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Title	AN UNEQUAL PAIR OF IDIOCHROMOSOMES IN THE TREE-CRICKET, OECANTHUS LONGICAUDA MATS. (With 11 Text-figures and 2 Plates)
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Citation	北海道帝國大學理學部紀要, 2(1), 1-35
Issue Date	1932-09
Doc URL	https://hdl.handle.net/2115/26946
Type	departmental bulletin paper
File Information	2(1)_P1-35.pdf



AN UNEQUAL PAIR OF IDIOCHROMOSOMES
IN THE TREE-CRICKET, *OECANTHUS*
LONGICAUDA MATS.¹⁾

BY

Sajiro MAKINO

(With 11 Text-figures and 2 Plates)

This paper deals with the chromosome of *Oecanthus longicauda*, an insect belonging to the family Gryllidae, which is quite unique among the Orthoptera in regard to the morphological aspect of the chromosomes. In spite of the extensive literature, that has appeared on the spermatogenesis of various Orthoptera, no author seems to have described such a peculiar type of chromosome as in the present species. Even in the allied species, *Oecanthus nigricornis*, which was recently reported by JOHNSON ('31), the chromosome shows nothing different from the usual type of the Orthoptera, so far as the form of chromosomes and the sex chromosome complex are concerned. At first sight the metaphase chromosomes of this species remind us of the chromosomes of the Hemiptera rather than those of the Orthoptera. Moreover, so far as known, the condition of the sex chromosome in the present species is quite exceptional and more interesting in comparison with the other orthopteran species, not only in the complex but also in the behavior during the growth period.

A short note on the chromosome of *Oecanthus longicauda* has already been published by OHMACHI ('27) who suggested the existence of the XY-pair of idiochromosomes by counting the number of the spermatogonial chromosomes. Unfortunately his obser-

1) Contribution No. 29 from the Zoological Institute, Faculty of Science, the Hokkaido Imperial University, Sapporo.

vation was not extended to the behavior of the XY-chromosome during the growth period and subsequent divisions of the auxocytes.

The present study was undertaken with a purpose of making clear the nature of the sex chromosome, as well as the entire history of the chromosome in general, in this interesting species of Orthoptera.

The investigation was made under the guidance of Prof. Dr. OGUMA to whom I wish to express my sincere gratitude for his examination of my findings and for his helpful criticism and suggestions. I also wish to thank Mr. H. FURUKAWA for identification of the species used in the present study.

Material and Methods

Oecanthus longicauda MATS., an Orthopteran of the family Gryllidae, is the unique representative of the genus *Oecanthus* in Japan¹⁾ and is widely distributed from the middle region of the main-land northward throughout Hokkaido but found more abundantly in the northern part (SHIRAKI '30). It is a pretty cricket of pale yellow color having a body length of 12 to 14 mm.

During the breeding season, which lasts rather a long period of time, from the last of August till the beginning of October, as HORI ('27) already reported, the adult males call their partners by a characteristic stridulating song. The adult tree-cricket is found upon any herbaceous plant in the field, $\frac{1}{2}$ -1 meter from the ground. The larvae, however, do not seem to live so high upon the plant as the adults, but dwell in bushes near the ground.

The specimens for the present study I collected at Sapporo in the latter part of August, 1931. The material employed consisted of about eighty larvae of 10 to 11 mm. in body length.

The testes, and ovaries as well, were taken out of the living insects by vivisection and immediately put into the fixatives. The

1) One more species, *Oecanthus indicus* is reported from Formosa Island, but this species is not recorded from the main-land of Japan.

testis of this insect is nearly spherical in shape, covered with a thin membrane-like fatty tissue, in contrast to the case of other grasshoppers and locusts, in which the seminal tubules are destitute of such covering. For this reason the best preservation of the chromosomes is to be obtained only when the covering is broken by means of fine needles, as attempted in the case of *Diestrammena* by the author ('31).

Although various fixing fluids, such as FLEMMING's, HERMANN's and BENDA's mixtures were employed, the best results were obtained by using MEVES' mitochondrial fixative. The formula¹⁾ is given as follows.

0.5% chromic acid in 1% NaCl-solution	15 c.c.
2% osmic acid	4 c.c.
Glacial acetic acid	1 drop

After fixation in this fluid for 20 to 24 hours, the objects were washed thoroughly in running water for the same length of time. After dehydration by passing through alcohol of various grades, the material was brought into absolute alcohol, which is then replaced by creosote-toluol, toluol, toluol-paraffin successively and at last imbedded in paraffin as usual. Sections were cut 13 to 15 micra in thickness. Staining was with HEIDENHAIN's iron-haematoxylin and light green. However, FLEMMING's triple staining and the combination of thionin and fuchsin as well, seemed to be more advantageous for some special purposes.

All the text-figures were drawn with the aid of ABBE's drawing apparatus at the level of the desk on which the microscope was set, using a 1.5 mm. ZEISS apochromatic objective and a K.20 compensating ocular. The magnification is uniformly about 4000 times. Considering from the relative magnitude, the cell-size in this insect

1) This is cited from ROMEIS, "*Taschenbuch der mikroskopischen Technik.*"

The modification of this formula, in which chromic acid is in 1 or 1.5% and with no trace of glacial acetic acid, seems to be adequate for the chromosomes of higher Vertebrata, e.g., Amphibia, as proved in my experiments, reported in the former papers ('32).

is much smaller as compared with other orthopteran insects and somewhat resembles that of the rat or mouse.

Descriptive

A. Idiochromosomes¹⁾

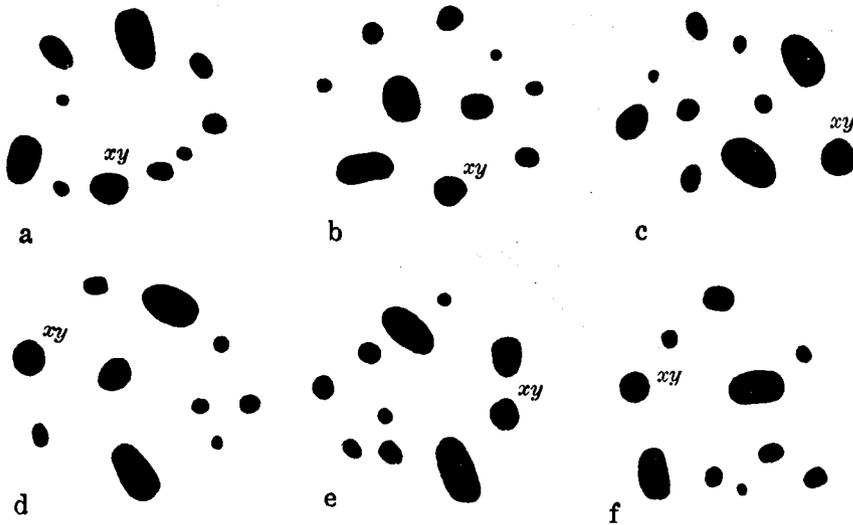
For convenience of explanation of the behavior of the idiochromosomes, the primary spermatocytes will be dealt with first.

1. *Idiochromosomes in the primary spermatocyte.*

The bivalent chromosomes of the first division constitute the metaphase plate in which every chromosome is separated from one another in distinct clearness as shown in Text-fig. 1. They are quite peculiar in arrangement upon the equator, contrary to the ordinary manner, in which the larger chromosomes encircle a central space where the smaller ones are scattered. In the present case, the small chromosomes are frequently found distributed in the peripheral zone of the equatorial plate without definite arrangement (Text-fig. 1, a-f and Figs. 37-39). Besides, the mode of arrangement of chromosomes is not so constant as in the dragonfly where their concentric ring arrangement was reported by OGUMA ('30) in the primary spermatocyte. In the majority of cases, however, the central space of the equatorial plane is occupied by either large or small chromosomes (Text-fig. 1, b-f), while it is rarely left blank without any chromosomes at all (Text-fig. 1, a).

In the polar view of the equatorial plate, the chromosomes show distinct and constant size differences. They are ten in number, of which four are distinctly large in contrast to the six remaining chromosomes (Text-fig. 1 and Figs. 37-39). Of these ten

1) The descriptive term "idiochromosomes" that will be employed throughout this paper, is based on WILSON's terminology ('65), in which he states as follows: "The term idiochromosomes will be applied to the two chromosomes, usually unequal in size which undergo a very late conjugation and subsequent asymmetrical distribution to the spermatid-nuclei."

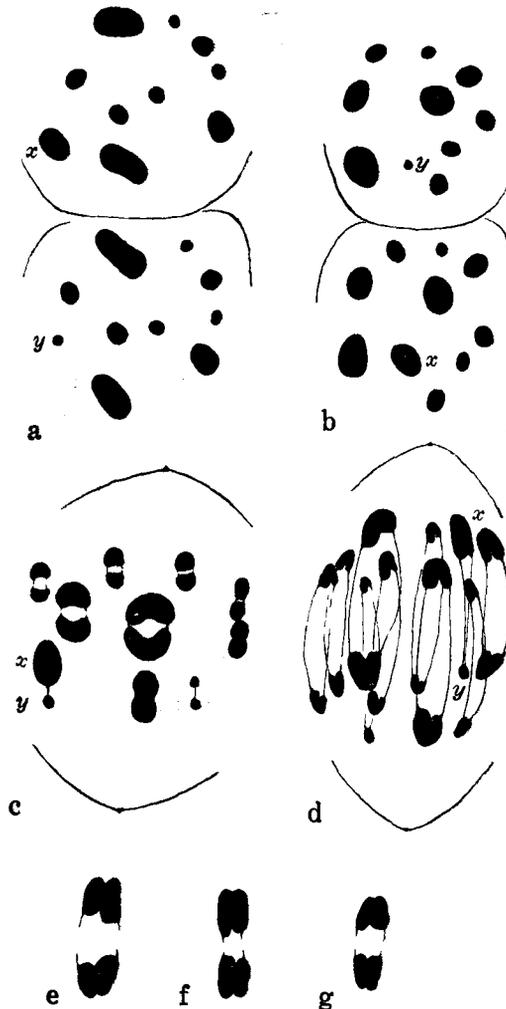


Text-fig. 1. a-f, Polar views of metaphase groups of chromosomes, primary spermatocyte division, 10 tetrads. The XY-chromosome (*xy*) always takes position in the peripheral part of the spindle.

chromosomes, nine take the ordinary bipartite appearance, representing ring or dumbbell-shape in lateral aspect. But the remaining one, which always occupies the peripheral position of the equator and belongs to the large category, shows a quite particular form. It presents in polar aspect rather a circular outline (Text-fig. 1, *xy*), while the other chromosomes of the large group are in the shape of short rods with blunt ends. When viewed from the side of the spindle, however, it is clearly recognized as an asymmetrical tetrad composed of unequal halves in striking contrast to the other nine tetrads, all of which have a symmetrical structure (Text-fig. 2, c-d and Figs. 22-23, *xy*; Figs. 40-42).

The inequality of size in the two composing parts of the asymmetrical tetrad is very striking; the large element attains a size, intermediate between the second and the third largest autosomes, while the small one is comparable with the smallest granule-like

chromosome. This size-relation of the components is to be accounted for only when we take the asymmetrical tetrad as the XY-bivalent, a heteromorphic tetrad of idiochromosomes, as fully established by study of the diploid groups and the growth period. In both two



Text-fig. 2. a-b, Polar views of daughter complexes of primary spermatocyte anaphase chromosomes. c-d, Side views of anaphase chromosomes of primary spermatocyte division. e-g, Three different anaphase chromosomes. The longitudinal splitting along the long axis is seen in each separating chromosome.

components of the idiochromosome-bivalent, the spindle fiber is constantly attached to their free ends, opposite the point where they come in contact, or in other words, they have telomitic fiber attachment. They are connected to each other end to end by means of fine linin fibers.

In anaphase, all the chromosomes alike divide transversely, and the daughter chromosomes appear like a pair of chromatids in close contact (Text-fig. 2, e-g). In well preserved preparations we can find such features not only in autosomes but also in the idiochromosome-bivalent (Text-fig. 2, d and Figs. 23-24). The ring tetrads, which are seen in the diakinetik stage or even in the metaphase side view, are, therefore, to be considered as composed of four rod-shaped chromatids, of which every two constitute a semi-circle in a similar manner in principle to the diaschistic tetrads commonly found in the Orthoptera (McCLUNG, '14), contrary to the view expressed by PAYNE ('14) for *Forficula*. On the other hand, they are not to be compared with the vertical ring tetrads in Amphibia. The mode of the tetrad formation will be described in detail in a later section. While the daughter halves of the autosome tetrads become separated from each other, the idiochromosome bivalent also disjoins into two unequal components as seen in unmistakable clearness in the side view of the spindle (Text-fig. 2, c and d). In fact, we know, after the present division, X and Y are distributed separately into two daughter cells or the secondary spermatocytes (Text-fig. 2, a-b, *xy*). As readily recognizable from the figures, each daughter cell possesses similarly ten chromosomes, of which one is strikingly larger in one cell, as compared with the corresponding one in another cell, representing X- and Y-component respectively. Thus, the present division produces two different classes of secondary spermatocytes, in spite of the equal number of chromosomes, in respect to the idiochromosomes.

Like the idiochromosome, the separation of chromatids is synchronous in all autosomes. The former neither lags behind nor pre-

cedes the autosomes (Text-fig. 2, c and d), although the precocious division of the XY-bivalent is known as a common phenomenon in the Hemiptera (WILSON), some Coleoptera (STEVENS, '09) and Diptera (STEVENS, '08). In the telophase, however, the X-component usually lags a little on the way passing to the pole and thereby stands independently from the other autosomes which have already become crowded at the pole (Figs. 25-26), though soon it reaches the latter. The formation of the chromosome vesicle never takes place in the X-chromosome as described by BAUMGARTNER ('04) about *Gryllus*.

2. *Idiochromosomes in the growth period.*

It is my purpose to trace the complete history of the chromatin nucleolus up to transformation into the idiochromosome-complex during the growth period. So the general history of the autochromatin during this period will be described briefly under the present topic.

In early stage of the growth period, the nucleus of the auxocyte is characterised by containing two, well-defined nucleoli, one of which represents the chromatin nucleolus (Figs. 4-5, *h*) and the other the plasmosome (*p*). The chromatin nucleolus is clearly distinguished from the plasmosome by its size and peculiar outline, as well as its more intense affinity to dye stuffs such as haematoxylin, safranin or thionin. The plasmosome, on the contrary, appears not so compact in texture as compared with the chromatin nucleolus and is nearly spherical in shape. Attention should be paid first to the destiny of this chromatin nucleolus.

After the final spermatogonial division, in which all the chromosomes are converted into irregular bodies with vague outline, there is always in the nucleus found only a single, large and spherical plasmosome stained homogeneously (Fig. 3), but the actual existence of the chromatin nucleolus cannot be proved. How the chromatin nucleolus above mentioned is brought about in the follow-

ing stage is quite obscure to me. So far as my material shows, the chromatin nucleolus can be traced backwards only up to the stage described above (Fig 4), but not be followed to the spermatogonial telophase as in the cases of some Hemiptera (*Lygaeus*, *Oncopeltus*, WILSON, '12) and mantis (OGUMA, '21). At any rate, in the earliest stage so far I could observe, the chromatin nucleolus takes a clavate form and comes to a position close to the nuclear wall (Figs. 4-5, *h*). Usually it lies widely separated from the plasmosome with a remarkable contrast in size and form (Fig. 4). In minor cases, however, they lie very closely together, nearly in contact as seen in Fig. 5. The association between the chromatin nucleolus and plasmosome which has been reported as occurring quite frequently in Coleoptera (STEVENS, '09) Diptera (STEVENS, '08) and *Gryllotalpa* (WINIWARTER, '27) could not be proved in the present case.

In parallel with advancement of growth, a remarkable change takes place in the nucleus in relation to the shape of the chromatin nucleolus, while the auto-chromatin still remains in diffused condition. In the first place, the chromatin nucleolus sends out a fine chromatic thread which appears as if budded out from it (Fig. 6, *h*). The chromatic thread grows longer step by step, probably pulled out from the main body of the chromatin nucleolus and attains at last a considerable length (Figs. 7-9, *h* and Fig. 43). The thread is stained uniformly to the main part and shows a sharply defined contour. Sometimes, undulation occurs in a slight degree along the long axis of the thread as shown in Figs. 7-8, *h*. But, there are observed no chromatin knobs whatever upon the thread. The main body of the chromatin nucleolus, from which the thread is drawn out, gradually becomes spherical and smaller in size, due possibly to the expenditure in the formation of the thread.

When its full length is attained in growth, the terminal part of the chromatic thread thickens so as to form a minute bead-like shape (Figs. 10-11 and Fig. 44). In this condition, accordingly, the chromatin nucleolus exhibits for the first time the distinction between the two components, of which the main part is, as the later

history shows, transformed into the X-element, while the bead-like body appended to the former is destined to be converted into the Y.

The thread part now begins to be shortened by condensation in making up the Y and the main body to be slightly elongated, until they are converted into the form characteristic to the idiochromosome-complex (Fig. 12, *xy*). Such transformation is completed in short duration in the earlier part of the growth period included under the stage "preleptotene," in which the auto-chromatin is still in confused appearance.

In the leptotene stage, which is clearly distinguishable from the preceding stage by having thin long threads intermingled with one another, the chromatin nucleolus does not show further transformation but remains in the form as given above (Fig. 13, *xy*). When the zygotene stage sets in the XY-complex migrates to the pole where the leptotene threads are to be polarized with the plasmosome (Fig. 14, *xy*). Sometimes, a longitudinal split appears at this time along the long axis of the X-element. After the chromatin threads grown gradually thicker and shorter into the pachytene condition give up their polarization at a pole of the nucleus and become scattered about through the nucleus, the XY-complex still remains in contact with the nuclear wall and always produces a clear longitudinal split along the long axis of the X-element (Fig. 15, *xy* and Fig. 45). In the diplotene stage when the pachytene threads begin to assume the appearance of tetrad, it still persists in a similar condition as found in the foregoing stage showing variation in minor degree, while the plasmosome decreases in its color affinity and commences to fade away (Fig. 16, *xy*, *p*, and Fig. 46). The XY-complex still further keeps its form unchanged, not only throughout the entire duration of the growth period but also during the prophase of the coming division, where the conspicuous ring tetrads are observable (Figs. 17-20).

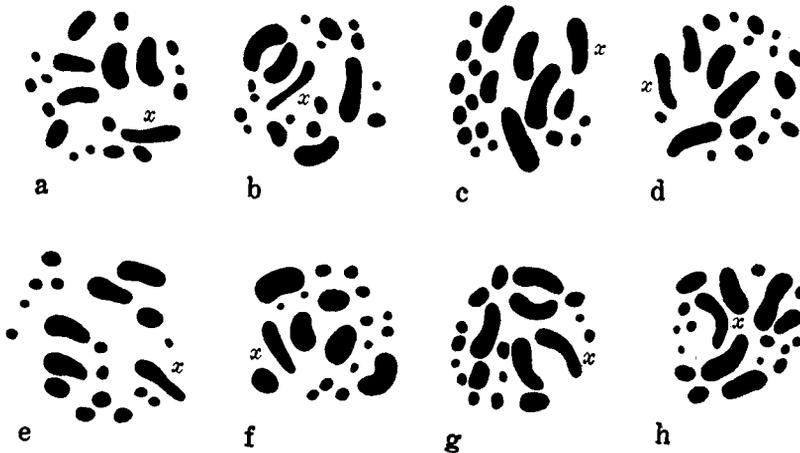
As the process of condensation is completed in autosome tetrads, the XY-complex assumes a definite shape, with which it comes into metaphase form (Figs. 19-20). The idiochromosome-

complex is exceedingly distinct from the autosome tetrads due to its characteristic form.

3. *Idiochromosomes in the spermatogonium.*

The relation of the chromosomes of the primary spermatocyte to those of the spermatogonium will be dealt with under this topic.

Though I could scarcely observe the metaphase spermatogonial chromosomes in the earliest generation on the material collected so late in the season, sufficient results still were obtained including the later generation of the spermatogonial division. A spermatogonium possesses twenty well defined chromosomes (Text-fig. 3, a-h). In none of the animal, in which I have made counts, was found any numerical deviation from the normal number. The chromosomes arrange on the equatorial plane in quite irregular fashion as comparable with the case of the primary spermatocyte division. So far as I am aware, there is no case in the Orthoptera, in which the chromosomes take similar arrangement as seen in the present species. The present



Text-fig. 3. a-h, Polar views of metaphase groups of chromosomes, spermatogonial division, 20 chromosomes, 7 large and 13 small. The X-chromosome (*x*) can be distinguished from the other autosomes owing to its slenderness in size, but the Y, which is considered to be one of the smallest granule-like chromosomes, can not be pointed out decidedly.

case is, therefore, to be noticed as a new type for the arrangement of orthopteran chromosomes. They do not take radial arrangement in the equatorial plate, in contrast to the usual case in Orthoptera in which larger ones surround the central space where the smaller ones lie. At first sight the equatorial plate of this species appears to be that of Hemiptera rather than Orthoptera, so far as the mode of chromosome arrangement is concerned. According to their size and form, the chromosomes are to be distinctly classified into two groups of chromosomes, large and small. The larger chromosomes are rod-shaped gently curved and seven in number. They are not equal in length but show slight differences, and most of them occupy the central part of the equator in their arrangement. The smaller ones are thirteen in number and range in shape from the extremely short rods with oblong contour to minute globules like a spot. They usually arrange themselves in the peripheral zone of the equatorial plane.

In division, each chromosome separates lengthwise into two daughter halves as shown in Fig. 2. It follows that the chromosomes have terminal fiber attachment.

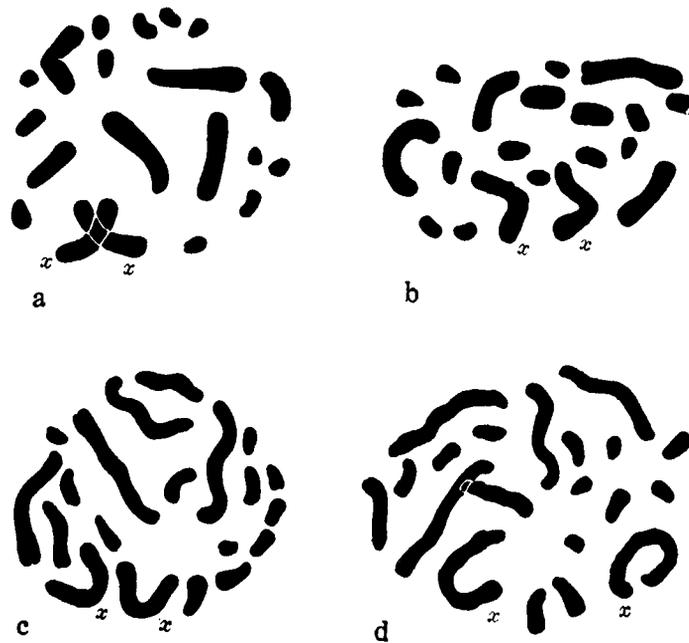
As already stated, both large and small chromosomes show similarly the odd number, as the former being seven and the latter thirteen. When pairing occurs in every two homologous mates, in the synaptic stage later on, one chromosome in each group, large and small, is left without a mate of like size. In other words, there are three homologous pairs of large chromosomes and six homologous pairs of small ones, and two remaining chromosomes without mates, of which one belongs to the category of large chromosomes and the other to that of the small ones. It is these two remaining chromosomes that constitute the X-Y pair of unequal size. I tried to make clear the homologous pairs by serial arrangement, though my attempt could not bring about any accurate results since the chromosomes are not constant both in form and in apparent size. If possible errors of identification will be taken into account, twenty chromosomes of a spermatogonium may be arranged into nine sym-

metrical pairs and one asymmetrical pair as shown in Text-fig. 9, *b*. It is needless to say that of the pair to the extreme right in this figure, the large chromosome is the X and the small one the Y. The X-chromosome thus identified is distinguished with extreme clearness from the other autosomes, owing to its thinner form, and the fact that it is sometimes curved (Text-fig. 3, a-h and Fig. 36, *x*). Its relative position in the equatorial arrangement does not seem to be constant; in some cases it is located in the peripheral zone while in others in the central part. Considering from size and form, the X-chromosome is not the largest one at all, even in a set of univalent chromosomes, not only in metaphase (Text-figs. 3 and 9) but also in prophase (Fig. 1, *x*). In this respect the present author hesitates to accept the view of OHMACHI ('27), who maintains that the X-chromosome is represented by the largest one in *Oecanthus longicauda*. So far as the present results go, the X seems to rank second or third in a serial arrangement of the univalent chromosomes. The Y-chromosome, on the other hand, is hardly to be distinguishable from the other small autosomes in the spermatogonium. It is certain, however, that the Y is one of the smallest granule-like chromosomes.

4. *Idiochromosomes in the diploid group of the female.*

To draw out the final conclusion on the significance of the idiochromosomes in relation to sex determination, it becomes quite necessary to examine them in the cells of the female line. For this purpose the follicle cells were employed as the most available material. In these cells I could find plenty of dividing figures of chromosomes, which enabled me to make clear the condition of idiochromosomes in the female diploid garniture. The female cells do not differ from the spermatogonia in arrangement and form of chromosomes. There are twenty chromosomes in every equatorial plate which I have made counts (Text-fig. 4, a-d.). The chromosomes can be distinctly divided into two groups of chromosomes, large and small, as in the spermatogonium, and the homologous pairs

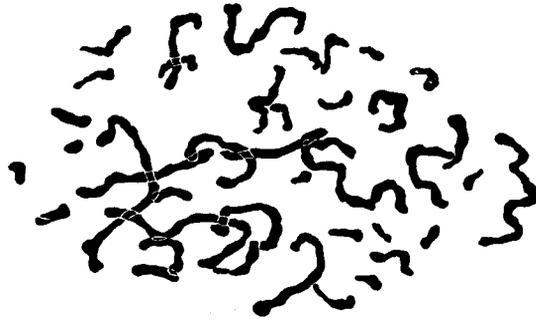
can easily be distinguished according to their size and form. The number of large chromosomes is eight and that of small ones twelve. Therefore, all of them constitute ten pairs of equal size, and there remains no chromosome without a mate of corresponding size. Compared with the male diploid group, the female has in reality



Text-fig. 4. a-d, Polar views of metaphase groups of chromosomes in the cells of follicle epithelia in female, 20 chromosomes, 8 large of which 2 are XX-chromosome pair (xx), and 12 small.

one more large chromosome identical with the X, instead of the Y; consequently there is not to be found a pair of unequal size as in the male (Text-fig. 9, a). These two X's are easily distinguished from the autosomes not only by their size and form but also by their relative position in the equatorial plates.

Though extremely seldom, I found some cases of giant cells in the follicle, in which tetraploid number of chromosomes was actually counted. There are forty chromosomes, of which sixteen are



Text-fig. 5. Polar view of chromosomes in the tetraploid cell of female follicle epithelium, early metaphase. 40 chromosomes, 16 large and 24 small.

large and the remaining twenty four are small as shown in Text-fig. 5. It probably resulted by a fusion of two daughter nuclei after mitosis as suggested by WILSON ('25).

5. *Idiochromosomes in the secondary spermatocyte.*

There is no distinct 'resting stage' between the two maturation divisions, and no nuclear vacuole is formed. The telophase chromosomes enter prophase of the next division *in situ*.

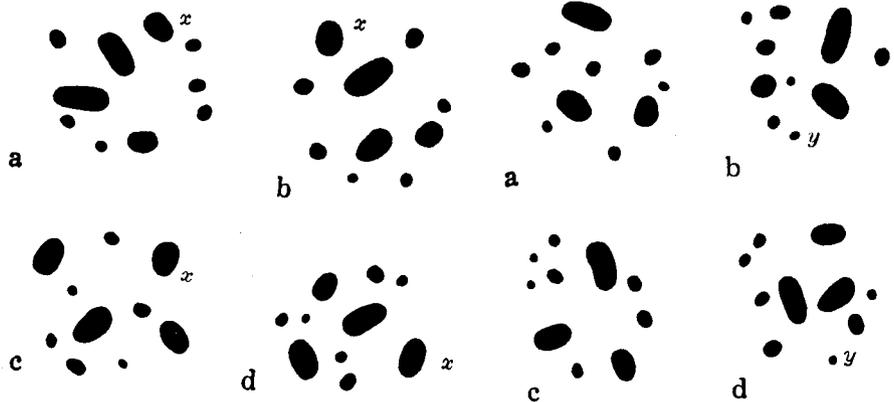
At the close of the first division, the telophase chromosomes are seen somewhat crowded together, retaining radial arrangement to some extent (Fig. 27). The chromosomes show no more regressive changes, neither do they fuse together nor disintegrate into fragments. We can count, at this time, ten chromosomes in every case without difficulty. When the cell body divides completely, the chromosomes become well separated again from one another (Fig. 28).

Throughout this period of interkinesis neither the X- nor Y-chromosome presents particular behavior, as compared with the other autosomes.

The interkinesis seems to be of rather brief duration, because the cysts contain the cells of every successive stage between the late anaphase of the first division and the metaphase of the second.

Towards the end of the prophase of the second division, a definite spindle is formed and the chromosomes spread further apart (Fig. 29).

There are found ten chromosomes in every equatorial plate of the secondary spermatocyte metaphase, in quite similar arrangement to the primary spermatocyte (Text-figs. 6-7). However, we can not fail to find that there are two different garnitures of chromosomes, the X- and Y-class, in the secondary spermatocytes, which are caused by the asymmetrical distribution of idiochromosomes in the first division. One kind of garniture consists of four large and six small chromosomes, one of the large chromosomes being identified by nothing but the X (Text-fig. 6, a-d and Figs. 47-48). In comparison with the chromosomes of the Y-class cell, the X-chromosome may be represented by the third largest one in the series of four large chromosomes, which lies always in the peripheral part of the equator. This fact can be seen by careful

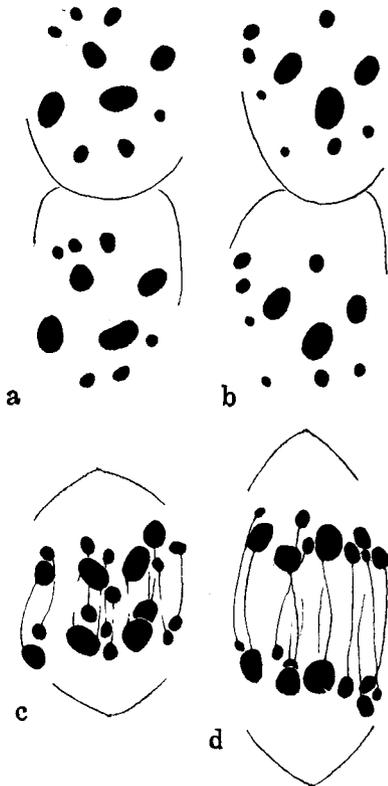


Text-fig. 6. a-d, Polar views of metaphase groups of chromosomes, secondary spermatocyte division, X-class, 10 dyads, 4 large and 6 small. *x*, X-chromosome.

Text-fig. 7. a-d, Polar views of metaphase groups of chromosomes, secondary spermatocyte division, Y-class, 10 dyads, 3 large and 7 small. *y*, Y-chromosome.

comparison with the large chromosomes in both kinds of cells. The other kind of garniture is, on the contrary, composed of three large and seven small chromosomes, of which the Y-chromosome may be indicated by one of the two smallest, granule-like chromosomes of nearly equal size, situated always at the peripheral part of the equator (Text-fig. 7, a-d and Fig. 49).

In the ensuing division, all the chromosomes divide equally and synchronously in both cases of the X- and Y-class, and there is found neither precession nor succession in particular chromosomes (Text-

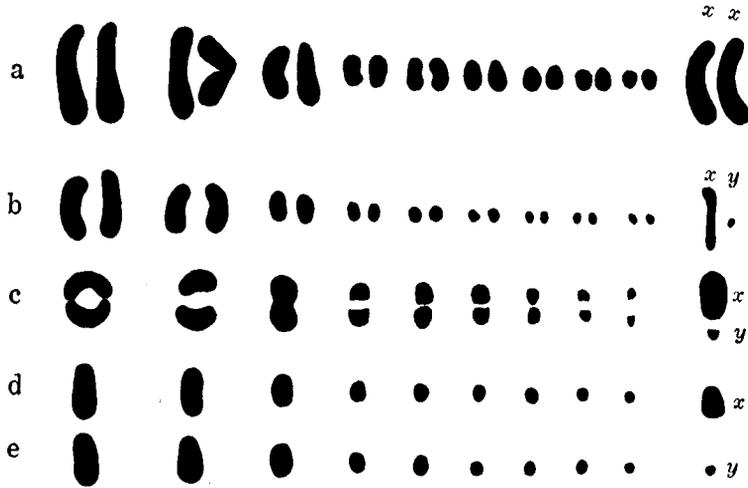


Text-fig. 8. a, Polar views of daughter complexes of secondary spermatocyte anaphase chromosomes, X-class, 10 monads in each plate. b, The same, Y-class. c, Side view of secondary spermatocyte anaphase, X-class. d, The same, Y-class.

fig. 8, c, d). In polar or slightly oblique view of the late anaphases, when both daughter groups are visible by changing foci in one section, the symmetrical distribution of all chromosomes may plainly be demonstrated either in the X-class or in the Y (Text-fig. 8, a, b). Each spermatid-nucleus thus produced receives ten chromosomes, one half the spermatogonial number; nevertheless they are to be classified into two classes in respect to the idiochromosomes.

In Text-fig. 9 I desire to indicate how the chromosomes maintain their relationship to each other in size and shape in different kinds of the male germ cells as in the female follicle cells. These arrangements show conspicuously not only the sexual differences, as readily recognized by a comparison of a and b, but also the distribution of the idiochromosomes during the matu-

ration divisions in *c*, *d* and *e*. There may be, of course, some errors to be accounted for in an arrangement of this fashion.



Text-fig. 9. a, Paired arrangement of supposed homologous mates of female diploid chromosomes from metaphase polar view. b, The same, spermatogonial chromosomes. c, Serial arrangement of chromosomes of primary spermatocyte from side view of early anaphase. d, Serial arrangement of chromosomes of secondary spermatocyte of X-class from metaphase polar view. e, Serial arrangement of chromosomes of secondary spermatocyte of Y-class from metaphase polar view.

6. *Idiochromosomes in the spermatid.*

In the late telophase of the second division, a formation of nuclear membrane takes place, within which the chromosomes begin to disintegrate (Fig. 30). Fig. 31 is a spermatid of a little later stage just described and shows the growth of the nucleus and the advancement of chromosome disintegration in comparison with the former. In cells of this stage, it is difficult to distinguish the idiochromosomes from the autosomes in the course of disintegration. Following this period, the disintegration of chromosomes continues still further until they almost completely disappear. In the latter part of this stage, the X- and Y-chromosome become recognizable

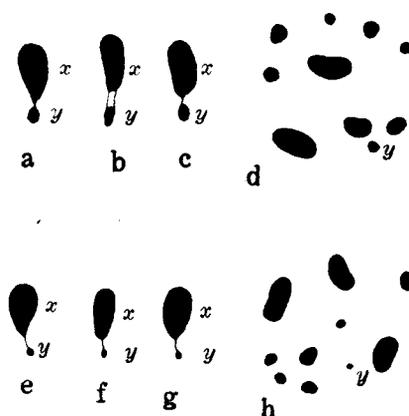
as a compact, deeply staining body with very sharp contour, together with a spherical-shaped plasmosome which is markedly distinguished from the former not only by its form but also by different affinity to dyes (Figs. 32-33). At this time, one can not fail to observe that there are two kinds of spermatids in respect to their chromatic inclusions. One of them is of the X-class in which the X-chromosome is found in the form of a deeply stained rod slightly curved (Fig. 32 and Fig. 50, *x*). The other is of the Y-class having the Y-chromosome of a minute granule-like shape instead of the X in the former kind (Fig. 33 and Fig. 51, *y*). This stage appears to be of rather long duration in the history of the spermatids, because in every cyst most of the spermatids are in this condition. Moreover, the appearance of the plasmosome would emphasize this supposition to a great extent.

Thus, with the X- and Y-chromosome we are able to follow them respectively as the chromatin nucleoli up to the early stages of transformation into spermatozoa (Figs. 34-35, *x*, *y*).

B. Size diminution of the Y-chromosome.

During the course of investigation I have found a few individuals with the Y-chromosome of considerably diminished size.

Before going further, we must call to mind that the size of the chromosomes may vary by several causes. Such variation always depends, on one hand, upon the method of fixation employed. For instance, after fixation in HERMANN's fluid the chromosomes appear larger than after the use of FLEMMING's solution. On the other hand, size variation is due to the staining method applied. Even if the same method is employed in staining, the duration of staining or the degree of differentiation produces surprising effects upon the apparent size of the chromosomes. From these reasons, I have examined, therefore, the slides prepared under the same condition; fixed in MEVES' mixture for 24 hours, mordanted in 4% aqueous solution of iron alum for 8 hours and stained with HEIDENHAIN's haematoxylin for 20 hours.



Text-fig. 10. Side and polar aspects of Y-chromosome showing size difference as found in different individuals. a-d, The most usual cases in size of Y-chromosome as found in a large number of specimens observed (a-c, side views of XY-bivalent in first division. d, polar view of second division). e-h, Views of Y-chromosome with diminished size as found infrequently in some specimens (e-g, side views of XY-bivalent in first division. h, polar view of second division).

In the majority of cases the Y-chromosome assumes the size as shown in Text-fig. 10, *a-d*, and this size may be taken as the original type of the Y-chromosome in the present species.

Extremely rare, though the cases may be, some individuals are found in which the Y-chromosome is reduced to about one-third the original size (Text-fig. 10, *e-h*, and Fig. 42). This size-relation of the Y-chromosome is constant in one and the same individual. Text-fig. 10, *e-h*, was drawn from one individual with the diminished Y-chromosome, to show how the Y keeps constant size through both the first and the second divisions. The reason why the diminution of the Y-chromosome is not due to chemical influence may be explained in the following ways: In comparison of chromosomes between two individuals, one of which has the Y-chromosome of original size and the other that of diminished size, we see that the X-chromosome remains unchanged (see Text-fig. 10, *a-c* and *e-g*); and, in several slides prepared from one and the same testis, the Y-chromosome always shows a similar condition of size.

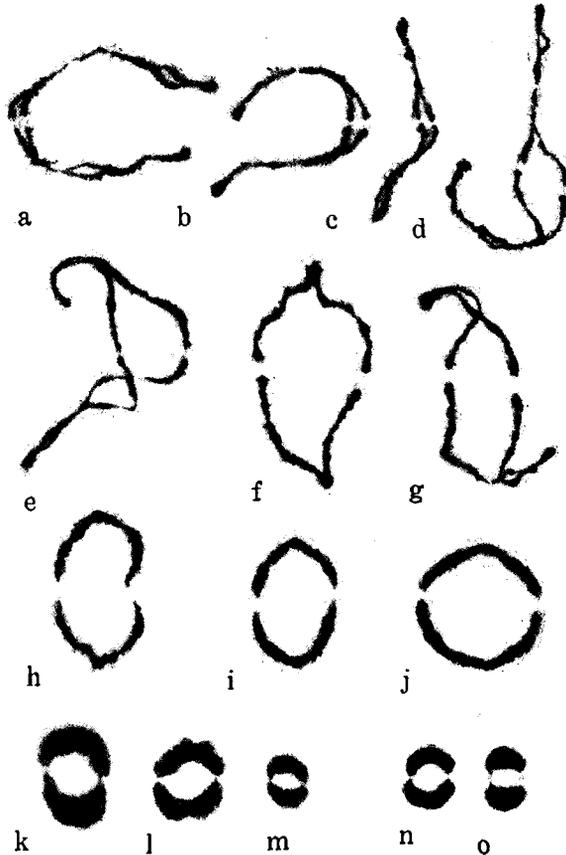
This evidence of size-diminution occurring in the Y-chromosome will give us some light on the question of the evolution of the sex chromosome, supporting WILSON's view ('11), when it is taken as a process of transformation of XY-type into XO which arises by the disappearance of the Y-element through gradual and progressive reduction. However, I hesitate to draw any definite conclusions from the above observations; since the material of this study is somewhat insufficient.

C. On the ring tetrad.

As already stated, all the tetrads, except two or three small rod-shaped ones, show typical ring structure in the prophase stage of the first division. The formation of these ring tetrads will be discussed in detail under the present topic.

It is clear, from close examination, that in every ring tetrad the chromatids from which the tetrad is composed, present a single nature, or in other words they do not provide any longitudinal splitting, or double nature. In this point they are completely different in principle from the so-called ring tetrads, as usually found in the Orthoptera and Amphibia. The ring is divided into two semi-circles by two median cross-sutures where they come in contact as shown in Text-fig. 11, *h-j*. Each of the semi-circles is in reality composed of two equal rods joined at a median point as shown later, although it is still obscure in this stage. As the chromatin condensation advances in every chromatid, the rings become gradually smaller and thicker (Text-fig. 11, *o-k* and Figs. 19-20). After the nuclear membrane fades away, they are brought about upon the equatorial plane of the coming division in vertical position with the median sutures on this plane (Text-fig. 2, *c*). Then they are divided into two composing semi-circles at anaphase of division. At this time, in the median point of every semi-circle a suture becomes visible for the first time, and a semi-circular daughter chromosome bends at this point producing double chromatids, which very frequently come in contact along their entire

length as shown in Text-fig. 2, *e-g*. Still later, the two chromatids thus closely applied with each other again open out to form simple V's (Text-fig. 2, *d*), representing biterminal attachment of the spindle fibers at the apex of the V. In the second division the V breaks into two composing rods.



Text-fig. 11. Various forms of chromosome threads during growth period, showing the formation of ring tetrads. a-d, Chromosome threads in late pachytene stage. e-g, Chromosome threads in half-way stage of ring formation, late diplotene stage. h-j, Ring tetrads in diakinesis. k-m, The same in late diakinesis. n-o, The same immediately after disappearance of nuclear membrane of primary spermatocyte.

From the above stated facts, the tetrad of this type may be considered as a modified form of the diaschistic tetrad with terminal attachment of fibers (WILSON, '25, p. 520). In other words, the

ring tetrad of the present case is derived from a diachistic tetrad in which the opening occurs between every two sister rods from where the two composing halves of the tetrad come in contact, as the sister rods become entirely separated except at the point of fiber attachment. This process of opening-out has already taken place in the earlier stage of the growth period far prior to the diakinesis (Text-fig. 11, a-g).

In *Forficula*, PAYNE ('14) described a ring tetrad of similar appearance but of different mode of formation, as compared with that of the present species. In brief, the ring tetrad in his case was formed by the gradual bending of a chromatin thread with a longitudinal split, as both free ends came in contact. This mode of ring tetrad formation has been known to occur in several species of Forficulidae as reported by MORGAN, W. P. ('28). So far as my material is concerned, the similar mode of tetrad formation is not actually observed in the case of *Oecanthus*. This fact compels us to contend that their conception might involve errors concerning the tetrad formation in *Forficula*, owing to their lack of knowledge on the complete history of the ring tetrad in the growth period, and on the correlation between the tetrad formation and spindle fiber attachment.

General considerations

1. *The form and arrangement of spermatogonial chromosomes.*

Considering from the morphological point of view, the spermatogonial chromosome of *Oecanthus longicauda* is quite sharply discriminated from that of the other orthopteran species, in the type of the sex chromosome as well as in the manner of the arrangement in the equatorial plate. In the present species the chromosome does not take a definite arrangement on the equator of metaphase, as the smaller ones are surrounded by the larger chromosomes which assume a rosette form in arrangement. In other words, they ar-

range themselves on the equator at random, without any definite relation in their arrangement. So far as I am aware, such a manner of arrangement has not been described in the spermatogonial chromosome by any author, who has studied the orthopteran spermatogenesis in general. McCLUNG ('14), as is well known, in the comparative study of the orthopteran chromosomes, established two types, *Hippicus* and *Stenobothrus* type, in the spermatogonial chromosome, both of which do not agree with the present case not only in the type of sex chromosome but also in the manner of chromosome arrangement. Even in the Gryllidae the present species should be a unique example, for, in all cases studied by BAUMGARTNER ('04), HONDA ('26), OHMACHI ('27, '29) and TATEISHI ('31), the chromosome shows nothing different from the species of the other orthopteran families in their metaphase arrangement.

One will recall, on the contrary, that a similar manner of chromosome arrangement as observed in *Oecanthus longicauda*, is usually known not in Orthoptera but in Dermaptera, Trichoptera and Hemiptera. At present, we know the chromosomes in several species belonging to Forficulidae of Dermaptera, studied by STEVENS ('10), PAYNE ('14) and MORGAN ('28), and in a few species of *Limnophilus* as the trichopteran representatives, recently investigated by KLINGSTEDT ('31). In these forms, however, all the chromosomes are of nearly equal size without any conspicuous size difference like the present species. So far as the size and form of chromosomes are concerned, the Hemiptera seem to be rather closely allied in appearance to the present species. Especially, the spermatogonial chromosomes of *Syromastes* and *Pyrrhocoris* (WILSON, '09) exhibit much similar appearance to *Oecanthus longicauda*, at least in their mode of arrangement.

2. Comparison of chromosomes in related species.

Now the important problem of comparing the chromosomes in two closely related species of *Oecanthus*, will be brought under consideration. In his paper concerning the cytoplasmic inclusions of

the Gryllidae, JOHNSON ('31)¹⁾ has shown as the diploid number, nineteen chromosomes in *Oecanthus nigricornis*, which consist of five large V-shaped, one of them being the X, and fourteen short rod-like ones. They arrange radially on the equatorial plate, the larger chromosomes occupying the peripheral part surrounding the smaller ones. The chromosomes of *Oecanthus nigricornis*, as stated above, represent a very similar feature in every respect to other Orthoptera, and it is selfevident that one can hardly find any correlated phenomena between these two related species, *Oecanthus nigricornis* and *O. longicauda*, in respect to chromosome morphology. This striking fact indicates further that the correlation between the chromosome morphology and the taxonomy does not appear in parallel as so often believed, and still further that at least in the present case, it will be impossible to discuss the taxonomical characteristics by means of the morphology of chromosomes.

3. Deviation of chromosome number.

In several species of the Gryllidae, chromosomes deviating from the normal number have been observed. HONDA ('26) reports a case in *Loxoblemmus arietulus*, in which he finds three kinds of deviated numbers, thirteen, fourteen, and fifteen, which are caused by the linkage of two non homologous autosomes. Recently a homologous evident is mentioned by TATEISHI ('31) in *Brachytrupes portentosus* which he explains as multiple chromosomes. But a similar occurrence of number deviation has not actually been observed in *Oecanthus longicauda*. Besides, in the present species a heteromorphic homologous pair of autosomes

1) In this paper, JOHNSON ('31) describes a peculiarly formed centriole which is V-shaped during the growth period to primary spermatocyte division and rod-form in the secondary spermatocyte. Whether a similar fact also occurs in the present species remains still obscure, as the staining methods applied in the present study are not available for the demonstration of the centriole. However, in some preparations, I could find at least the "terminal vesicle," a related structure of the V-shaped centriole, which seemed to be of the same nature with that described by JOHNSON. In the near future, I wish to study critically the structure of the centriole of the present species.

as described in *Nemobius yezoensis* by OHMACHI ('27), which seems to be of the same nature as those reported by CAROTHERS ('16) and ROBERTSON ('15), can not be detected.

4. *The XY-chromosome.*

With regard to the sex chromosome of the Orthoptera, there are three types which have been hitherto investigated, all in agreement to be of male heterogamety. The first of them is the XO-type and the majority of cases studied belongs to this type. The second type is composed of the multiple X-Y chromosomes. One example of this type is observed in the mantis, *Paratenodera* and *Tenodera* by OGUMA ('21) and the other one is reported in a species of the mole-cricket, *Gryllotalpa borealis* by PAYNE ('16). The last one is the XY-type, which is confirmed in *Gryllotalpa vulgaris* from Belgium and Germany by WINIWARTER ('27) and in *Oecanthus longicauda* by the present author.

As just stated, there have been reported only two cases which belong to the XY-type among the Orthoptera, *Gryllotalpa vulgaris* and *Oecanthus longicauda*. WINIWARTER ('27) counts twelve chromosomes in the spermatogonium of *Gryllotalpa vulgaris* which consist of eleven V-shaped and one granule-like Y-chromosome. The X-chromosome of this species is represented by a V-shaped body of intermediate size with median fiber attachment, while the Y-chromosome can easily be pointed out among the other autosomes by its extremely small size, in striking contrast to the case of *Oecanthus longicauda*.

According to OHMACHI ('27), the shape of the X-chromosome is that of a V in most cases of the Gryllidae. This seems to be so with the species belonging to allied families such as *Diestrammena* (MAKINO, '31) and *Gryllotalpa* (PAYNE, '16, WINIWARTER, '27, OHMACHI, '29). In this respect the rod-shaped X-chromosome in *Oecanthus longicauda* is quite exceptional, with the case of *Cirtoriphus ritzema* in which OHMACHI ('27) has found a similar condition.

The size difference between the X- and Y-chromosome of *Oecanthus longicauda* is marked and in the lateral aspect of the maturation division the XY-bivalent is similar in form to that of some species of Coleoptera (STEVENS, '09), Diptera (STEVENS, '08) and Hemiptera (WILSON). In the Dermaptera, on the contrary, the inequality of the XY-chromosome is not so great and in some of the Hemiptera (WILSON), *Nezara*, *Mineus*, *Oncopeltus*, they become nearly equal.

As the case may be in the Orthoptera, Coleoptera, Diptera and Mammalia, the XY-chromosome of *Oecanthus longicauda* also undergoes preheterokinesis in the maturation division and hence two kinds of secondary spermatocytes are produced. They show neither precession nor succession in division, although these conditions of sex chromosomes are the most usual in other forms. There is nothing unusual observed about the division of the XY-chromosome in the present species.

Among the greater number of observed cases, the mantis affords the only example most parallel with the present result, in respect to the mode of development of the sex chromosomes during the growth period, in spite of duplicity of the X-chromosome. In either case, the ultimate form of the sex chromosome complex results from differentiation of one component from the other, with which the former was formerly combined, constituting a single chromatin nucleolus. In the present species, however, the process of transformation is completed already in the preleptotene stage, while in the mantis, it takes place in the later diakinetik nucleus. It has been repeatedly observed in other orthopteran forms, that the association between the chromatin nucleolus and plasmosome very frequently occurs during the growth period, but it has never occurred in the present material. In *Gryllotalpa vulgaris*, WINIWARTER ('27) describes and figures an elongated rod-shaped chromatic body, sometimes constricted in the middle region and closely attached to a plasmosome at one end, as an XY-complex which develops into XY-bivalent in the following division. STEVENS ('10) and PAYNE ('14),

in studies on *Forficula*, found that the single nucleolus transforms into an unequal pair of heterochromosomes during late prophase of the first division. In some other species of Dermaptera, MORGAN ('28) shows that there are two deeply staining nucleoli in the nucleus of the early growth period, and later they unite into a single nucleolus from which there arises the XY-complex in the first division. STEVENS ('08, '09), in many species of Diptera and Coleoptera, observes two chromatin nucleoli of large and small size in the growth period, which are well separated from each other, and closely associated with a plasmosome. In most cases of the Hemiptera, as explained by WILSON, the X and Y often remain separate throughout the whole growth period. KORNHAUSER ('14) discovered a peculiar example in *Enchenopa binotata*, in which a single straight non-granular and deeply staining thread is united with a broader and less deeply staining thread at one end in the leptotene nucleus. And it is this thread which is transformed into the XY-pair in later stages.

It is interesting to compare the XY-chromosome of *Oecanthus longicauda* with that of the rat studied by MINOUCHI ('28) as both have apparently the same structure in heterochromosome-complex. In comparing these two cases, I found first that they are in accordance with each other in having the terminal fiber attachment. In the rat, the chromatin nucleolus consists of two different substances, deeply stained coiled chromatin thread and the ground substance lightly stained, in early growth period. Later, the coiled chromatin thread becomes thicker and free from the ground substance, and finally the complete heterochromosome-complex, composed of a long, slightly curved rod (X) and a short rod (Y), appended at one end of the former, is formed. In the metaphase of the first division the heteromorphic tetrad of XY lies always in the periphery of the equatorial plane, X being parallel to it, Y almost perpendicular. X conjugates at the end of the fiber attachment with the free end of Y in the first division. Thus the

mode of formation of the XY-complex in the rat is almost entirely different from that of *Oecanthus* notwithstanding that the structure is apparently similar.

Summary

1. The chromosomes of the spermatogonium do not arrange in the general form of a rosette but are irregular in their metaphase arrangement. They are twenty in number, of which seven are large and thirteen small. The large chromosomes are all slightly curved rod-shaped and the small ones range in shape from somewhat oblong blocks to minute granule-like ones. The X-chromosome, one of the large chromosomes, can be distinguished from the other autosomes due to its slenderness in form, and the Y is considered as one of the smallest granule-like chromosomes, though there is difficulty in absolute identification.

2. In the follicle cells of female individuals, twenty chromosomes are observed. They are composed of eight large and twelve small chromosomes, corresponding to the male diploid group. An equal pair of idiochromosomes (XX) can be distinguished from the other autosomes by their relative size and form. Infrequently a giant cell, having tetraploid chromosome number is observed.

3. During the growth period, the complete history of the XY-complex can be followed, from a single chromatin nucleolus up to the ultimate form.

4. The primary spermatocyte possesses ten tetrads, of which four are distinctly large in contrast to the remaining six chromosomes. A heteromorphic tetrad of the XY-chromosome is found situated at the periphery of the equator. All the tetrads separate reductionally in division and neither precession nor succession of the idiochromosomes is actually present.

5. There is no 'resting stage' between the first and second divisions; no nuclear vacuole is formed and the chromosomes retain their individuality throughout.

6. As a result of asymmetrical distribution of the XY-chromosome, there are produced two kinds of secondary spermatocytes, ten in number, one of which contains X-chromosome, the other Y. All the dyads separate equationally in division.

7. The X-chromosome and the Y as well, can respectively be followed as the distinct chromatin nucleolus in the spermatid-nuclei.

8. Although rare, there are some individuals, in which the Y-chromosome is reduced to about one-third the original size. This size-relation of the Y-chromosome is constant in one and the same individual.

9. The ring tetrads seen in the diakinetik nucleus are considered to be the modified form of the diaschistic tetrads with terminal fiber attachment.

February, 1932

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Explanatory notes to Pl. I.

All the figures of this plate were drawn at the level of the desk on which the microscope was set, with the aid of ABBE's drawing apparatus. ZEISS apochromatic objective 1.5 mm. and compensating ocular K. 20, t.l. 160 mm. The magnification is about 4000 times. Figs. 3 to 18 show the plasmosome and chromatin nucleolus (XY-complex) in detail and the structure of the chromatin threads in rough sketch.

Abbreviations: *p*, plasmosome or true nucleolus. *h*, heterokaryosome or chromatin nucleolus. *x*, X-chromosome. *y*, Y-chromosome.

Fig. 1. The nucleus of the later spermatogonium in late prophase.

Fig. 2. Side view of the anaphase chromosomes of the spermatogonium.

Fig. 3. The nucleus of the final spermatogonium in the resting stage. Only one nucleolus (plasmosome) is seen.

Fig. 4. The nucleus of the primary spermatocyte in the preleptotene stage. The plasmosome and chromatin nucleolus are seen widely apart.

Fig. 5. The same. Two nucleoli are seen nearly attached.

Figs. 6-11. Views showing the transformation of a single chromatin nucleolus into the XY-complex, in the preleptotene nuclei.

Fig. 12. The chromatin nucleolus (XY-complex) in the preleptotene nucleus of the later stage.

Fig. 13. The same in the leptotene nucleus.

Fig. 14. The same in the zygotene nucleus.

Fig. 15. The same in the pachytene nucleus. The double nature is seen in the X-component.

Fig. 16. The same in the diplotene nucleus.

Figs. 17-20. The nuclei of the primary spermatocyte in diakinesis. 10 tetrads are seen. A heteromorphic tetrad of the XY-chromosome can be distinguished from the ring tetrads of the autosomes with conspicuous clearness. Figs. 17-18, early diakinesis. Figs. 19-20, late diakinesis.

Explanatory notes to Pl. II.

All the figures of this plate, except Figs. 36 to 51, were made under the same magnification and in a similar manner to the previous plate. Figs. 27 to 35 show the nucleus in detail and the other cytoplasmic structure in rough sketch.

Figs. 36 to 51 are photomicrographs. The magnification is about 2000 times.

Abbreviations: *p*, plasmosome. *x*, X-chromosome. *y*, Y-chromosome. *c*, chromatoid body. *m*, mitochondria. *n*, "Nebenkern."

Fig. 21. Polar view of the primary spermatocyte chromosomes in early anaphase. Separating components in black and gray.

Fig. 22. Side view of the primary spermatocyte chromosomes in metaphase.

Fig. 23. The same in anaphase. The anaphasic duality is seen in each separating chromosome.

Fig. 24. XY-bivalent in early anaphase of the primary spermatocyte division. The longitudinal splitting along the long axis is seen in both the X- and Y-elements.

Figs. 25-26. Side views of the telophase of the primary spermatocytes. Frequently the X-chromosome goes to the pole slightly behind the autosomes.

Fig. 27. Side view of the late telophase of the primary spermatocyte. Under this condition, the nucleus immediately passes into the metaphase of the second division without the resting stage and consequently neither the disintegration of chromosomes nor the formation of nuclear membrane takes place.

Fig. 28. The prophase of the secondary spermatocyte division from side view.

Fig. 29. The following stage of the same.

Fig. 30. The telophase of the secondary spermatocyte division.

Fig. 31. The spermatid before transformation begins.

Figs. 32-33. The early stages in the transformation of the spermatid. The X-element and Y as well, are detected by the peculiarity in the form of the deeply stained rod or dot, while the other autosomes undergo disintegration. A plasmosome of spherical shape is always seen in the spermatid of these stages. Fig. 32, X-class. Fig. 33, Y-class.

Figs. 34-35. Still later stages in the transformation of the spermatid. Fig. 34, X-class. Fig. 35, Y-class.

Fig. 36. Polar view of the equatorial plate of the spermatogonium.

Figs. 37-39. Polar views of the equatorial plates of the primary spermatocytes. 10 tetrads are seen in each plate.

Figs. 40-41. Side views of the XY-bivalent in the first division. The most usual size of Y-chromosome is shown.

Fig. 42. The same. The diminished size of Y-chromosome is shown.

Fig. 43. The chromatin nucleolus in the preleptotene nucleus. The plasmosome cannot be seen in this focus.

Fig. 44. The same.

Fig. 45. The chromatin nucleolus (XY-complex) in the early pachytene nucleus.

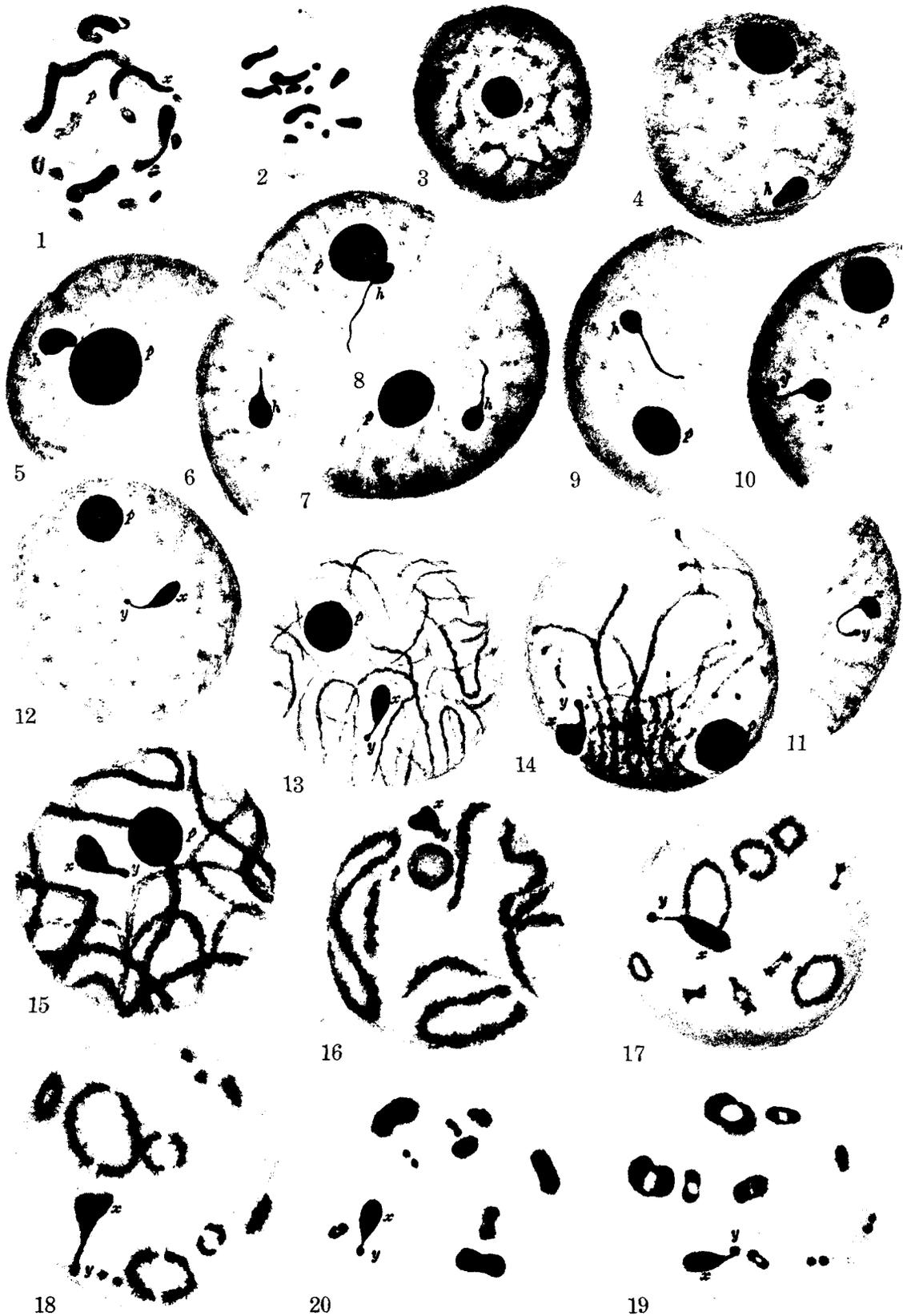
Fig. 46. The same in the diplotene nucleus.

Figs. 47-48. Polar views of the equatorial plates of the secondary spermatocytes, X-class. 10 dyads, 4 large and 6 small.

Fig. 49. The same, Y-class. 10 dyads, 3 large and 7 small.

Fig. 50. The spermatid in the early stage of transformation, X-class.

Fig. 51. The same, Y-class.



S. Makino del.



S. Makino del.