried, and frequently affords an excellent fixation. Extensive shrinkage in the melted paraffin is sure to follow the use of sublimate mixtures unless celloidin is used to support the soft tissue. This double infiltration of celloidin, followed by paraffin, has been found the best method of securing clear and accurate figures, for, because of the lessened shrinkage, the elements are not crowded together and rendered indistinct. This circumstance is particularly fortunate in the case of the Locustid cells, where the nuclear elements are so numerous and crowded.

The stains employed are the iron-hæmatoxylin of Heidenhain and the safranin-gentian violet-orange combination of Flemming. For general purposes, nothing excels the hæmatoxylin

stain, but it is frequently advantageous to trace the chemical changes undergone by the different cell elements in the process of mitosis, and the aniline stain above mentioned serves excellently for this. Kernschwarz has also been found a valuable stain for some purposes.

III. NOMENCLATURE.

The terminology as outlined in a former paper (**17**) will be followed in the present one.

IV. OBSERVATIONS.

(*a*) *General Form and Structure of the Testes.*

The testes of the Locustidæ are paired structures lying in the anterior dorsal portion of the abdomen. Each organ is made up of numerous short follicles, which are bound together by a connective tissue investment. In adult animals the testes are a bright yellow color, while in nymphs the color varies from white in the youngest to yellow in the oldest. The pigment is lodged in the connective tissue sheath about the testis, and is seen in sections as irregularly rounded masses in the cytoplasm.

(*b*) *The Spermatogonia.*

No further discussion of the spermatogonia will be given here than is necessary for an understanding of the derivation of the first spermatocytes. As appears to be universally the case, the second spermatogonia, in their last generation at least, are much reduced in size as compared with the primary spermatogonia that preceded them and with the first spermatocytes that arise from them. The entire cell stains dark with almost all stains and, as the nucleus occupies nearly the whole cell body, the chromatin appears relatively large in amount. A cyst of spermatogonia, therefore, looks as if composed almost entirely of chromatin aggregated into rounded masses—the nuclei.

The chromosomes are of the rod type, and divide longitudinally in each mitosis. The number of chromosomes is large and could not be determined with absolute certainty, but a number of careful enumerations makes it evident that there are most probably thirty-three. In most species of Locustids, one chromosome is easily distinguished from the others by its larger size and tardy division in the act of metakinesis. This is the

element as described for *Xiphidium*, which passes into the first spermatocyte as a formed chromosome, while its fellows break up into the spireme.

In the anaphase the chromosomes are drawn away from the equator, and extend lengthwise of the spindle as long rods. During the telophase the disintegration of the chromosomes takes place rapidly, and, for a time, the individual chromosomes may be distinguished in the loose masses of chromomeres. This distinction, however, is soon lost, and the nuclear vesicle becomes covered with fine and apparently unrelated chromomeres. It is at this point that the transformation of the cells from second spermatogonia to first spermatocytes takes place. So long as the chromosomes are present in the somatic number, we have to deal with spermatogonia, but when the disintegrating process comes upon them and they are lost to view as distinct entities, then is reached the end of destructive spermatogonial changes, and upon their reconstruction they are chromosomes of the spermatocytes.

(*c*) *The First Spermatocytes.*

The main features characterizing the next steps in the process are the rapid increase in size of the cell and nucleus, and the arrangement of the chromomeres into a fine thread or threads (figs. 2–4). This is well called the growth stage, for all parts of the cell engage in the work of regaining the ground lost during the period of multiplication in the secondary spermatogonia. As a result of this metabolic activity, the first spermatocytes at the end of the prophase have reached a volume often as much as ten times that possessed by the last generation of the secondary spermatogonia from which they were derived. Nucleus and cytoplasm, in about an equal degree, participate in this enlargement, and, at the end of the period, present an appearance much different from that of the spermatogonia. This consists most strikingly in the greater clearness of all the parts, due to the increased amount of hyaloplasm which separates by greater distances the more solid structures of the cell.

In the nucleus, for instance, the chromatin aggregates are now definitely apparent, and each stands free and clear except for connecting threads of linin. The cytoplasm, likewise, instead of showing a coarsely granular aspect, exhibits a clearly

reticular structure, with such large intervening hyaloplasmic areas as to suggest an almost alveolar structure, especially in the later stages (figs. 3–9). This increased amount of fluid becomes evident by an examination of sections under even a low power of the microscope, principally by the lessened density of the general stain in the cell.

A peculiarity of the archoplasm in these early prophases is the persistence manifested by the spindle fibers of the previous generations. Often connecting fibers may be seen, joining cell to cell, as has been described by many writers, but, in addition to this, the spindle remains of more remote ancestral mitoses show themselves. In figure 3 is represented a cross-section through three persisting spindles of as many generations. Their age is suggested by size and intensity of stain, both factors being least marked in the oldest structure.

Centrosomes and astral radiation do not present themselves with the prominence and frequency of such structures in corresponding cells in *Hippiscus*.

The main interest of these studies, however, attaches to the movements of the chromatin granules. As was suggested in an earlier paper (**17**), it is only by an understanding of the constructive processes in the prophase that we can appreciate the structure and changes of the chromosomes in the metaphase. It is to this period in the history of the chromosomes that I have given the most attention and to which I will devote the most space in the record of observations.

Apparently the chromomeres resulting from the disintegration of the spermatogonial chromosomes are loosely scattered through the nucleus, so that no formed structure is to be seen. With the increase in size of the cell, however, a linear arrangement of the elements becomes apparent, so that it seems as if a thread is formed. Whether this is continuous or segmented it is not possible to determine. The large amount of chromatin and the tortuous course of the filaments put a solution of the problem beyond the range of assured observation. It is with much regret that this fact is recognized, for one of the most important questions connected with the maturation mitoses hinges upon the method by which the chromosomes, as such, are derived from those of the spermatogonia. Upon this point the evidence of the ordinary chromosomes of these cells would,

if anything, tend to confirm the view that there is a possibility of complete rearrangement of the chromomeres in the different chromosomes. Concerning this, however, the accessory chromosome is much more conclusive and convincing, as will be shown later.

Disregarding the relations of the chromosomes of the two generations, it is evident that from the material of the spermatogonial elements there is formed the thread of the spermatocyte prophase. As indicated in figures 3 and 4, this is at first composed of a single series of chromomeres. But in a slightly later stage, represented by figure 5, it becomes plain that the thread is wider and at the same time double. A careful investigation will show that the halves of the thread are exact duplicates of each other, each granule of the one having its mate in the other. There is but one conclusion to be derived from the appearances just described, which is that the double thread is formed by a longitudinal division, granule by granule, of the original filament. The evidence afforded, not only by the Locustids, but by all the Orthoptera, is unequivocal on this point. The cleavage of the thread is not exaggerated in the accompanying figures, and is distinctly in evidence even under ordinary conditions of illumination and magnification.

Much controversy has recently arisen among both botanists and zoologists concerning an appearance of the chromatin in the prophase, which has received the common designation "synapsis," by which is meant, usually, a one-sided contraction of the chromatin in the nuclear vesicle. No such stage in the nucleus could be found in *Hippiscus*, and it is likewise absent in the Locustid cells. I therefore repeat the assertion made in the previous paper (**17**), that in properly fixed material derived from Orthopteran sources the first spermatocyte prophase shows no unilateral massing of the chromatin.

Shortly after the formation of the double spireme, it is to be seen that the thread is no longer—even if it was previously—continuous, but is composed of segments (figs. 5–10). So early as this it is possible to observe that the segments are of very unequal lengths. The extent of this inequality may be gathered by consulting figures 6 and 7. Even in this early stage the real structure of the segments may be determined, and in

those favorably situated the quadripartite nature of the future chromosomes manifests itself very distinctly.

This important stage in the history of the first spermatocyte chromosomes first received attention at the hands of Paulmier in his studies upon *Anasa*. Almost at the same time I found structures in the Orthopteran spermatocytes so nearly identical that it would be impossible to distinguish any marked difference between them. The Locustid material, equally with the Acridian, permits an exact determination of the chromosome structures, which later become so masked as to be indeterminate.

The interest attaching to the construction of the spermatocyte chromosomes is so great as to warrant an account of the process, although, in general, it is largely a repetition of what has been given for *Anasa* and *Hippiscus*. As early as the stage represented in figure 6, it becomes noticeable that the chromatids near the middle of the thread tend to diverge from each other, leaving a diamond-shaped space. This becomes more pronounced, and it is soon seen that each half of the thread is broken across at the same level, resulting in the production of a chromosome of four parts. Still retaining their general shape, these segments shorten and broaden until they are almost the size of the metaphase chromosome.

All variations conceivable upon the wider separation of the halves along the longitudinal split, the movement of the parts upon the line of separation at right angles to the original cleft, or of approximation and rotation of the free segmented ends are found. Thus do we get the cross-shaped, the double-V, the figure-of-8, the Y-shaped and ring figures, in figure 11. Many of the rings give the impression, upon superficial examination, of loops with their free ends crossed. A careful examination will always reveal the fact, however, that what appears to be the crossed ends is really the middle portion of the segment, with the chromatids drawn out along the plane of the cross-division. In segments that are favorably placed, there is never any difficulty in correlating the structures with the typical one of a cross-split lengthwise of each arm.

The quadripartite nature of the chromatin segments may be determined, as already indicated, almost as soon as the longitudinal split occurs. From this time on until the chromosomes

are divided in the metaphase, it is possible to trace the formation of the tetrad chromosomes and to be sure of the relation existing between the longitudinal and cross planes of separation. As evidence of the existence of a longitudinal division of the chromatin thread and of the sequence of the two divisions, I do not see how more could be asked of any material. In the early prophase the greatly elongated and granular thread becomes twice split, once along its length and once across it. As the cell ages, a continuously closer approximation of the chromomeres occurs, without obliterating the lines of separation between the four parts of the segment; accompanying this, the segment becomes shorter and thicker, and the previously existing linear arrangement of the chromomeres is superseded. When the segments have reached approximately the size of the definitive chromosomes of the metaphase, the nuclear membrane disappears and distinction between cytosome and nucleus is lost. As a coincident step, the formerly granular segments become homogeneous in structure by the disappearance of the chromomeres as individual structures; all lines of separation between parts are lost to view, so that an examination of the formed element would betray no indication of composite structure. But, having traced the formation of the chromosomes in this way, one is at no loss to identify each part of the preexisting quadripartite chromatin segment. This is possible because, while all trace of internal structure is gone, the general outline is retained and the crosses and rings of the early stages are still, even up to the metaphase, crosses and rings.

Having traced the formation of the ordinary chromosomes through the various stages of the prophase, I should like to return to the beginning again and bring up to a like degree of development the aberrant element which I have called the accessory chromosome. This has already been given in general outline in my first paper upon *Xiphidium* (**16**), but a number of important observations since made render a general discussion desirable.

I have not yet found it possible to make a detailed study of the spermatogonia of the Locustids, as was done for the Acrididæ by Sutton in this laboratory, but sufficient observations have been made to be assured that the accessory chromosome

participates normally in the mitoses of the secondary spermatogonia. It is here distinctly visible because of its large size, which causes it to extend down to the equatorial plate, while the other chromosomes are in a late anaphase.

At the close of the spermatogonial divisions, when the disruptive processes reduce the other chromosomes to masses of chromomeres in which chromosome identities are not apparent, the accessory chromosome, with apparently more cohesive vigor than the others, retains its general form and is at all times distinguishable. It is marked off from the others, not only by persistence of form, but also by the difference in staining reaction, this being such as is usually exhibited by chromatin when concentrated into homogeneous masses. While studying the cells of *Xiphidium*, I noticed that, at one stage, this color reaction changed somewhat and more nearly approached that of the diffused chromatin. At this time the accessory chromosome had the form of a flattened, apparently fenestrated, plate. I have been fortunate enough, in preparations of *Orchesticus*, to discover that the accessory is really at this time in the form of a long, coiled thread (fig. 5). It is thus seen that, even in respect to the spireme stage, the accessory chromosome is comparable to the others, the only difference being that the diffusion of the chromomeres is less, and the independence of the element greater, than is the case with the other chromosomes.

As the chromatin segments shorten and thicken, the thread of the accessory likewise increases in diameter at the expense of its length, and is finally observable in various degrees of contortion, as shown in figure 12. By the time the chromosomes are ready for division, the accessory has assumed a form very similar to that it shows in the spermatogonia. With the establishment of the equatorial plate, *the accessory moves to one pole of the spindle and there remains undivided during the first spermatocyte mitosis.* It is accordingly a member of only one second spermatocyte resulting from the division of each first spermatocyte.

Returning to the group of chromosomes preparing for metakinesis, we find that in their earlier stages they lie so that their longer diameter is in the equatorial plate, while attached to the enlargement in the center of each, representing the point of separation laid out for the second spermatocyte division, are

the mantle fibers running to the centrosomes. The changes now ensuing are easily decipherable, because the chromosomes do not all undergo division at the same time. Since the main differences at present existing between insect spermatologists relate to the sequence of the divisions in the spermatocyte mitoses, I shall again describe the process, although it is identical with that already given for *Hippiscus*.

The necessity for a thorough understanding of the chromosome construction here becomes evident. Knowing how the chromatids were associated in the chromosomes, one can follow understandingly their movements during metakinesis.

It is first to be noted that the chromosomes lie with their longer axis in the equatorial plate. This, as we have seen, is the plane along which the longitudinal cleft occurred, so that a separation in this way means the longitudinal division of the chromosomes in the first spermatocyte. This is, in reality, what occurs. The contracting mantle fibers attached to the middle of the segments drag the adhering chromatids apart without at any time exposing a separating space. It is in this way that in the beginning the longer axes are at right angles to the spindle axis and at the end parallel with it, while during intermediate periods crosses with arms of varying length exist (figs. 13, 14).

The previously disguised lines of separation become at once visible in the daughter chromosomes, for, instead of remaining closely apposed, as formerly, the chromatids spring apart at the free ends and the chromosomes pass through the anaphase as V-shaped bodies instead of as simple rods. The space thus disclosed represents that which separates what would be the ancestral spermatogonial chromosomes, assuming that the reduced number occurs by the end-to-end union of chromosomes of the secondary spermatogonia. As already stated, the accessory chromosome does not divide at this time.

At the end of the anaphase we find the ordinary chromosomes massed at the poles of the cell, and, in addition, at one the undivided accessory chromosome. The second spermatocytes are therefore of two kinds, one possessing the accessory chromosome and the other not. One additional feature of interest that becomes apparent during the migration of the daughter chromosomes to the poles is the retarded division of one

of the elements (figs. 22–24). Some cysts contain cells that almost invariably exhibit this peculiarity. The lagging chromosome is always one of the small ones, but whether the same in each case could not be determined.

In the telophase, the main interest is centered in the question as to whether there is a loss of identity of the chromosomes or not. The evidence afforded by the Locustid cells is strongly in favor of the conception of persisting elements. As is usually the case, I believe, the chromosomes, when not under the active influence of the archoplasm, loosen up, and their homogeneous structure gives way to the granular appearance noticeable in the prophase. Although the chromosomes become closely massed and granular, their outlines can usually be distinguished (figs. 23–27). The accessory chromosome does not change its form and structure at this time (figs. 25, 27). The telophase ends with the ingrowth of the dividing cell-wall, and the second spermatocyte mitotic figure is established without any real prophase. Between the two generations it is evident that there exists no such thing as a "rest stage."

(*d*) *The Second Spermatocytes.*

In the metaphase of the second spermatocyte are formed exact duplicates of the chromosomes seen in the anaphase of the first spermatocyte. These arrange themselves radially in the equatorial plate, one chromatid immediately above the other, so that the plane separating the halves is at right angles to the spindle axis. Mantle fibers attach to the inner ends of the chromatids at the point at which, in all probability, the fibers of the first spermatocyte were connected. I am inclined to regard this as true because the opposite ends, during the anaphase, seemed to be mutually repulsive.

The spindle itself is small and weak as compared with that of the first spermatocyte, and does not long survive the anaphase condition. The material composing it, however, persists as the nebenkern of the spermatid.

A marked difference between the second spermatocytes that contain the accessory chromosome and those which do not is observable. In the metaphase, the element, already longitudinally split in the prophase of the first spermatocyte, projects from the equatorial plate for some distance into the cytoplasm.

It is very much larger than most of the other chromosomes, as may be seen in figure 28. It divides readily in metakinesis, and its chromatids travel to the poles with those of the other chromosomes, but, on account of their greater length, project downward from the mass (fig. 31). Here, as always, the accessory stubbornly maintains its independence, and can be seen extending out from the mass of other chromosomes at each end of the mother cell (fig. 32).

The division of the other class of second spermatocytes is, of course, unaccompanied by modifications due to the presence of the accessory chromosome. Aside from this, no difference between cells of the two classes is noticeable.

To summarize, we may say, that resulting from the division of each first spermatocyte are two second spermatocytes, one of which contains an accessory chromosome while the other does not. The second spermatocyte containing the accessory divides, and with it the accessory, so that each of the spermatids derived from it contains a chromatid from the accessory. The other second spermatocyte, not containing the accessory, also divides, producing two spermatids in which the accessory is absent. Thus half of the spermatids contain accessory chromosomes while the other half does not.

(*e*) *Number of Chromosomes.*

The enumeration of the chromatic elements, while a very important part of any study upon the nucleus, is unsatisfactory at the best. If there is any great number of chromosomes in the cell, it is impossible to secure a determination of it in a lateral view of the metaphase, because the elements overlie one another so as to render their distinction very uncertain. A polar view is much more desirable, but even here one is never certain that all the elements are represented, or that only entire chromosomes of one cell are present. The first of these contingencies arises from the fact that, in the event of a cell being cut in two, some of the chromosomes may drop out and not appear in the sections; or, if still on the slide, and in a small group, they may lie so close to a mass of chromosomes in another cell as to be confused with them. An excess in number may be found if a portion of the chromosomes have already divided in the equatorial plate, while the remainder are still united (*cf.* fig. 19),

or if one or two from the fragment of another cell are in the neighborhood. All these embarrassments are increased when an independent structure like the accessory chromosome is present. These difficulties exist when the conditions are most favorable, *i. e.*, when the chromosomes are arranged in the equatorial plate; they become practically insurmountable during any other stage of mitosis by the intertwining of the chromatic segments or by fusion of chromosomes in later stages.

Because of these considerations, I do not put implicit confidence in conclusions drawn from numerical relations when they involve the question of whether or not there is a difference of one chromosome between two cells. What I have to say, therefore, concerning the numbers of chromosomes in the different cell generations of the Locustid testis, I must state as my best judgment in the matter, based upon the most careful observations I could make upon cells showing the elements with the greatest clearness. While I regard them as in all probability correct, I do not rely so thoroughly upon them as I do upon observations of structural details, and have therefore based no conclusions upon numerical relations alone.

As is stated elsewhere, the number of chromosomes in the spermatogonia appears to be thirty-three. This was ascertained by selecting the clearest possible cases of the metaphase that could be found and drawing them under the *camera lucida*. Subsequent countings were made, and in most of the cells thirty-three chromosomes were found. An inspection of figure 1 will show that there is a characteristic arrangement of the chromatin bodies, the larger ones being on the outside of the group, the smaller within. Amongst the large ones, it was impossible to distinguish the accessory chromosome, but a lateral view of the anaphase shows it clearly. From the fact that it was a single element in the spermatogonia, it was to be expected that an uneven number of chromosomes would appear in this cell generation.

In the spermatocytes, as in the spermatogonia, the polar view of the metaphase was the stage selected for use in counting the chromatin elements. A large number of cases showed that sixteen and seventeen were the prevailing numbers. The smaller of these is easily accounted for when it is recalled that the accessory chromosome is at one pole of the spindle, and would

very often lie in another section, where it would not be possible to be sure of its relations. I am convinced from these counts that seventeen is the reduced number in the first spermatocyte, sixteen of the elements being ordinary chromosomes, the other one being the accessory chromosome which has come over unaltered from the spermatogonia. This coincides with the theoretically expected number, deduced from the independently determined number of spermatogonial elements.

In view of the divergences found in insect spermatogenesis, the established theory that the reduced number of chromosomes is exactly half the normal or somatic number is not a strictly accurate one, for in this case the reduction is from thirty-three to seventeen. Similar instances may be found in the forms investigated by Montgomery and de Sinéty.

When we come to consider the second spermatocytes, spermatids, and spermatozoa, it is necessary to divide them into two classes, because of the unequal apportionment of the accessory chromosome consequent upon its remaining undivided in the first spermatocyte mitosis. There are formed, accordingly, two numerically equal classes of second spermatocytes—those containing sixteen chromosomes plus the accessory chromosome, and those with merely the sixteen chromosomes. The members of each of these classes divide and double their kind, forming spermatids marked as were the second spermatocytes—one class with seventeen chromatic elements, and the other with sixteen. From these, by the usual transformations, are derived the mature male elements, which are thus of two distinct kinds.

(*f*) *Spermatids.*

The limits set to this paper preclude anything more than passing mention of the spermatids. As stated above, cells at this stage of development are of two classes, depending upon the presence or absence of the accessory chromosome. The distinction thus set up continues to exist visibly far through the transformation stages of the spermatid, by reason of the persisting independence of the accessory chromosome. Of the dual nature of the spermatids I was very early convinced, because the accessory chromosome is so strikingly displayed by the nuclei in which it exists that it is impossible to overlook its absence in a large proportion of the cells. As to the

certainty of this partial distribution in the transforming spermatozoa, I am rendered positive by the most careful and painstaking study. This is valuable corroboration of the observed fact that the accessory chromosome remains undivided in one of the spermatocyte mitoses.

V. COMPARISONS AND CONCLUSIONS.

The literature relating to the spermatocytes of insects was reviewed at some length in my previous paper upon the history of these cells in the Acrididæ (**17**). It is not my purpose to go over this same ground again except in so far as increased knowledge makes it necessary. More recent papers by Montgomery, Wilcox and others will, however, be discussed in detail. The policy previously announced, of restricting comparisons to results derived from insects, will again be adhered to. I believe that the main features of the maturation divisions are essentially the same in all insects, and I desire to see this belief either well established or overthrown. If it can be demonstrated that so large a class as the insects are characterized by a common process, it will be a firm basis upon which to conduct further comparative studies into more comprehensive groups. On the contrary, if it is shown that there is no type, even in the class, then it is useless to seek agreements between widely removed species.

(*a*) *Nomenclature.*

A necessary basis for any comparative work is a common terminology. Confusion inevitably follows the loose application of names to the structures compared. This is perhaps unavoidable in the early stages of an investigation, but should be overcome as soon as possible. There is surely no reason for continuing uncertainty after terms have received general acceptance. Believing this, I feel called upon to repeat my criticisms of Montgomery's application of the well-accepted terms "prophase," "metaphase," "anaphase," and "telophase."

In reply to my previous objection directed against this part of his work, Montgomery acknowledges the validity of the criticism so far as it relates to the metaphase, but denies the application to the other phases, particularly to the anaphase. He alleges in support of his position that the introduction of an unusual condition, the "synapsis," makes it impossible to cor-

relate strictly the stages of the germ-cells with those of ordinary divisions. Upon this point I must again disagree with him. It is impossible for any known modification of the prophase to change the essential character of the anaphase, so as to make it precede instead of follow the metaphase. This stage marks the movements of the chromosomes from the equatorial plate to the poles, and terminates when they are massed around the centrosomes. How can the "synapsis" in the least affect the duration or character of this process? It is apparent enough, I think, that Montgomery's subphases of the "anaphase" do not belong to this portion of the mitotic cycle at all, but are really portions of the telophase of the spermatogonia and prophase of the first spermatocyte. Further, it may be noted that, even were these subphases properly included in the anaphase, they would belong to the spermatogonia and not to the spermatocytes.

Montgomery himself seems to be rather uncertain of the position of his "anaphase." In the first paper, upon *Euchistus* (**12**), it was put down as the anaphase of the first spermatocyte; in his later paper (**14**), upon *Peripatus*, it is recorded as the anaphase of the spermatogonia. Still more confusing is his use of the "telophases," for in the article upon *Peripatus* (**14**) it is, in the "Contents," placed as a substage of the spermatogonial anaphase, and in the body of the work, page 307, as the telophase of the spermatocyte! Neither the anaphase nor the telophase can, by any possible construction of their proper meanings, be made to apply to the "growth period" of the germ cycle, as Montgomery insists; they are the last stages of the "division period," in reality. The prophase of the first spermatocyte is the initial stage in the constructive process marking the growth period.

Montgomery's translocation of the terms makes the "synapsis" occur in the anaphase. This is manifestly an impossible condition of the chromatin at this time, and his figures show definitely enough that it is a prophase, or, at the earliest, a spermatogonial telophase, that witnesses the contraction of the chromatin. The objection urged in my earlier paper (**17**) to the use of the term as a designation for the mere contracted condition of the chromatin cannot apply to Montgomery's latest use of it; for he here recognizes the justice of my contention

that it was primarily designed to indicate the fusion of the spermatogonial chromosome to produce the chromosomes of the spermatocyte. He states this clearly in the following words: "Moore (1895) first gave the name 'synaptic phase' to that stage in the growth period of Elasmobranchs when the reduction in the number of chromosomes takes place. Accordingly, the criterion of the synapsis stage is, first of all, the combination of univalent chromosomes to form bivalent ones; whether the chromosomes are then densely grouped or not is of secondary importance."

(*b*) *The Spermatocytes of the Locustidæ and Acrididæ.*

The formation of the first spermatocyte chromosome gives us an insight into the later changes undergone by these elements such as cannot be obtained in any other way. The great importance attaching to this part of the spermatogonial process renders it desirable to exhaust every effort in obtaining a knowledge of the actual changes here taking place. This thought has been held constantly in mind during the progress of these investigations, and every point of resemblance or of difference between the various species studied has received careful attention. Despite variations in details, however, I must state that the essential features of the maturation divisions are the same in all species of the Orthoptera examined. It is true that as yet only two families, the Acrididæ and the Locustidæ, have been worked out in a detailed way, but the close agreement between these raises a strong presumption in favor of the general prevalence of the type. The processes of the two families have already been described in detail, but it will perhaps be well to call particular attention to some points worthy of mention.

The general appearance of the material derived from the two families is quite different in sections. Even the hastiest observation will show this. The spermatocytes of the Locustid testis are much smaller, denser and more deeply staining than those of the Acrididæ. The relative quantity of chromatin is greater, so that it is possible by microscopical examination of a section to tell whether it was prepared from Locustid or Acrididan material.

The transformation from the telophase of the last spermatogonial division to the prophase of the first spermatocyte is marked by practically the same changes in both families. It

is to be observed, however, that the derivation of the spireme from the disintegrating chromosomes of the previous generation is not so clearly indicated in the Locustid cells, and it was for this reason that in the examination of *Xiphidium* I was not able to determine certainly that the accessory chromosome came over from the spermatogonia into the spermatocytes as a formed element. Upon this point, as upon others, my later material is clearer, and I was able to reconcile the appearances in the two families. In both, unfortunately, it has been found impossible to determine the exact origin of the first spermatocyte chromosomes.

In connection with the transformation of the chromatin from the spermatogonial condition to that of the spermatocyte, we must take notice of that stage which is commonly denominated the "synapsis." The evidence afforded by the Orthopteran cells is entirely negative regarding this. In properly fixed material there is no distortion of the chromatin in the nucleus at any time. It would, if present, be particularly easy to observe, as was stated in my previous paper, for during the entire winter the spermatocytes exist in the spireme stage, and in a longitudinal section of a follicle all stages may be discerned. On the other hand, in poorly fixed or hastily prepared material the synapsis is present, and always in such a form as to indicate its artificial character. What is here said regarding the synapsis refer to the appearance commonly thus designated, but, as has already been stated, such an application of the term does not meet the spirit of the definition as intended by Moore (**20**). A fusion of the spermatogonial chromosomes of some sort must certainly occur, but that it is always marked by a unilateral massing of chromosomes, I deny.

During the prophase the chromatin segments in the cells of *Orchesticus* and other species of the Locustids are heavier, more granular and denser than they are in *Hippiscus*. It is to be observed, also, that there is a greater variation in the size of the elements. This fact is observable from the earliest appearance of definite segments down through both the spermatocyte mitoses. This disproportion may be such that one chromosome will exceed another in the same cell by twenty or thirty times its volume. We have here, as is pointed out in another place, a strong proof concerning the individuality of the chromosomes,

for in some species it is possible to distinguish a particular chromosome in all the spermatocytes. This is strikingly the case in *Anabrus*, where there is always one chromosome very much larger than any of the others. It exceeds in size even the accessory chromosome, and might be mistaken for it were it not for the difference in form. It is, however, typically a tetrad, and shows the four chromatids, while the accessory chromosome exhibits the usual spermatogonial condition.

As was indicated under the head of "Observations," the prophase tetrad characteristic of *Anasa* and *Hippiscus* is again exemplified in the Locustid cells. So close is the resemblance of the maturation chromosomes of these various insect cells in their early stages, that I now regard it as practically established that they are commonly present in all insect spermatocytes. No more important evidence regarding chromosome structure and behavior can be obtained than that afforded by these elements. Particularly are the ring figures of value in the determination of the sequence of the longitudinal and cross divisions, and upon this point the material from the two families is equally convincing and positive in demonstrating that the first spermatocyte mitosis witnesses a separation along the longitudinal cleft of the spireme thread.

I should like to emphasize the fact that the chromosomes in both the Orthopteran families studied have been carefully traced from their earlier appearance down to the time of their dissolution in the spermatid through such a gradual series of changes that there can be no reasonable doubt of the accuracy of the conclusion set forth in these papers. The Orthopteran material possesses one distinct advantage over the Hemipteran, in that the point of cross-division is always marked by the same sort of a protuberance as is to be distinguished in the early chromatin segments. When the two free ends of the element are brought around to form a closed ring, the last particle of doubt regarding the position of the planes of separation marked out for the two spermatocyte divisions is dispelled.

This diagnostic character seems to be lacking in the chromosomes of the Hemiptera, and Paulmier, in his work on *Anasa*, depends for his criteria of orientation upon the relative lengths of the chromosome axes. Such a feature would be valueless in Orthopteran cells, because, as has been shown, the chromatids

move upon each other in such a way as to exactly reverse the preexisting relation between the axes. How applicable this observation may be to conditions in the Hemipteran cells, I do not know; but, judging from the great resemblance of the elements in the prophase, it would seem most reasonable to expect a similarity of the divisions.

Paulmier (**22**) advances the suggestion that in the double-V figures we may find a structure that will serve to reconcile the divergent accounts concerning the longitudinal and cross divisions of the tetrads. The only way in which this might be accomplished would be to suppose that each of the interspaces represents a longitudinal cleavage of the thread, the first being at right angles to the second. I have given this suggestion careful consideration, and find no evidence to support it. The double Vs are only of rare occurrence, the common element being a straight rod, in the center of which is a diamond-shaped clear spot representing the two planes of division laid out for the spermatocyte mitoses. If two longitudinal divisions occur, one must precede the other considerably and the resulting halves become mutually repulsive, so that they move apart and lie in one plane with only a slight connection at the point of final separation. Moreover, the second cleavage must begin at the opposite end of the segment and proceed in a reverse direction from the first. Not only this, but the first spermatocyte mitosis divides the elements along what is generally conceded to be the longitudinal split, and this must necessarily succeed the supposititious first longitudinal cleavage by some time. Without going into a consideration of these points, I may say that they suggest such deviation from normal processes that only extensive and acccurate observations would make Paulmier's suggestion worthy of further consideration.

(*c*) *Formation of the Tetrads.*

In my former paper I reviewed the results obtained by Montgomery upon the Hemiptera, but further notice of his work will now be necessary, since on almost every important point relating to chromosome structure he has changed his opinion. His late extensive comparative study upon the Hemipteran cells, as well as that upon *Peripatus*, will at the same time receive consideration.

It appears from Montgomery's account that at the point

where the Orthoptera are least valuable in demonstrating chromosomal relations the Hemiptera and *Peripatus* are most convincing. I refer here to the derivation of the first spermatocyte chromosomes from the chromatin of the spermatogonia. He claims to have observed the union by pairs of the secondary spermatogonial chromosomes during the anaphase (his synapsis) so clearly as to be positive of this fusion. I hope this may be verified, for it offers a logical explanation of the process of reduction, and is a confirmation of what has previously been assumed true without sufficient basis in observed fact, as was suggested in my paper on *Hippiscus*. This, if established, would also be a strong support of the theory relating to the constancy of the chromosomes. If this true synapsis is accomplished at this time, however, it must be noted that it occurs during the last phase of the final spermatogonial mitosis, and is not an act of the spermatocyte prophase. But as to the exact location of this point no contention need be made, for it is conceivable that the time of its occurrence might vary considerably without affecting the essential nature of the process.

With regard to such an origin of the first spermatocyte chromosomes, there is an important difference to be noted between the earlier and later work of Montgomery, and one which he fails to mention. In his paper (**12**) upon *Euchistus* he states the matter as follows: "But in the post synapsis we do not find seven chromosomes, the definitive number present in the spermatocyte divisions, but a smaller number; hence, in the synapsis the true (*i. e.*, exactly half) reduction of the chromosomes does not take place, but the number is reduced to less than one-half." This statement is based, he says, upon a most careful and painstaking enumeration of the chromatic segments in a number of nuclei, and is unhesitatingly declared correct.

In his later paper, on the contrary, he is just as positive that the definitive reduction is here accomplished, for he says: "Since then I have been able to demonstrate that this numerical reduction is effected in the synapsis by the union into seven pairs of the fourteen chromosomes, each of the seven bivalent chromosomes (pairs) being composed of two univalent chromosomes joined end to end." This statement is made without adducing any specific proof, as was formerly done. By what means we are to reconcile these diametrically opposite state-

ments Montgomery does not say. He, however, insists that he has always known that the fusion by pairs takes place. How this was to be brought about under his previous assumption that one of the fourteen spermatogonial chromosomes became removed from participation in the usual processes of the cell to form a "chromatin nucleolus," he fails to state. Until the confusion is cleared up by corroborative evidence on one side or the other, a most important part of Montgomery's work must still be regarded as uncertain.

Despite his recognition of the fusion of the chromosomes in the synapsis as the essential feature of this stage, Montgomery is insistent upon the concentration of the chromatin as its distinguishing characteristic. Regarding this he says: "McClung considers the appearance of the synapsis stage as artefacts. It is hardly necessary to reply to this criticism, since in all *Metazoa* where the spermatogenesis has been carefully examined, with the exception of certain *Amphibia*, the dense massing of the chromosomes (?) in the synapsis stage has been shown to be a perfectly normal phenomenon."

Concerning two points in this statement I wish to take exception. First, as was suggested in my previous paper (**17**), the term synapsis is usually applied to a condition of the prophase in which the apparently unsegmented spireme exists. It must be remembered that most investigators consider that the reduction of the chromosomal number takes place by the segmentation of a spireme into half the usual number of segments. In the second place, I must resent the implication that the work done in this laboratory is not "carefully" conducted. Many "*Metazoa*" have been examined "carefully," and in none has the "synapsis" occurred when the material was well fixed and prepared. It has, moreover, been found possible to produce the appearance at will. One case of this kind is sufficient to raise the presumption that it may not be normal even when constantly found in certain preparations. I have not, however, absolutely denied the possibility of such an occurrence, because it is conceivable that from the telophase of the preceding division the massing of the chromosomes may persist during their elongation. My contention is that the appearance is not a constant or necessary condition in "all the *Metazoa*," and this I have proven.

In rather striking contrast to the work of Montgomery, in which an effort is made to formulate a typical process for the entire *Metazoa* from the study of a single order, is that of Wilcox, wherein a general denial of any apparent system in the maturation divisions of animals is based practically upon the study of a single species. As was stated in my former paper, I regard Wilcox entirely in error upon the vital point of his theory of tetrad formation, not by "forced interpretation" of his own views, but by an actual examination of the object upon which he worked. There is no point upon which Orthopteran material affords more indisputable evidence than upon the occurrence of the longitudinal division of the chromatin thread in the early prophase. My statement regarding Wilcox's position on this subject was in no sense "misdirected criticism," but an actual statement of fact; it was not an attempt to explain away "abundant and evident cases which cannot be made to fit into the scheme," but simply the presentation of proof that *one* case was wrongly interpreted.

Wilcox claims the distinction of being the first and only investigator to doubt the hypothesis that longitudinal and cross divisions of the chromatic thread produce chromosomes of a different character. It is perhaps well that this is so, in view of the reasoning by which such a distinction is secured. Upon his own unconfirmed and disputed statement that there is no longitudinal division of the spireme, Wilcox presumes to disparage the accepted view of practically all cytologists. The constructive thought of the last two decades is summarily disposed of by this author in the following language: "The whole question, therefore, whether a certain division is longitudinal or transverse loses its practical significance, since the theoretical interpretation which has long been placed upon these divisions is shown to be impossible and absurd!" The showing alluded to consists in the statement that the chromosomes consist of an indefinite number of granules, which cannot be expected to arrange themselves in any order, and which, therefore, may be divided in any way without affecting the results.

Laying aside for a moment the question as to the occurrence of a longitudinal division, we may well inquire whether the belief that, "In view of this manner of the formation of the chromosomes (by the aggregation of the chromomeres), it

seems absurd to assume that the separation of an individual chromosome by one plane could be quantitative while the separation by another plane was qualitative," is well founded. At the basis of such an assumption lies the implication that any definite arrangement of chromomeres is impossible; for if any definite order were possible, then the supposed argument against the longitudinal disposition of the chromomeres would be invalid.

The argument of Wilcox is therefore directed against order in general, and not against order in any one particular, as he would have it appear. For it must be admitted that if it is possible for the scattered chromatic granules of the early prophase to arrange themselves at all (and this even Wilcox does not deny), it is equally possible for them to come together in a definite order. That they do this is amply evidenced by the fact that later they appear in definite groups or chromosomes. It is to be noted, moreover, that the later investigations tend to suggest that the apparently unorganized chromatic granules in the first spermatocyte prophase are really bound together and represent merely a diffuse condition of the spermatogonial chromosomes.

Wilcox's chief error, however, is not to be sought in speculative theories, but rather in his faulty observations. He repeatedly denies the occurrence of any longitudinal split in the chromatic thread of the first spermatocyte prophase. That he is mistaken here I am thoroughly convinced, both from a study of his own object and from investigations upon many other species of the same family. At the present time, also, practically every spermatologist is aligned in support of the view denounced by Wilcox. For a while Wilcox had some backing, but most of those who advocated only cross-divisions of the thread have later been able to demonstrate the longitudinal cleavage in better prepared material.

There is general acceptance of the opinion that the chromomeres of the last secondary spermatogonia appear in a linear arrangement to form what is commonly known as the "spireme." Wilcox declared that while in a very fine condition this thread breaks across into segments, which unite by pairs to form the chromosomes of the first spermatocyte. The great majority of other investigators are unanimous in the opinion

that this fine thread, made up of granules, becomes double by the division of each granule individually, thus producing a double thread. Thus it is that the two halves of a longitudinally divided chromosome are made equivalent, not by the sifting apart of preexisting granules, but by the division of these after they are arranged in a linear series. It need hardly be mentioned that the formation of the thread has here a reason for existence which is entirely lacking according to Wilcox's scheme.

This much space has been devoted to Wilcox's statements, not because they present any arguments against the generally accepted views of his fellow workers, but because he represents a rapidly lessening minority which is content to work in a very limited field and to resort for the explanation of diverse results to the very convenient theory that great differences may be expected in the normal processes of even closely related forms. One needs only to glance at the work of all insect spermatologists to see how closely the agreement now is upon the important points of the process. This accordance of results Wilcox notes, but interprets in his own way, which may be regarded as not exactly complimentary to the skill and judgment of his colaborers. "It is only necessary," he says, "to refer to any recent publication on the subject to find examples of this attempt to force the divergent processes in different species to fit the same formula." This is certainly a very easy and convenient way to dispose of the accumulated observations of the many careful investigators who have come to an agreement upon the important questions under discussion, but I venture to think will hardly satisfy any one except its sponsor.

After handing in this article for publication, I fortunately secured a copy of the paper by R. de Sinéty (**37**) in which the spermatogenesis of various Orthopteran species is described. I regret that the available time is so short that I shall not be able to bestow upon this contribution to insect spermatogenesis the attention it deserves, but I shall try at least to consider the principal points wherein a difference exists between the results of de Sinéty and of myself.

It is unfortunate that we have here a further complication of the problem concerning the character of the two maturation divisions in insects. At this time it had begun to appear as if

there was every possibility of insect spermatologists coming to an agreement with regard to the maturation processes. Indeed, with the exception of Wilcox, who occupies a unique and solitary position in the field, workers upon the subject are committed to a belief in the occurrence of a cross and a longitudinal division of the chromosomes in the spermatocyte mitoses. The sole difference of opinion relates to the sequence of the divisions. We have now to consider in connection with insects the remaining possibility in tetrad formation—that of two longitudinal divisions—which finds an advocate in de Sinéty.

Because of a thorough acquaintance with the forms upon which this author has worked, I do not hesitate to say that he is entirely mistaken with regard to the character of the second spermatocyte division. I am convinced of this because of the fact that in the early period of my work upon Orthopteran spermatogenesis I was inclined to place just such an interpretation upon the phenomena encountered in the spermatocytes of the Acrididæ as does de Sinéty. I soon became convinced, however, that I was proceeding upon a wrong assumption, and abandoned it in favor of the one which more extended observation taught me is correct. I hope to demonstrate here the ground for my plain statement that de Sinéty is in error upon the question of a double longitudinal division of the chromatin thread during the formation of the tetrads in insect spermatocytes.

It is fortunate that our author has properly appreciated the value of the early prophase in the determination of the structure of the first spermatocyte chromosomes, for we are here upon common ground, and need only compare like stages in order to reach our conclusions. As will be recalled, the statement is made elsewhere in this paper that the typical chromosome of the first spermatocyte is an approximately straight rod, split longitudinally, and again cleft in its middle by a second fissure at right angles to the first. Such an element is represented in figures 15*a*, 17, D and E of my paper upon the Acrididæ, and in figures 7, 9, 11 and 38 of the present one. Although this is extremely common, and, as the photomicrographs show, undeniably present, de Sinéty does not figure it at all. The nearest approach to such a structure is found in figure 123*c*, where a

cross with two nearly equal arms is represented. My interpretation of this figure, based upon a great number of careful observations, is that this represents merely an extension of the shorter arms at the expense of the longer ones. In support of this, I have stated that all intermediate stages between a rod with a mere enlargement at the center and a cross with equal arms could be found. How, according to de Sinéty's conception of overlying free elements, could these structures be explained?

It is not necessary, however, to have these gradations in order to disprove the theory under discussion. One needs only to carefully examine one of these crosses to be convinced that the two arms lie in one plane where they intersect, and are not superimposed one upon the other as de Sinéty shows in his figure 123. Our author clearly realizes the importance of the cross, as may be judged by the following quotation:

"La croix est de toutes ces figures celle dont la genèse peut le plus facilement donner lieu à des interprétations en sens contraire.—C'est précisément pour cette raison que nous croyons devoir l'étudier spécialement au point de vue critique, persuadé que, cette figure une fois rattachée à une théorie, les autres doivent en suivre le sort."

It is unfortunate, therefore, that he was not able to trace the formation of the element in its very early stages and through the various modifications which connect it with the typical rod already described.

As the simplest modification of this basic form, we find the one where it is evident that the change consists merely in a flexure of the rod at the weak spot in its center. Such forms are shown in figure 14 of my former paper (**17**) and in figures 8, 9 and 11 of this one, but are not illustrated by de Sinéty. It occasionally happens that in chromosomes of this character the halves diverge widely at the center, producing the double-Vs of Paulmier, as is represented in figure 14 of my paper upon the Acrididæ (**17**) and in figure 8 of the present one. These structures are not shown by de Sinéty and would be difficult to explain in agreement with his conception of the tetrad.

I have consistently placed great reliance upon the frequent ring-shaped chromosomes in determining the structure of the first spermatocyte elements, and have no occasion to change my opinion of them since examining the work of de Sinéty.

This investigator joins issue with me upon my interpretation of these structures, and states his attitude in the following language:

"McClung fait grand fond, pour appuyer son interprétation, sur une forme spéciale, la forme en anneau, qui pour lui dérive du bâtonnet $\frac{a' b'}{a' b'}$, supposé placé transversalement sur le fuseau, inséré par son milieu et incurvé en dehors jusqu'à rapprochement et soudure de ses extrémités.

"Le chromosome en anneau est en effet très fréquent chez les acridiens; mais il nous a été possible d'en reconstituer l'histoire, grâce à des détails qui ne semblent pas s'être rencontrés dans les figures de McClung. On se souvient que nous avons établi les deux points suivants en complet désaccord avec la théorie de l'auteur américain:

"1. Les deux moitiés de l'anneau proviennent de la première division longitudinale.

"2. L'insertion est terminale."

With equal emphasis, I must deny that the enclosed space in the ring represents any plane of division in the chromatin thread; and that the insertion of the spindle fibers is at any place except at the center of what would be the typical rod-shaped chromosome were the ring straightened out. We encounter in de Sinéty's interpretation of these rings the very error against which I was careful to caution elsewhere in this paper, *i. e., of regarding the points where the fibers are attached as the crossed ends of a simple segment.* This mistake de Sinéty has made, and has thereby vitiated all his conclusions concerning the structure of the tetrads. It is not necessary to repeat here the proof which I have brought forward in support of my views. No one, I am sure, will find difficulty in reducing the various forms of chromosomes found in the first spermatocytes to the type of a doubly split rod, in which one plane of division is parallel to the long axis and the other at right angles to it. The explanation offered by de Sinéty requires us to conceive a doubly split rod in which one separating space may vary indefinitely while the other is constant. There is here no common type, but an infinitely variable one, which differs with every modification of the interspace between the first pair of chromatids in each chromosome.

As a constructive basis for the foundation of his theory of a double longitudinal division, de Sinéty uses particularly the chromosomes of *Œdipoda* (*Hippiscus*) *miniata*, represented in figures 129 and 130, concerning which he says:

"Survient le phénomène exceptionnellement important de la seconde division

longitudinale; nous regardons comme un point capital dans notre travail d'en mettre l'existence hors de doute et pour cela nous désirons ne faire appel qu'à des images extrêmement claires. Nous considérons comme telles les fig. 129 et 130 rapprochées l'une de l'autre.

"Il est de toute évidence que le chromosome *a*, fig. 130, n'est que le chromosome de même désignation, fig. 129, dont les deux anses jumelles se sont clivées. De même, le chromosome en forme de boucle, *c*, fig. 129, dont les deux branches représentent, comme nous l'avons fait remarquer, deux anses jumelles, se retrouve avec un clivage très évident en *d*, fig. 123. On pourrait faire les mêmes rapprochements entre *b*, fig. 105, et *a*, fig. 107; ici, le clivage est moins avancé, mais les granules sont nettement divisés."

I am obliged to confess that I have never seen in other species of this genus any appearances that would incline me to place an interpretation upon them such as does our author upon these. I would venture to suggest, on the contrary, that the chromosomes represented in figure 129 have not as yet demonstrated any division, but show merely irregular spaces between chromosomes. At even an earlier stage (figs. 5, 37, and 38), I have shown the formation of the tetrads by means of simultaneous cross and longitudinal divisions so clearly that presumed successive divisions, as represented by de Sinéty, cannot be regarded as occurring.

Finally, I would emphasize the fact mentioned in connection with the discussion of the cross-shaped chromosomes, that where the elements of one of these compound chromosomes intersect *they lie in one plane, and are not superimposed upon each other*, as de Sinéty's theory demands and as his figures represent. This was shown clearly in Paulmier's figures as well as in my own, and is even more clearly demonstrated, if posssible, in the very long, slender chromosomes of the myriapods, which I have observed in Mr. Blackman's preparations. This, and the continuity of the chromatin in contiguous arms of the cross, is alone sufficient to disprove de Sinéty's theory, and, fortunately, is easily demonstrated. This same fault of de Sinéty's is encountered, in another form, in his discussion of the ring figures. He asserts that the halves of the rings are pulled past each other while they lie in the plane of the spindle axis. Herein my observations fail entirely to agree with his. The rings lie in the plane of the equator, and no elements of the mitotic figure show a lateral displacement of the separating halves equal to the width of the chromosome when viewed in this plane.

(*d*) *The Spermatocyte Divisions.*

I approach a discussion of Montgomery's conclusions regarding the form of the chromosomes in the first spermatocyte, and the sequence of their divisions, with considerable hesitation, because of the difficulty I experience in appreciating his exact position. This is due, not to any lack of positive statements on his part, but to the partial contradictions that result from his frequent changes of opinion. The most important statement in his first paper upon *Euchistus* reads as follows: "From the resting stage of the first spermatocyte to the formation of the spermatid, there is absolutely no longitudinal division of the chromosomes. I have studied hundreds of nuclei in these stages, and at the first with a hope of finding a trace of such a process, but observation shows that all divisions of the chromatin elements are transverse divisions."

This would certainly seem to be as strong a stand as one could take upon the subject, but in later papers Montgomery assumes with equal assurance the opposing position, which holds for a longitudinal division. Regarding this he says: "During the synapsis stage the chromosomes become split longitudinally, as was first shown by Paulmier (1898, 1899) for *Anasa*—a process that I had overlooked (!) in my former paper (1898)." Throughout his later investigations this hypothesis serves as the basis of all his theories, and the careful longitudinal division of the thread is assigned an important *role* in the maturation process. So far as positive assertions to the contrary are concerned, a general acceptance of the theoretical importance attaching to this act is to be supposed.

Notwithstanding this, I find nowhere in his later writings any statement that he abandons the conception formerly entertained regarding the non-importance of the longitudinal cleavage. This attitude is indicated in the following language: "If it can be proved that the mode of division of a chromosome, *i. e.*, the axis of the line of division, is merely a function of its chromomeres, then it would be of no theoretical value whether the division be longitudinal (equation) or transverse (reduction). But it happens that the postulated difference forms one of the main premises of Weismann's theoretical superstructure. On account of the differences observed in different objects in regard to the modes of division of the chromo-

somes, it would appear that the differences have no theoretical value, but that the halving of the mass of chromatin is the process of importance—the standpoint taken by Hertwig.

"In the two reduction divisions the chromosomes may split by two longitudinal divisions, by two transverse divisions, by one longitudinal and one transverse division, or by one division (longitudinal or transverse) preceded or followed by an elimination division. The facts show already that there is no general uniformity in the mode of division of the chromosomes in the reduction mitoses. The long line of observations on different objects show this to be the case, and demonstrates that the expected uniformity does not occur."

Herein lies the essential conclusion of the work upon *Pentatoma*, which, so far as a specific retraction is concerned, stands yet. If this be abandoned, then the first work upon the chromatin structure of *Pentatoma* is practically discredited, for Montgomery has definitely retreated from his positions concerning the absence of the "chromatin nucleolus" in the spermatogonia, the non-occurrence of a longitudinal cleft in the spireme thread, the lack of an equational division of the chromatin in the spermatocyte, the origin of the "chromatin nucleolus," and the fragmentation of the "chromatin nucleolus." In addition to these specifically acknowledged errors, we may infer that Montgomery (**12**) considers himself at fault in his views upon the production of chromosomes from the "three to six chromatin loops" by breaking apart in the prophase, and upon the occurrence of both longitudinal and cross divisions of ordinary chromosomes in the same mitosis. The observations recorded in his last paper (**15**) upon the production of the spermatocyte chromosomes by the end-to-end union of those in the last spermatogonial division warrant this assumption.

It follows from all this that we may practically disregard Montgomery's earlier work upon chromosomal structure and take his views as expressed in the later papers (**14, 15**) as representing his opinions upon the subject. These later theories are largely the result of his investigations upon *Peripatus*, but they seem to be carried over and applied to the Hemiptera without essential modifications, and we may regard this concept as applicable to the forms studied by him.

I called attention in my previous paper to the fact that, by

many investigators, the definitive form of the chromosome is used as the basis for determining the direction and sequence of the chromosome divisions. This fact and the danger attending the practice was partly realized by Montgomery in his work upon *Euchistus* (**12**), for he devotes considerable space to a consideration of the prophase segments, but in determining the character of the second spermatocyte division he regards only the formed element. With respect to this he says: "And now a fact may be determined which is of the greatest importance in estimating the morphological value of the second division of the chromosomes. While the latter are still parallel to the axis of the spindle, there may be clearly seen in some cases a transverse constriction on some of the chromosomes, so that they already acquire a dumb-bell shape." This constriction is not correlated with any similar one on the prophase elements, and is here observed for the first time.

In his paper upon *Peripatus*, however, he definitely supports the contention that it is only in the prophase of the first spermatocyte that we can learn the construction of the chromosomes, for he says: "The early stages in the prophase are of the greatest importance in determining the exact constitution of the chromosomes of the first maturation division. . . . Since, then, as has been shown in another section of the present paper, the split of the univalent chromosome of the second spermatocyte is a true longitudinal split, corresponding perfectly in position with the longitudinal split of the early prophase, it follows that the univalent chromosome does not become turned upon its axis to take its place on the equator of the spindle." Orientation is in both spermatocytes based, accordingly, upon planes determined in the prophase. Upon this point Paulmier and Montgomery, as students of Hemipteran spermatogenesis, are now agreed, and their results correspond with observations made upon Orthopteran cells.

It is upon the sequence of divisions in the spermatocyte that differences now exist between these investigators and myself. In my previous paper I took occasion to elaborate the proof in support of my position regarding the early occurrences of the longitudinal division in the Orthopteran spermatocytes. Montgomery follows Paulmier in ascribing the reduction division to the first spermatocyte, and takes no account of my results upon

Hippiscus. The objections that I previously urged against Paulmier's conclusions apply equally well to Montgomery's. Until the chromosomes are traced in a more detailed way through the prophase to the metaphase, I shall consider the presumption against the occurrence of the cross-division in the first spermatocyte mitosis. In this I believe that I am justified by the definite proof of my position brought forward in the work upon *Hippiscus*. Here, it may be recalled, I observed and photographed in the same mitosis all stages of movement by the chromatids along the plane of the longitudinal split. In addition, I was able to locate definitely the position of the future cross-division in the ring figures, so that it is impossible to mistake the character of the first division in them. These two proofs I consider incontrovertible so far as they apply to the Orthopteran families studied.

Paulmier judged the planes of the division by the relative lengths of the chromosome axes, but, as I pointed out, this is not conclusive unless it can be shown that they have not shifted, as it is possible for them to do, during the prophase. The value of the ring figure, which is formed at such an early stage that it would be impossible for the shifting of the axis to occur, is here evident.

Montgomery finds these rings in *Peripatus*, and realizes the importance of their evidence in determining the planes of division, but places his conclusions upon a much more insecure footing than those founded upon the Orthopteran cells, because of the criterion used in determining which point represents the junction of the paired chromosomes. The diagnostic feature he uses is the linin connection persisting between the "central ends" of the chromosome, which holds them together until the "distal fibers" connect with the centrosomes and cause the rupture of the "central" fiber. Since the whole of his elaborate theory regarding the continuance of the linin spireme is practically a theoretical conception with little basis in observed fact, the value of such proof cannot compare with that furnished by the definitely formed chromosomes themselves in the Orthopteran cells.

In view of all these facts, I think it must still be held an open question as to which is the reduction and which the equation division in the Hemipteran spermatocytes, although it is not to

be doubted that the probability of the first spermatocyte being witness of the reduction division is much increased when thus interpreted by two independent observers.

(*e*) *The Accessory Chromosome.*

I have already, in another paper (**19**), taken up a comparative study of the accessory chromosome in different insect spermatocytes, and shall not be obliged, for that reason, to enter into a very lengthy discussion of the subject here. The great interest attaching to this structure, however, compels me to consider the work that has been done since the manuscript of the earlier article was sent in for publication. This review will concern, very largely, the investigations of Montgomery upon a considerable number of Hemipteran species, which are set forth in his paper under the pretentious title "A Study of the Chromosomes in the Germ Cells of Metazoa."

In his first work upon *Euchistus*, Montgomery describes a cell element under the name "chromatin nucleolus" which corresponded so closely to my accessory chromosome that I concluded the two structures were identical. These similarities were, the origin from a spermatogonial chromosome, the integrity and constancy of staining power and position during the spermatocyte prophase, and participation in the division act during metakinesis of a spermatocyte.

Among the numerous changes of opinion recorded by Montgomery in his latest work, there are several relating to his "chromatin nucleolus" that materially alter the aspect of the question. Perhaps the most important of these concerns the origin of the element. I was some time in determining that the accessory chromosome is a spermatogonial chromosome which divides in the spermatogonia with the other chromatin elements and comes over into the first spermatocyte as a formed structure. The work of Sutton upon the early history of the element in *Brachystola*, however, was convincing in this respect and confirmed me in the opinion I had already formed. I therefore gave Montgomery the credit for this discovery, and set it down as strong confirmation of the assumption that we were dealing with similar structures in the two orders of insects.

Upon this point Montgomery now completely reverses himself, and declares that his "chromatin nucleolus" is not a spermatogonial chromosome, but may be noted in the earlier

generations as a nucleolar structure, which, however, divides in metakinesis. The most important feature to be noted in this connection is the fact that the structure does not exist as a simple element, but is observed as a number of granules, and that this number varies considerably in different species. These granules fuse during the "synapsis stage," as do the chromosomes, to produce in the spermatocyte half the number of "chromatin nucleoli" that were present in the spermatogonia. In this respect the "chromatin nucleolus" differs radically from the accessory chromosome, which has the same valence in both cell generations. The indefinite number and insignificant size of Montgomery's structures are other characters that point to extensive differences between them and the accessory chromosome.

In his work upon *Peripatus*, Montgomery states that in restudying his preparations of *Euchistus* he observes a continuous linin spireme which involves the "chromatin nucleolus" as well as the chromosomes. Here, again, there is a difference between the Hemipteran element and the accessory chromosome; for the latter is entirely free from linin connections in the prophase and is usually surrounded by a hyaloplasmic investment.

According to Montgomery, also, his "chromatin nucleolus" usually takes part in both spermatocyte mitoses. In this respect there exists an essential difference between his element and that found in the Orthoptera, for, after extended and most critical studies, I have become convinced that only one division takes place in the spermatocytes. In those cases where Montgomery admits but a single division, it is stated to occur in the first spermatocyte, while in the Orthoptera the accessory chromosome remains undivided here and is halved in the second spermatocyte.

If, therefore, Montgomery's recent observations are correct, it must follow, I think, that his "chromatin nucleolus" and the accessory chromosome are different structures. I am free to admit, however, that his statements are far from convincing. So much dependence is placed upon the numerical relationships of elements that are admittedly very minute, and so little corroborative proof is given, that I entertain serious doubts as to the accuracy of the observations. In this connection I would

suggest a comparison between the figures of the "chromatin nucleolus" in the first paper upon *Euchistus* (figs. 55–68) (**12**) and those in the last one (figs. 1–17) (**15**). The showing here made would alone be sufficient to raise a question as to the nature of the "chromatin nucleolus," and until further evidence is forthcoming the character of the peculiarly modified chromosomes in the spermatocyte of the Hemiptera must remain in doubt.

Aside from definite retractions that Montgomery has made regarding his earlier views on the character of the "chromatin nucleolus," there are noticable different attitudes toward it in his earlier and later works. Thus, in his lecture at Woods Holl (**13***a*), we find the following: "These remarkable 'nucleolar' structures which stain like chromatin have been observed by numerous writers, but as yet no satisfactory description has been given of their mode of origin. They have been observed by me in spermatocytes of various insects, in hypodermal and other cells of *Carpocapsa*, and in follicle cells of the testicles of *Plethodon* and *Mus*." At this early stage of Montgomery's investigations it is apparent that he views his "chromatin nucleolus" primarily as a nucleolus with chromatic origin and characters, but the fact is equally apparent that he now regards it primarily as a "chromosome" with nucleolar attributes. This is made evident in his recent definition, which reads: "The chromatin nucleoli are morphologically chromosomes, undergoing division in mitosis like the other chromosomes, but differing from them in the rest stage by preserving a definite (usually rounded) form."

What has here been said regarding the "chromatin nucleolus" applies to those structures in *Euchistus* and other Hemiptera to which Montgomery has given the name without qualification. According to his definition, however, there is present in the cells of *Protenor* and other species another form, the "chromosome x." Not only by inference is this classification operative, but by direct statement we learn that Montgomery regards this element as a member of the class of bodies which he calls "chromatin nucleoli." In speaking of *Protenor* chromosomes, he says: "This is the only case in the Hemiptera where one chromosome becomes differentiated into a

'chromatin nucleolus' for the first time in the spermatocyte generation."

The noteworthy thing about this "chromosome x" is the fact that in every essential detail it corresponds to the accessory chromosome of the Orthoptera. It is a spermatogonial chromosome that comes over intact into the spermatocyte; it retains its form and staining power unchanged through the prophase of the spermatocyte; it divides in only one of the spermatocyte mitoses; and is a large and conspicuous element of the cell at all times.

This "chromosome x" agrees just as closely in its description to the accessory chromosome as do the ordinary ones of the two orders, and, if Montgomery's account is correct, there would seem to be no reason for doubting their identity. In two respects, however, there are differences between these structures. First, it is to be noted that the "chromosome x" divides in the first spermatocyte, while the accessory chromosome undergoes separation in the second spermatocyte. Should Montgomery's observations prove correct, it would yet indicate no fundamental difference in the character of the element, for the result is the same whether division takes place in the first or second mitosis. In either event, one-half the spermatozoa are provided with the odd chromosome while the remaining half are not.

The second point of difference would seem to be a more serious one. Montgomery states that during the spermatogonial mitosis the "chromosome x" regularly divides as do all the other chromosomes, *i. e.*, longitudinally. In the spermatocyte mitosis, however, the element is broken across, and the longitudinal split, which is apparent in the early stages, disappears and is not utilized in division. We have here the remarkable occurrence of a chromosome entirely unchanged in its structure, but merely differing in its surroundings, which, instead of dividing along the plane marked out for it, as it has done in all preceding mitoses, breaks across after it is a formed element. An occurrence of this kind, so different from the usual method of division, would require strong proof to establish it, and this, in my opinion, Montgomery has not brought forward.

A criticism of the degeneration theory as advocated by Paulmier and Montgomery has already been given (**17**), so that it

would not be necessary to consider it here except in so far as it has been modified since its promulgation. As a rule, Montgomery refers to his "chromatin nucleoli" throughout his late paper (**15**) as degenerating chromosomes, but in discussing their function specifically he makes important changes in this conception. These are stated as follows: "When we find, accordingly, the mutual apposition of them (true nucleoli) to chromatin nucleoli, it would be permissible to conclude that the chromatin nucleoli are chromosomes which are especially concerned with nucleolar metabolism. And this, I think, would be the correct interpretation. The chromatin nucleoli are in that sense degenerate that they no longer behave like the other chromosomes in the rest stages, but they would be specialized for a metabolic function; and from this point of view they would certainly seem to be much more than degenerate organs."

It is difficult to comment upon a contradictory statement like this; but, fortunately, it is not necessary to do so, since it carries with it its own refutation. The conception of a chromosome specialized in the direction of increased metabolic activity as being in the process of disappearing from the species can hardly be regarded seriously.

Taking everything into consideration, it may be said that Montgomery's work upon the Hemiptera has left the subject in a very disturbed condition, and any prospect of a complete agreement between the accessory chromosome of the Orthoptera and the "chromatin nucleolus" of the Hemiptera is made more remote than was previously the case. This, I think, is largely due to the inferior character of the Hemipteran material, which has lead to misconception of phenomena that are clearly marked in Orthopteran cells.

It is gratifying to note that the recent work of de Sinéty (**37**) practically corroborates the conclusions herein set forth regarding the history of the accessory chromosome. Aside from failure to observe the important spireme condition of this element in the first spermatocyte prophase, de Sinéty describes the same series of processes with scarcely an exception. His summary contains the following account of the accessory chromosome:

"Le 'chromosome accessoire,' découvert par McClung chez *Xiphidium fasciatum*, se retrouve chez les locustiens que nous avons étudiés. Chez *Orphania*, il se divise dans les spermatogonies en deux masses volumineuses et

allongés, que l'on reconnaît dans les nucléoles, également volumineux et allongés, des spermatocytes de premier ordre en prophase. A la métaphase de la première cinèse, on le trouve situé excentriquement et plus près de l'un des pôles; *il va tout entier a l'une des cellules-filles.* Dans celle-ci, il se divise comme un chromosome ordinaire, d'où il suit que *sur quatre spermatides formant la descendance d'un spermatocyte, deux se trouvent privilegiees.* Par ce partage inégal, non réalisé dans *Xiphidium fasciatum*, d'après McClung, le chromosome spécial d'Orphania rappelle celui des hémiptères."

A like series of processes is recognized in the Phasmids.

As is elsewhere explained in this paper, the occurrence of two divisions of the accessory chromosome in *Xiphidium*, which was mentioned as a possible occurrence in my preliminary paper, is shown not to take place. While it is much more difficult to demonstrate the undivided condition of the accessory chromosome in one of the spermatocyte mitoses of *Xiphidium* than it is in the cells of *Orchesticus*, *Anabrus*, *Scudderia*, and *Microcentrum*, I am convinced that it does not differ from the other Locustids in this respect.

We may therefore feel assured that our knowledge of the morphological character of the accessory chromosome in the Orthoptera is fairly well established. This gives us a good base from which to conduct further comparative studies into other groups, and it is to be hoped that our knowledge of this element will rapidly increase.

Unfortunately, de Sinéty has chosen to add another name to the already overburdened list of synonyms, and "chromosome spécial" now takes its place in the literature of insect spermatogenesis. The reason for adding this name—

"Il reçu successivement les noms de 'accessory chromosome' (McClung), 'small chromosome' (Paulmier), 'chromatin nucleolus' (?), 'chromosome *x*' (Montgomery). Nous avons préféré éviter ces appellations, qui semblent toutes supposer une signification qui n'a jamais été définie ou s'appuyer sur des caractères plus ou moins secondaires, pour adopter un nom indifférent, celui de 'chromosome spécial,' nous conformant à l'idée de Wilson, pour qui c'est un 'extra chromosome,'"

would seem to be at least insufficient, since "accessory chromosome" can scarcely be regarded as implying any more primary or secondary function than can "chromosome spécial."

(*f*) *Individuality of the Chromosomes.*

In each of my preceding papers I took the opportunity to point out the fact that, even were the accessory chromosome

of no other value, it would certainly be worthy of study for the light it throws upon the question of the individuality of the chromosomes. On this point Montgomery has much to say in his late paper (**15**). I think it cannot be questioned that we have here indisputable proof that at least one chromosome may be identified through all the cell generations of the testis. While this does not prove that chromosomes are persisting and independent structures, it does evidence the fact that they may be, and greatly strengthens the hypothesis that they are.

In addition to the evidence here offered by the accessory chromosome, there must be noted that derived from a study of spermatocytes in which there is always present one ordinary chromosome that greatly exceeds the others in size. Such a condition is found in the cells of *Anabrus*. The disproportion in size of the elements is here so striking that it would be impossible to fail in distinguishing the giant chromosome. In each of the spermatocytes of *Anabrus* there are therefore two chromosomes which are plainly recognizable. It may be observed further that the remaining chromosomes are quite different in size, and it may be possible within reasonable limits of certainty to pick out one or more other chromosomes in each cell. Unless this could be done for each element, however, it would not definitely prove that all the chromosomes are distinct and recognizable structures. The actual recognition of two elements in each cell of the same generation and its ancestors or descendants in other generations goes far, however, to render probable the individuality of each chromosome.

Beyond this point studies upon the Orthopteran cells will not permit me to go; but Montgomery has been fortunate enough to find in *Peripatus* an object in which he considers it possible to demonstrate the continuity of the chromosomes from one generation to another, and their fusion by pairs in the early history of the spermatocyte to bring about the reduced number. This is, in the main, a logical conclusion to my own work, and I am therefore bound to regard his results as probably correct. While doing this, however, I recognize that the absolute proof he brings forward in support of his hypothesis is very slight. I consider any deductions based upon observations of linin structures as very insecure, and it is upon these that Montgomery principally relies to demonstrate his theory. Further

observations upon the behavior of the chromosomes between the spermatogonia and the spermatocytes in objects favorable for study will be awaited with interest. In the meantime it must be conceded that the work upon insect spermatogenesis has at least lent strong support to the theory of the individuality of the chromosomes in general and has definitely shown that there is such a thing in some instances.

(*g*) *Nucleoli.*

Considerable importance is attached by some investigators to the nuclear structures, properly called plasmasomes, that occur in the spermatocytes. It is probable that there are marked differences between the cells of various species in regard to the occurrence of these bodies, for in the Orthoptera they either do not appear at all, or, if present, they are minute and inconspicuous. This fact would tend to disprove any theory which would attach a fundamental importance to these structures, such as is conceived for the chromatin. The Orthopteran cells do not allow any observations which would add to our positive knowledge of the nucleoli, and I include this brief statement merely for the negative value it may possess.

(*h*) *Rest Stage.*

In his first paper upon *Euchistus*, Montgomery assigns an important and conspicuous place to the "rest stage" among his numerous subphases preceding the first spermatocyte mitosis. As a result of his later comparative work upon the Hemiptera, however, we learn that in certain families no trace of such a condition of diffusion on the part of the chromatin is observable, from which we conclude that "accordingly such a stage would appear to have no broad significance." It has already been announced that nothing like a rest stage intervenes between the spermatogonia and spermatocytes of the Orthoptera, and the work of most investigators would tend to indicate that it is the exception rather than the rule. In those cases where such a condition of the nucleus exists, it would seem to be true that nothing more unusual than an excessive diffusion of the spermatogonial chromosomes occurs, and this is of hardly sufficient importance to receive a special designation.

The existence of a rest stage between the first and second spermatocytes is also negatived by the conditions found in the

Orthopteran cells. The formation of chromosomes in the prophase of the first spermatocyte that are already prepared for two divisions would *a priori* render improbable the intervention of a rest stage here; and the actual observed persistence of the chromosomes, as such, through the telophase of the first spermatocyte and through the modified prophase of the second spermatocyte gives actual proof in support of the view that commonly prevails regarding the suppression of the second spermatocyte rest stage.

Observations upon numerous species tend to show that the behavior of the chromatin during the period between the two spermatocyte mitoses varies considerably with the species and even within the species itself. The amount of diffusion would, in some measure, seem to be related to the form of the chromosomes and to vary correspondingly in those individuals where the chromosomes are of diverse forms. Thus, where the elements of the second spermatocyte metaphase appear as short double rods, the amount of diffusion is slight, and the individual chromosomes may be distinguished throughout the telophase of the first spermatocyte; but in those cases where the members of the mitotic figure are much elongated the diffusion is more extensive and the distinction between elements is made difficult or impossible. Since these two conditions may prevail in the same testis, it is probably only a question as to the extent of elongation on the part of each chromosome. In those cases where the elements become very much extended the appearance of the resting condition would be simulated closely, while, on the contrary, chromosomes consisting of spherical or short cylindrical chromatids would never give a suggestion of such a stage. In this we may find, I think, an explanation for those cases in which a rest stage is described as occurring between the spermatocyte generations.

VI. SUMMARY.

1. The secondary spermatogonia are much reduced in size at the end of their divisions and the cytoplasm is very small in amount. The rod-shaped chromosomes number thirty-three, and, of these, one is to be distinguished from its fellows by greater size and slower division.

2. From the substance of the disintegrated spermatogonial

chromosomes, the tetrads of the first spermatocytes are formed. It was impossible to determine the relation of the elements of the two generations, but the changes are rapid and there is no intervening resting condition of the nucleus.

3. It could not be determined whether or not the spireme is continuous. A longitudinal split appears very early, and shortly after the chromatin segments may be seen. These soon betray at their centers an indication of the cross-division, producing crosses with arms that may vary considerably in relative lengths. No reason was found for considering both divisions longitudinal.

4. The typical element is granular and more or less rod-shaped, with the longitudinal division merely indicated by a narrow line, and with but slight elongation of the chromatids along the plane of the cross-division. Various modifications of this occur, by which the longitudinal cleft is much increased in width at the center, the cross-arms are greatly extended, or approximation of the ends of the rod brought about, producing a ring.

5. The definitive chromosomes of the metaphase are produced by a concentration of the prophase elements, whereby they become shorter, heavier, and entirely homogeneous in structure. Distinct lines of division between the chromatids are not visible, but the tetrad character of the elements is readily established by observing the steps in their formation.

6. The accessory chromosome early becomes distinguishable because of its peripheral position and strong tendency to stain with safranin, while the remaining chromatin takes the gentian violet by Flemming's three-color method. At first it appears as a homogeneous plate, but later this is seen to be a closely coiled thread. As the chromatin segments shorten and broaden to form the chromosomes of the mitotic figure, this thread also grows shorter and heavier until it forms an element of essentially the same character as that of the spermatogonial chromosome from which it was derived.

7. Upon the establishment of the mitotic figure, the chromosomes arrange themselves in the equatorial plate with their longer axis perpendicular to the spindle axis. Division of the elements is not synchronous, so that all stages of the chromatid movements may be observed in one nucleus. By this means it

is possible to determine that separation of the chromosomes takes place along the plane which marked the longitudinal division of the prophase thread in such a way that the chromatids show no clear interspaces. The individual chromosome near the end of its division has the same form as that with which it started, except for the difference that the chromatids are now in contact for the greater part of their length along the plane of their cross-division. As the daughter chromosomes separate, this line of division comes into evidence through the springing apart of the two chromatids now composing each chromosome. The result is the formation of two V-shaped chromosomes with mantle fibers attached to their apices. The accessory chromosome does not participate in this division, but passes unchanged to one pole of the spindle.

8. By reason of the action of the accessory chromosome in the first spermatocyte mitosis, there are produced two numerically equal classes of second spermatocytes—(*a*) those containing sixteen dyad chromosomes and an undivided accessory chromosome, and (*b*) those with merely the sixteen dyad elements. In both cases the mitotic figure quickly reforms without an intervening rest stage in which the chromosomes lose their identity. There is a loosening up of the chromomeres in all the elements except the accessory chromosome, so that they have a structure and staining reaction similar to that of the first spermatocyte chromosomes just before they enter the metaphase. The dyads of the first spermatocyte telophase, and of the succeeding and greatly abbreviated second spermatocyte prophase, are quite as definite structures as are the chromosomes of the first spermatocyte prophase.

9. All the chromosomes of the second spermatocyte are paired structures and divide in a similar way. The spindle is small and weak as compared with that of the first spermatocyte, and the chromosomes arrange themselves radially on its periphery in such a way that the pairs lie in the plane of the spindle axis with their joined ends inward. The space between the chromatids represents the line of cross-division observable in the prophase segments of the first spermatocyte, and their separation accordingly represents a reduction division. The accessory chromosome, on the contrary, divides along the plane marking the longitudinal cleft of the spermatogonial spireme.

10. From each first spermatocyte there are formed, by two divisions, four spermatids, of which two are distinguished from the remaining pair by the possession of an extra chromosome in addition to the number—sixteen—common to them all. Both classes undergo a like series of transformations by which they become mature spermatozoa. These are necessarily of two kinds; and it is believed that those containing the accessory chromosome, in the act of fertilizing the egg, determine that the germ-cells of the embryo shall be sexually male, or like themselves, while those from which it is absent are unable to impress their sex upon the egg and assist in producing female embryos.

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DESCRIPTION OF FIGURES.

Drawings were made with a *camera lucida*, the optical combination being a 1–16 B. & L. objective and a Watson "Holoscopic" ocular No. 7. Details were studied with a Zeiss 2-mm. apochromat, N. A. 1.30. As reduced in reproduction, an enlargement of 1500 diameters exists. Photomicrographs, excepting those of figures 37 and 38, were made by the use of the arc light and horizontal camera. The exceptions represent illumination by ordinary diffuse daylight. In all cases the lenses used were the Zeiss 2 mm., N. A. 1.30 objective and projection oculars. A Watson "Parachromatic" oil-immersion condenser of 1.30 N. A. was employed to illuminate the objects. In use it was stopped down to between .75 N. A. and 1.0 N. A.

Explanation of Plate VII.

FIG. 1. Pole view of spermatogonial metaphase, showing the thirty-three chromosomes. It will be observed that the chromosomes are of unequal sizes, and that the large ones arrange themselves in a circle on the outside of the figure.

FIG. 2. Very young spermatocyte. The chromatin derived from the breaking down of the spermatogonial chromosomes in a diffuse condition, with no trace of a linear arrangement. The accessory chromosome x on the periphery of the nucleus, darkly staining and homogeneous.

FIG. 3. Early stage in the formation of the spireme. In the cytoplasm the remains of the spermatogonial spindle. The cell has entered upon the growth period.

FIG. 4. A later stage in the spireme formation. The accessory chromosome larger and more flattened. A surface view shows it as an apparently fenestrated plate. The remains of the two spermatogonial spindles still persisting.

FIG. 5. First appearance of definite chromosomes. One shown entire with longitudinal and cross-divisions marked. The accessory chromosome is here seen to be in a spireme condition.

FIG. 6. Condition of the chromosomes after further contraction of the early segments. As here shown, they are more granular than is usually the case.

FIG. 7. Common types of the prophase chromosomes.

FIG. 8. A cell in which one of the chromosomes has its halves widely separated along the longitudinal division, forming Paulmier's double-V figure.

FIG. 9. In this cell may be seen the variation in form and size of the early spermatocyte chromosomes.

FIG. 10. Two cells of the late prophase, with the chromosomes at almost the extreme degree of concentration.

FIG. 11. Chromosomes of cells in the stage shown in figure 10. These represent the different types of rings, crosses, etc., commonly observed in first spermatocytes just before the formation of the mitotic figure.

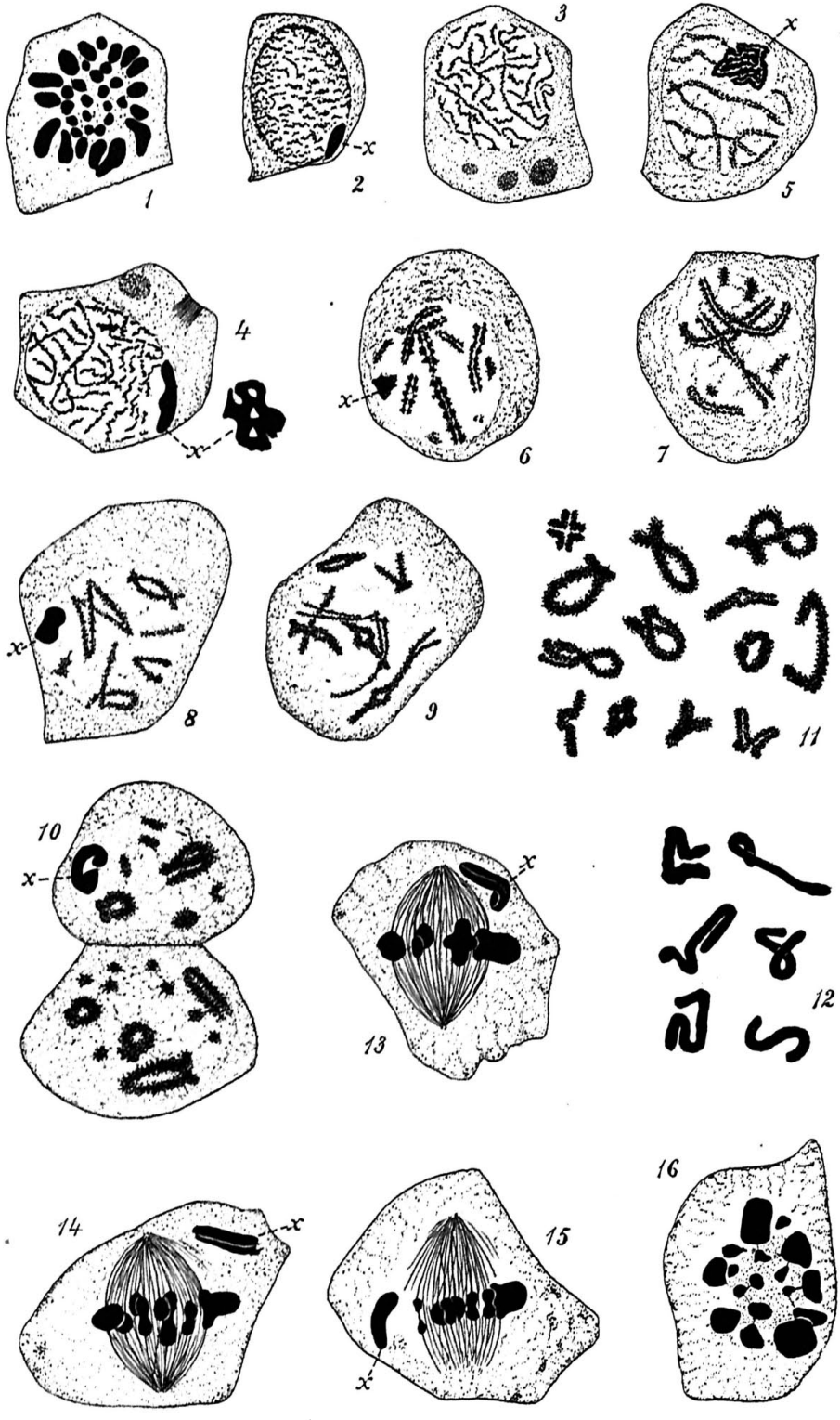
FIG. 12. Different forms assumed by the accessory chromosome in the prophase of the first spermatocytes of *Xiphidium*.

FIG. 13. Metaphase of the first spermatocyte. The accessory chromosome is seen at one pole of the spindle, to which it has moved before the separation of the chromatids of the remaining chromosomes.

FIG. 14. Another cell in about the same stage as that represented in the preceding figure.

FIG. 15. A first spermatocyte metaphase in which the accessory chromosome has not as yet moved to the pole of the spindle. This is uncommon in *Orchesticus*, but frequent in *Anabrus*.

FIG. 16. Pole view of a first spermatocyte metaphase, showing seventeen chromosomes. The variation in size of the elements, so marked in the spermatogonia, is even more pronounced here. This is a cell similar to that of figure 15, in which the accessory chromosome lies in the equatorial plate.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Explanation of Plate VIII.

FIG. 17. Two cells in metaphase—a pole view of one and an oblique view of the other. The accessory chromosome does not show in the former, the cell being such a one as is represented in figures 14 and 15.

FIG. 18. Pole view of another cell, showing but sixteen chromosomes.

FIG. 19. Early anaphase of the first spermatocyte, with the accessory chromosome already at one pole.

FIG. 20. Mid-anaphase, with the giant chromosome still undivided.

FIG. 21. Later anaphase, in which the accessory chromosome is seen at the lower pole. This figure shows, also, the character and extent of the intercellular material.

FIG. 22. Later anaphase. The accessory chromosome at the upper pole. An undivided chromosome lying between the groups of daughter chromosomes.

FIG. 23. About the stage of figure 22, but the lagging chromosome has divided.

FIG. 24. Very late anaphase. Here, again, the lagging chromosome is divided.

FIG. 25. Pole view of first spermatocyte telophase, showing the accessory chromosome at one side of the daughter chromosomes.

FIG. 26. Pole view of a cell in the same stage as that represented in figure 25. Here, however, the accessory chromosome is not present.

FIG. 27. Lateral view of telophase, with the accessory chromosome in the lower daughter-cell.

FIG. 28. Fragment of second spermatocyte, showing the chromosomes in metaphase. The relative sizes of the accessory chromosome and the remaining chromosomes is well shown.

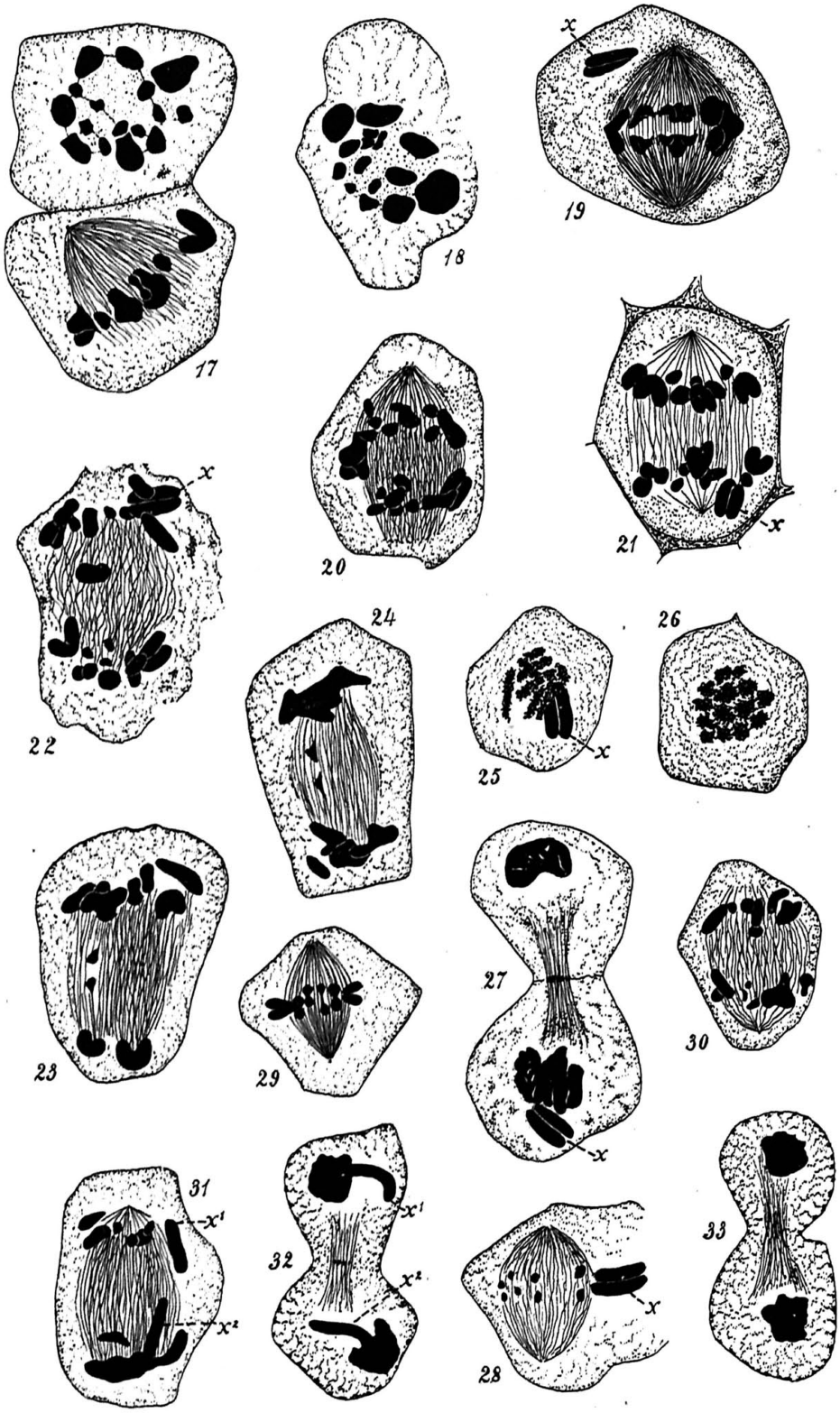
FIG. 29. Metaphase of a second spermatocyte, in which the accessory chromosome is not present.

FIG. 30. Anaphase of second spermatocyte, in which there is no accessory chromosome.

FIG. 31. Anaphase of second spermatocyte, where the accessory chromosome is present—x^1 and x^2.

FIG. 32. Telophase of the same class of second spermatocytes. The accessory chromosome extends out from the mass of chromosomes at each pole—x^1 and x^2.

FIG. 33. Telophase of the class of second spermatocytes from which the accessory chromosome is absent.


17
18
x
19
20
21
x
22
x
24
25
x
26
23
29
27
x
30
31
x¹
x²
32
x¹
x²
28
x
33

Explanation of Plate IX.

FIG. 34. Photomicrograph of early spireme stage of first spermatocyte, showing peripheral position of the accessory chromosome x. At the left, secondary spermatogonia, last generation. $\times 1300$.

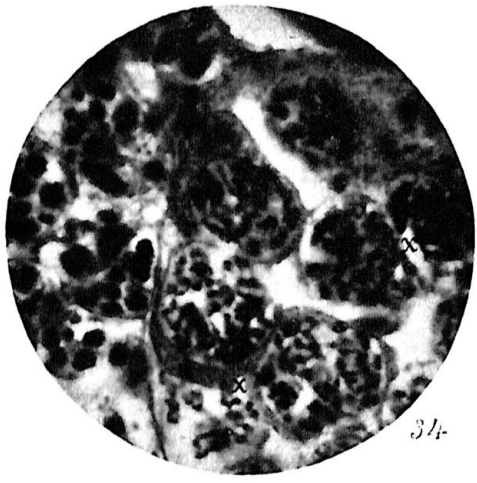
FIG. 35. A late prophase, showing accessory chromosome x, and spindle remains s (*cf.* figs. 3 and 4). $\times 1300$.

FIG. 36. Coarse spireme of first spermatocyte. $\times 1300$.

FIG. 37. Prophase, with chromosomes in the form of long segments. At a, the cell drawn in figure 9. In the cyst at the left are spermatocytes in a later stage, with the chromosomes homogeneous. $\times 1000$.

FIG. 38. Prophase with segments divided longitudinally and across. At a is is one shown *en face*. Accessory chromosome at x. $\times 1000$.

FIG. 39. Metaphase and anaphase of first spermatocyte. The accessory chromosome x at one pole of the spindle. Lagging chromosome at c. $\times 1300$.



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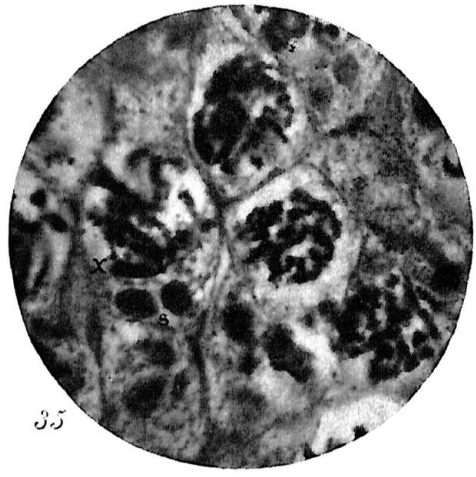

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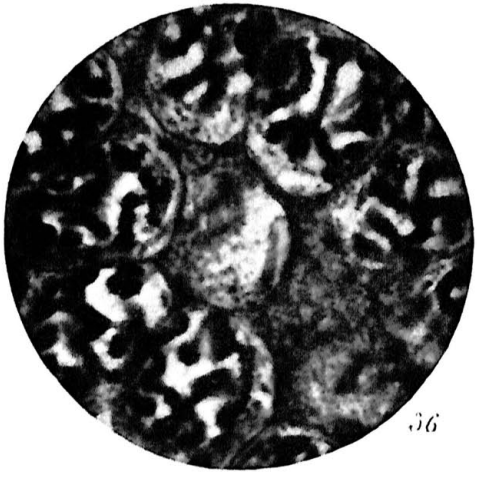

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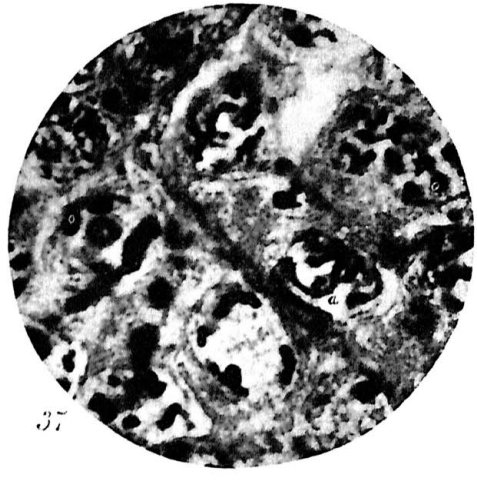

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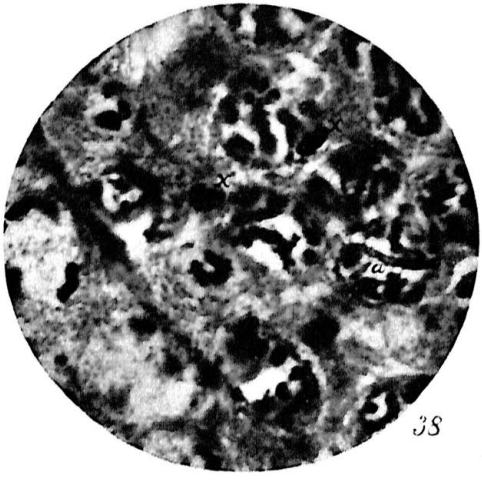

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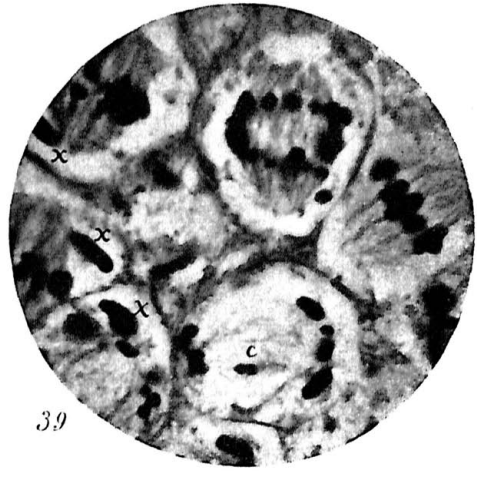

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Explanation of Plate X.

FIG. 40. Anaphase of first spermatocyte. Accessory chromosome x at one pole. The form of chromosome in the anaphase well shown. The lagging chromosome c seen in two cells. $\times 1300$.

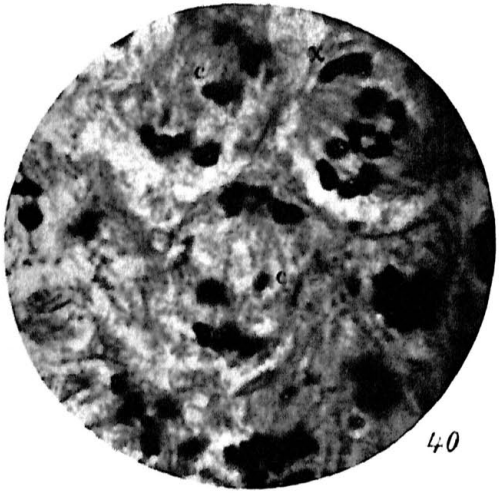
FIG. 41. Anaphase of the first spermatocyte, showing the longitudinally divided condition of the accessory chromosome x in the cell near the center. Compare with the accessory chromosome in the metaphase of second spermatocyte, figure 43. $\times 1300$.

FIG. 42. Second spermatocyte in metaphase. In most of the cells the focus is upon the ends of the chromosomes, but in one a side view is obtainable. Compare with the chromosome of the upper cell in figure 40. No accessory chromosome in most of the cells in focus. $\times 1300$.

FIG. 43. Second spermatocyte metaphase and spermatids. Note the relative sizes of the accessory chromosome and the other chromosomes. In the spermatids the accessory chromosome has taken its place on the periphery of the nucleus in the same way that it does in the prophase of the first spermatocyte. $\times 1300$.

FIG. 44. Anaphase of the second spermatocyte, showing the accessory chromosome x separated. Other cells in metaphase. $\times 1300$.

FIG. 45. Telophase of the second spermatocyte. Two daughter-cells with persisting spindle between, showing the accessory chromosome x in each. Other nuclei in focus show no accessory chromosomes. $\times 1300$.



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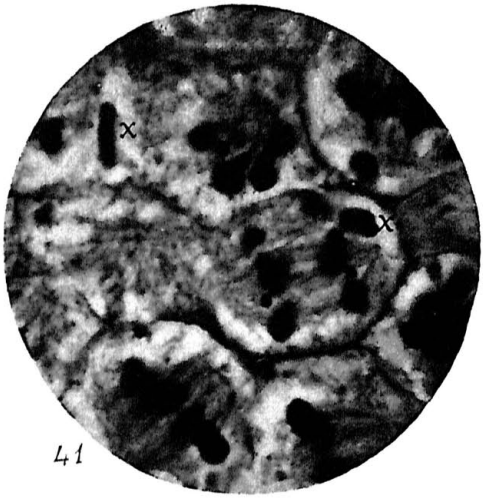

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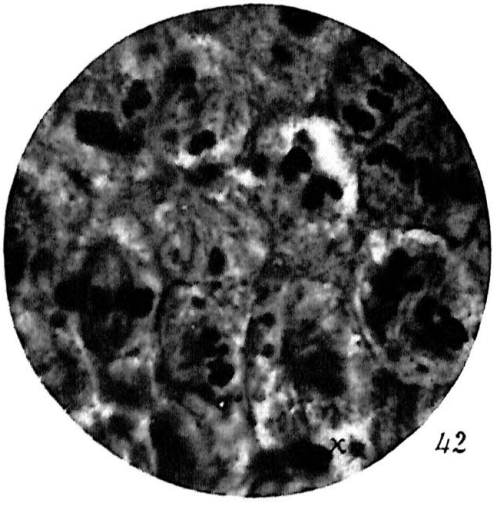

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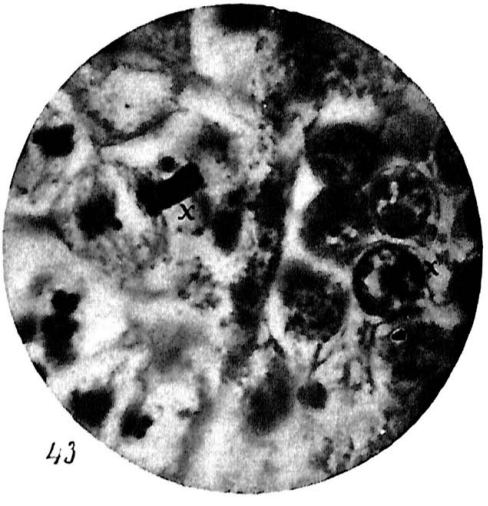

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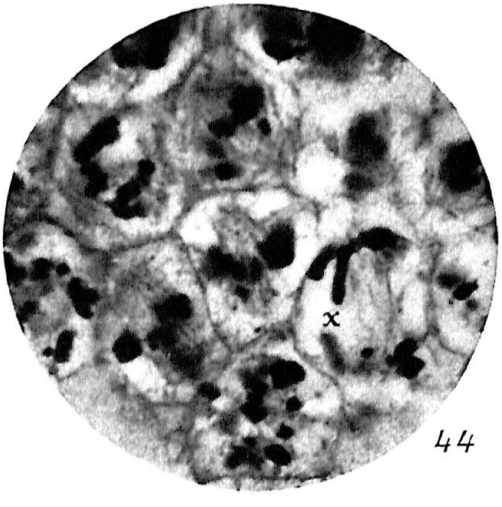

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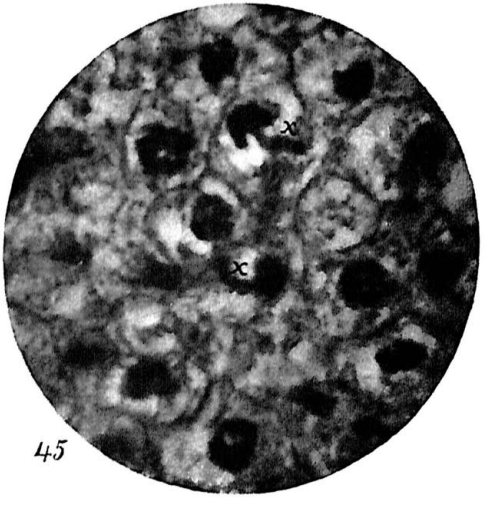

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