MEIOSIS IN THE MALE OF THE BRAZILIAN SCORPION

TITYUS BAHIENSIS

By

S. de Toledo Piza Jor.

Escola Superior de Agricultura «Luiz de Queiroz»
University of S. Paulo, Brazil

(Thirty-six figures)

I — THE NORMAL MEIOSIS

A — **Introduction** — While the history of the chondriosomes and Golgi bodies in the germ cells of scorpions has been worked by some modern investigators (WILSON 1916, 1931; NATH 1925; WILSON and POLLISTER 1937), the same cannot be said with regard to the history of the chromosomes. During the long period of rapid progress in Cytology beginning with the rediscovery of the Mendelian laws of heredity, in 1900, excepting some fragmentary accounts by the present writer (PIZA 1939, 1939a, 1940, 1941, 1942) the spermatogenesis in scorpions only once has received a more or less complete treatment. It was SOKOLOW (1913), in whose paper the early literature is referred to, who published a detailed account on the behavior of the chromosomes in the different phases of meiosis in two distinct species of scorpions, namely in Euscorpius carpathicus and Buthus eupeus.

Probably the great number of chromosomes and their small size is responsible for this situation. The discovery by the present writer of a remarkable species (Tityus bahiensis) having only six large chromosomes brings the scorpions to a prominent place among the animals useful in cytological investigations, specially if we consider that to the genus Tityus belong more than ninety species not at all investigated and that

the scorpions generally can be managed and manipuled very easily in laboratory.

In the present paper, aside from the description of the normal spermatogenesis, some highly interesting special cases due to spontaneous fragmentation and reciprocal traslocations of chromosomes will receive treatment.

B — **Methods** — After cutting off the sting segment of the tail the animal is opened alive and fixed by means of pins on the wax or paraffin botton of a Petri dish and then covered with Ringer solution. The testis is rapidly dissected out and transferred to the fixing solution. As fixatives Navashin, Bouin-urea as recommended by Carothers for insects, Allen-Baur modification of Bouin, and San Felice fluid were used with good results. The two latter, however, gave always finer spindle figures. The material was embedded in paraffin and sectioned at 6 to 10 micra. Sections were stained with iron-haematoxylin and gentian violet. The acetocarmine or aceto-orcein smear method has given very beautiful slides.

Tityus bahiensis can be collected for study at any time but the best results were obtained with the individuals captured in the period from October to February.

C — **Acknowledgments** — It is a pleasure to express my best thanks to Prof. Franz Schrader and Dr. S. Hughes-Schrader, both of Columbia University, N. Y., for valuable suggestions and for their kindness in reading the manuscript. They are, however, in no way responsible for the opinions or interpretations that this paper contains.

D — **The reproductive organs** — The reproductive organs of the male Tityus bahiensis were described in a previous paper (PIZA 1939b). Latter (PIZA 1939c), subjecting living scorpions to mechanical local excitations of the genital opening by means of a laboratory needle or producing in them a generalized excitation by electric current applied to different parts of the body, it was possible to demonstrate experimentally that the two lateral and independent chitinous pieces which have been

described as distinct copulatory organs and considered by PAWLOWSKY (see for reference WERNER 1935) as pieces of support for the paraxial organs, are in reality hemipenes — since these pieces, comming out from a single orifice unite laterally to each other to form a tube, the actual penis, full of sperm fluid.

The testis is composed of a long thin U-shaped tube whose branches, connected by short transverse segments to a median longitudinal tube, open separately into the lateral organs. All parts of the testis, whether longitudinal or transverse, have spermatogenic function. Only the terminal portions of the U-shaped tube, when full of sperm cells, lose almost completely their gametogenic activity, converting themselves into supplementary seminal vesicles. Nevertheless, one or other phase of meiosis may be observed in the wall os these parts.

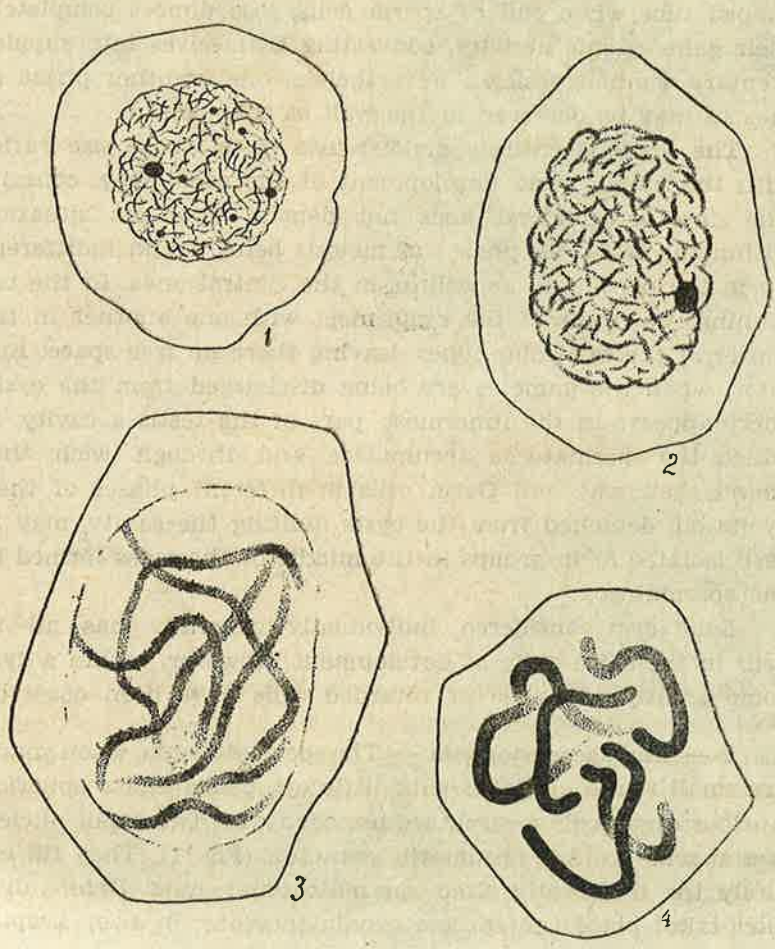
The testis is entirely divided into cysts whose size varies with the number and development of the cells they contain. The state of each cyst does not depend upon its situation within the testis, all phases of meiosis being found indifferently in the peripheral as well as in the central ones. In the beginning, the walls of the cysts meet with one another in the center of the testicular tubes, leaving there no free space. But, later, when the gametes are being discharged from the cysts, there appears in the innermost part of the testis a cavity in which the spermatozoa accumulate and through wich they found their way out. Germ cells in different phases of their evolution, detached from the cysts limiting the cavity, may be seen isolated or in groups in the middle of the mass formed by the spermatozoa.

Each cyst considered individually generally has all its cells in the same state of development. However, within a cyst, some greatly advanced or retarded cells have been observed.

E — The spermatogonia — The spermatogonia, when young, are small round elements with little cytoplasm and a spherical nucleus of reticulate structure provided with two small nucleoli and some minute chromatin granules. (Fig. 1). They fill entirely the differently sized spermatogonial cysts. Before division takes place the spermatogonia increase in size, keeping

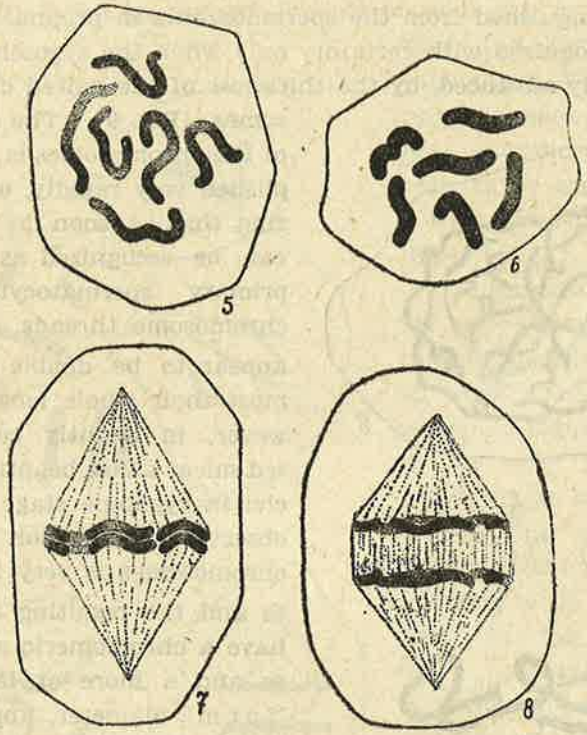
however more or less the same nucleoplasmic relation. (Fig. 2). The nucleoli disappear one after the other and the primitive reticulum becomes more and more distinct.

The spermatogonial prophase presents nothing of particular interest. As in the ordinary mitosis the nuclear thread becomes progressively shorter and correspondingly thicker. (Fig. 3, 4 and 5). The chromosomes are 6 in number. From the late prophase until metaphase the duplicity of the individual threads may be recognized more and more easily. The metaphase chromosomes are clearly divided by a wide longitudinal



(Explanation of figures at the end of this article)

split. (Fig. 7). They are large elements little different in size, always bent or sinuous in the most varied manner. (Fig. 6). In no phases of the spermatogonial division can the centrosomes be clearly observed. However, at metaphase and anaphase, in the most favorable conditions, a very faint corpuscle, just in the limit of visibility, can ben detected at the poles. (Figs. 7 and 8).

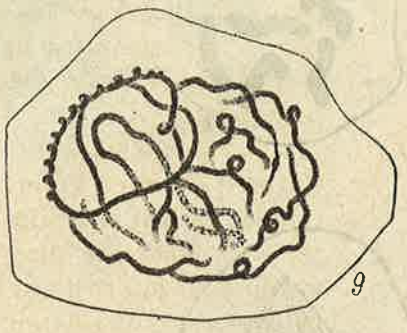


The metaphase chromosomes are distributed in the equatorial plane without any apparent order. There they are oriented in such a manner that the plane of the equator passes through the median split dividing them. The spindle is made up of extremely fine fibrils spread out along the entire poleward surface of the chromosomes.

During anaphase the divided chromosomes form two symmetrical lots which separate parallelly. (Fig. 8). The daughter

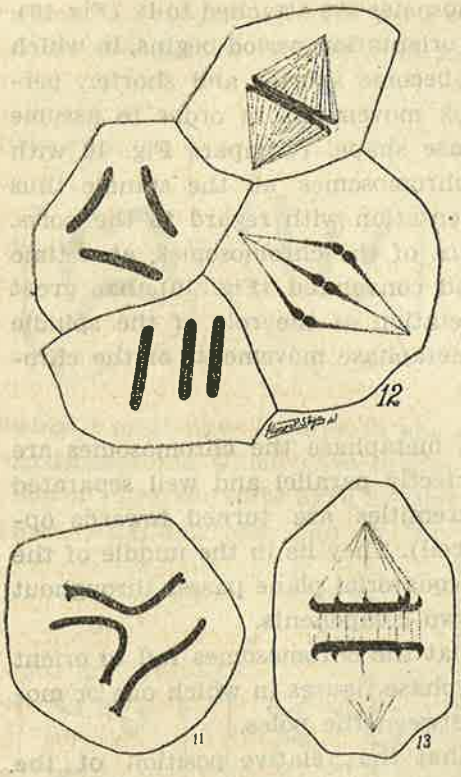
chromosomes maintain the shape of the chromosomes they came from, as if the equatorial plane were a material plane which after being split carried toward the poles the chromosomes lying on its independent halves.

F — First meiotic division — a) The spermatocytes — The primary spermatocytes in leptotene stage cannot be easily distinguished from the spermatogonia in prophase. They can be recognized with certainty only when the zygotene phase is greatly advanced, by the thickness of the paired chromosomes. (Fig. 9). The pairing of the chromosomes is accomplished very rapidly, considering that as soon as a cell can be recognized as a true primary spermatocyte the chromosome threads already appear to be double in almost their whole length. However, in slightly compressed smear slides beautiful nuclei in zygotene stage can be observed. The union of the chromosomes is very intimate and the resulting threads have a chromomeric structure and a more or less uniform diameter, appearing clearly spiralized in some smear preparations. These double threads describe a complicated course within the nucleus. From the end of the zygotene stage till metaphase the pachynemes become progressively shorter and thicker, simultaneously becoming more smooth in outline. (Figs. 10, 11 and 12).


9


10

This continuous process cannot be divided into distinct phases. From the moment the chromosomes become individually distinct, their double nature, marked in the beginning by a lighter and extremely fine median line, appears more and more clearly, until a very pronounced split separates the two members of each pair. This occurs generally when the chromosomes still are much longer than they are in metaphase. From this point on the components of each pair separate farther and farther from each other. When metaphase is reached the paired chromosomes are seen to be separated by a wide space. (Fig. 12, upper cell).



Chiasmata have never been observed at any time in meiosis. Therefore, what holds the chromosomes together in side by side association seems to be nothing else than a generalized mutual attraction. It is likewise to be remarked here, that the chromosomes, once they begin to separate at prometaphase,never reverse this process. On the contrary, the more the bivalents contract, the more their components separate from each other. Since the nuclear membrane has already disapeared, there is likewise no peripheral distribution of chromosomes. Consequently, so far as I can see, there is here nothing comparable to the diakinesis of ordinary meiosis nor to the diplotene stage as it generally occurs with the cromosomes of other animals.

Divergence at both extremities of the bivalents can be observed before metaphase is reached. (Figs. 10 and 11).

b) Prometaphase — It is not possible to determine exactly the moment in which the nuclear membrane breaks down and disappears. The centrosomes, in their turn, cannot be observed before they have reached the poles, probably because they are not accompanied by astral rays and separate very quickly. At the poles they appear as small, easily visible granules. At this time the spindle is already completely formed and the still very long chromosomes are attached to it. (Fig. 10). Now, a long and remarkable orientation period begins, in which the chromosomes, as they become shorter and shorter, perform important and complex movements in order to assume their characteristic metaphase shape. (Compare Fig. 10 with 12). The insertion of the chromosomes at the spindle thus greatly anticipates their orientation with regard to the poles. So premature an attachment of the chromosomes, at a time when they are still long and convoluted (Fig. 10), has great significance for the interpretation of the role of the spindle fibres in the pro- and postmetaphase movements of the chromosomes.

c) Metaphase — At metaphase the chromosomes are typically formed by two perfectly parallel and well separated rods, whose homologous extremities are turned towards opposite poles. (Fig. 12, upper cell). They lie in the middle of the cell in such a way, that the equatorial plane passes throughout the space separating their two components.

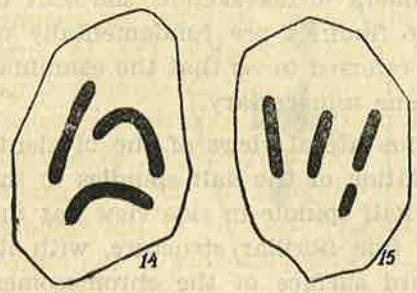
Sometimes it happens that the chromosomes fail to orient normally, giving rise to metaphase figures in which one or more bivalents are stretched between the poles.

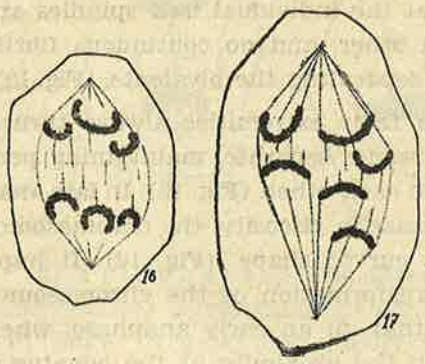
The polar views show that the relative position of the straight or curved bivalents may be various. They can be disposed parallelly or not, as well as in triangle. (Fig. 12). Two of the bivalents are approximately of the same length, the third being a little longer. Viewed from the poles the components of each pair present a very fine and light median line,

difficult to see, thus revealing that each is already split. (Fig. 12, cells at left). The end views of the bivalents confirm that they are really quadripartite. (Fig. 12, lower right).

Metaphase chromosomes with a very marked constriction; as well as chromosomes that have broken into two pieces have occasionally been found. (Figs. 14 and 15). Different kinds of bridges have also been observed, though rarely. (Figs. 18, 19 and 20).

d) Anomalies of pairing — Different kinds of anomalies in pairing have been observed in single spermatocytes as well as in many or in all spermatocytes belonging to the same cyst. When the anomaly is shown by a single spermatocyte, it probably was caused by something occurring in the last division of a spermatogonium preceding meiosis. When, on the contrary, many cells in a cyst show the same anomaly, the phenomenon determining it must have occured in one of the earlier division of the spermatogonia which gave origin to the whole cyst. Anomalies of different sorts have at times been observed in the same cyst,l affecting one, two or the three chromosome pairs. All these irregularities are interpreted (PIZA 1942a) as being due to breakage of the chromosomes, followed or not followed by readjustment of the fragments. Among these, the following are the most frequent: a) chromosomes paired at the extremities and more or less widely open in the middle; b) chromosomes pai-





red in the middle of the body and more or less widely separated at both ends; c) chromosomes paired in one half of the body and unpaired in the other half; d) chromosomes entirely devoid of pairing ability, lying apart in the cell.

It is to be emphasized here that the paired segments whatever may be their length, orient normally with regard to the poles, while the unpaired parts of the chromosomes seem to be immune to any orienting influence, assuming freely any position in the cell.

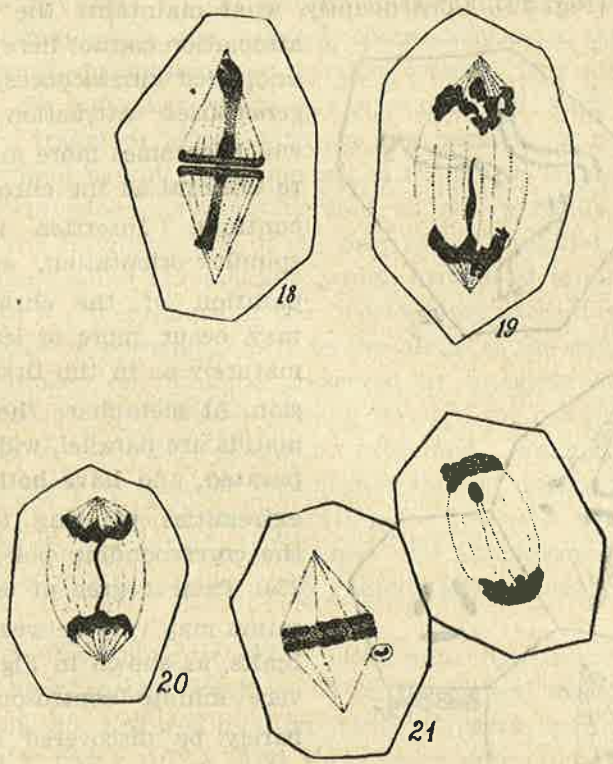
Dr. S. HUGHES-SCHRADER has written me that she observed identical abnormal behavior of the chromosomes in material I have brougth to her and kindly offered to put her slides at my disposal. The camera lucida sketchs she sent to me showed however that her findings are fundamentally of the same nature as those just referred to, so that the examination of her preparations became unnecessary.

e) The spindle — The lateral views of the bivalents permit the analysis of the relation of the half spindles to the chromosomes. The individual half spindle in side view has the form of a triangular film of fine fibrillar structure, with its base along the entire poleward surface of the chromosomes. This film is limited by two terminal fibrils stretched between the pole and the extremities of the chromosomes. Due to the quadripartite condition of the chromosomes the fibrils attached at their ends show a remarkable thickness when the chromosomes are viewed from the extremities. (Fig. 12, lower right cell). Such a view reveals that the individual half spindles are entirely independent of each other, and no continuous fibrils can be detected in the space separating the bivalents. (Fig. 12).

f) Anaphase — With their extremities always turned toward the poles, the chromosomes separate, maintaining perfect parallelism with regard to each other. (Fig. 13). In late anaphase, probably due to a decrease in viscosity, the chromosomes assume a very characteristic curved shape. (Fig. 16). It happens sometimes that this transformation of the chromosomes begins very prematurely, so that, in an early anaphase, when the chromosomes are still in the proximity of the equatorial

plane, they show already a more or less irregular surface and a very pronounced arch shaped form. The asynchronous movements of the chromosomes, repeatedly observed, are in support of the complete independence of the individual half spindles. (Fig. 17).

The chromosomes at the end of anaphase seem to be clearly divided into their two chromatids. In lateral view, the chromosomes already far advanced in their transformation, very often present themselves as irregular more or less elongated chromatin masses characteristically bent toward the poles.

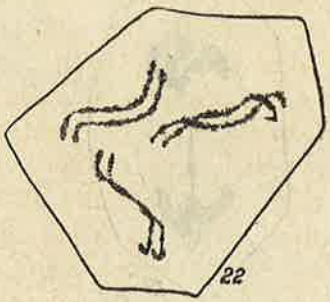


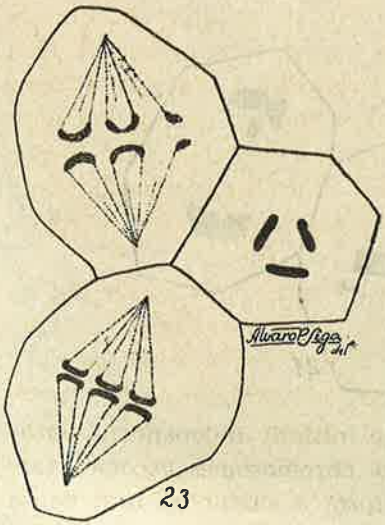
g) Telophase — The three initially independent chromatin masses corresponding to the chromosomes become more and more irregular and finally form a single, at first dense and then loose mass, without any distinct individual bounde-

ries. The interzonal fibres developed during anaphase give rise to a narrow stem body.

h) Interphase — During the short interphase the chromosomes form threads.

G — **Second meiotic division** — The prometaphase of the second division of the spermatocytes is a phase of great theoretical interest. The chromosomes are composed of two chromatids perfectly separated throughout their whole length and more or less loosely twisted around each other. Sometimes they appear as two independent strands, side by side, but well separated. (Fig. 22). Consequently, what maintains the paired association cannot here be the undivided kinetochores, but a generalized attraction force which becomes more and more uniform as the chromatids contract. Insertion in the spindle, orientation, and separation of the chromatids may occur more or less prematurely as in the first division. At metaphase the chromatids are parallel, widely separated, and have both their extremities pointing towards the corresponding pole. (Fig. 23). Their degree of condensation may vary between wide limits, as shown in Fig. 23. A very minute centrosome can barely be discovered at the poles. During separation at anaphase the chromatids assume a very pronounced arch shaped form.





II — SOME SPECIAL CASES

In an animal like Tityus, with chromosomes provided with a kinetochore at each end, spontaneous aberrations due mainly to bridges formation followed by breakage of the chromosomes might well be expected. Fortunately, so singular a morphological peculiarity in chromosomal structure is largely compensated by an accurate orientation mechanism, which prevents almost completely the formation of bridges. These, however, have been encoutered in a rather low percentage of the cases examined.

Besides some typical bridges formed during anaphase of the spermatogonia and primary spermatocytes, a very singular case of chromosomal connection between two adnate cells just in the metaphase stage was once observed. Each cell possessed a fragment paired with the end segment of a monovalent element passing from one cell to the other. This extraordinary occurrence may be interpreted, at least partially, as being due to the fragmentation of one only of the daughter halves of a spermatogonial chromosome, which instead of orienting normally in the metaphase of the division just preceding meiosis, would stretch perpendicularly to the plane of the equator. How the unbroken chromosome preserved its integrity during the resting stage of the nuclei, passing through the cytoplasm from one cell to the other, cannot be explained. We may at least assume that the nuclear membranes formed during telophase as well as the membrane separating the two cells were incomplete at the point where they met the chromosomal thread. This observation was made in acetocarmine smear preparation.

In a previous paper (PIZA 1940) some very interesting cases of spontaneous breakage of chromosomes, some followed and some not followed by interchange of parts, were described. In a later paper (PIZA 1942) these cases were cited in support of the view that homologous chromosomes attract one another as wholes and not point by point as postulated by the gene theory. In this report two new, highly significant cases, in which a quite different mode of cross association of recipro-

cally interchanged chromosomes was discovered, will receive treatment. These cases were already presented as proof for the dorso-ventrality of the chromosomes (PIZA 1942) but have not yet been fully described.

A — FIRST CASE: ONE CROSS AND ONE ROD, BRIEFLY THE XI CASE

Since in Tityus anaphase can begin before the chromosomes have reached their final condensation, it is difficult to determine the ultimate size of the chromosomes. However, in case XI, in situation considered as the most favorable for size estimation, two spermatogonial chromosomes proved to be smaller than the rest. In form and behavior these chromosomes are entirely comparable to those of normal individuals. In prophase of the first spermatocytes division also no differences from the normal can be pointed out. In the prometaphase, however, a long bivalent like those of the corresponding normal phase appears at the side of an equally long cross association of the other two bivalents. (Figs. 24 and 25). At that time, as in normal cases, the chromosomes are already inserted in the spindle. Orientation is here too of long duration. But, when it ends at the time the chromosomes have reached the maximum condensation, the elements of the cross — a short cross with three arms of about the same length and a fourth much smaller one, show something highly interesting in the mutual relations of their components. The associated chromosomes, instead of keeping the same parallelism at the different arms of the cross, as was so clearly observed in another case (PIZA 1940), behave in the present one in a somewhat different way, so that the cross, in polar view, shows two arms with parallel and two with superposed elements. (Fig. 36). This particular behavior has been interpreted (PIZA 1942, 1943) as being due to a reversal of dorso-ventrality in two components of the cross. The pairing which is the consequence of such reversal is diagrammed in figure 26 A, in which the white chromosome becomes superimposed on the arms of two other chromosomal compo-

nents in order to bring its pairing surface into apposition with the reversed segments. The type of configuration shown in figure 26 B is the espected when no reversal of dorso-ventrality takes place.

It is evident that the cross, as we know it at metaphase, represents the end result of a process of pairing begun much earlier at prophase. I ám firmly convinced that the actual mode of union of the chromosomes is that shown in figure 26 A. The superposition in two arms of the cross is merely the consequence of a torsion determined by the contraction of the element passing across them and to which they are united. The premature dissolution of the union of the chromosomes at the shortest arm of the cross, repeatedly observed, seems to be accountable for by the effect of the torsion just referred to.

Sometimes three or even all four arms of the cross, in polar view, seem to be formed by more or less perfectly superposed elements, which may be attributed to the transmission of the torsion effect to the whole block of chromosomes. Generally, however, two branches of the cross are correctly oriented at metaphase. Notwithstanding that, separation of the paired elements goes on normally, giving rise to secondary spermatocytes provided with three chromosomes. Bridges have but unfrequently been observed, showing that only rarely some components of the cross cannot separate regularly.

Which branches of the cross show parallel association or cross association depends on chance. Thus, the shortest arm of the cross, which can be distinguished much more easily than the other three, appears very often in parallel as well as in cross association.

B — SECOND CASE: ONE CROSS AND THREE RODS, SHORTLY THE XIII CASE

The present case is undoubtedly much more interesting than the preceding one, because of the larger number of chromosomes coming into play. Reference to this case has been made previously (PIZA 1942, 1943), and will now be described.

The spermatogonia before metaphase cannot be distin-

guished by any particular feature from those of normal individuals. At metaphase, however, instead of the six long, bent or sinuous chromosomes of the normal set, there are ten shorter chromosomes which in lateral view are split like the normal ones. A little before the chromosomes have assumed their final form, it is easier to recognize that they are different in size. (Fig. 27).

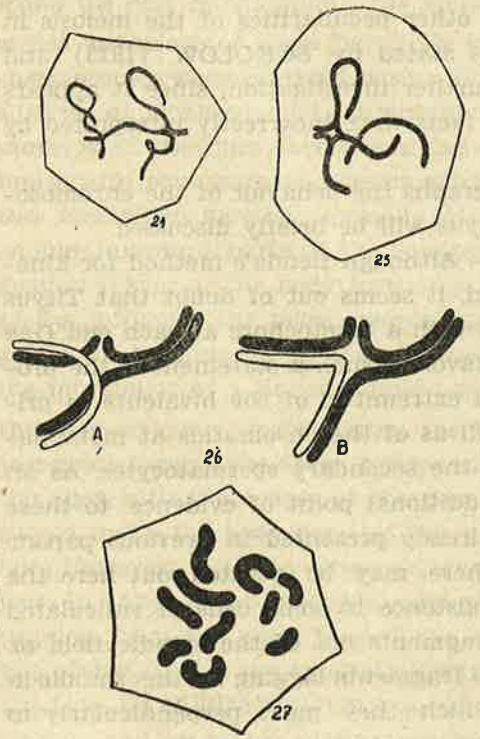
Prophase of the first spermatocyte division exactly parallels the normal. At prometaphase, however, three long bivalent threads and an equally long cross association much like that of the first case, can be distinguished in the primary spermatocytes. (Fig. 28). As in the former case or in the normal ones, all chromosomes at this time are already attached to the spindle. At metaphase these chromosomes appear forming a cross of exactly the same nature of that in the preceding case as well as three rods (two equal in size and the third much smaller than the other two), whose arrangement in the cell is random. (Fig. 29). The cross as well as the rod-shaped chromosomes usually orients as in the preceding case. However, abnormal orientation has sometimes been observed. Thus, crosses presenting two arms extended on the equatorial plane and the other two directed to the opposite poles, as well as one, two or all three rods disposed parallel to the axis of the spindle, were repeatedly found.

Chromosomes provided with deep constrictions exposing them to subsequent fragmentation, fragments with a single attachment lagging on the spindle or entirely destitute of kinetochore and thereupon vesiculated (Fig. 21), different kinds of abnormal pairing, and several sorts of aberrant behavior of the chromosomes have also been encountered.

Usually the chromosomes separate without any hinderance, giving rise to secondary spermatocytes with five small chromosomes of different sizes. (Fig. 32).

Cysts of tetraploid primary spermatocytes have been observed. The chromosomes of these cells behave in the expected manner, entering into bivalent or polyvalent associations. The formation of one cross or two independent ones or of a

very confused association of the eight elements belonging to both crosses, together with bivalent, trivalent or tetravalent groups of chromosomes are common occurrence. One of the less complicated metaphase plates is that represented in the figure 31. These tetraploid spermatocytes have their origin probably in spermatogonia whose chromosomal division was not followed by cell division. Indeed, spermatogonia with double chromosome number have been found in this individual as well as in normal ones. Occasionally primary spermatocytes were found, not only in the present case, whose chromosomes, after being separated in the beginning of anaphase, entered into telophase transformation without leaving the equaorial region of the cell, thus giving origin to tetraploid interphase cells. These cells, when they enter again into kinetic activity, they produce diploid secondary spermatocytes.



The secondary spermatocytes divide normally. Some cells provided with ten smaller chromosomes have been found, though rarely. (Fig. 35). These cells seem to originate from an early lapsing of the mutual attraction force which holds the chromatids together, so that each chromatid appears at metaphase as if it were an independent chromosome. In this circumstance it is highly probable that at anaphase an irregular distribution of the chromatids occurs. Abnormal orientation of the chromosomes was often seen. (Figs. 33 and 34).

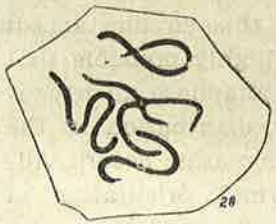
One cyst was found in the present case, whose primary spermatocytes showed at metaphase two crosses and only one rod, which means that, as in he case of the first cross, a new interchange of parts between chromosomes belonging to two of the three independent pairs of this individual gave rise to another cross. (Fig. 30). This, however, appears to be a little different than the first one in having one of its branches not much smaller than the other three.

III — DISCUSSION

The mode of origin of the tetradiform bodies which give rise to the spermatogonial chromosomes, the prophase of the first meiotic division during which the universal zigotene phase fails to occur, the pachytene stage being considered the result of the progressive contraction of a single unpaired thread, the transverse division of the chromosomes in the secondary spermatocytes, and many other peculiarities of the meiosis in Euscorpius and Buthus as stated by SOKOLOW (1913) and CARNOY (1885) require further investigation, since it appears that several fundamental facts were incorrectly interpreted by these authors.

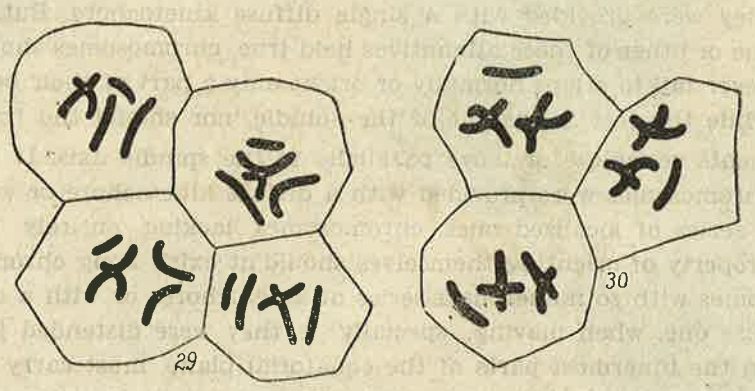
In the following paragraphs the behavior of the chromosomes during meiosis in Tityus will be briefly discussed.

a) **The kinetochores** — Although Benda's method for kinetochore has not been tried, it seems out of doubt that Tityus chromosomes are provided with a kinetochore at each end. One of the best arguments in favor of such a statement is the pronounced repulsion at both extremities of the bivalents in primary spermatocytes as well as of the chromatids at metaphase and early anaphase of the secondary spermatocytes. As an additional point of evidence to those already presented in previous papers, there may be pointed out here the existence in some cells of vesiculated fragments out of the spindle field or of fragments lagging in the spindle in which they move perpendicularly to the equatorial plane. The latter are



considered as having a single kinetochore localized at the end turned toward the pole, while the former must be entirely devoid of kinetochores. The suggestion made by HUGHES-SCHRADER and RIS (1941) that Tityus chromosomes may have a diffuse kinetochore or at least a series of individualized kinetochores distributed along their entire length is contredicted not only by the behavior of the fragments just referred to, but also by the whole history of the normal chromosomes. If we assume that each spindle fibre observed in the poleward side of the chromosomes is the material expression of the activity of a localized kinetochore, then the kinetochores must be extremely numerous and the chromosomes would behave as if they were provided with a single diffuse kinetochore. But, if one or other of these altenatives held true, chromosomes should never fail to orient normally or orient only a part of their body while the rest hangs out of the spindle, nor should the fragments vesiculate or move parallelly to the spindle axis. If the chromosomes were provided with a diffuse kinetochore or with a series of localized ones, chromosomes lacking entirely the property of orienting themselves should'nt exist. Long chromosomes with so numerous a series of kinetochores or with a diffuse one, when moving, specially if they were distended just on the innermost parts of the equatorial plane, must carry the median region of the body forwardly and not backwardly, since the influence the poles exercise upon them must be stronger in the median region than in the extremities. Besides that, the movement of a linear supple body like a chromosome within a fluid cone, starting from the base on which it is lying, and going toward the apex (being easier in the line connecting the apex with the center of the base than in any other line), should make the body bend at the point correspondig to that line. However, this was never observed with Tityus chromosomes. In spite of the probable existence of a peripheral flow running from the poles toward the equator of the cells — as was observed long ago by ZIMMERMANN (1890) in the skin of the frog, by NUSBAUM (1893) in the endoderm of the frog's embryo and by many other investigators during cleavage of

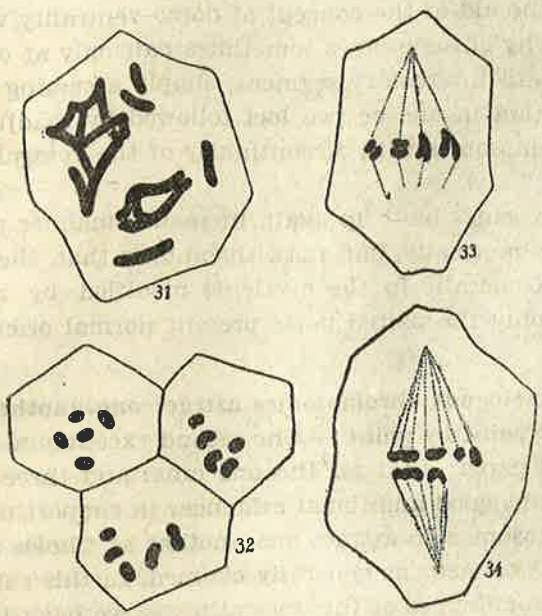
the egg of several animals (see for references CHAMBERS 1924) — a flow wose influence would oppose the movement of the chromosome extremities in the opposite direction, the chromosomes of Tityus move always with both ends turned toward the poles. It thus becomes evident that if the chromosomes possessed a series of kinetochores, those localized at the ends must be more powerful than the others or, if the kinetochore was of the diffuse type, it must be assumed that its kinetic activity is greater at the exremities. In the one as well as in the other case the chromosomes would behave as if they were provided with a single localized kinetochore at each end.



b) The spindle — The evidences in hand speaking conclusively against the existence of more than two terminal kinetochores, the assumption was made, in order to account for the presence of a half spindle component along the entire body of the chromosomes, that the kinetochores, in spite of being localized, may have a diffuse activity. (PIZA 1942, 1943). With such an assumption in mind and admitting that a chromosome may lose the extremity it sometimes carry attached to it like a small satellite, we are prepared for understanding on the one side how a minute fragment could produce spindle fibres and move as the unbroken chromosomes do, and, on the other side, why chromosomes do exist which can produce spindle fibres and orient normally only at one end.

Vesiculated fragments have been considered as being derived from the median region of the chromosomes and thus devoid of kinetochores, while the fragments moving parallelly to the spindle axis were interpreted as end pieces carrying a single kinetochore.

The very premature insertion of the chromosomes at the spindle first referred to in my short account on the meiosis in Tityus (PIZA 1939) (and recently observed by HUGHES-SCHRADER (1942) in Nautococcus), affords a strong argument in support of the idea fully discussed in another paper (PIZA 1943) of the complete uselessness of the spindle fibres in moving or orienting the chromosomes.



c) **Dorso-ventrality of the chromosomes** — If the chromosomes are truly differentiated in one ventral (kinetochore) side and one dorsal side, as was asserted by PIZA (1940), and if they pair with each other only by the ventral side, the broken chromosomes which interchange parts may pair in two diffe-

rent ways to form cross association. Since the broken pieces may join one another with or without a previous rotation, so that the ventral part of one may alternate with, or continue the ventral part of the other, crosses with crossed branches are to be expected as well as crosses with the four branches perfectly parallel. (Fig. 26).

Parallel crosses denoting that the ventral parts of the rejoined fragments are turned to the same side, were encountered in a testis previously described. (PIZA 1940). Crosses with crossed branches as represented in Fig. 26, A, showing, on the contrary, that he fragments have been united with the ventral parts turned to opposite sides, are typical of the two cases dealt with in he present paper.

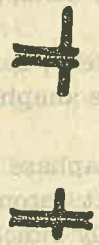
With the aid of the concept of dorso-ventrality we can understand why chromosomes sometimes pair only at one or both ends or at an intercalary segment, simply assuming that after fragmentation in one or two loci followed by readjustment of parts, chromosomes with discontinuity of the ventral side have resulted.

Chromosomes built up again in such a manner may orient and move abnormally, but, notwithstanding that, they separate regularly. Generally in the bivalents modified by rotation of segments, only the paired parts present normal orientation.

d) **Homologous chromosomes attract one another as wholes and not point by point** — The second exceptional case treated in this paper, that is, the one cross and three rods case (XIII), brings good additional evidences in support of the view that chromosomes do attract one another as wholes and by no means locus to locus as generally claimed. In this case, instead of the 6 chromosomes of the normal testis, we have 10 chromosomes, two of which belonging to different pairs exchanged parts giving rise to a cross of four elements, while the remaining six form the three rods of the primary spermatocytes. To judge by the behavior of the chromosomes at metaphase and early anaphase, the components of the cross as well as the rod-shaped bivalents are provided with a kinetochore at each

end. It is difficult to decide whether at least some of the chromosomes present are exactly the same as those of the normal testis. For purposes of discussion we can assume that the elements associated in the cross and in one of the rod-shaped bivalents are the six normal chromosomes of Tityus. Then, the other four elements forming the two remaining pairs can be nothing but whole or partial repeats of the normal chromosomes. Therefore, if the attraction point by point prevail, why do the repeated loci never enter into association with the corresponding loci of the normal chromosomes? In answering this question let me refer to the case of a testis already discussed (PIZA 1942), exhibiting spermatogonia with 18 chromosomes and primary spermatocytes with 9 regular pairs. In spite of the indubitable presence of many repeated loci, an association higher than bivalent was never found in this testis. To explain this fact we have to assume that the 18 chromosomes in question, whether normal or modified by fragmentations (followed or not by readjustment or loss of parts), constituted 9 different pairs notwithstanding the presence of the same loci in many of them. Thereupon, when two entirely homologous chromosomes are in the presence of each other they attract one another as wholes, being not at all influenced by the existence of homologous parts in other chromosomes. Thus, any modification affecting in the same way two homologous chromosomes in a lot, originates a new pair of elements which show no longer affinities to the chromosomes to which they were homologous before the modification. Due to this, two minute fragments may behave as a distinct pair, never going to join with the corresponding parts of other chromosomes, provided that they are entirely homologous and have a kinetochore. However, centric fragments may pair with the corresponding parts of an entire chromosome to which they are homologous, provided that the


35


36

fragments in question have no independent homologous partners. When more than two entirely homologous chromosomes are present in the same cell, as was observed in the tetraploid cyst of the XIII case, they behave as in the known cases of polysomy or polyploidy.

SUMMARY

1) Tityus chromosomes are provided with a kinetochore at each end. Due to the activity of the kinetochores a half spindle component is formed along the entire body of the chromosomes. Notwithstanding that, the kinetic activity remains stronger at the extremities of the chromosomes, determining a pronounced repulsion of these extremities at metaphase of the spermacytes.

2) Chromosomes separate parallelly, having both ends turned to the correspondig pole. At late anaphase they assume a curved shape.

3) Paired chromosomes at metaphase are well separated, being each composed of two distinct chromatids. A very faint light line along their body is the only indication that the components of a pair are double elements.

4) At prophase of the first meiotic division typical diplotene and diakinesis stages cannot be recognized.

5) Chromosomes are never connected by chiasmata.

6) Chromatids at prometaphase of the secondary spermatocytes are perfectly separated throughout their entire length, so that what maintains them side by side is nothing else than a generalized mutual attraction.

7) The spindle fibres develop very precociously, playing no part in the orientation of the chromosomes, nor in their movements.

8) The chromosomes are dorso-ventrally differentiated, uniting with each other in pairing by their ventral (kinetochore) side.

9) Homologous chromosomes attract one another as wholes and not point by point as generally claimed.

Observation:

My first paper on meiosis in Tityus (PIZA 1939) has appeared with such a quantity of faults, that its re-impression was considered necessary. However, in consequence of the war, the journal which published it has been interrupted so that a republication of the corrected text became unpossible.

The use at some places of "chromtids" for "chromosomes" and of "chiasma" to designate also the cross configuration resulting from reciprocal translocation, were the principal mistakes.

LITERATURE CITED

CARNOY, J. B. 1885 — La cytodiérèse chez les arthropodes. **La Cellule,** 1: 191-440.

CHAMBERS, R. 1924 — The physical structure of protoplasm as determined by micro-dissection. **Cowdry's General Cytology,** 237-309.

HUGHES-SCHRADER, S. and H. RIS 1941 — The difuse spindle attachment of coccids, verified by the mitotic behavior of individual chromosome fragments. **J. Exp. Zool.** 87: 429-456.

HUGHES-SCHRADER, S. 1942 — The chromosomes of Nautococcus schraderae Vays. and the meiotic division figure of male llaveiine coccids. **J. Morph. 70**: 261-299.

NATH, V. 1925 — Mitochondria and sperm-tail formation with particular reference to moth, scorpions and centipedes. **Quart. Jour. Mic. Sc. 69**: 643-659.

NUSBAUM, J. 1893 — Über die Verteilung der Pigmentkörnchen bei Karyokinese. **Anat. Anz. 8**: 666-668.

PIZA, S. de TOLEDO, Jr. 1939 — Comportamento dos cromossômios na primeira divisão do espermatócito do Tityus bahiensis. **Scientia Genetica. 1**: 255-261.

PIZA, S. de TOLEDO, Jr. 1939a — Considerações em tôrno da meiose do Tityus bahiensis (Scorpiones-Buthidae) e uma nova teoria sôbre a movimentação dos cromossômios. **Jor. de Agron. 2**: 342-370.

PIZA, S. de TOLEDO, Jr. 1939b — Observações sôbre o apare-

lho reprodutor e a reprodução do Tityus bahiensis (Scorpiones: Buthidae-Isometrinae). **Jorn. de Agron.** 2: 49-56.

PIZA, S. de TOLEDO, Jr. 1939c — Estudos anatômicos em escorpiões brasileiros. II — Sôbre os órgãos copuladores dos escorpiões. **Jorn. de Agron.** 2: 285-288.

PIZA, S. de TOLEDO, Jr. 1940 — Poliploidia natural em Tityus bahiensis (Scorpiones) associada a aberrações cromossômicas espontâneas. **Rev. de Biol. e Hig.** 10: 143-155.

PIZA, S. de TOLEDO, Jr. 1941 — Chromosomes with two spindle attachments in the brazilian scorpion (Tityus bahiensis Perty). **Jour. of Hered.** 32: 423-426.

PIZA, S. de TOLEDO, Jr. 1942 — Dorso-ventralidade dos cromossômios. **Rev. de Agric.** 17: 154-168.

PIZA, S. de TOLEDO, Jr. 1943 — The uselessness of the spindle fibers for moving the chromosomes. **Amer. Nat.** (in press).

SOKOLOW, I. 1913 — Untersuchungen über die Spermatogenese bei den Arachniden. I — Über die Spermatogenese der Skorpione. **Arch. f. Zellf.** 9: 399-432.

WERNER, F. 1935 — Scorpiones, Pedipalpi. **Bronn's Klassen u. Ordnung d. Tierreiches.** 5. Bd. IV Abt. 8. Buch. 490 pgs.

WILSON, E. B. 1916 — The distribution of the chondriosomes to the spermatozoa in scorpions. **Pros. Nat. Acad. Sci.** 2: 321-324.

WILSON, E. B. 1931 — The distribution of the sperm-forming materials in scorpions. **J. Morph.** 52: 429-483.

WILSON, E. B. and A. W. POLLISTER 1937 — Observation on sperm formation in the centrurid scorpions with special reference to the Golgi material. **J. Morph.** 60: 407-443.

ZIMMERMANN, K. W. 1890 — Über die Theilung der Pigmentzellen speciell der verästelten intraepithelialen. **Arch. f. mikr. Anat.** 36: 404-410.

EXPLANATION OF FIGURES

Fig. 1 — Young spermatogonium. (x 8000).

Fig. 2 — Early prophase of the spermatogonium. (x 5800).

Fig. 3 — Late prophase of the spermatogonium. (x 4300).

Fig. 4 — Prometaphase of the spermatogonium. (x 3500).
Fig. 5 — Advanced prometaphase of the spermatogonium. (x 3200).
Fig. 6 — Metaphase of the spermatogonium in polar view. (x 2700).
Fig. 7 — Metaphase of the spermatogonium in lateral view. (x 3300).
Fig. 8 — Anaphase of the spermatogonium. (x 3300).
Fig. 9 — Prophase of the primary spermatocyte. (x 3600).
Fig. 10 — Prometaphase of the primary spermatocytes in side view, showing long chromosomes already attached at the spindle. (x 4200).
Fig. 11 — Late prometaphase of the primary spermatocyte in polar view. (x 2800).
Fig. 12 — Metaphase of the primary spermatocytes in polar, in lateral and in end view. (x 2600).
Fig. 13 — Anaphase of the primary spermatocyte. (x 3200).
Fig. 14 — Late prometaphase of a primary spermatocyte showing a chromosome with a submedian constriction. (x 3000).
Fig. 15 — Metaphase of the primary spermatocyte showing a fragmented chromosome. (x 3000).
Fig. 16 — Late anaphase of the primary spermatocyte in lateral view. (x 2300).
Fig. 17 — Late anaphase of the primary spermatocyte showing asynchronous movement of the chromosomes. (x 3000).
Fig. 18 — Metaphase of the primary spermatocyte in lateral view showing one of the bivalents stretched between the poles with two weaker subterminal regions. This bivalent will most probably give rise to two independent pairs of end fragments and to one pair of median fragment devoid of kinetochore. (x 2800).
Fig. 19 — Primary spermatocyte showing a broken bridge. (x 2800).
Fig. 20 — Primary spermatocyte showing an unbroken bridge. (x 2700).

Fig. 21 — Metaphase of the primary spermatocyte in side view with a median fragment devoid of kinetochore and vesiculated and telophase with an end fragment provided with a single kinetochore and moving parallelly to the spindle axis. (x 2700).

Fig. 22 — Prometaphase of the secondary spermatocyte showing twisted and parallel chromatids. (x 4000).

Fig. 23 — Secondary spermatocyte in early anaphase and in metaphase (polar and lateral views). (x 3300).

Fig. 24 — Early prometaphase of the primary spermatocyte of the XI case. (x 2300).

Fig. 25 — Late prometaphase of the primary spermatocyte of the XI case.

Fig. 26 — Diagramm to show the cross association of the chromosomes with crossed (A) and parallel (B) arms. (x 4000).

Fig. 27 — Late prometaphase of the spermatogonium of the XIII case. (x 2600).

Fig. 28 — Early prometaphase of the primary spermatocyte of the XIII case. (x 3000).

Fig. 29 — Four primary spermatocytes of the XIII case in metaphase. (x 2500).

Fig. 30 — Three primary spermatocytes with two crosses and one rod (XXI) at metaphase.

Fig. 31 — Metaphase of a tetraploid primary spermatocyte of the XIII case. (x 2800).

Fig. 32 — Secondary spermatocytes of the XIII case at metaphase. (x 1000).

Fig. 33 — Abnormal orientation of the chromosomes of the secondary spermatocyte of the XIII case. (x 3400).

Fig. 34 — Abnormal orientation of the chromosomes of the secondary spermatocytes of the XIII case. (x 3500).

Fig. 35 — Metaphase of the secondary spermatocyte of the XIII case with ten chromosomes. (x 3200).

Fig. 36 — Diagramm to show a polar view of crosses with two oriented and two unoriented arms. (x 1400).