# A CASE OF SPONTANEOUS END-TO-END PERMANENT UNION OF TWO NON HOMOLOGOUS CHROMOSOMES IN THE BRAZILIAN SCORPION TITYUS BAHIENSIS ACCOMPANIED BY IRREGULARITIES IN PAIRING

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*(With 42 figures)*

**INTRODUCTION** — Due probably to the presence of a kinetochore at each end, chromosomes of Tityus bahiensis, our most common Scorpion species, are frequently subjected to spontaneous fragmentation. The resulting end-fragments, whatever may be their length, having a terminal kinetochore, may continue to live as independent chromosomes or fuse with another fragment, while median pieces, devoid of kinetochores, when not attached to the broken end of a centric fragment, vesiculate and disappear. Interchange of parts, when occurs, gives rise at metaphase of the first meiotic division to very beautiful cross configurations.

The normal behavior of Tityus chromosomes at meiosis, briefly reported in a series of papers (PIZA 1939, 1939a, 1941) and fully described in a more extensive one (PIZA 1943, 1943a), needs to be summarized here in order to facilitate a comparative apreciation of the facts to be referred to in the present article.

The six variously bent or sinuous chromosomes of the spermatogonia give origin at metaphase of the first division of the spermatocytes to three rod-shaped bivalents, whose components notwithstanding the complete absence of chiasmata stay long time side by side. The paired chromosomes are perfectly parallel to each other along their entire length (a condition necessary to their subsequent separation), showing but their

corresponding ends turned to opposite poles. A very faint longitudinal line seen on the middle of the chromosomes when they are loocked from the poles indicates that they are already split along their entire length. Indeed, in top view the bivalents present an evident quadripartite aspect. At anaphase the chromosomes separate at first parallelly and then assuming a more or less pronounced curved shape. At second metaphase the sister chromatids appear so separated as the paired chromosomes at the first one. They are likewise parallel to each other and separate in the same way as the chromosomes of the primary spermatocytes did.

Abnormal pairing due to interchange of parts or to structural modifications of the chromosomes has repeatedly been found in isolated cells of the testis and sometimes in all the cells of many cysts. (PIZA 1943, 1943a, 1943b). In some instances, however, numerical and morphological variations of the chromosomes having appeared in all the cells of the whole testis, created special cases in which the abnormality became a normal situation for the affected individuals. Two of these cases, described as the one cross and one rod case (XI) and the one cross and three rods case (XIII) respectively, constituted part of the subject of a very recent paper. (PIZA, 1943, 1943a). In the present one, a new and highly interesting case of a spontaneous fusion of two chromosomes by one of their ends, will receive treatment.

**The one double and four single chromosomes case (Shortly the WI case)** — This is the case of an adult male, normal in its morphological characters, collected in the neighbourhood of my laboratory in the month of June, whose testis was almost entirely constituted by cells carrying a permanent new type of chromosomal plate. (This case was referred to under f) in my last publication (PIZA, 1943b)).

MATERIAL AND METHODS — Since Tityus chrmosomes have shown to be an excellent material for investigation, every male individual brought to the laboratory is as soon as pos-

sible dissected in Ringer solution and its testis divided into two unequal parts, the larger being immediately transferred to the fixative and the smaller smeared in aceto-carmine or aceto-orcein. If the smear preparation reveals nothing unusual in the chromosomal constitution of the individual, the fixed material is imbedded in paraffin and the block kept in our block collection. If, on the contrary, something appears which proves to be interesting for investigation, the prepared block is sectioned at a convenient thickness and the sections treated by the selected method.

In the present case aceto-orcein was employed for smearing, Baur's modification of Allen-Bouin was used as fixative and Heidenhain's hematoxylin as dye. Sections were made at 3 micra.

THE SPERMATOGONIA — Compared with the normal, the spermatogonia in the present case differ in having instead of the six approximately equal chromosomes, five chromosomes, four of which being of the ordinary size and the fifth a little more than twice longer. (Fig. 1). The first impression got from the examination of the smear preparation was that the double sized chromosome has been originated from an end-to-end union of two ordinary chromosomes, a view which was soon confirmed by the study of the primary spermatocytes. In the morphology of this chromosome nothing indicates the place where fusion occurred, so that by the examination of its body only, without taking into account its length nor indirect evidences, no one would detect its compound nature. By the study of the spermatogonia it is not possible to discover whether the compound chromosome is the result of the union of two homologous or non homologous chromosomes. The behavior of the chromosmes in the first meiotic division of the spermatocytes, makes, however, the matter clear.

Some extremely rare spermatogonia provided with the six chromosomes of the normal testis have been found, showing in this manner that the type of anomaly dealt with in this paper is not of an absolutely general occurrence.

THE PRIMARY SPERMATOCYTES — The analysis of the prophase of the first meiotic division of the spermatocytes do not help in the understanding of the actual pairing modus of the chromosomes. However, some inability for pairing seems to be manifest at places where in normal individuals the chromosomes use to be intimately united. Metaphase, on the contrary, offers the best condition for an accurate analysis of the manner in which the chromosomes have been paired. Indeed, in the most favorable situations one can easily observe that a long chromosome paired at each half of its body with a different partner and an independent bivalent are always present in the metaphase plates. This observation makes clear that the attached chromosomes are not homologous or in other words that they belong to different pairs. Sometimes the group formed by the fused chromosomes and their partners appears regularly stretched on the plane of the equator, permitting thus a more accurate observation of their mutual relations as well as of the way in which they are inserted at the spindle. (Figs. 2, 3, 20, 22). Lateral views show that orientation is quite correct, the independent chromosomes laying at the same side of the compound one. They all have their ends turned toward the correspondig pole, the latter behaving as if it was a single element. In spite of the achromatic apparatus being less markadely developed than in normal cases, spindle fibres connecting the poles with the extremities of the chromosomes could be detected. Since fusion of the attached chromosomes has certainly been preceded by subterminal breakage in both of them, no indication of the presence of kinetochore in the middle of the body of the compound element has accordingly been found. Never the compound chromosome showed a median angular prominence directed to the pole to which it is connected or assumed a double-arch shape revealing the existence os kinetochores also in the middle of its body. The very few situations in which this chromosome formed a wide median bow toward the same pole to which its extremities were looking are not conclusive. (Fig. 25). These exceptional aspects may rather be due to secondary mechanical causes than to a specific

influence of the pole on the median part of the chromosome. In addition, the repeated formation of anaphasic bridges speaks in favor of the absence of functional kinetochores in the middle of its body. (Figs. 29, 30, 31). To judge by the length of the compound chromosome, the pieces detached from the fused extermities of its components should have been extremely small to be preserved as independent centric fragments.


Accordingly, no such fragments have been found anywhere in the whole testis. It becomes thus difficult to find an adequate explanation for so terminal a breakage. Probably, some structural alteration affecting previously the extremity of the chromosomes did favor the elimination of a minute centric granule incapable of living independently. (To live and behave as a true chromosome a fragment needs to have something more than a kinetochore). In support of that view is the fact of chromosomes carrying a granular end-piece attached to them like a small satellite, referred to in a previous paper (PIZA 1943, 1943a). If we assume that this end-piece may fi-

nish by becoming entirely free, a condition sets up which enables us to understand what actually occurred in the case dealt with in the present paper. However, the possibility of the existence of some residual kinetic activity in the middle of the compound chromosome due to a residue of the insertion area at least in one of the fused chromosomes is not completely excluded specially in consequence of the confused situation present in the majority of the cells.

Sometimes the compound chromosome seems exactly twice longer than its partners. Generally, however, it appears still longer, leaving in the middle of its body a wide unpaired segment. (Figs. 5 to 11). To explain this fact one must consider that the prometaphase chromosomes of the tetravalente group being paired but not united or connected by chiasmata can accomplish their contraction at different rates independently from each other. In accordance with the length of the unpaired weaker median region, the compound chromosome is submetted to different kinds of bending or torsion at that place, which make obscure the mutual relations of the components of the group. In the less complicated situations like that represented in the Figs. 4 to 6, 8 to 10, as well as in those in which the group is stretched in the plane of the equator (Figs. 20 to 23, 25) the independent chromosomes appear at the same side of the compound one, showing in this way that the attached chromosomes have been fused with the pairing surface turned to the same side. (See the concept of dorso-ventrality developed by PIZA specially in 1942).

One of the most common configurations of the tetravalent group is that in which the compound chromosome is uniformly curved, being paired at both ends with a segment of its partners generally longer than the half of their length. Hence, a figure results which can be compared with a more or less regular M or W. (Fig. 5). A thorough examination of these frequent aspects has shown that the pairing ability of the chromosomes is greatly altered in the innermost region of the group. The more or less pronounced incapacity for pairing in the segment of the chromosomes corresponding to the median

part of the compound element may very well be attributed to the failure of the kinetochore in this region. Moreover, when the chromosomes show to be regularly paired also at their proximal extremity (Figs. 4, 6, 12) it cannot be inferred from this that the pairing ability is equally developed there. It is highly probable that having paired regularly with the centric half of their compound partner, the independent chromosomes may, at metaphase, stretch passively on the pairing surface of the other half. What seems to be true is that pairing is regular only in a segment corresponding approximately to the distal half of the individual chromosomes.

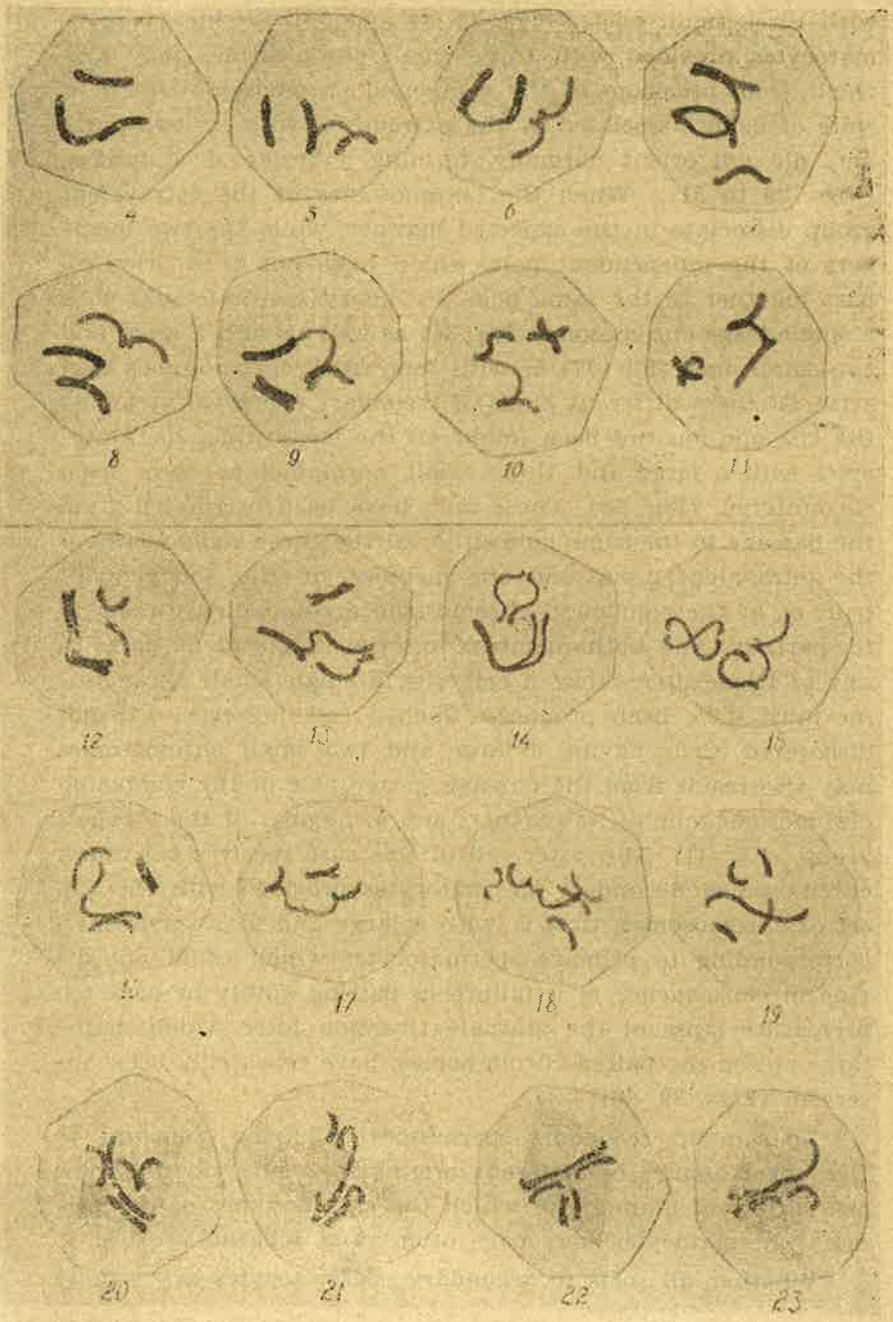
Normal pairing has been considered as being due to a generalized attraction force developed by the kinetochores and spread out all over the body of the chromosomes. This force, as inferred from many metaphase plates, when not completely unprevailing at the median region of the tetravalent group, became at least evidently weaker there. (Figs. 7, 10). Moreover, in the present case, the attraction force which maintains the paired chromosomes united lapses much earlier than normally, so that the chromosomes often separate before their orientation in the spindle has been accomplished. (Figs, 16 to 19). Therefore, chromosomes can move at anaphase without any order, going at random to the one or the other pole. So premature a lapsing in the pairing force very often coincides with an equally premature separation of the chromatids which complicates the situation in the group.

As it was pointed out in a previous paper (PIZA 1943b) an identical behaviar of the chromosomes observed by BRIEGER and GRANER (1943) in smear preparations in which some cells in normal metaphase stage were also present led them to a completely erroneous interpretation of the meiotic prophase in Tityus. Knowing nothing about the different kinds of abnormalities commonly encountered in the testis of this highly interesting scorpion, the authors did not suspect that cells detached from normal and abnormal cysts were present in the same preparation and thus, disposing the observed phases in the order which appeared to them as corresponding to the nor-

mal sequence of the meiotic phenomena gave an entirely false description of the pairing modus of Tityus chromosomes.

Now, let us tell something about the independent bivalent. The chromosomes forming it sometimes show not only regularity in pairing, but also in orientation. (Figs. 4, 5, 7, 9, 16, 23). When this happens, anaphasic separation occurs in the expected manner. Generally, however, these chromosomes appear unpaired, either at the same place or very far apart in the cell. (Figs. 3, 10, 11, 12, 22, 25, 27,). In the former case all evidence indicates that they have been previously paired and became later separated from each other in consequence of a premature lapse of the pairing force. In the latter, it is not unprobable that they never have been paired before. In the one as well as in the other case the chromosomes do not orient normally moving randomly to different poles or both to the same one. But, since there is no evidence of any alteration in the chromosomal structure, it can be assumed that the kinetochores although present may undergo changes of different degrees in their physiological activities, losing sometimes entirely the functions they are charged with in normal situations. It is interesting to note here that this inability of the kinetochores to perform their activity in pairing and orientation of the chromosomes may be determined by some conditions established in the cell as a system, considering that not rarely all the chromosomes are scattered in the cell without any order. (Fig. 19). When this happens the cells may not divide, giving rise to a secondary spermatocyte provided with a full set of chromosomes. (Figs. 39, 40).

THE SECONDARY SPERMATOCYTES — When orientation in the primary spermatocytes has been accomplished in a manner which may be considered as normal for the present case, the compound chromosome and one member of the independent pair pass to one pole while the two chromosomes paired with it together with the other member of the independen pair pass to the opposite pole. Thus, two secondary spermatocytes arise, one with a large and a small chromosome and other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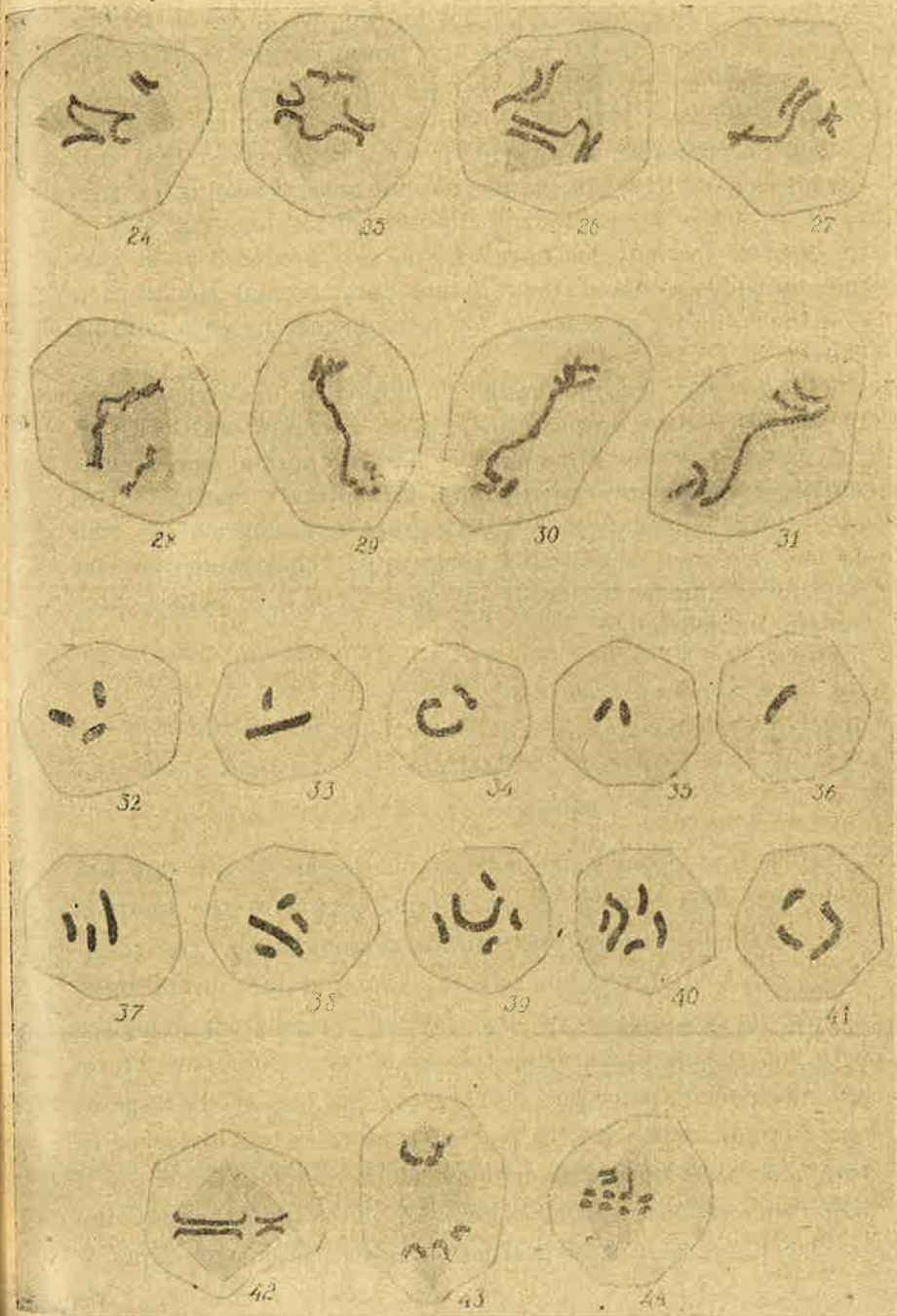
with three small ones. (Figs. 33, 34 and 32). Secondary spermatocytes provided with three small chromosomes may also result from breakage of the compound chromosome which in spite of being associated at the extremities with its two partners did not orient normally, forming a bridge at anaphase. (Figs. 29 to 31). When the chromosomes of the tetravalent group dissociate in the expected manner, while the two members of the independent pair, which have not been oriented, pass together to the same pole, secondary spermatocytes with a single large chromosome (Fig. 36) as well as with a large and two small ones (Fig. 37) or with four small chromosomes may arise. Of these different kinds of secondary spermatocytes only the last one has not been found. In the meanwhile, spermatocytes with a large and three small chromosomes have been encountered. (Fig. 38). These may have been originated from the passage to the same pole either of the three components of the tetravalent group end one member of the independent pair, or of the compound chromosome accompanied by one of its partners and both members of the independent pair. In any of these alternatives a cell with a single small chromosome must have been produced. Such a cell, however, was not discovered. Cells having a large and two small chromosomes may also result from the passage to one pole of the compound chromosome, one of its partners and a member of the bivalent group. (Fig. 41). The sister cell in this case receives two small chromosomes. Secondary spermatocytes provided with the full set of chromosomes, that is, with a large and four small ones, corresponding to primary spermatocytes which could not divide in consequence of a failure in pairing ability or of a too premature lapse of the mutual attraction force which maintains united the paired chromosomes, have repeatedly been observed. (Figs. 39, 40).

To sum up, secondary spermatocytes having from one to five chromosomes of different origins frequently found, show the variety of manners in which the chromosomes of the primary spermatocytes may pair, orient and separate.

Whether all sorts of secondary spermatocytes are equally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capable of regular division is not known. We have no serious reasons to doubt of this possibility. However, only some of the kinds described in this paper have been found in different phases of mitosis. (Fig. 42 to 44). In those in which the compound chromosome is present it can be observed that this chromosome behaves likely the normal ones, revealing no trace of an active kinetochore in its attached ends. (Fig. 42).

Mature spermatozoa have been largely produced as in normal individuals. About their actual chromosomal constitution and their vitality or capacity for fecundating the eggs nothing can be said here.

Only a very few spermatogonia limited to a restricted area in the testis have scaped the kind of anomaly dealt with in this paper, showing a metaphase plate of normal appearence. Those spermatogonia gave origin to primary spermatocytes with three independent bivalents apparently normal as well as to some ones abnormally constituted. They were however in so low a number to permit any analysis of their actual chromosomal constitution.

## SUMMARY

A case of spontaneous end-to-end fusion of two non homologous chromosomes in the spermatogonia of the scorpion Tityus bahiensis is described in the present paper. The resulting compound chromosome, twice so long as the normal ones, appears at metaphase of the primary spermatocytes paired with both its partners, while the other two chromosomes form an independent pair. Due probably to the loss of the kinetochore of the attached ends pairing is more or less disturbed in the median region of the compound chromosome. Generally it pairs only at the centric extremities with approximately the corresponding half of its partners. Due to a premature lapse of

the pairing force, orientation may be seriously disturbed, so that separation of the paired chromosomes, not only of the tetravalent but also of the bivalent group, may accomplish in different ways, giving rise to secondary spermatocytes provided with from one to four chromosomes. Sometimes separation of the chromosomes fails to occur and a secondary spermatocyte with a full set of chromosomes is thus formed. In the most regular situations the compound chromosome paired at the same side with its partners stretches on the plane of the equator. When this happens and the independent bivalent orients normally one of the resulting secondary spermatocytes receives the compound chromosome and one member of the independent pair, while the other receives both partners of the long chromosome together with the other member of the bivalent group. In a very limited area of the testis some spermatogonia provided with six apparently normal chromosomes and some primary spermatocytes with three independent bivalents have been found. These cells, however, were too few in number to permit an analysis of their actual chromosomal constitution. In some of them a new type of anomaly seemed to be present in the chromosomes.

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## EXPLANATION OF FIGURES

All figures in this paper are free-hand drawings made with Leitz's 8 x B oc. and 1/12 oil im. obj. and therefore correspond only approximately to the given magnification.

Fig. 1 — Three spermatogonia showing a long compound chromosome and the four normal ones. (Aceto-orcein, × 2100).

Figs. 2 and 3 — Metaphase of the primary spermatocytes showing the compound chromosome paired at the same side with its independent partners. In 3 the other two chromosomes are unpaired and very far apart in the cell. (Aceto-orcein, × 2800).

Figs. 4 to 19 — Different metaphase and prometaphase plates of the primary spermatocytes showing the mutual relation of the chromosomes of the tetravalent group as well as of the bivalent. In 19 all the chromosomes are unpaired. (Sect. Hemat. × 2400).

Figs. 20 to 27 — Primary spermatocytes at metaphase in lateral views showing orientation of the chromosomes and their insertion at the spindle, especially in the tetravalent group. (Sect. Hemat. × 2400).

Fig. 28 — Lateral view of a primary spermatocyte showing very long chromosomes already attached at the spindle. (Sect. Hemat. × 2400).

Figs. 29 to 31 — Primary spermatocytes in lateral view with the compound chromosome forming a bridge between the poles. (Sect. Hemat. × 2400).

Figs. 32 to 41 — Several kinds of secondary spermatocytes originated from different distribution of the chromosomes of the primary spermatocytes. Figs. 39 and 40 with a complete set of chromosomes. (Sect. Hemat. × 2000).

Figs. 42 to 44 — Division of the secondary spermatocytes. In 42 the compound chromosome shows no indication of the existence of kinetochores in the middle of its body. In 44 all five chromosomes resulting from an undivided primary spermatocyte are present. (Sect. Hemat. × 2300).