A Study of the Karyotype of the Corixid, Krizousacorixa Femorata GUERIN (Hemiptera-Corixidae)

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A Study of the Karyotype of the Corixid, 
Krizousacorixa femorata GUERIN (Hemiptera-Corixidae)

by

Maureen Marie Kleba

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
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INTRODUCTION

The purpose of my research was (1) to determine the number of chromosomes in the corixid *Krizousacorixa femorata* (Guerin) and to study the arrangement, distribution, orientation, and mode of division of the chromosomes during the first and second meiotic division in the spermatoocytes of this organism. The chromosomes of *K. femorata* were of interest due to its polymorphic character of dextrality and sinistrality (Peters, 1963). *K. femorata* has symmetrical females and asymmetrical males. The asymmetry is caused by the location of the strigil and the direction of the genital capsule: in the dextral males, the genital capsule points to the right, and the strigil is located on the right margin of the sixth segment, while in the sinistral males the genital capsule points to the left and the strigil is located on the left margin of the sixth segment. It was thought that a karyological study of meiotic divisions during spermatogenesis might contribute to a better understanding of the mechanisms responsible for this polymorphic condition.
REVIEW OF LITERATURE

A convenient approach to compare nuclei of different species is to identify individual chromosomes during mitosis or meiosis. They then appear as cylindroids and can be stained intensely with basic dyes. This makes it possible to determine their number, relative size, morphological appearance, distribution during mitosis and meiosis, and, if electron microscopy is applied, their internal organization.

Spermatogenesis

Insect testis has proven excellent material for the study of chromosomes during meiosis. The presence of a large number of synchronously dividing cells in the insect testis makes this material one of the most favorable for the study of cell division by light and electron microscopy, since one single preparation can supply a number of almost identical stages of meiosis. Many reports have appeared in recent years on various aspects of mitosis and meiosis during insect spermatogenesis as observed by phase contrast (Kawamura, 1957), polarizing microscope (Inoue, 1953), and electron microscopy (Phillips, 1970).

The testes in the vertebrates and insects are composed of numerous sperm tubules converging toward common ducts leading the mature sperm to the exterior. Since spermatogenesis is a continuous process, it is possible to see at one time various stages of sperm development in the
same sperm tubules. Within the tubules there is an orderly arrangement of cells in different phases of development. The testicular follicles can be either spherical or tubular (Phillips, 1970). Tubular follicles are found, e.g., in grasshoppers (Phillips, 1970) and in the object of my investigation, K. femorata. The anterior end of the tubules contains the spermatogonial cells which are undergoing proliferation by a succession of mitoses. Cells in more advanced stages of growth and maturation, i.e., primary and secondary spermatocytes, are found further down the tubule. Mature spermatozoa are found in the posterior tubular end (Lima-de-Farís, 1959).

The sperm tubules have many cysts containing clones of germinal cells embedded in large epithelial cells (Baccetti and Bairati, 1964; Cantacuzene, 1968). Within a given cyst the meiotic divisions are synchronous.

Karyotype of the Corixidae

Although the number of chromosomes can vary widely in the Heteroptera, from 4-51 (Makino, 1951), some families have a fairly constant chromosome number. Of more than 100 species that have been studied in the family Pentatomidae, 85% have a diploid number of 14 chromosomes - 12 autosomes and 2 sex chromosomes (Schrader, 1958). The chromosome number for members of the family Notonectidae is 24 or 26 (Jande, 1961), while the characteristic diploid number for the family Corixidae is 24, admitting only one exception, Cymatia bonsdorffi SHLB. (Slack, 1938) which has a diploid number of 26. K. femorata, which is the
object of this investigation, is a member of the family Corixidae. A more detailed description of the karyotypes of various species of the Corixidae is given below.

Prokofiewa (1933) studied the karyotypes of 11 species of Corixidae, placing major emphasis on *Corixa distincta* (Fieb.) and comparing all other species to it. In *Corixa distincta* 13 "Chromatinelemente" (chromatin elements) appear during the first meiotic metaphase (=MI). Twelve are regularly arranged in a circle while one small chromosome, named "m-chromosome" (= microchromosome) by Prokofiewa, is located in the center of the ring. The "chromatin elements" of the peripheral ring represent 10 bivalents and 2 univalents. The univalents are sex chromosomes (X and Y) which during early prophase of the first meiotic division are closely associated with one another, become separated in late diakinesis, and then remain as two separate chromosomes in the metaphase plate. Among the 10 bivalents there are 3 large ones (M) of which one exceeds the others by far, 6 medium size (A), one small one (a), and finally one which is very small (m), in the middle of the plate. *Corixa distincta* therefore has the following karyotype formula: 1M, 2M, 6A, a, m, X, Y. As the conjugated "m-chromosomes" are separated somewhat earlier than the other autosomes, namely, toward the end of prophase, two single microchromosomes rather than the one bivalent are sometimes identified in the center of the plate. Such an early separation of microchromosomes is characteristic of the Hemiptera and it has been described by Wilson (1907, 1909, 1932) for some Coreidae
(Metapodius terminalis DALL., Banasa calva, and Banasa dimidiata), Browne (1916) for some Notonectidae (N. glauca, N. shooterii, and N. irrata), et al. The sex chromosomes of Corixa distincta are of medium size and slightly different from each other in dimension. Although they are not connected during metaphase I, they are always found next to each other (Prokofiewa, 1933).

In a side view of the first meiotic spindle the bivalents appear dumbbell shaped. The constriction of the bivalents represents the point where two conjugated chromosomes touch each other. During meiotic division, the autosomal bivalents are oriented so that the two pairing chromosomes face opposite ends of the spindle. The result is that the first meiotic division is reductional for the autosomes.

The sex chromosomes during the first meiosis are always found next to each other and attached to the peripheral spindle fibers. However, as they do not join to form bivalents they are oriented so that during the first meiotic division they divide equationally. Only at the subsequent interkinesis do the sex chromosomes unite and form a bivalent, thus reducing the number of distinct chromosomal units from 13, found during the first meiotic metaphase, to 12 at the second. An examination of the second meiotic metaphase plate shows a similar, though not quite as regular arrangement of chromosomes in a ring. During this division all the autosomes, including the small "m-chromosomes", are located in the periphery of the ring, while the XY bivalent is found in the center of the plate. A side view of the spindle clearly shows the dumbbell shape of the XY bivalent and its asymmetry due to
the difference in size between the X and the Y chromosome.

The other species studied by Prokofiewa conform more or less to this general description. Their chromosomal characteristics are briefly as follows:

**Corixa striata** (L.)

The karyotype is: 1M, 4M, 4A, a, m, X, Y. The sex chromosomes are almost equal in size and of similar dimension as the A-type autosomes.

**Corixa fosarum** (Leach.)

Characteristic of this species is the large size of the "M" chromosomes. The karyotype formula is: 1M, 2M, 6A, a, m, X, Y. Both sex chromosomes during the first metaphase are close to one another and form a very asymmetric bivalent. The Y chromosome is the smallest in the ring and it is not much larger than the "m-chromosome". The X chromosome belongs to the class composed of medium size (=A) chromosomes. The arrangement of chromosomes in a ring is not always as regular as in *C. distincta* and one of the middle size chromosomes may frequently be found in the middle.

**Corixa falleni** (Fieb.)

The karyotype formula is: 1M, 4M, 4A, a, m, X, Y. The X chromosome is of medium size while the Y chromosome is the smallest.
**Corixa fabricii** (Fieb.)

The karyotype formula is: 3M, 6A, a, m, X, Y. The sex chromosomes lie so close together that they look like one asymmetrical body. The X chromosome is of medium size, while the Y chromosome is small. The "m-chromosome" in this species is so minute that it is barely visible.

**Corixa semistriata** (Fieb.)

The number of chromosomes and their arrangement is the same as in *C. distincta*. The X chromosome is of approximately the same size as the middle-size autosomes, while the Y chromosome is of approximately the same size as the small autosomes.

**Callicorixa praeusta** (Fieb.)

Peculiar to this species is the location of the X and Y chromosomes. During late prophase they migrate far away from each other and appear separate from each other in the first meiotic spindle. The karyotype formula is: 1M, 3M, 5A, a, m, X, Y.

**Callicorixa wollastoni** (Dgl. Sc.)

The chromosomes of this species exhibit an irregular arrangement because a small chromosome (either a or Y) is often found within the ring.

**Corixa sahlbergi** (Fieb.)

The equatorial plates of the first and second division as well as the
chromosomes themselves are much larger than in all previous species. Among the 12 peripheral chromosomes of metaphase I, the sex chromosomes are difficult to distinguish since in late diakinesis they are so far separate from each other that they enter metaphase as two separate entities. The karyotype formula is 1M, 1M, 5A, a, m, X, Y.

Corixa linnei (Fieb.)

The karyotype of the first and second division is like that of Corixa sahlbergi, except that the regularity of the ring-shaped arrangement is disturbed by the central location of "a" or Y chromosome. The "m-chromosomes" are comparatively large.

Macrocorixa dentipes (Thoms.)

In this species the chromosomes are not regularly arranged in a ring, and the X and the Y chromosome cannot be identified. During the first meiotic division M. dentipes has 13 distinct chromosomal units, all much bigger than in other species. During the second meiotic metaphase 12 chromosomal units can be distinguished, one of which is extremely large. There is no difference in size between the sex chromosomes.

Comparison of karyotype of the Corixidae with that in other families of Hemiptera-Heteroptera

Among the publications which deal with cytological aspects of the Hemiptera-Heteroptera, there are a number of papers which afford a
comparison of the Corixidae with other families in this order, such as the families Notonectidae and Naucorididae. The karyotype of the Notonectidae, e.g., N. glauca, N. irrorata, and N. shooterii (Browne, 1916), and of Naucoris cimicoides among the Naucoridae (Steopoe, 1929) is quite similar to that of the Corixidae to the extent that even the chromosome number is the same. Their chromosomes for the most part appear as oval or elongated bodies. During meiotic prophase they are arranged in a ring. In the Corixidae, as in a number of other heteropteran families, there are "microchromosomes" (Prokofiewa, 1933) present which during the first metaphase are almost always found in the center of the ring and which approach opposite spindle poles sooner than the other chromosomes (Prokofiewa, 1933).
MATERIALS AND METHODS

Light microscopy.

During the course of my work I made squash preparations from approximately 500 male specimens, using different techniques of fixation and staining. Unfortunately, most of these methods were inadequate. Approximately 30 specimens were eventually processed with the method which I finally adopted and which is as follows. Testes from fifth (last) instar nymphs and young male adults of *Krizousacorixa femorata* were dissected out and placed for 5 min. in modified isopropyl Carnoy's fixative, containing 1 part glacial acetic acid, 3 parts absolute isopropyl alcohol (Ueshima, 1963). The testes were then removed and placed on a glass slide in a few drops of 2% aceto-orcein stain (La Cour, 1941), covered with a cover slip, and pressed lightly. The edges of the coverslip were sealed with paraffin. Shortly after addition of the stain, the squashes were examined under phase contrast with a Zeiss Photomicroscope. For sectioning, some testes were fixed with a saturated aqueous solution of picric acid and embedded in paraffin. Deparaffinized sections were stained with iron hematoxylin according to Heidenhain (1896). Photomicrographs were made on KB 14 film at magnifications of 250-500x. A green filter (VG-9, Zeiss) was used to increase phase contrast.
Electron microscopy.

Testes were fixed for 1 hr. at 4°C in 4% glutaraldehyde (Sabatani, Bensch, and Barrnett, 1963) containing 0.05M cacodylate buffer (pH-7.2) and 0.01 M CaCl₂. The tissue was postfixed in 1% osmium tetroxide containing 0.05M cacodylate buffer (pH-7.2) for 1 hr. at 4°C. After dehydration with ethanol, the specimens were perfused with propylene oxide for 15 min. The propylene oxide was then progressively replaced with Epon 812 (Luft, 1961). Silver and golden sections were collected on carbon-coated grids. For staining, the grids were floated face down for 5 min. on uranyl acetate (Watson, 1958), washed with distilled water, and floated upside-down on lead citrate solution (Reynolds, 1963). The specimens were examined with an RCA EMU 3F-2 electron microscope using a 50 KV voltage. Electron micrographs were generally taken at magnifications of 8,000-14,000 times.
RESULTS

Karyotype

One striking phenomenon observed in a study of spermatogenesis in *K. femorata* is the high degree of synchrony with which the various stages of spermatogenesis are found within specific regions of the sperm tubules. Only one stage of spermatogenesis is found in a particular region at any given time and, if this stage is one of meiosis, e.g., meiotic metaphase, each nucleus is at a stage that is indistinguishable from that of any other nucleus (Figs. 3, 4, and 5).

Different regions of the sperm tubules within one testis show different stages of sperm development. Nuclei in the pachytene stage (Figs. 1 and 2) may be found, e.g., in the anterior end of the tubules while more advanced stages of meiosis are found in lower tubular regions. A dense, heterochromatic body can be distinguished during pachytene which is regularly found next to the disappearing nucleolus and which is characteristic for all the Corixidae (Slack, 1938). This body, according to Slack (1938) represents the two closely associated heterochromatic sex chromosomes (*X* and *Y*).

With the approach of the first meiotic metaphase the chromosomes become more condensed and they become arranged in form of a ring (Figs. 3-7) on the periphery of the spindle, with the exception of a pair of
very small chromosomes (similar and probably identical to those called "m-chromosomes" by Prokofiewa, 1933) which are found in the center of the ring. The chromosomes forming the ring are all closely paired and most of them appear as dumbbell shaped bivalents. Two of the chromosomes in the ring are only loosely associated, and one of them is a little thicker than the other. These are, according to the references available (e.g., Slack, 1938; Prokofiewa, 1931 and 1933; White, 1945) the sex chromosomes (X and Y). A slight degree of asynchrony is apparent with regard to meiotic division between different chromosomes within one meiotic figure; i.e., the central pair of "m-chromosomes" are sometimes found separated in preparations which were not excessively squashed, at a time when all other autosomal pairs are still in the stage of diakinesis. This suggests that the movement of the "m-chromosomes" toward opposite poles might begin slightly earlier than that of the other chromosomes (Fig. 9).

The shape and arrangement of the autosomes and the sex chromosomes during the first meiotic metaphase is shown in Figs. 6-12. Thirteen chromosomal units are visible in the first metaphase plate. These include 11 autosomal bivalents and the two separate sex chromosomes (X and Y chromosome). The karyotype formula conforms well to that established by Prokofiewa (1933) for Corixa distincta. Among the autosomal bivalents (Fig. 13) there are 3 large ones (M, numbers 1-3), 6 of medium size (A, numbers 4-9), one small bivalent (a, number 10), and one which is very small, composed of the two "m-chromosomes" (number 12).
All autosomal bivalents, except the "m-chromosomes" are dumbbell shaped (Fig. 27). The sex chromosomes (Fig. 13, number 11) are of medium size and similar in length. They are slightly thicker at the ends than in the middle (Fig. 27). Although they are parallel and close to one another, there is no point of contact between them. The chromosomes of which the bivalents are composed are paired at one point of terminalization only or at two points which are very close to each other. Pairs of bivalents are found in which the homologous chromosomes are paired at two points. The smallest pair of autosomes, the central "m-chromosomes", seem to be paired at only one terminalization point.

This behavior of the chromosomes during the two meiotic divisions is a result of their orientation toward the spindle. During the metaphase of the first meiotic division, the autosomes are very closely paired, at terminal points, and the two pairing chromosomes of which each bivalent is composed are oriented toward different poles of the spindle (Figs. 8-12, and 14). The sex chromosomes are only loosely paired and they are oriented parallel to the long axis of the spindle in such a way that the two chromatids of each sex chromosome face different spindle poles. As a result, the autosomal bivalents undergo a reductional division during first metaphase while the sex chromosomes are divided equationally, with each daughter nucleus receiving one chromatid of each. The same has been found for other Corixidae by Prokofiewa (1931, 1933).

In the squash preparations, the pair of "m-chromosomes" always tend to be arranged perpendicular to the long axis of the spindle, i.e.,
parallel to the plane of the chromosomal ring. This would at first make it a little difficult to understand why the first meiotic division of this pair should be a reductional one like that of the other autosomes. However, it is obvious in sections that this difficulty does not exist (Fig. 19): the "m-chromosomes" are arranged parallel to the longitudinal axis of the spindle and the position seen in the squash preparations is an artifact produced by the treatment. Sometimes both "m-chromosomes" are seen in the center of the metaphase ring (Fig. 19) indicating that in at least a few cases the "m-chromosomes" are oriented parallel to each other rather than in the typical end to end association.

Since the squashing procedure which I employed might produce artifacts, such as the breakage of thin chromosomes, I compared the number of chromosomes observed in the squash preparations with that found in stained sections and found no difference for the number of autosomes (Figs. 17 and 18). The bivalent nature of the autosomes was not apparent in these sections since they were oriented parallel to the equatorial plate. The X and the Y chromosome lie extremely close to one another and appear as one asymmetrical body in the sections. At anaphase (Figs. 14-16) the separating bivalents remain connected with one another by thin filaments until a distance between the anaphase plates of approximately 4 μ is reached. The nature of these connections is not obvious. Some authors believe, although without having cytochemical evidence, that these connections represent thin chromatin elements from both bivalents which remain paired, even during the anaphase movement, at the points of terminalization of the previous chiasmata. However, there is
also reason to believe (see below under "Discussion") that membrane elements surrounding the chromosomes are involved in the formation and long persistence of these filamentous connections. It might be significant in this connection that the connecting filaments appear to be of approximately the same thickness and have the same intensity of contrast under the phase microscope as the nuclear membrane seen in Fig. 16. The connections eventually come apart as the anaphase movement of the chromosomes toward the poles proceeds, and they are found (Fig. 16) as thin filaments which are trailing behind the chromosomes which continue to move further toward the poles as the division proceeds.

At the second metaphase (Figs. 20-22) the orientation of the chromosomes is drastically changed. All autosomes, including the small "m-chromosome", are now located in the peripheral ring. In the center of the ring we find a large rod shaped unit which seems to be composed of two parts, one of which is thicker and, under phase contrast, appears darker than the other. As the ring is composed of only 11 chromosomes we believe that this central body is of chromosomal nature. As the sex chromosomes have not yet undergone meiosis, it is most likely that this unit, which is so obviously asymmetric, represents the two closely paired sex chromosomes. Although we did not observe the final division of the metaphase plate, it seems likely that the central pair now undergoes a reductional division.
During the spermatogonial divisions and later on, during meiotic divisions, the individual chromosomes (Figs. 23-26) are surrounded by membrane elements. These elements are similar in appearance to elements of the nuclear membrane. Their thickness is approximately 300 Å. There are large gaps between these pieces, as seen, e.g., in Figs. 23 and 24.

In other preparations, such as those shown in Figs. 25 and 26, the membrane surrounding the chromosomes is coherent except for small holes which are similar in size and appearance to holes seen in the nuclear membrane (DuPraw, 1968), and which might be remnants of the larger gaps seen at earlier stages between the loosely assembled pieces of the membrane elements.
DISCUSSION

Division synchrony

Division synchrony of the high degree of precision which is found in spermatogenesis of *K. femorata* and other organisms is frequently found in groups of cells which originated from one single cell by a sequence of divisions. This natural synchrony is found, for instance, during early cleavage in sea urchin eggs. The synchrony of spermatogonial as well as spermatocyte divisions might well belong to this category. However, a second possibility, which we cannot exclude, is suggested by the synchrony of divisions observed, e.g., in plant endosperm (Jungers, 1931), in multinucleated giant amoebae (Kudo, 1947), in insect eggs during superficial cleavage (Sonnenblick, 1950), and in cells which were artificially fused with one another (Johnson and Harris, 1969). In these cases, division synchrony between nuclei which share the same cytoplasm is probably the response of all the nuclei to the same unknown factors to which they are all exposed simultaneously and some of which might be involved in controlling the time of mitosis. This type of mechanism might well be responsible for division synchrony in spermatogenesis provided that small intercellular, cytoplasmic bridges exist between the cells affording an exchange of substances. Such cytoplasmic bridges resulting from incomplete cytokinesis during spermatogenesis have
been found (Baccetti and Bairati, 1964; Hoage and Kessel, 1968) and make each clone of germinal cells a functional syncytium. Only a specific electronmicroscopic study could determine if such connections exist in K. femorata.

Morphological appearance and behavior of chromosomes during meiosis

(1) Reductive versus equational division

The distribution of chromosomes during meiotic divisions in most organisms (Du Praw, 1968) occurs in such a way that the first meiotic division results in a reduction of the diploid number of chromosomes to a haploid level, while the second meiotic division is equational. A number of exceptions to this rule have been found in the order Hemiptera. For instance, in all coccids and aphids (suborder Homoptera) investigated, the first meiotic division according to Brown and Cleveland (1968) is equational ("meiotic inversion"; Brown and Cleveland, 1968). In K. femorata and in other heteropterans (Wilson, 1907 and 1912; Schrader and Hughes-Schrader, 1958; Wolfe and John, 1965) the autosomal bivalents divide reductionally during the first meiotic division and equationally during the second one, while the sex chromosomes divide equationally first and reductionally next. This differential behavior is due to the different orientation of the autosomes and the sex chromosomes toward the spindle poles during the first meiotic division (MI). Since two homologous, closely paired autosomes face different spindle poles during MI, the first division results in a reduction to a haploid number. The
sex chromosomes, on the other hand, are not closely associated during MI, although they are oriented parallel to each other. Their orientation toward the spindle poles is such that the two chromatids of each sex chromosome face different poles, resulting in an equational division during MI. It would be interesting to know more about the fine structure of the sex chromosomes for, if the above explanation is correct, and if what in a squash preparation appears as one dumbbell shaped "chromosome" actually represents two chromatids, it follows that the longitudinal axis of the sex chromosomes is not identical with the line which one could draw from one "end" to the other. A hypothesis will be offered further below, in a discussion of the chromosomal membranes, to account for the different orientation of the sex chromosomes and autosomes during MI.

The specimens which were used in the above studies for making the squash preparations were selected at random, without preference for either sinistrals or dextrals. All squash preparations differed slightly from one another with regard to the appearance (shape, staining intensity, and intensity of phase contrast of single chromosomes, as seen, e.g., in Figs. 3-12. This was the case even for different nuclei of the same bug and was probably due to such uncontrollable factors as the degree of squashing and the different orientation of metaphase plates relative to the surface of the coverslip and the microscope slide. Among the approximately 30 specimens from which suitable, i.e., well-spread, undistorted, squash preparations were obtained, only two were sinistrals. No difference between the sinistrals and dextrals was
found in these squashes with regard to the number of chromosomes. Slight variations between squashes in the morphological appearance of the chromosomes in my opinion were not greater than the variations which one finds between squash preparations from different dextrals (Figs. 3-9) and even between different meiotic metaphases found within one squash preparation from the same gonad (Figs. 3-5). Hence, if any difference should exist between the karyotypes of sinistral and dextral males, this difference might be more subtle than it would have to be in order to be evident by comparison of the squash preparations used in this investigation. A detailed study of a greater number of sinistral males with the techniques which I used as well as with electron microscopy might therefore be useful.

(2) Terminalization

During diplotene, which I did not observe in my preparations, the chromosomes, which were previously paired along their whole length, move apart from one another and then remain associated at a few points, the chiasmata (DuPraw, 1968). During the following stage of diakinesis, the paired chromosomes become progressively shorter, and the chiasmata move slowly to the ends of the chromosomes. This process is called "terminalization" (DuPraw, 1968). Terminalization in the majority of organisms (DuPraw, 1968) results in the formation of a bivalent in which the homologous chromosomes remain paired with each other at two points which are at opposite ends of each chromosome.

The process of terminalization differs from the above in _K. femorata_
and in other hemipterans. During the first meiotic metaphase the dumbbell shaped bivalents give the impression that only one end of each of the homologous chromosomes of which they are composed is paired with the corresponding end of the other chromosome and that the pairing chromosomes are oriented parallel to the long axis of the spindle. This view has been put forward for other hemipterans by Schrader (1935), Hughes-Schrader and Schrader (1961), and by Wolfe and John (1965). According to Schrader (1935) there is only one terminalization point for each pairing chromosome in the Hemiptera. The other free end begins to move to the poles ahead of time and, as a result, the chromosomes which previously were oriented at an angle of 90° toward the long axis of the spindle, begin to assume a position parallel to the spindle axis. Thus, the meiotic chromosomes which are holokinetic during mitotic divisions (Hughes-Schrader and Ris, 1941), i.e., devoid of a defined kinetochore region, become telokinetic as they approach meiosis (Schrader, 1935).

Despite this evidence, and despite our own pictures obtained with the light microscope, another view of the orientation and terminalization of the autosomal chromosomes in K. femorata is possible. As pointed out above, it is likely that, when we are looking at a sex chromosome in a squash preparation, we are actually looking at two chromatids of what appears to be one dumbbell shaped chromosome. This means that what geometrically appears as the long axis of a sex chromosome, going from one "end" to the other, is actually a view of the two chromatids. It is therefore equally possible that the appearance of the bivalents as seen in the light microscope is deceiving and that each of the pairing autosomes,
although they appear to be oriented parallel to the spindle axis, with one end of each chromosome free and the other one attached to the corresponding point of terminalization of the other chromosome, is actually of an extremely distorted U-shape, with the two ends of the U so close to each other that they appear like one point of terminalization, whereas in reality both ends of each chromosome might still be attached to the two corresponding points of the other homologous chromosome. There is at least one piece of evidence in the literature suggesting that this interpretation might be possible and that each chromosome has indeed two terminalization points. Buck (1967) in a paper on *Rhodnius prolixus* presents one picture (his Fig. 6) which shows the ultrastructure of a chromosome during meiosis. This picture suggest that each of the pairing chromosomes has two points of terminalization.

(3) **Chromosomal membranes**

Membranes having similar dimensions as the nuclear membrane have been found around single chromosomes of other Hemiptera, e.g., in *Rhodnius prolixus* (Buck, 1967). The presence of membranes might play a role in determining the mode of division of different types of chromosomes during the first meiosis. Buck (1967) has found that in *R. prolixus* the two pairing chromosomes of a bivalent are completely surrounded by membranes. If this were also the case in *K. femorata*, as I have assumed in the Fig. 28, the presence of a membrane around the bivalents could account for the long lasting persistence of thin, low-contrast
connections between the segregating chromosomal bivalents in this organism (Figs. 14 and 15). Furthermore, in view of the fact that no membrane connection is seen between the sex chromosomes, the presence or absence of a connecting membrane might influence the orientation of chromosomes toward the spindle and this in turn could account for the difference between the behavior of the sex chromosomes and that of the autosomal bivalents during meiosis I and, consequently during meiosis II.
SUMMARY

Some aspects of karyotype and chromosome structure during spermatogenesis in *K. femorata* were investigated (1) by light microscopy in aceto-orcein stained squash preparations, and (2) by electron microscopy. The number of chromosomes and their behavior during meiosis conformed to the general pattern found for other members of Corixidae family. Eleven autosomal bivalents were found during the first meiotic division and two closely adjacent, but separate sex chromosomes. The sex chromosomes and 10 of the 11 autosomal bivalents are arranged in a ring. One autosomal bivalent is extremely small and located in the center of the ring. The bivalents and the sex chromosomes are arranged so that the first meiotic division is reductional for the autosomes and equational for the sex chromosomes. During the second meiotic division, all the autosomes are arranged in the form of a ring, and the sex chromosomes are found as a bivalent in the center of the ring. The chromosomes are surrounded by a membrane which is similar in appearance to the nuclear membrane.
ILLUSTRATIONS

PLATE I

Figs. 1 and 2

Pairing of homologous chromosomes during pachytene. Note knoblike enlargement (arrow) at end of chromosome in Fig. 1.

S, heterochromatic sex chromosomes

N, disappearing nucleolus

Magnifications: Fig. 1, x 3840
            Fig. 2, x 1024
PLATES II, III, AND IV

Figs. 3, 4, and 5

Metaphase of the first meiotic division. Typical appearance of preparations which were sufficiently but not excessively squashed. The cells in this preparation are still intact. Note synchrony of division. All squash preparations used for Figs. 3-5 as well as for Figs. 6-13 were obtained from dextral males.

Magnifications: Fig. 3, x 1400
            Fig. 4, x 2040
            Fig. 5, x 2040
Figs. 6 and 7

First meiotic metaphase showing typical ring configuration with bivalent autosomes and loosely paired, univalent sex chromosomes in the periphery (S), and one pair of small autosomes ("m-chromosomes", Prokofiewa, 1933) in the center. The chromosomes are still oriented toward the anticipated direction of anaphase movement. The homologous chromosomes of each pair of autosomal bivalents are facing different spindle poles whereas the sex chromosomes are oriented in such a way that the two chromatids of each sex chromosome face different poles of the spindle. No difference was found between sinistrals and dextrals.

Magnification: x 3840
PLATE VI

Figs. 8 and 9

First meiotic metaphase. In Fig. 9, the bivalent pair of small chromosomes in the center has already separated. Note typical orientation of XY pair (S) in all three plates.

Magnification: x 1790
Squash preparations of chromosomal plates which apparently were not oriented parallel to the surface of the microslide. As a result, the orientation of all chromosomes in these preparations is still indicative of the direction of their anticipated movement toward the anaphase poles during the first meiotic division (approximately up - down). The sex chromosomes are oriented parallel to each other and to the spindle axis.

Magnification: x 3000
Upper two rows: Idiogram of autosomal bivalents and pair of sex chromosomes (X,Y) during first meiotic metaphase. All chromosomes shown in this idiogram are from the same metaphase plate.

No. 11, sex chromosomes
No. 12, central chromosomes

Lower left: Central chromosomes from another squash preparation showing connection between the two central "m-chromosomes".

Magnification: x 3000
PLATE IX

Figs. 14, 15, and 16

First meiotic division, side view.

Fig. 14

Metaphase. Bivalents connected by thin strands which might represent chromatin filaments from both chromosomes which are connected at the terminalization points of chiasmata. Each chromosome is composed of two chromatids.

Magnification: x 3000

Fig. 15

Anaphase. Connection between paired chromosomes not yet severed. Note slight asynchrony among different nuclei.

Magnification: x 1180

Fig. 16

Anaphase. The trailing ends of the connections seen at an earlier
stage between the separating bivalents show approximately the same intensity of phase contrast as the nuclear membranes. This preparation does not allow one to determine if the filaments seen between the chromosomes, in Figs. 14 and 15, or trailing behind them, in Fig. 16, are 
(1) chromatin threads, or (2) thin hoses of membrane material or (3) composed of both.

N, nucleus

Magnification: x 3000
PLATE X

Figs. 17, 18, and 19

Sections through equatorial plates of first metaphase. The arrows in Figs. 17 and 18 point at the XY pair. Both plates contain 10 peripheral, clumped bivalents, the peripheral XY pair, and the central "m-chromosome" bivalent. Fig. 19 shows the orientation of the "m-chromosome" parallel to the spindle axis (arrow).

Magnifications: Fig. 17, x 1680
Fig. 18, x 1000
Fig. 19, x 1000
Figs. 20, 21, and 22

Second meiotic division. The autosomes divide equationally and are oriented with their chromatids facing opposite poles of the spindle. The X and the Y chromosomes are now paired end to end and oriented so that they face different spindle poles. The small "m-chromosome" is not seen in Fig. 20. In Figs. 21 and 22 the "m-chromosome" (arrows) is located among the other peripheral autosomes, while the XY pair is in the center.

S, X and Y chromosome

Magnification: x 3000
PLATE XII

Figs. 23 and 24

Chromosomes surrounded by membrane (arrow). The same preparation is presented at two different magnifications to show (1) the general orientation (Fig. 23) of the chromosomes toward the spindle and centriole, and (2) the appearance of the chromosomal membrane (Fig. 24). The function and significance of vesicles and multilayered bodies in these figures is unknown. The large gaps between membrane pieces suggest that at this stage the chromosomal membrane is still in a stage of being assembled.

H, gap between membrane elements

Magnifications: Fig. 23, x 24,000

Fig. 24, x 36,000
PLATE XIII

Fig. 25

Chromosome surrounded by membrane. Probably a spermatocyte division, as above. Arrows point at holes in the membrane.

Magnification: x 12,800

Fig. 26

Chromosome almost completely surrounded by membrane. The chromosomal membrane is coherent and there are only a few small holes (arrow). This is probably a stage of meiotic division.

Magnification: x 18,000
Arrangement of a few representative chromosomes ($X$, $Y$ and two autosomes found in the ring in e.g., Fig. 9), with regard to the direction of movement (arrows) during the first meiotic division. $X$ and $Y$ are oriented parallel to each other in such a way that the first meiotic division will be an equational division, e.g., the two chromatids of each sex chromosome face different spindle poles. The autosomes are oriented in such a way that the two chromatids of each autosome face the same pole of the meiotic spindle. Note absence of connections between $X$ and $Y$. The arrows indicate the direction of movement at anaphase.

This drawing of a section through a bivalent represents a hypothetical interpretation of the arrangement of autosomes seen in Fig. 14 and 27. Two interpretations are offered. Both suggest that the pairing autosomes are surrounded by a common membrane. The drawing on the left assumes that both chromatids of each autosome as well as the pairing autosomes are surrounded by one common membrane, while the drawing on the right assumes that the two chromatids of a given autosome at the time of pairing are separated by the same membrane which surrounds
the chromatids from the two pairing autosomes that are facing each other. It is assumed that the section goes through a plane which is at some distance from the points of terminalization. The presence of a common membrane around the pairing autosomes, including the small central autosomes, and the absence of a similar membrane around the sex chromosomes might have an effect upon their orientation toward the spindle and the poles during the first meiotic division and might thereby determine whether this division is a reductional division, as is the case for the autosomal bivalents, or an equational division as is the case for the sex chromosomes.

c', c'', chromatids belonging to a given chromosome
LITERATURE CITED


Plate X

Fig. 17

Fig. 18

Fig. 19
APPROVAL SHEET

The thesis submitted by Maureen Marie Kleba has been read and approved by the director of the thesis.

Furthermore, the final copies have been examined by the director and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

[Signature]

Date: 5/21/90

Signature of Advisor: W. Peterson