THE CHROMOSOMES OF FOUR WILD SPECIES OF MURIDAE[1)]

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(With three Plates and one Textfigure)

As mentioned in his previous paper, the present author has been engaged these several years in the study of the chromosome morphology of Muridae found in the wild state in Japan. The result now to be described in this paper constitutes the second report in his serial studies, of which the first, on *Apodemus* was published last year (OGUMA '34). Due probably to facility in securing material the domestic forms of rats and mice have continuously been employed by investigators since TAFANI ('89) first published his accounts on *Mus musculus*, but our knowledge has consequently been much limited, being scarcely extended to any wild species, until CROSS ('31) recently attempted a comparative study of rodents.

Even viewed from a different angle, this group of small rodents can certainly be thought interesting objects to advance our observation on the chromosome, because of being the sole representative of mammals in which any genetic study may easily be carried on, since they raise their progeny without difficulty in comparatively large numbers under confinement. In fact, SWEZY ('28) obtained from rats a very interesting and important result by crossing experiments between those having different kinds of chromosome garniture. DARLINGTON and others, on the other hand, have reported cytological evidence which strongly emphasizes their genetic interpretation on

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chiasmata produced by crossing over between chromatids. The present study, however, has not been undertaken with any genetic purpose, but with the expectation to explore the actual state of chromosomes as they are, merely from the view point of comparative morphology.

At this opportunity the author wishes to express his hearty thanks to Prof. AOKI of Taihoku University, by whom all species of Muridae have been taxonomically identified so far as the present study is concerned. He is also indebted to Dr. TOKUDA of Kyoto University, from whom he has received occasional information concerning the names as altered by modern mammalogists.

1. The Black Rat, *Rattus rattus* L.

A world wide distributed species, *Rattus rattus* L. involves two well known subspecies, *R. rattus rattus* L. and *R. rattus alexandrinus* GEOFF., the former being characterized by having slate-gray fur in contrast to the latter, which is reddish brown throughout the dorsal area of the body. In the history of the chromosome investigation of this species one finds a few classical works, which seem to be quite insufficient to allow a close discussion in comparison with results obtained by improved methods of fixation as developed by modern cytologists for particular use in Mammalian cells. Thus there are only two recent studies of importance, in which the chromosomes are analysed morphologically and compared with those of species closely related. One is the work of PINCUS ('27) and the other is that of CROSS ('31). It is a matter of importance not to be overlooked that the former author obtained his results from observation on *R. rattus rattus*, while the latter has carried on his work on the different subspecies, *R. rattus alexandrinus*, and that the chromosome number was estimated as forty by both investigators quite uneffected by any racial characteristics. However, the fact which the present author is going to discuss hereafter in this paper[1] is somewhat

1) A short note has already been forewarded in the fourth annual meeting of Zoological Society of Japan ('28).

different from that recorded by the above two authors. The chromosome of the black rat caught in Japan does accord with that of the American individuals neither in number nor in the form. Furthermore, the writer has discoverd a definite structure in the X-chromosome, different from any cases ever previously recorded in any species of Muridae.

The material consisted of a pair of testes of *Rattus rattus rattus* captured at Sapporo. When castrated the testicular tissue was cut into small fragments and treated by the double fixing method of CARNOY-FLEMMING, first recommended by OGUMA and KIHARA ('23). The sections were stained partly by HEIDENHAIN's iron haematoxylin and partly by the improved method of FLEMMING's triple staining. In order to obtain an accurate figure of the minute structure of the X-chromosome the former was always better than the latter.

The Spermatogonial Chromosome. In certain seminal tubules a portion was found where the spermatogonial division was taking place synchronously in a great number of cells. Consequently we could very easily discover the metaphase plates favourable to a count of the number of chromosomes and to observation of their individual form quite distinctly. The chromosomes (Fig. 1–4) take a typical radial arrangement and the number is constantly 42. This number evidently differs from that reported in the American species, in which both PINCUS ('28) and CROSS ('31) similarly reported 40 chromosomes, and coincides, on the contrary, with that of the Norway rat, *Rattus norvegicus*. No numerical variation was noticed in this respect so far as the present material is concerned. It is self evident, therefore, that the number 42 should be accepted as the definitive number of chromosomes in the black rat dealt with in the present study.

The chromosomes appear in such an univalent state like simple rods with a taper end to which the spindle fibre attaches (telomitic in terminology). They vary, however, in length to a great extent from the shortest to the longest following a gradatory order. Suggested from the number of chromosomes the sex-chromosome should

be made up of X and Y, and it is easily proved whenever the primary spermatocytes are brought into view. But they present no particular form in contradistinction from autosomes so long as they remain in the spermatogonium.

Now, let us consider why the chromosome number is so different from the known cases, as mentioned already. In order to explain this numerical discrepancy it is absolutely necessary to compare from all directions the chromosomes recorded by previous authors with those described in the present study. PINCUS ('27) states that in *Rattus rattus* one finds invariably three pairs of large U-chromosomes by which this species is sharply distinguished from *Rattus norvegicus*. We know a fact, however, quite different in respect to the number of such chromosomes in descriptions of CROSS ('31), who counts similarly 40 chromosomes in *Rattus rattus*, but reports only two of them as being represented by V's.

Our present knowledge compels us to accept the view that every chromosome is characterized by a definite mode of spindle fibre attachment and thereby the form, V or rod, was originally determined. Under some circumstances, of course, there may be found apparent V's or U-shaped chromosomes resulting from a temporary bending in rod chromosomes with terminal fibre attachment. Experienced observers, however, will not confuse such cases with true atelomitic chromosomes of V or U-shape. So the difference appearing in the morphology of chromosomes as reported by two authors (PINCUS and CROSS) in the different materials of the same species seems apparently to be derived from the racial differences in taxonomy as CROSS ('31) states that the material he employed belongs to *Rattus rattus alexandrinus*, while the material of PINCUS belonged to *Rattus rattus rattus* (CROSS p. 379). Unfortunately, the present author can hardly support his view with satisfaction, as the chromosome figures drawn by PINCUS are not absolutely clear to match with ours, in spite of his emphasis concerning his excellent fixation. There remains a doubt whether the three V's do actually and constantly exist, so long as we consider our figures shown in this paper.

Compared with the figures of PINCUS, those shown by CROSS ('31) seem very clear to support his conclusion concerning chromosome morphology. As stated already, he found only two V's of large size in a diploid garniture of chromosomes, and one must note that this fact was observed in the subspecies, *Rattus rattus alexandrinus*. From the present study, it will be absolutely clear that in another subspecies, *Rattus rattus rattus*, the chromosomes are represented without exception by the rod shape but they are two more in number than the preceding case. When comparison is made of these two different results, obtained from two different subspecies belonging to one and the same species, one recollects at once the theory of chromosome linkage, initiated by the finding of WOOLSEY ('15) and ROBERTSON ('16) in Orthoptera and elaborated later by MATTHEY ('31, '33) in his great work on Reptilian chromosomes. More recently MAKINO ('34) tried to solve in a similar way the numerical difference of chromosomes existing between Japanese and American species of urodele Amphibia. It is by no means unreasonable, therefore, to suppose that linkage takes place in a certain two pairs of rod chromosomes in *Rattus rattus rattus* of the present case to give rise to two V's, instead of four rods, and thus to constitute ultimately the chromosome garniture of *Rattus rattus alexandrinus*.

The Chromosome in the Primary Spermatocyte. Twenty one bivalent chromosomes are found in the primary spermatocyte including X-Y complex (Figs. 5–7). The larger kind of chromosomes, from 8 to 10 in number, assume the form of horizontal ring tetrads or a thick V-shape when the distal ends of chromatids open, while the remaining ones appear like crosses or rods. In mode of equatorial arrangement these chromosomes show nothing particular compared with the recorded cases of any kind of Muridae. In brief, the ring tetrads of large size are arranged peripherally surrounding the smaller ones. The sex-chromosome is invariably found in the outer zone with large rings.

It is somewhat noteworthy that in every tetrad the terminal parts of the chromatids, where the spindle fibres attach always

swell up into a globule in which the proximal or polar granule is included. Some portion of the chromatin matter annexed to the polar granule condenses, as it seems to the writer, into a globule and become distinguished from the remaining portion of the chromatid by marked constriction. This structure is most prominent in preparations stained by haematoxylin (Fig. 10), but is not marked in safranin preparations (Fig. 8).

The Sex-Chromosome. Though almost entirely impossible to identify in the spermatogonial garniture of chromosomes, there are two sex-chromosomes, X and Y, without the slightest doubt in the present species as in the case of *Rattus norvegicus*, as is readily expected from the even number of spermatogonial chromosomes as well as from the actual finding of their conjugated figure in the primary spermatocyte (Figs. 5–7). The X-chromosome is characterized by a diffused configuration with a more or less vague contour, and seems to be about four times longer than the Y-chromosome, with which the former is connected at its proximal extremity (Figs. 9–11). It lies, as stated above, in the most peripheral part of the spindle together with the larger tetrads, and stretches with its long axis along a radius of the equatorial plane. The Y-chromosome, on the contrary, is always stained as deep as the autosomes with a sharp contour. In these respects too, the black rat does not present features different from what has hitherto been known in the Norway rat.

Now attention should be given to the structure of the X-chromosome. Speaking morphologically it evidently belongs to a rod chromosome having the fibre attachment at the proximal tip. In preparations, considered as of the best fixation, a definite structure is discovered. In the first place, it presents a segmental structure as being divided into three parts by two constrictions (Figs. 5, 6, 10 and 11). In the second place, the proximal segment, in which the proximal or polar granule lies, is sharply distinguished from the remaining two by showing a more compact texture. This segment thus characterized takes the appearance of a knob which is called the proximal knob in the following descriptions. The remaining two parts show,

on the other hand, a typical vague contour, though frequently they become indistinguishable from the proximal knob not only in structure but also in staining reaction (Figs. 7, 8 and 9).

The X-chromosome segregates from the Y in the first division, and the segmental structures of the X-chromosome as described above, at least in preparations well fixed and adequately stained, can be followed continuously up to the telophase (Fig. 12) through anaphase (Fig. 11). The author wishes to discuss this structure in detail comparing with the case of the Norway rat later on.

2. The Norway Rat, *Rattus norvegicus* ERXLEBEN

The Norway rat, *Rattus norvegicus* ERX., has been the favorite material for investigators intending to study the cells from the side of cytology. Thus many important works, beside classical ones, have hitherto been published by ALLEN ('18), PINCUS ('27), PAINTER ('28), MINOUCHI ('28), BRYDEN ('32) and KOLLER and DARLINGTON ('34). But the studies were undertaken, without exception, on the albino forms, *Rattus norvegicus albus*, and not on the wild forms, except the work of SWEZY ('28) who observed two kinds of chromosome garnitures in both wild and domestic forms. Much interested in the report published by the latter author, together with the purpose to complete the serial studies of chromosomes in the wild species of Muridae, the present author has gathered wild Norway rats in connection with the other species here dealt with.

The material belongs to the subspecies, *Rattus norvegicus norvegicus* ERX., with brown fur. The study was undertaken merely on the cells of testes, which after castration were fixed partly with FLEMMING's mixture without glacial acetic acid and partly with CHAMPY's fluid as modern cytologists recommend. The sections were stained only with HEIDENHAIN's iron haematoxylin.

The Spermatogonial Chromosome. Many figures of dividing spermatogonia are found along the wall of the seminal tubules, and one of the most typical metaphase figures is shown in Fig. 14. The equatorial plate contains forty-two chromosomes of rod shape. They

show a gradual reduction of length from the longest to the shortest. In these respects, therefore, the wild Norway rat can not be distinguished from the domesticated albino rat, in which the chromosome number seems to be settled as forty-two by recent studies. This number of chromosomes is, at the same time, just equal to that of the black rat at least in the material dealt with in the present study. The only one characteristic by which these two allied species may be distinguished from each other rests upon the fact that there is a great difference between them in the length of the longest chromosomes. In the black rat the ratio in length of the longest pair to that of the next longest ones is approximately 3 : 2.5, while in the Norway rat it is about 5 : 3. A nearly similar ratio is also noticed in the figures shown by ALLEN ('18), BRYDEN ('32), and MINOUCHI ('28) in the albino rat, though not in those by PAINTER ('28). So the longest chromosomes always show a conspicuous configuration in the spermatogonial garniture in the Norway rat compared with the case of the black rat (compare Fig. 14 with Figs. 1–4). If the crossing be futile, as has hitherto been found in experiments between these two species, and if it be due to the difference of chromosomes, consideration must first be given to the morphological difference of the longest chromosomes.

The Chromosome of the Primary Spermatocyte. In a metaphase plate (Fig. 15) are found twenty-one bivalent chromosomes, twenty tetrads and an X-Y complex. Their structure and mode of arrangement resemble greatly those of the black rat, excepting the tetrad of largest size. The latter sometimes assumes the form of a simple ring (Fig. 15) like the majority of the remaining larger tetrads, but more frequently takes the form of a compound ring tetrad with three or more chiasmata as KOLLER and DARLINGTON believe. One such example is drawn in Fig. 17. Ring tetrads with two opposite ends opened as shown in Fig. 18 are also found instead of simple rings. Similar occurrence has also been recorded in the albino rat by previous investigators (ALLEN '18, MINOUCHI '28, KOLLER and DARLINGTON '34). We have never found, on the cont-

rary, tetrads of such structure in the black rat, in which the chromosomes are not sufficiently long to make up such complicated configurations.

The Sex-Chromosome. Two kinds of sex-chromosomes are also found in the present species as in the preceding form, and show, after conjugation, a similar complex appearance in the metaphase plate of the primary spermatocyte. There is only one fact to which it may be necessary to call the readers' attention, that the X-chromosome again displays the tripartite structure as observed in the black rat. It is shown quite clearly in the annexed figures. In Fig. 15 the X-chromosome, extending horizontally upon the equator, can be sharply distinguished from autosome tetrads not only by its elongate form but also by its paler color, and the entire body acquires segmentation into three parts. Upon looking at Fig. 16 then, moreover, the formation of the proximal knob will be recognized in absolute clearness in striking contrast to the distal segments which still remain in loose texture. Of course there are cases in which the proximal segment does not take the appearance of a globular knob. Even in these cases that segment will be distinguished from the distal segments by the different grade of staining reaction (Fig. 18).

There are two different views concerning the constitution and the behavior of the sex-chromosomes in the Norway rat. The first is that which was believed by MINOUCHI ('28) who described for the first time the detailed structure of the sex-chromosome in relation to mode of conjugation as well as the fibre attachment. So far as he states and illustrates, each kind of the sex-chromosomes is telomitic, and they are connected together in such a way that the telomitic end of X is associated with Y at its one end free from the proximal granule. We find an excellent demonstration in his Fig. 30, in which the proximal granules are seen in absolute clearness in both kinds of sex-chromosomes. In connection with the problem of the mode of association between X and Y, he made clear how they develop in the stages prior to metaphase. The sex-chromosome originates in his observation in a karyosome, in which first appears a chromatin

thread of spiral form and thereafter it differentiates into the X-Y complex as seen in metaphase. The facts, essentially similar to this, have also been reported by the same author in *Nyctereutes* ('29) and *Canis* ('28).

Strikingly adverse to such findings of MINOUCHI ('28), a series of facts, quite different in important points, have been announced by KOLLER and DARLINGTON ('34) in the same species of rat. Under their interpretations the fibre attachment of sex-chromosomes is subterminal, instead of terminal, in both kinds, X and Y, and they conjugate side by side at first, during the early half of the growing period. At the pachytene period the X-Y chromosome is seen as paired thick threads of inequal length, instead of a karyosome, as shown in their Text-fig. 15. The chromatin threads thus conjugated produce one or two chiasmata, in favor of the theory of crossing over of genes, before they separate from each other in the following periods of the first maturating division. They distinguish in the X-chromosome the pairing segment, which comes in actual contact with Y, from the differential segment, which remains without direct connection with Y throughout the developing stages. The mode of segregation of the sex-chromosomes differs, as they believe, according to the positions where the chiasmata actually occur: in the majority of cases (90%) they separate pre-reductionally at the first division, while in the minority (10%) however the segregation takes place in the second division post-reductionally. The similar view seems to have been adopted also by other geneticists not only in the Norway rat (BRYDEN '32) but also in the mouse (CREW and KOLLER '32). CUTRIGHT ('32) holds the same opinion too as to the mode of conjugation of the sex-chromosomes in the mouse (*Mus musculus albula*), although no example of post-reduction has been illustrated.

Considering the structure of the X-chromosome observed in the present study in the two species of rats, the pairing segment of KOLLER and DARLINGTON ('34) evidently corresponds with the proximal knob as elsewhere described in both species, and their differential segment to our distal segments. The only difference

rests merely on the fact that in our case the distal segment is further subdivided into two parts. But this difference is by no means so important as that which exists in regard to the mode of conjugation between X and Y. So far as the present study is concerned, at least the X-chromosome takes an appearance of a chromatin nucleolus, elongate and flattened to some extent, from which the X-chromosome of the final shape develops out, as if in support of the view of MINOUCHI ('28). In his previous study on a field mouse, *Apodemus speciosus*, the present author ('34) arrived at the conclusion that the X-chromosome constitutes, during the growing period of the first division, a chromosome vesicle in which the chromatin matter condenses, in course of the maturating process, along the wall of the vesicle assuming gradually the characteristic chromosome form. The examples to strengthen this view are shown here from preparations of black rat stained with safranin (Fig. 13). In either *a* or *b* in this figure one can readily notice the marginal condensation of the chromatin matter similarly to the case of *Apodemus*. Though not very conspicuous in its degree the chromatin thread shows a spiral configuration as MINOUCHI ('28) describes. As more important to be noticed, the proximal granule, to which the spindle fibre attaches later, appears at a certain point, a slight distance from the true extremity of the thread. In other words, the fibre attachment may likely be subterminal as KOLLER and DARLINGTON believe. This terminal short part separated by the proximal granule, on one hand, may be taken for the Y-chromosome conjugated telosynaptically with X by any one who considers that the Y-chromosome is involved in a chromatin nucleolus with the X. However, if one refers to the case of *Apodemus* above mentioned, on the other hand, the small part in question seems to correspond with the short arm of the X-chromosome having two arms of different length, not representing the Y (cf. OGUMA '34). If such interpretation be correct, therefore, the proximal knob, as described elsewhere in this paper, would have a constitution of double nature, the proximal granule plus that small part of chromosome thread in question. For the part of the Y-

chromosome, the present material offers only a little evidence, available in support of its atelomitic nature as KOLLER and DARLINGTON ('34) state. As one example let Fig. 11 be observed, in which the Y-chromosome apparently presents subterminal attachment of fibre though not sufficient enough to establish this view undoubtedly.

Whatever the fibre attachment may be, the X-chromosome exhibits the nature of a chromosome vesicle in both species of rats dealt with as in *Apodemus*, and the vesicle is naturally taken for a chromatin nucleolus (karyosome) so far as it is deeply and homogeneously stained by any dye. Considering the evidence above mentioned the present author hesitates to approve at once the view proposed chiefly by geneticists, who maintain that the sex-chromosomes conjugate parasynaptically in the form of threads like autosomes. We expect, on the contrary, that the Y-chromosome may possibly constitute a different chromosome vesicle of smaller size than that of the X, at least during a certain part of the growing period, and that they conjugate at last during prophase of the first division, as we always found the conjugated figure in nucleus of diakinesis.

It is a problem, very important and particularly interesting from the genetic point of view, whether the X-Y complex may or may not segregate in the first maturating division independently from the autosomes as KOLLER and DARLINGTON believe (see above). So far as the present study shows the two components of the complex invariably separate from each other at the first division. In not even one case of this division the author has succeeded to discover such occurrence as the X-Y complex being divided into two equal halves as observed by KOLLER and DARLINGTON ('34) in the Norway rat and by CREW and KOLLER ('32) in the mouse.

3. The House Mouse, *Mus molossinus* TEMM. et SCHLEG.

Over twenty papers have been published concerning mice by previous authors (see OGUMA and MAKINO '32) who describe the chromosome to more or less extent. There are, of course, found works

to be supposed insufficient as compared with the results obtained by modern cytologists through their improved techniques. Still there are, as representatives of the best work, the papers of MASUI ('32), COX ('26), PAINTER ('27, '28), MINOUCHI ('28), CREW and KOLLER ('32) and CUTRIGHT ('32) whose material is seemingly to be classified into two different kinds from the taxonomical point of view. The ordinary house mouse, *Mus musculus,* was adopted as material by PAINTER ('28) and CREW and KOLLER, COX and CUTRIGHT, while the Japanese white and dancing mice (*Mus wagneri*) were used by MINOUCHI and PAINTER ('27). In results of study, however, it may be seen that there exists no essential difference between these two species so far as chromosome morphology is concerned, as all authors have informed the presence of forty chromosomes of rod shape in their diploid garniture.

In Japan there is a wild mouse, very nearly related to but different from those above mentioned, inhabiting a very wide range from Hokkaido to Kiushu, and known in Hokkaido as one of the most harmful animals in the household. It is the mouse having the scientific name *Mus molossinus,* on which the present study has been carried on.

The mice were captured always in the interior of our dwelling houses though often caught at the farms near the university. The testes were employed for study after usual treatment of fixation by CHAMPY's fluid and sections were stained with HEIDENHAIN's iron haematoxylin. In the preparations thus treated many interesting figures of cytoplasmic inclusions could be observed in addition to the chromosomal elements, but descriptions and discussions will be confined in this paper merely to the chromosomes.

The Spermatogonial Chromosome. Counting the chromosomes exhibited in the metaphase plates it is ascertained that the definite number of a spermatogonial garniture of chromosomes is forty. In this respect no difference is recognized in comparison with other allied species studied. Speaking generally the cell size, and the diameter of a nucleus as well, seems much larger than that of the

rats already described. Accordingly the chromosomes, when spread widely in metaphase of division, are very clearly observed as shown, for example, in Fig. 19. All forty chromosomes, as readily recognized, belong without exception to the rod type in their morphology, and taper in most cases towards their proximal ends. A characteristic by which the present species differs strikingly from rats (*Rattus*) may be the relative length of the smaller kinds of chromosomes. They show as in rats a gradual reduction of length from the longest to the shortest. But the rate of reduction seems not so remarkable as in rats. It follows that the bivalent chromosomes of the primary spermatocytes show no marked size difference with one another as will be stated below. The even number of chromosomes, 40, evidently announces the presence of X and Y as sex-chromosomes, although it is almost impossible to identify them with certainty in such diploid group of chromosomes. The Y-chromosome, however, may possibly be represented by the shortest one.

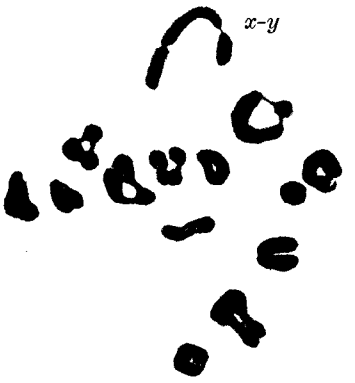
The Chromosome of the Primary Spermatocyte. Expected from the number of spermatogonial chromosomes, there are invariably found twenty chromosomes of bivalent nature in the metaphase plate of the primary spermatocyte (Fig. 20). Of these twenty bivalents one is represented by the X-Y complex and the remainder by autosome tetrads which assume the form of a ring, a cross or a thick rod, arranged in the usual way with the larger ones surrounding the smaller. All tetrads of larger size belong to the simple horizontal rings in their structure as in the case of rats, but show a somewhat different appearance. First, the chromatids, from which the tetrad is composed, are rather thick and eventually short, often opened at the distal end of association, resulting in a V-figure in polar aspect. Owing to the thickness of the chromatids the round space left between two of them usually becomes reduced. If there occurs much more swelling from any cause in chromatids, this space is converted into a narrow slit as the cases shown by MINOUCHI ('28) and CUTRIGHT ('32). As instance can be presumed, moreover, where the swelling advances still further. Then the median

slit would finally disappear, in consequence of fusion of chromatids, as in the figures shown by MASUI ('23) or COX ('26). Examples of a similar appearance are also frequently found in our preparations in which fixation seems not to be the best in every respect. The tetrads, therefore, are not to be considered of different structure from ordinary ring tetrads, in spite of their peculiar appearance. Even in the largest one the occurrence of compound chiasmata could not be found though their existence has already been proved in the Norway rat (cf. supra) and in the mouse by CREW and KOLLER ('32).

A structure, much more conspicuous and important than that above described, is the formation of the proximal knob of large size in every four chromatids from which a tetrad is composed. The occurrence of similar structure has already noticed in the case of the black rat, in which the terminal parts of every two chromatids become fused to constitute a single knob, at least in appearance. But in the present case the formation of the proximal knob takes place in every single chromatid, whereby in the polar aspect of a ring tetrad there are usually found two distinct knobs in intimate connection at the proximal end of the tetrad. By this structure the tetrad of the mouse seems to be distinctly characterized in contradistinction from that of the rat.

The Sex-Chromosome. Similarly to autosome tetrads, the X-Y complex shows a marked difference from that of the rat. It lies not exclusively in the most peripheral row of tetrads in metaphase arrangement of the first division as in the case of rats, but frequently in the interior of the equatorial plate (Fig. 20). The reason why it takes such different position in arrangement, however, will readily be recognized if one closely observes the structure of the complex, in relation particularly to the fibre attachment on the X-chromosome. In the mouse now dealt with, just like all cases recorded by previous authors in other species of mice, the X-Y complex extends the whole body upon a straight line in parallel to the spindle axis, at least in metaphase of the first division (Figs. 21 and 22). The fibre attachment on the X-chromosome is known to be the extremity, opposite

to where the Y-chromosome comes into contact with it because it is obviously noticeable that at the end first mentioned one or a pair of polar granules develop (Fig. 21). In most cases the X-chromosome is stained much paler than the Y, which is as deeply colored as the autosomes, and presents no tripartite structure as observed in any species of rats, but the form of a simple rod or clavate shape with gradual thickening towards the distal extremity (Fig. 22). In one case, as shown in Textfig., the terminal part of the X-chromosome is marked off by a constriction. A similar figure can be found in PAINTER's work (see his Fig. 20, '27).



Textfig. The chromosomes immediately before equatorial arrangement of primary spermatocyte (not all chromosomes are drawn). Note the structure of sex-chromosome (*x-y*).

The magnitude of the Y-chromosome, relative to that of the X, is seemingly much bigger than in the case of rats, but it may vary in certain individuals. For instance, one individual was found, in which the relative size of Y is strongly reduced as shown in Fig. 22. This size reduction constantly occurs in that individual, and this consequently strengthens our hypothesis to a great extent that the Y-chromosome possesses in general a tendency to grow smaller due probably to casting off some portion of chromatin, from which it is composed, as proved by the fact observed in *Oecanthus* by MAKINO ('32).

From what has been recorded by previous authors on chromosomes of mice, two interesting points should be taken now under consideration. The one is the mode of conjugation of sex-chromosomes during the growing period of primary spermatocytes, and the other is the occurrence of a chromatin nucleolus from which an autosome tetrad of the largest size develops out. Contrary to the case of rats, a side-by-side union of the sex-chromosomes has repeat-

edly been described by PAINTER ('27), MINOUCHI ('28), CUTRIGHT ('32), and CREW and KOLLER ('32), though the present author still has a doubt on this point in comparison with the other species he actually observed. On the other hand, CUTRIGHT ('32) expresses the opinion that the chromatin nucleolus found in the prophase nucleus converts finally into the largest tetrad. If it be surely a credible fact, his finding should throw light to a great extent upon the knowledge hitherto accumulated from investigation of Mammalian cells, for in no case does the autosome assume a form of chromatin nucleolus independently from the sex-chromosomes. Unfortunately the present study has not succeeded in thorough tracing of the entire course of transformation of sex-chromosomes during the growing period up to metaphase. The author finds some data in favour of CUTRIGHT's opinion, but some others, on the other hand, quite adverse to his view. Further discussion, therefore, will better be postponed until the study will have been completed.

The X-chromosome segregates from Y at the first division, in striking contrast to the results obtained by CREW and KOLLER ('32) who expect with all probability that segregation takes place at the second division. In the present material no one fact was observed to support their view. In fact, the X unexceptionally separates from the Y at the first division, and enters one daughter nucleus in which the Y does not migrate. Thus we never find in every secondary spermatocyte (Figs. 23 and 24) the co-existence of both kinds of sex-chromosomes, as expected from the equational distribution at the first division postulated by CREW and KOLLER.

4. The Vole, *Evotomys* (*Clethriomys*) *bedfordiae* THOMAS

The vole now to be described under the last section of this serial study, does not belong to the subfamily Murinae, in which rats and mice hitherto studied are included, but to the different subfamily Microtinae, regarding the chromosomes of which we have had little knowledge, excepting some fragmental data from the genus *Microtus* reported by ATHIAS ('12) and CROSS ('31). In the present species

the chromosomes were thoroughly studied and accordingly their morphological nature has become very clear after close observation of all kinds of male germ cells in successive stages of development. In brief, the results evidently reveal that this kind of vole differs so much from Murinae in morphology of chromosomes, as to parallel the difference of the taxonomical characteristics.

This vole is known to distribute in a rather confined area, north from the Tsugaru Strait, but it is found abundantly in Hokkaido as well as in Sakhalin. They were caught in numbers by means of traps settled in the woods near Sapporo, and the testes were employed for study after adequate treatment. Both FLEMMING's and CHAMPY's fluids were applied for fixation but the staining was confined merely to iron haematoxylin after HEIDENHAIN.

The Spermatogonial Chromosome. The spermatogonial chromosomes were observed in a great number of cells composing the terminal parts of seminal tubules. Two metaphase figures of large size, in which the overlapping occurs in chromosomes in least degree, are shown in Figs. 25 and 26. The chromosomes constitute a garniture of the spermatogonium with a definite odd number, fifty five, in striking contrast to the preceding three species, though there is another example of odd number in *Apodemus* (see OGUMA '34). This number suggests at the same time the absence of the Y-chromosome. All fifty-five chromosomes are represented by rods from the morphological point of view, though some are strongly bent and some others undulating, but varying in length showing a gradatory order as usual. Readily noticeable from these two figures the homologous chromosomes, of large size at least, are found situated near by each other. Thus it is possible without much difficulty to distinguish those constituting pairs in a certain number as labelled for example in the figures, and this results in the finding of the X-chromosome in such diploid group of chromosomes. The X-chromosome thus identified seems to be represented by one of the larger kinds. The exact magnitude is, however, likely impossible of determination as it shows in this stage not so compact an appearance as the autosomes,

without a more or less sharp contour. It will be expected, therefore, that it may sometimes assume much smaller size when strongly condensed.

The Chromosome of the Primary Spermatocyte. The metaphase plate of the primary spermatocyte is composed of twenty-eight chromosomes, of which one represents of course the X-chromosome and the remaining ones the tetrads of autosomes. The ring tetrads seem to be less in number in comparison with cases of Murinae—usually three or four (Fig. 27), some of them still showing a tendency to open out at the distal end of chromatid communication. Only one that descended from the longest pair in a diploid group, invariably assumes the typical ring, expressing often a suggestive structure of occurrence of a distal chiasma (Fig. 28), even in the case where all remaining tetrads are V-shaped by giving up the distal communication, probably due to extreme condensation of the chromatids, as Fig. 29. In the latter case the formation of the proximal knobs becomes conspicuous as the case of *Mus*. The tetrads, smaller than those that form the rings, take the form of cross or V-shape when viewed from a pole of division, while the smallest kinds assume the form of short rods.

The Sex-Chromosome. Among large tetrads in peripheral arrangement of a metaphase plate is found the X-chromosome with its characteristic appearance (Figs. 27–31). The X-chromosome in this stage varies widely in its magnitude, corresponding to its structure, in different cells. In some case, Fig. 27 for instance, it takes the appearance, indistinguishable at first sight from some of the ordinary tetrads of a compressed ring, and is stained nearly as deep as the latter. More frequently, however, it swells up to an ovoid shape with the proximal end tapered, and absorbs though to a variable degree, much less amount of dye than the autosome tetrads, presenting an appearance very like a chromosome vesicle, in which the marginal condensation of chromatin takes place in varying degree. In Fig. 28 the condensation occurs in such way as to make up a clavate body, bent along the margin, while in Figs. 29 and 30 it advances in no

way producing a definite form but plainly diffused. Corresponding to the mode and grade of condensation, the X-chromosome shows a marked variation in its apparent magnitude. When the condensation occurs uniformly in the X of vesicular nature the ultimate size of the chromosome becomes considerably smaller in comparison with the case where the condensation takes place in a definite form. Though seldom in occurrence, such a case as shown in Fig. 31 has actually been observed, in which firstly the size is extremely large, and secondly the condensed portion of chromatin assumes, the form of a string or ribbon with approximately uniform breadth in something like the appearance seen in case of the black rat (Fig. 13 *a* and *b*). The only point by which the present case may be distinguished from the latter is that the chromatin string thus produced is continuous through its entire length. This phenomenon should be accounted for, if it be presumed that a precocious division and separation of the chromatin units as chromomeres has taken place already in the chromosome of vesicular nature and thus separated chromatin substance then separately condensed along both sides of the vesicle. In other words, the chromatin loop illustrated as continuous in Fig. 31, is composed in reality of a pair of daughter chromosomes which will actually disjoin at the second division.

A fact which requires special attention is the constant occurrence of the proximal granule, independently from the variable form of condensed chromatin. This granule takes a definite position on the chromosome at its proximal end tapering in more or less degree, and it never grows into a proximal knob but remains as a small granule.

From what has hitherto been observed in a serial and comparative study of the sex-chromosome of Muridae, it seems to be most logical to conclude that the X-chromosome generally possesses a vesicular nature, in which the chromatin condensation takes place in various ways, grades and times in course of maturating divisions. In rats (*Rattus*) the duration of the vesicular construction is confined merely to the growing period, but in *Apodemus* and *Evotomys*,

where no mating Y is present, it extends further up to metaphase. The reason why the X-chromosome remains so long destitute of compact appearance in the latter case may probably be accounted for by the absence of the Y-chromosome with which to conjugate.

The X-chromosome runs, without division, to either one pole of the first division resulting in two kinds of secondary spermatocytes in relation to chromosomes. Some cases may actually be found of twenty eight chromosomes (Figs. 32 and 33) and the others of twenty seven (Figs. 34 and 35) due to absence of X-chromosome.

Summary

1. The results obtained from the study on four wild species of Muridae, *Rattus rattus, Rattus norvegicus, Mus molossinus* and *Evotomys bedfordiae,* were reported with special reference to the structure and behavior of the sex-chromosomes.

2. The chromosome numbers of four species studied are 42, 42, 40 and 55 respectively, and there were found no forms other than simple rod in the chromosomes of all species.

3. In three species belonging to the subfamily Murinae the sex-chromosomes are represented by X and Y of different size, while one included in the other subfamily Microtinae possesses no Y-chromosome.

4. The X-chromosome shows the nature of chromosome vesicle, at least during the growing period in *Rattus* and up to the time of metaphase in *Evotomys.*

5. Consequently the view of investigators, who maintain that conjugation of sex-chromosomes takes place early in the pachytene stage in form of thick threads like autosomes, can not be accepted.

6. The X-chromosome when paired with Y separates without exception from the latter at the first division, and in case where the Y is wanting it runs, without division, to one pole of that division. The post-reduction of sex-chromosomes could not be ascertained to exist in the four species now reported. At present, therefore, the

case of *Apodemus* previously recorded by the present author seems to be the sole example in which the post-reduction of the sex-chromosome actually takes place so far as hitherto studied species of Muridae are concerned.

Literature

1918 ALLEN, E. Studies on cell division in the albino rat (*Mus norvegicus albus*). Journ. Morph., Vol. 31.

1932 BRYDEN, W. Cytogenetic studies on the rat. Somatic chromosome complex, meiosis and chiasma frequency. Journ. Gen., Vol. 26.

1926 COX, E. The chromosomes of the house mouse. Journ. Morph. Phys., Vol. 43.

1932 CREW, F. A. E. & KOLLER, P. CH. The sex incidence of chiasma frequency and genetical crossing-over in the mouse. Journ. Gen., Vol. 26.

1931 CROSS, J. C. A comparative study of the chromosomes of rodents. Journ. Morph., Vol. 52.

1932 CUTRIGHT, P. R. The spermatogenesis of the mouse (*Mus musculus* var. *albula*). Journ. Morph., Vol. 54.

1934 KOLLER, P. CH. & DARLINGTON, C. D. The genetical and mechanical properties of the sex-chromosomes 1. *Rattus norvegicus*, ♂. Journ. Gen., Vol. 29.

1932 MAKINO, S. An unequal pair of idiochromosomes in the tree-cricket, *Oecanthus longicauda* MATS. Journ. Fac. Sci. Hokkaido Imp. Univ., Vol. 2.

1934 Id.—— Supplementary notes on Urodelan chromosomes. (Japanese abstract). Zool. Mag. Tokyo., Vol. 46.

1923 MASUI, K. The spermatogenesis of domestic mammals. III. The spermatogenesis of the mouse and of the rabbit. Journ. Col. Agr. Tokyo Imp. Univ., Vol. 8.

1931 MATTHEY, R. Chromosomes de Reptiles Sauriens, Ophidiens, Cheloniens. L'évolution de la formule chromosomiale chez les Sauriens. Rev. Suisse Zool., T. 38.

1933 Id.—— Nouvelle contribution à l'étude des chromosomes chez les Sauriens. Rev. Suisse Zool., T. 40.

1928a MINOUCHI, O. Spermatogenesis of the albino rat (*Mus norvegicus albus*). Jap. Journ. Zool., Vol. 1.

1928b Id.—— The spermatogenesis of the dog, with special reference to meiosis. Jap. Journ. Zool., Vol. 1.

1928c Id.—— On the chromosomes of the domestic mouse (*Mus wagneri albula*). Jap. Journ. Zool., Vol. 1.

1929 Id.—— On the spermatogenesis of the racoon dog (*Nyctereutes viverrinus*), with special reference to the sex-chromosomes. Cytologia, Vol. 1.

1934 OGUMA, K. A new type of the mammalian sex-chromosome, found in a field mouse, *Apodemus speciosus*. Cytologia, Vol. 5.

1923 OGUMA, K et KIHARA, H. Etude des chromosomes chez l'homme. Arch. Biol., T. 33.

1932 OGUMA, K. & MAKINO, S. A revised check-list of the chromosome number in Vertebrata. Journ. Gen., Vol. 26.

1927 PAINTER, TH. The chromosome constitution of Gates' 'Non-disjunction' (v–o) mice. Genetics, Vol. 12.

1928 Id. —— A comparison of the chromosomes of the rat and mouse with reference to the question of chromosome homology in mammals. Genetics, Vol. 13.

1927 PINCUS, G. A comparative study of the chromosomes of the Norway rat (*Rattus norvegicus* ERXL.) and the black rat (*Rattus rattus* L.). Journ. Morph., Vol. 44.

1916 ROBERTSON, W. R. B. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. Journ. Morph., Vol. 27.

1928 SWEZY, O. On the existence of two chromosome numbers in a mixed rat strain. Journ. Exp. Zool., Vol. 51.

1915 WOOLSEY, C. I. Linkage of chromosomes correlated with reduction in numbers among the species of a genus, also within a species of the Locustidae. Biol. Bull., Vol. 28.

Plate II

Explanation of Plate II

The figures were drawn by aid of Abbe's drawing apparatus under magnification approximately 5000 times upon the desk surface where the image falls, using Zeiss Apochromat 1.5 mm. and compensating ocular 18.

Rattus rattus rattus

Figs. 1–4. Spermatogonial chromosomes (polar view).

Figs. 5–7. Chromosomes of primary spermatocytes (polar view).

Fig. 8. The same in profile (stained by FLEMMING's triple method).

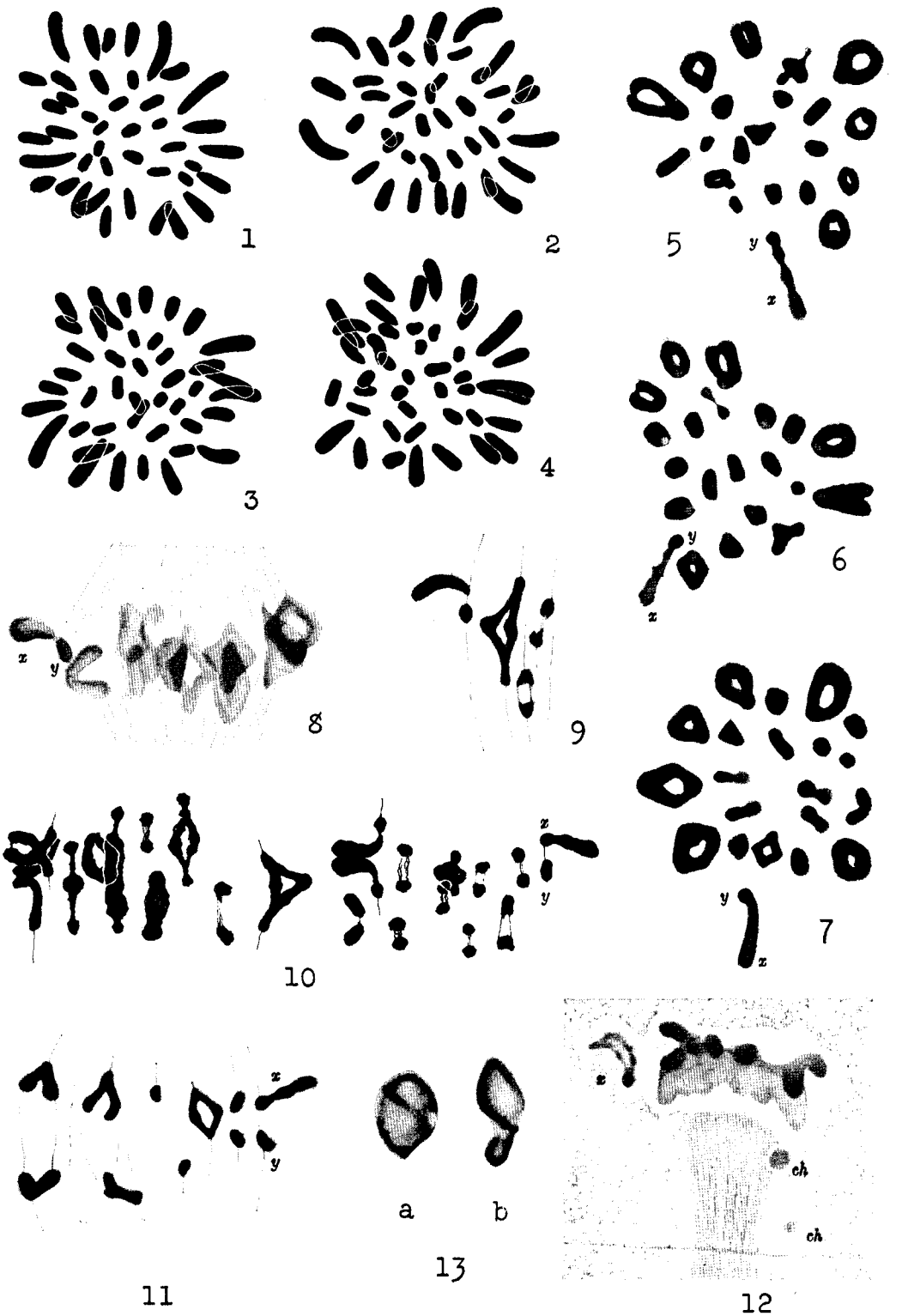
Fig. 9. A part of the first spindle, including three tetrads and sex-chromosome complex (profile).

Fig. 10. Early anaphasic figures of all 21 bivalent chromosomes belonging to one and the same nucleus (profile).

Fig. 11. Anaphasic figures, shortly advanced (profile).

Fig. 12. A telophase group of daughter chromosomes (stained by FLEMMING's triple method); note the X-chromosome constitutes a distinct chromosome vesicle retaining the original tripartite structure further on, and the longitudinal splitting becomes clear. *ch*, chromatoid bodies.

Fig. 13. Chromatin nucleoli from two different nuclei in late stage of growing period.



K. Oguma : The Chromosomes of Four Wild Species of Muridae.

Plate III

Explanation of Plate III

Rattus norvegicus

Fig. 14. Spermatogonial chromosomes in metaphase (polar view).

Fig. 15. Chromosomes of primary spermatocyte in metaphase (polar view).

Fig. 16. A lateral view of the first spindle.

Fig. 17. A large tetrad in form of compound ring.

Fig. 18. X-Y complex and a largest tetrad in profile.

Mus molossinus

Fig. 19. Spermatogonial chromosomes in metaphase (polar view).

Fig. 20. Chromosomes of primary spermatocyte in metaphase (polar view).

Fig. 21. Chromosomes of primary spermatocyte in metaphase, showing X-Y complex.

Fig. 22. The same from an individual in which Y-chromosome is much reduced in size.

Fig. 23. Chromosomes of secondary spermatocyte of X-class (polar view).

Fig. 24. The same of Y-class.

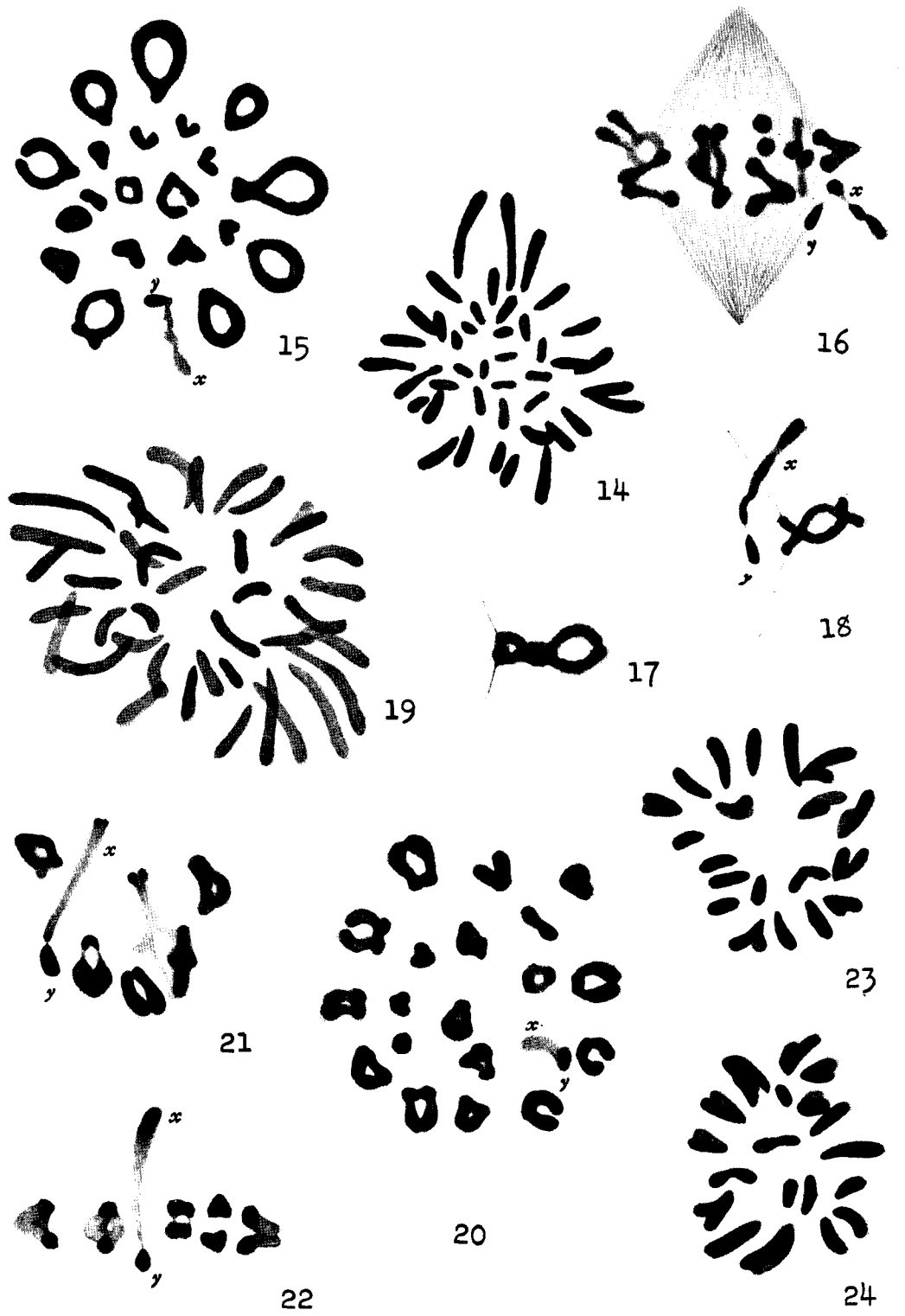


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Plate IV

Explanation of Plate IV

Evotomys (*Clethriomys*) *bedfordiae*

Figs. 25–26. Spermatogonial chromosomes in metaphase (polar view).

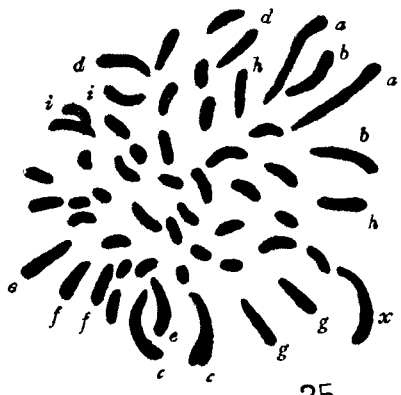
Figs. 27–29. Chromosomes of primary spermatocytes in metaphase. (polar view but Fig. 28 slightly oblique).

Fig. 30. A part of first spindle including X-univalent and 4 autosome tetrads.

Fig. 31. An oblique view of metaphase chromosomes of primary spermatocyte (not all the chromosomes are drawn).

Figs. 32–33. Chromosomes of secondary spermatocytes of X-class (polar view). Note the number 28.

Figs. 34–35. The same of the class without X (polar view). Note the number 27.



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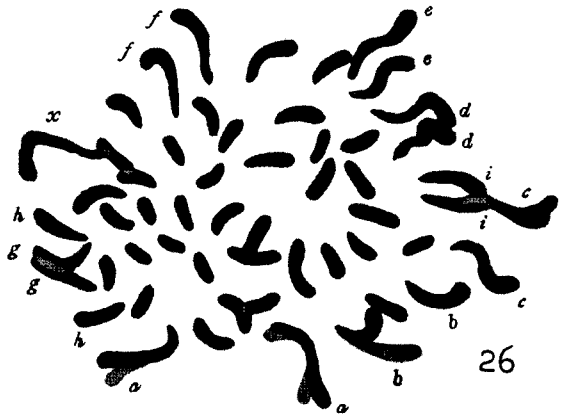

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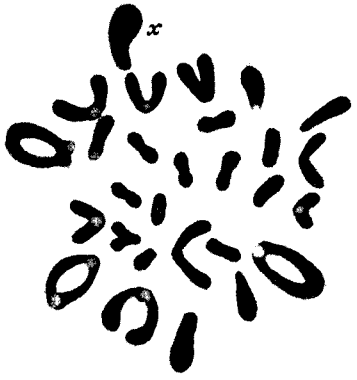

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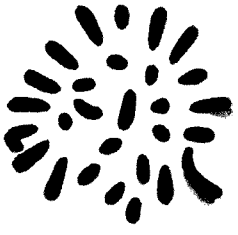

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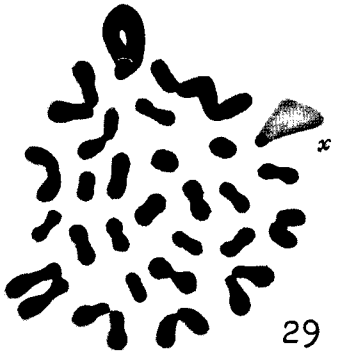

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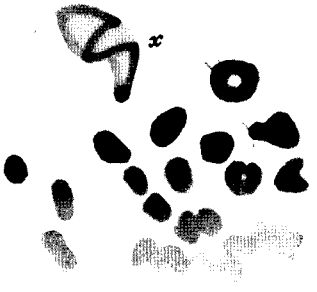

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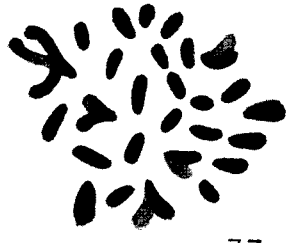

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