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# Studies on the Murine Chromosomes III. A Comparative Study of Chromosomes in Five Species of *Rattus*<sup>1)</sup>

By

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(With 130 Figures)

The present paper deals with the morphological study of the chromosomes in five species of rats belonging to the genus *Rattus* found in Japan, undertaken from the comparative standpoint. Reference to the literature (cf. Oguma and Makino '37) indicates that the majority of former works regarding the chromosomes of *Rattus* have been carried out with the Norway rat, *Rattus norvegicus*, on both, the domesticated albino variety common in our laboratories and the wild form, only a few having been concerned with the black rat, *Rattus rattus*. Our knowledge has thus been confined to the above at present, and we have been ignorant on the chromosomes of any other species of *Rattus*.

The species observed in this study are (1) *Rattus norvegicus*, (2) *R. rattus*, (3) *R. losea*, (4) *R. fulvescens coxinga*, and (5) *R. confucianus culturatus*. The former two species are well known as the cosmopolitan house rats with world wide distribution and always live having a close connection with man. The latter three forms are not indigenous to Japan-Hondo, and their distribution in Japan is confined to Taiwan (Formosa) where they are found in fields and highlands.

The present paper consists of two main parts. In the above five species the form, number and other morphological characteristics of chromosomes, and the morphology of the sex chromosomes are chief subjects to be treated in Part I. Part II concerns with the

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comparative morphology of the chromosomes, devoted with the purpose of finding out, if possible, the specific characteristics of the chromosomes in the studied species. The chromosomes of the five species were morphologically analysed and compared on the basis of the following three points: the number of chromosomes, the relative size of the autosomal elements, and the behaviour and some morphological characters of the sex chromosomes.

The brief accounts on the sex chromosomes of the five species herein concerned have been formerly given by Tateishi ('35) and Makino ('42b) in the form of a preliminary note.

The work has been done under Prof. K. Oguma whose valuable suggestions and helpful criticism given during the course of the work are heartfully acknowledged here. For collection of rats used the author is greatly indebted the good offices of Dr. Marq. Y. Yamashina (Tōkyō), Prof. B. Aoki of the Taihoku University, Assist. Prof. S. Tateishi and Mr. R. Tanaka of the same University. The expenses in collecting the rats studied in this work were defrayed for the most part by the Scientific Research Expenditure of the Department of Education.

### Material and Method

With the testicular material coming from the following five species of *Rattus* belonging to the Murinae of the Muridae, the present investigation was carried out: (1) *Rattus norvegicus norvegicus* (Berkenhout), (2) *R. rattus rattus* (L.), (3) *R. losea* (Swinhoe), (4) *R. fulvescens coxinga* (Swinhoe), and (5) *R. confucianus culturatus* (Thomas). The former two species are well known cosmopolitan forms, familiar to man as house dwellers and sometimes as field rats. They are found in all of the Japanese islands from Karahuto to Taiwan. *R. losea* is one of the most dominant field rats found in Taiwan, mainly inhabiting the entire lowlands. *R. fulvescens coxinga* and *R. confucianus culturatus* are the remarkable upland forms found through the whole island of Taiwan. According to Aoki and Tanaka ('41), *R. f. coxinga* is distributed from the foot of the mountains up to a height of 8,000 ft., but neither as low as the level land nor reaching 10,000 ft., while *R. c. culturatus* is found in uplands mostly higher than 5,000 ft., without descending, according to them, below 5,000-6,000 ft., in the

northern and 7,000–8,000 ft., in the central and southern parts of Taiwan.

With the exception of *R. norvegicus*, which were obtained in Hokkaido, Okinawa and Taiwan, all the other forms adopted as material were collected in Taiwan by the author during his sojourn there taking place in spring of 1940. They were mostly trapped in fields. The testes were removed alive in every case, cut into small pieces and dropped in the fixative. For fixative Flemming's strong solution with no trace of acetic acid was exclusively adopted. For staining the material was subjected to Heidenhain's iron-haematoxylin method with light-green as the counter stain.

## PART I. OBSERVATIONS

### 1. *Rattus norvegicus norvegicus* (Berkenhout)

A considerable number of the wild form of this species coming from various localities, such as Sapporo (Hokkaido), Taihoku (Taiwan) and Naha (Okinawa) were used as the material in this study. In addition to them, the domesticated albino rats derived from the Wistar strain and the common laboratory colony also furnished the material.

The former study made by the author (Makino '42 a) which was related to the question as to how morphological difference or likeness occurs in the chromosomes between the wild form and its domesticated albino variety of this species, revealed that so far as the general morphological characteristics of the chromosomes are concerned, the chromosomes of the wild rat are quite identical in all characters with those of the domesticated albino form, and further that there is no visible difference in the chromosomes between the individuals coming from different localities. In the present paper, therefore, the generally accepted accounts on the chromosomes of this species both in the wild and domesticated forms, may be given with special attention towards the comparison of chromosomes with the other species dealt with in this study.

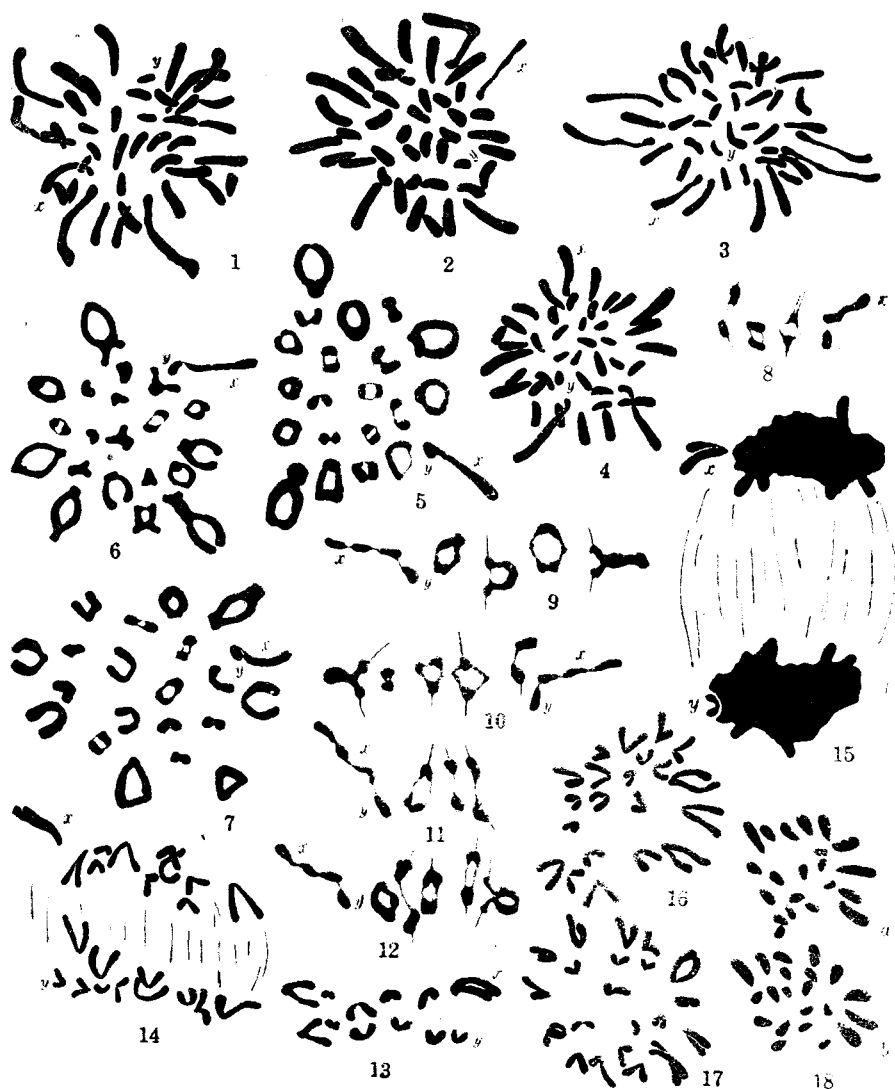
Starting with the work of Moore appeared as early as 1893, a considerable amount of work has been made on the chromosomes of this species, probably on account of the facility of getting the material, (cf. Oguma & Makino '37, Makino '42 a), but it is only

quite recently that the morphology of the chromosomes has been satisfactorily studied, the results being owed to the following investigators, Pincus '27, Painter '28, Minouchi '28, Swezy '28, Bryden '32, Koller & Darlington '34, Oguma '35, Tateishi '35, Matthey '36, Allen '40.

*Spermatogonial chromosomes.* In both of the wild form and the domesticated albino the number of chromosomes was found to be 42 in diploid (in the spermatogonia) and 21 in haploid (in the primary and secondary spermatocytes) without any fluctuation, being in a complete agreement with the reported number by the previous authors<sup>1)</sup> as given above. The chromosomes are generally found to be of a simple rod-type from their apparent configuration (Figs. 1-4). Sometimes there are found in the complex some larger chromosomes which are characterized by a satellite-like globular part forming their inner terminals. After examination of a large number of cases through the process of division it seemed to the author that the terminal globular part is not a true satellite but a segment of the chromosome which assumes a temporal and superficial swelling. In no case observed the chromosomes under consideration show any kind of constriction between the globular part and the rest of the chromosome, which is regarded as the attachment constriction for the spindle fibres. Occasionally the globular part of the chromosome shows a slight bending, but no constriction was present at the bent part. And further, viewed from the behaviour of the chromosomes in the primary and secondary spermatocytes during the process of division, there is found no positive evidence to show that they are true satellite chromosomes. In view of these considerations, though the inner essential structure of these chromosomes cannot be stated at present with certainty, they may be accepted, according to the general conception, as simple rod-shaped

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1) In the study of a hybrid colony of rats obtained by interracial crosses of the domesticated albinos with the wild rats, Swezy ('28) reported two kinds of individuals, one showing a diploid number of 42 and haploid numbers of 21 and 31, and the other possessing 62 diploid and 31 and 21 haploid numbers. The evidences indicated contain many doubtful and questionable points for which no adequate explanation is applicable. All the conditions have been considered referring to her original descriptions and drawings, and the present author comes to the conclusion that the observations made by Swezy ('28) cannot be regarded as valid and no reliable result can be expected therefrom. Full discussion in this respect was made in the author's previous papers (Makino '41, '42a).



Figs. 1-18. Chromosomes of *Rattus norvegicus norvegicus*.  $\times 3400$ . x: X-chrom. y: Y-chrom. Figs. 1-4, Spermatogonial chromosomes. Figs. 5-7, Chromosomes of the primary spermatocytes. Figs. 8-12, Side views of the primary spermatocyte metaphases, showing the XY complex. Figs. 13-14, Anaphases of the first division, showing the segregation of the X and Y. Fig. 15, Telophase of the same. Fig. 16, Chromosomes of the secondary spermatocyte, probably X-class. Fig. 17, The same, Y-class. Fig. 18 a-b, Sister complexes at anaphase of the secondary spermatocyte division.

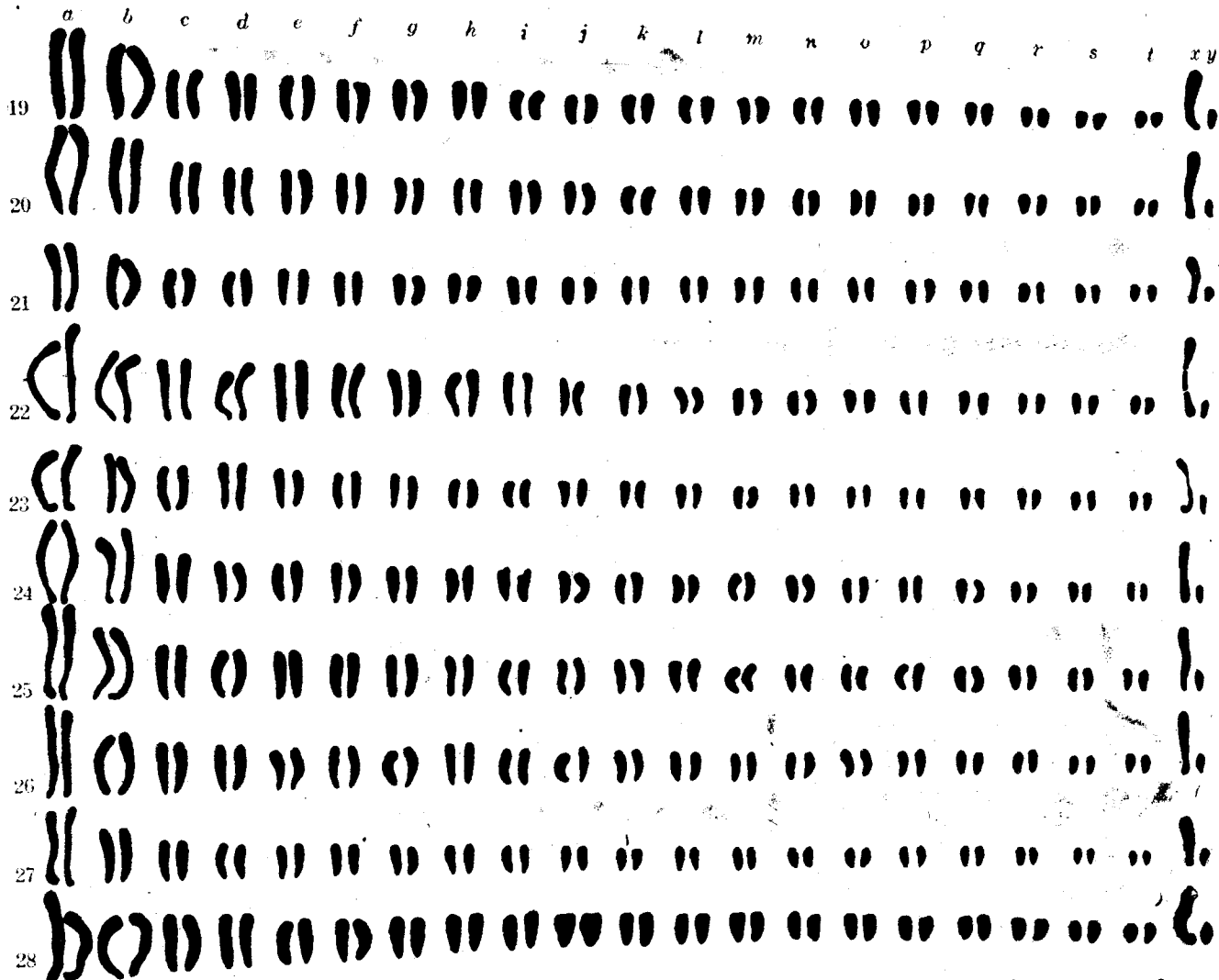
chromosomes having the fibre attachment at their inner terminals. According to application of the new terminology advocated by Oguma ('42 a), therefore, the chromosomes of this species are all comprised in the category of the orthotelomitic chromosome (*Chromosoma orthotelomiticum*)<sup>1)</sup>.

As above mentioned, the chromosomes are all simple rod-type and therefore, they bear no distinguishable characteristic feature other than that of length. The morphological analysis of chromosomes was made according to the procedure adopted by the author in the former studies (Makino '41, '42 a) and the serial alignments of the chromosomes were produced, in which the supposed homologous pairs of chromosomes were ranged in order of length. As shown in Figs. 19-28, each series thus established shows 20 homologous pairs of autosomes ranging from *a* to *t*, and an unequal pair of the sex chromosomes made up of the X (large) and the Y (small) elements placed at the extreme right. Of the autosome pairs, the first and second ones (*a*'s and *b*'s) are occupied by prominently large sized chromosomes, and from the third largest pair (*c*'s) downwards the chromosomes form a closely graded series, no outstanding element being present.

By a close examination on a large number of the metaphase figures the identification of the X chromosomes becomes possible among the members of the complex by its delicate but characteristic feature and by its relative magnitude. The X takes usually a more slender feature than autosomal elements, being loose in texture, and sometimes shows characteristic constrictions. The X thus determined is represented by the chromosomes ranging between the second (*b*'s) and third (*c*'s) largest autosome pairs in relative size. When all elements are compared, the X ranks third, being slightly longer than the *c*'s. The Y chromosome is not very easy to be identified in the diploid complement. The paired alignments of chromosomes indicate

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1) According to a new terminology recently offered by Oguma ('42 a), which was based on the study of the essential structure of the chromosome, the name *Chromosoma orthotelomiticum* is applied to the chromosome having a terminal fibre attachment (*i. e.* the telomitic chromosome in the old terminology) and *Chromosoma syntelomiticum* to the chromosome of a non-terminal attachment which has long been designated as the atelomitic chromosome.



Figs. 19-28. Chromosomes of *Rattus norvegicus norvegicus*. Paired alignments of homologous mates from spermatogonial chromosomes in a descending order. Figs. 19-21, from the albinos of the Wistar strain. Figs. 22-23, from the common albino strain. Figs. 24-25, from Taihoku specimens (wild). Figs. 26-27, from Sapporo specimens (wild). Fig. 28, from Naha specimen (wild).



that the Y approximately simulates in size the members of the smallest pair (*t*'s), sometimes being somewhat slender in outline<sup>1</sup>).

*Spermatocyte chromosomes.* The primary spermatocyte division shows at metaphase 21 well defined bivalent chromosomes of varying size, 20 of them being autosomal tetrads and the remaining one is the XY complex with heteromorphic structure, composed of the elongate rod-shaped X and the small Y in end-to-end conjugation (Figs. 5-7). The larger tetrads are distributed in the peripheral zone in the equatorial arrangement and take the form of a horizontal ring or a thick V. As pointed by Oguma ('35) the largest tetrad appears in most of cases in the form of a compound ring shape characterized by three or more chiasmata. The XY complex always lies in the periphery together with the larger tetrads. The smaller ones occupying the central space of the equatorial plate are found in the shape of a cross or a horse-shoe. Viewed from their structural nature it is evident that all the tetrads are to be regarded as the diaschistic tetrad possessing orthotelomitic fibre attachment, which are similar in structure to those widely known in the orthopteran insects.

The mode of conjugation taking place between the X and Y chromosomes was studied in detail by Minouchi ('28) in the albino form, and the result of this study supports his view. As clearly observed in the lateral configuration as indicated in Figs. 8-12, the X lies horizontally in the equatorial plate with its long axis parallel to the plate, directing its attachment end towards the centre of the spindle. To the inner extremity of the X, the Y element comes to conjugate by a delicate connecting thread. The Y stands perpendicular to the equatorial plate and to its free end the spindle fibres are constantly attached.

Noticeable is the tripartite structure displayed by the X element, which was first pointed out by Oguma ('35, '37 a, b) in

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1) Pincus ('27) and Painter ('28) expressed the view that the X is represented by one of the medium sized elements and the Y is one of the smaller (but not the smallest) elements, probably corresponding in length to the members of the 15th pair. As pointed out in the study of *Mus* by the author (Makino '41, pp. 313-314), the classical method of study adopted by these American authors cannot produce any accurate result which admits a precise morphological study of chromosomes. The evidences indicated by them involve no exactness and are no longer appreciable at present.

some rodents and man. The X is provided with two transverse constrictions which divide its entire body into three successive segments, proximal, median and distal segments (Figs. 8-12). All segments look like to have nearly equal size and the proximal segment in which the attachment locus of the spindle fibres lies, assumes a rather compact texture. The exact locus of the fibre attachment in the X is difficult to determine. The finding of the present observation falls in the view that it seems to locate near the tip of the proximal segment of the X. The tripartite structure of the X has been found at present to be the characteristic and fundamental structure generally found in the mammalian X chromosome, and its occurrence has been broadened over several other forms of mammals by the present author (Makino '41, '42 a, b, '43 a, b, c). In none of the cases observed the variations in size and behaviour of the X and Y as described by Allen ('40) have been encountered at all in the present study.

The separation of chromosomes at the first division anaphase has taken place quite synchronously and there is no element which lags or precedes the others (Figs. 13-14). All elements appear, after separation, in the form of two corresponding single V's which are composed of identical chromatids united at the inner end of the fibre attachment. In the first division the separation of the X and Y has constantly taken place without any one exception, migrating to the opposite poles (Figs. 13-15). Thus the first division results in the production of two kinds of secondary spermatocytes, each being equal in number showing 21, but differing in the constitution of the sex-chromosome (Figs. 16-17). One of them includes the X chromosome and the other the Y. All the chromosomes (dyads) at metaphase assume the shape of a single V, giving the identical configuration to those found in anaphase of the previous division. It is evident that every one consists of two identical chromatids produced by splitting and joined at the inner end. In division they separate each into a single rod-shaped chromatid, the monad (Fig. 18). All the conditions seen in the meiotic chromosomes of this species agree on the whole with those observed in *Mus* by the author (Makino '41).

Except Minouchi ('28) and Oguma ('35), the previous workers such as Pincus ('27), Painter ('28), Swezy ('28), Bryden ('32), Koller & Darlington ('34) and Allen ('40), have not been successful

in preserving the chromosomes of the meiotic divisions in a natural state. The chromosomes given in their figures are all very far from indisputably clear and involve much obscurity. It is likely, therefore, that many misconceptions and controversies might have been produced from observations of chromosomes being in such an aberrant state. In fact, the behaviour of the chromosomes during meiotic divisions, particularly that of the sex chromosomes, has been the subject of repeated discussions among the previous authors. Noticeable is a great variation in shape, size and behaviour of the X and Y reported by the previous authors. The cases are found in the papers of Pincus ('27), Bryden ('32), Koller & Darlington ('34) and Allen ('40). Pincus ('27) figures a considerable variation in shape and size of X and Y according to different cells. Allen ('40) likely describes a number of variations in size and behaviour of the X. Such cases as above described, however, are very questionable to be regarded as the natural and fundamental feature of the X and Y. Referring to their original papers, it becomes evident that these questionable figures are no other than the aberrant configurations which were caused by the technical procedure. One cannot overlook the fact that the chromosome pictures, the subjects of our observations, are those derived from the material prepared by artificial treatment with reagents. It is very likely that irregular deformation and abnormal orientation of the chromosomes protruded from the equatorial plate possibly arise artificially due to inadequate method of fixation. The same may be said with the cases reported by Bryden ('32) and Koller & Darlington ('34). These authors emphasized that the equational segregation of the XY-chromosome occurs in a few case of the first division. The skillful observer may not doubt to see that the figures given by Koller & Darlington ('34) as the equational separation of the XY are nothing but the irregularly protruded chromosomes, caused by faulty fixation. Their hypothesis established on the basis of such a very erroneous finding will no longer be appreciable at all. The evidences presented in the study of *Mus* by the author (Makino '41, pp. 338-346) are sufficient to emphasize this point to the greatest extent. A statistical examination on the segregation of the X and Y in the first division undertaken by the present author, which was carried out on clear anaphasic figures found in the testes of wild and domesticated forms, results in the conclusion that all of the examined cases showed to be

reductional as regards the separation of the X and Y, as shown in the following table.<sup>1)</sup>

TABLE I. Segregation of XY in the first division of *R. norvegicus*

Reductional	Equational
152	0

As above mentioned, a series of these events reported by the previous investigators are all by no means the natural and fundamental features characteristic to the observed species, but are only artefacts induced by inadequate technique. After of all, the author wishes to repeat here that the microscopical figures under our observations are those derived from the material fixed and treated with various reagents. In view of this fact, therefore, it is very necessary, before making any conclusive statement, to bear in mind the occurrence of artefact configurations in the material under observation in greater or less degree, which were caused by the technical procedure. It is certain that the most part of controversies among the investigators in the mammalian cytology can find their cause in the observation and interpretation of artefact products.

## 2. *Rattus rattus* (L.)

This is also a cosmopolitan species together with the former *R. norvegicus*, having a world-wide distribution travelling in companionship with human agency. It is said however, that this species is becoming progressively smaller in number towards the north. The present species is clearly distinctive from *R. norvegicus* in having a relatively longer tail, larger ears and smaller hind feet. There are recorded from Japan two other subspecies of this form, *R. r. alexandrinus* and *R. r. rufescens*. They are distinguishable from one another only by trivial characters relating to coloration. After a close study carried out on a large number of specimens coming from various localities, Tokuda ('41) expressed the view that all the Japanese representatives, in spite of their great variability in coloration, are to be referred to one and the same subspecies.

So far as the literature shows, five papers have appeared relating

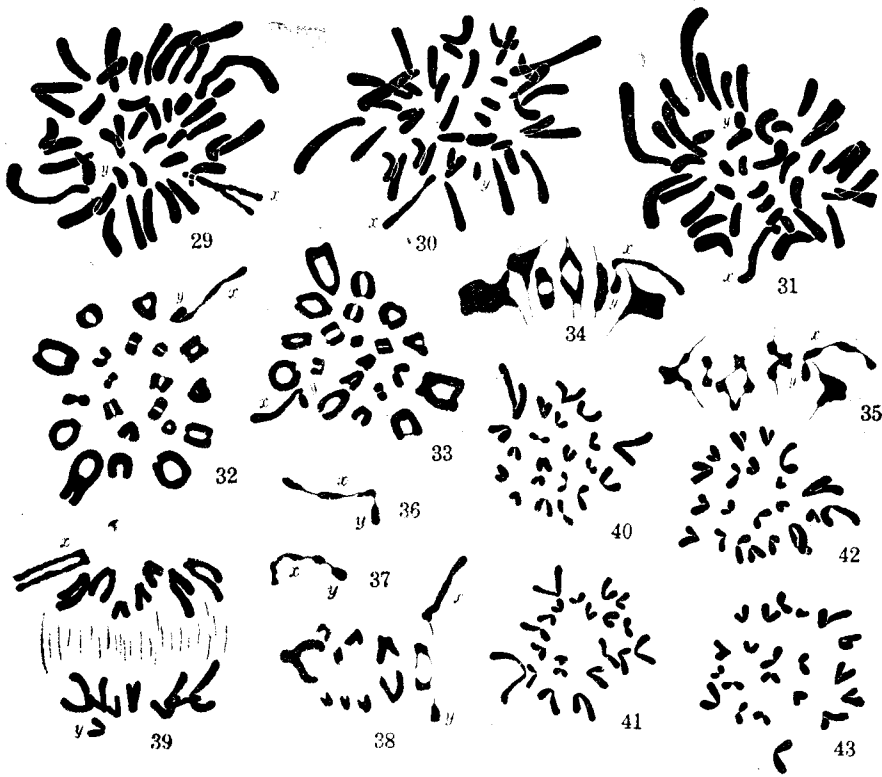
1) The same has also been shown in the case of *Rattus rattus* (cf. Table II).

to the chromosomes of the black rat, *Rattus rattus*. Van Hoof ('11) was the first to describe the chromosomes of the black rat, reporting so a low number of chromosomes as 24 in diploid and 16 in haploid. Later Pincus ('27) undertook a comparative study of chromosomes in *R. norvegicus* and *R. rattus*, in which he showed 42 chromosomes as diploid for the former and 40 chromosomes for the latter species. Then Cross ('31), working with *R. r. alexandrinus*, gave the diploid number as likewise 40 for this form. Contrary to these previous investigations, Oguma ('35) announced the different view that the chromosomes of *R. r. rattus* are entirely identical to that of *R. norvegicus* in number and general morphological characters, establishing 42 in diploid and 21 in haploid. Tateishi ('35) reached to the same conclusion in the study with *R. r. rufescens*.

The following observations were based on the material coming from four individuals of which three were collected in Taihoku and one in Kobé.

*Spermatogonial chromosomes.* As already pointed out by Oguma ('35), the general morphological features of the chromosomes of this species are found on the whole to be quite identical in essential points to those of the former species, *R. norvegicus*, and the number of chromosomes is likewise 42 in diploid, all of them being of orthotelomitic nature (Figs. 29-31). There is present no evidence for the existence of the V-shaped chromosome. The careful study reveals that the only difference distinguishable occurs in the relative magnitude of some autosomal elements. In this respect reference may be made to the later section.

The morphological analysis of the chromosomes was done by means of the mating up of the homologous elements by comparison of their shape and size under microscope. The paired chromosomes are arranged in the serial alignment in the order of size. Examples are shown in Figs. 103-107. Each series represents 20 autosomal pairs of equal size ranging from *a* to *t* and an unequal pair composed of the X and Y at the extreme right. As occurred in the former species, the first and second largest pairs (*a*'s and *b*'s) are provided with the outstandingly large elements, and from the third pair downwards the chromosomes diminish in length showing a graded seriation. Any noticeable differences are not found in the chromosomal constitution between the specimens collected in Taihoku and Kobe.



Figs. 29-43. Chromosomes of *Rattus rattus rattus*.  $\times 3400$ . *x*: X-chrom. *y*: Y-chrom. Figs. 29-31, Spermatogonial chromosomes. Figs. 32-33, Primary spermatocyte chromosomes. Figs. 34-35, Side views of the primary spermatocyte metaphases, showing the XY complex. Figs. 36-37, XY complexes in the primary spermatocyte metaphases. Figs. 38-39, Anaphases of the first division showing the segregation of the X and Y. Figs. 40-41, Secondary spermatocyte chromosomes, probably X-class. Figs. 42-43, The same, Y-class.

When the individual chromosomes are carefully examined, especially in the serial alignment, it is obvious that the X element ranks third largest in length ranging between the *b*'s and *c*'s in magnitude, while the Y is nearly identical in size with the members of the smallest pair (*t*'s), bearing sometimes slender outline. The X assumes a rather loose texture and sometimes represents characteristic constrictions, being divided into three consecutive segments in a linear series. All the conditions shown by the X and Y elements of this species thus exhibit nothing noticeably different

from those in *R. norvegicus*, not only in the apparent morphological feature but also in the relative magnitude. The result obtained by Pincus ('27) indicating that the obvious difference between *R. rattus* and *R. norvegicus* lies in the nature of the X-Y pair, is by no means an appreciable one but may be explicable as an effect of faulty fixation.

On the basis of the above observations, some mention should be made here concerning the different views afforded by the previous authors for this species. As formerly stated, Pincus ('27) and Cross ('31) gave the diploid number of chromosomes of the black rat as 40. In spite of the uniformity in the chromosome number, the formative elements of the complex are dissimilar according to the two authors. According to Pincus ('27) there are found three pairs of large V-shaped chromosomes besides the remaining rod-shaped ones in the diploid complement, while Cross ('31) reported only one such pair. The material used by Pincus ('27) is *Rattus rattus rattus* and that by Cross ('31) *R. r. alexandrinus*. Such a difference in the chromosomal composition, however, is, as it seems to the author, too great to be interpreted as the chromosomal difference between the different subspecies: no logical cytological explanation can be found to interpret such a condition. Referring to the figures given by these authors upon which their conclusions were based, it becomes evident at once that the chromosomes are not indisputably clear-cut to admit the accurate study of morphology and the exact counting of the number, since the chromosomes are clumped together with each other, without showing any definite outline. With such an insufficient material, the accurate observation may be difficult for any skillful observer. In view of this fact it seems certain that these two authors made error in the study of morphology and in the counting of the number, on account of the clumped condition of the chromosomes. As shown by Oguma ('35) and also by this study, the number of chromosomes of the black rat, *Rattus rattus rattus*, was evinced to be invariably 42 in diploid and 21 in haploid, so far as the chromosomes were quite clear without any shadow of obscurity. And further it was shown that no variation occurred in the number of chromosomes among the individuals obtained in different localities. Tateishi ('35) attained to the same conclusion in the study of *R. r. rufescens*. The evidences

offered by Pincus ('27) and Cross ('31) are by no means fundamental, but can be explained by the effect of fixation.

*Spermatocyte chromosomes.* The primary spermatocyte metaphase shows 21 bivalent chromosomes consisting of 20 autosomal tetrads and an XY bivalent which is very prominent on account of its heteromorphic structure (Figs. 32-33). The morphology of the tetrads has been fully described by Oguma ('35), so that no further detailed description is required here. As in the case of the former species, the larger tetrads assume the form of a horizontal ring distributing in the peripheral zone of the equatorial plate and surrounding those of the smaller size which assume the shape of a horse-shoe, a cross and a thick V. As compared with *R. norvegicus*, remarkable and noticeable is the fact that there is entirely absent the compound ring tetrad in this species, whereas in *R. norvegicus* one of the larger tetrads always appears in the form of a compound ring holding three or more chiasmata. As already noticed by Oguma ('35) this seems to be the characteristic feature of *R. rattus*.

The XY bivalent is found lying always in the periphery of the equatorial plate. As is the case with *R. norvegicus*, the X is characterized in lying parallel to the equator in an elongated form, and to its inner end where the spindle fibres attach, the Y comes to associate (Figs. 34-35). The latter stands perpendicular to the equator. The tripartite structure of the X is likewise distinct as given, for example, in Figs. 35 to 37. Thus the morphological composition of the XY bivalent is not essentially different from *R. norvegicus*.

In the first division the X constantly disjoins from the Y without exception (Figs. 38-39). The result of the statistical research for the anaphase segregation of the XY bivalent in the first division, based on a total of 164 anaphasic figures, shows the unexceptional separation of the XY (see Table II).

TABLE II. Segregation of XY in the first division of *R. rattus*

Reductional	Equational
164	0

There are produced, as the result of the first division, two kinds of secondary spermatocytes, being equal in the number of chromo-



somes but different in the constitution of the sex chromosomes contained. One of them is the X-class cell containing a large X element in addition to 20 autosomal dyads (Figs. 40–41), and the other is the Y-class cell possessing the constitution of 20 autosomes and a small Y (Figs. 42–43). The morphology of the chromosomes of the secondary spermatocyte is entirely similar to that of the former species and no special statement will be required to be added here. The erroneous chromosome number reported by Pincus ('27) as 20 in haploid, is undoubtedly accounted for as the result of faulty preservation. By reference to the figures given in his paper (Pincus '27), it is understood at once that many misconceptions would be born from the observations of the chromosomes crowded into a clumped condition, showing no definite configuration.

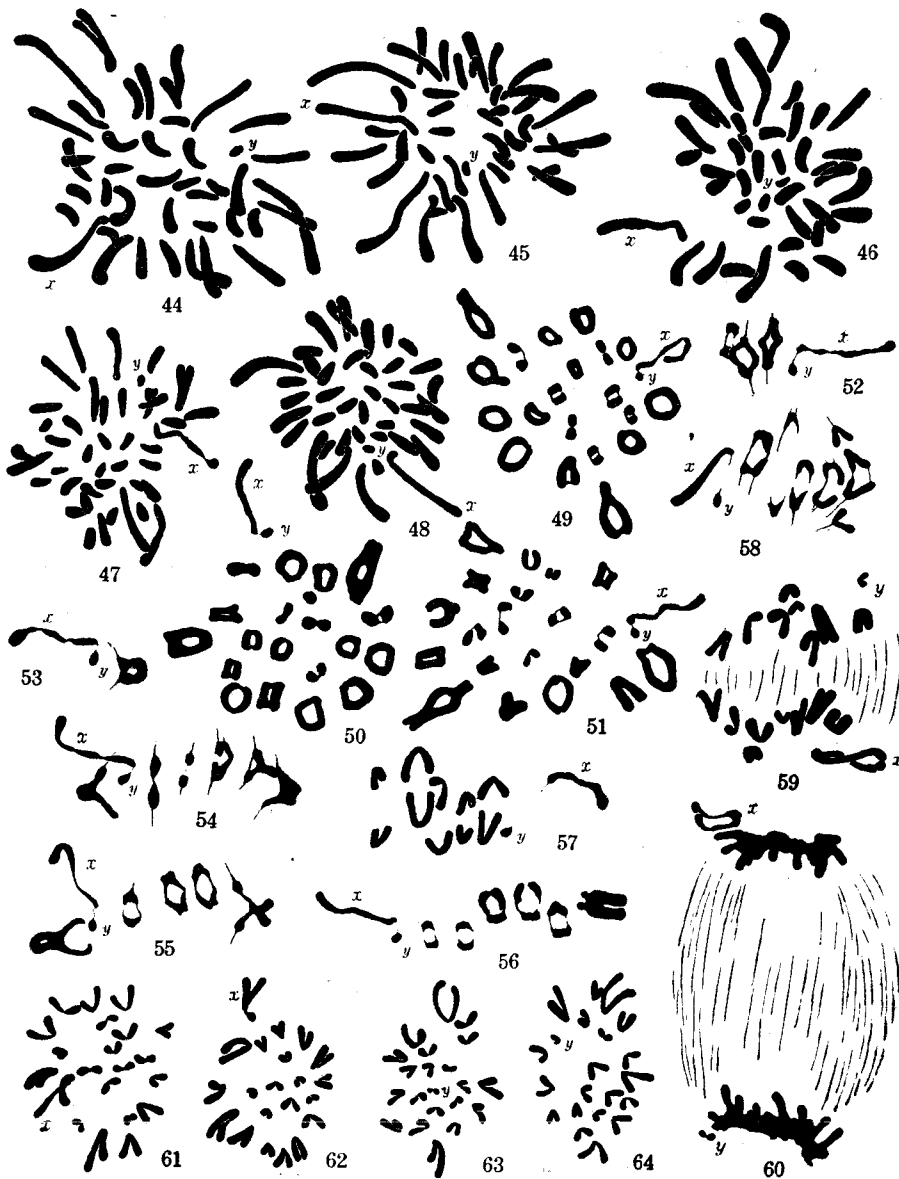
### 3. *Rattus losea* (Swinhoe)

This species is a common field rat in Taiwan inhabiting the entire lowlands. This is taxonomically a close ally to *R. rattus* and carries a number of very related characters. The testes derived from five individuals which were collected in Taihoku and Kōsyun provided the present material.

The chromosome number of this rat has been briefly reported first by Tateishi ('35) and then by the present author (Makino '42 b) in the form of a preliminary report.

*Spermatogonial chromosomes.* The material supplied a large number of metaphases. The general observations upon them show that the apparent morphological features of chromosomes in this species fairly resemble those existing in the former two species not only in the number but also in the form. The number of chromosomes is invariably 42 being the same as that of the former species (Figs. 44–48). In general all the elements appear as simple rod-type of orthotelomitic nature. A close examination of the individual chromosomes, however, reveals that some appreciable differences, by which the present species is distinguishable from the former two, lie in the relative magnitude of certain chromosomes and in the structural feature of the X and Y elements, as described below.

As in the former cases, the serial alignments of the chromosomes were made after pairing of the homologous mates and by arranging them in the descending order, to make the morphological comparison



Figs. 44-64. Chromosomes of *Rattus losea*.  $\times 3400$ . x: X-chrom. y: Y-chrom. Figs. 44-48, Spermatogonial chromosomes. Figs. 49-51, Primary spermatocyte chromosomes. Figs. 52-56, Side views of the primary spermatocyte metaphases, showing the XY complex. Figs. 57-59, Anaphases of the first division showing the segregation of the X and Y. Fig. 60, Telophase of the first division showing the segregation of the X and Y. Figs. 61-62, Secondary spermatocyte chromosomes, X-class. Figs. 63-64, The same, Y-class.

of chromosomes evident. All the chromosomes are arranged into 20 pairs of autosomal elements ranging from *a* to *t*, and the XY pair which is unequal in size. Examples are given in Figs. 108-112. By reference to these figures it becomes apparent that the members of the first largest pair (*a*'s) are quite prominent in being extremely large sized, outstanding among the rest, and that from the second largest pair (*b*'s) to the fourth largest pair (*d*'s) the elements exhibit no striking variation in length, diminishing very slightly, and from the fifth largest (*e*'s) downwards the members show a closely graded diminution of size.

Contrary to the cases in the former species, the X chromosome of this species is represented by an extremely large sized element which probably corresponds in length to the members of the first largest pair (*a*'s), while the Y is the smallest of all and attains the size approximately one-half the members of the smallest autosome pair (*t*'s). The X is usually characterized by a loose texture and displays sometimes two constrictions being divided into three segments. One more noticeable feature of the X is that it gently bends at a point a little distant from its extreme tip (see *x* in Figs. 44-48 and Fig. 123). In regards to the question whether the locus of the fibre attachment lies at the curved point of the X or not, in other words, whether the X is syntelomitic or not, the present study offers no positive evidence. But in the observations of the first meiotic division a few pictures, though not sufficiently valid, were found which tend to show that the attachment locus of the X lies at a portion a little apart from its extreme end.

*Spermatocyte chromosomes.* The primary spermatocytes of this species are not different from those of the former two species in showing at metaphase 21 bivalent chromosomes which consist of 20 autosomal tetrads and a heteromorphic tetrad of the XY complex (Figs. 49-51). The morphology of the autosomal tetrads is also alike to that observed in the former cases, and larger tetrads found in the periphery of the equator assume the form of a horizontal ring and a thick V, surrounding in the central space those of the smaller size having the shape of a ring, a cross or a horse-shoe. As occurred in *R. norvegicus*, the largest tetrad of this species seems to take likewise the form of a compound ring.

Being apart from the autosomal tetrads, the feature of the XY complex is rather noteworthy for this species, though the mode of

conjugation between the X and Y shows nothing different in principle from that in the former cases. The X element is very remarkable by having an extremely elongated feature and lies on the equatorial plate stretching over its whole length with its inner end of the fibre attachment towards the centre of the spindle. The Y element, which is very minute in size, comes to contact by a thin thread with the X at its inner extremity (Figs. 52-56 and 124-125). The size relation existing between the X and Y is thus very striking in the present species, and it is not comparable with either of the former two cases.

The tripartite segmentary structure is also evident in the X of this species too, as given in Figs. 52 to 54 for example. On account of this structure, coupled with its very elongated form, the X generally displays a light undulation.

In the first division all the tetrads segregate into their component halves; the XY bivalent also does the same without any exception, the X and Y passing to the opposite poles. Figs. 57 to 60 indicate this feature beyond the slightest doubt. Thus the first division is reductional for the X and Y, and this results in the production of two sorts of secondary spermatocytes, (a) those having 20 autosomal dyads plus the X element (Figs. 61-62) and (b) those possessing an equal number of autosomal dyads plus the Y (Figs. 63-64). In well preserved condition the X, as well as the Y, are easily distinguishable from the rest of the complex, on account of their characteristic shape and size.

#### 4. *Rattus fulvescens coxinga* (Swinhoe)

This rat is the mountainous form only found in Taiwan, extending from the foot of mountains to an elevation of about 8,000 ft. (Aoki & Tanaka '41). This is quite remarkable in having well developed spiny hairs in the dorsal part of the pelage and the long bicolored tail. In several taxonomical characteristics this species is pronouncedly dissimilar from the foregoing species of *Rattus*. The chromosomal constitution of this form was found, as mentioned below, to be incompatible with those in the foregoing rats, in parallel to the systematic condition.

The brief notes on the chromosomes of this species have pre-

viously been given by Tateishi ('35)<sup>1)</sup> and the present author (Makino '42 b). The material for the present investigation was provided through the kind offices of Assist. Prof. S. Tateishi of the Taihoku Imperial University, Taiwan.

*Spermatogonial chromosomes.* The present species pronouncedly differs from the foregoing species of rats in possessing 46 chromosomes in the diploid complex, being four more than the latter (Figs. 65-68). In respect of the general morphological character of the individual chromosomes, however, there is found a close agreement among them, since the individual elements appear in the form of a simple rod of usual orthotelomitic nature, and there is no evidence for the presence of the V-shaped element.

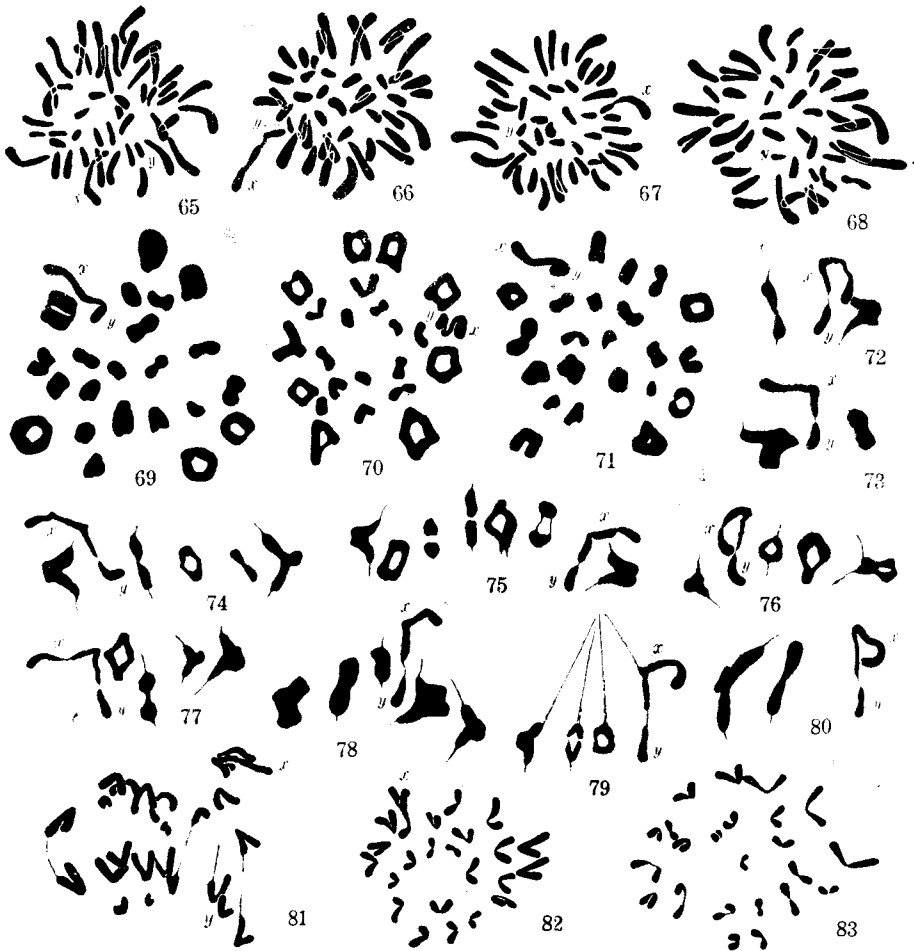
The morphological analysis of the diploid chromosomes was done by plotting them in the alignment arrangement of the approximate order, after mating up of the homologous mates by means of intimate observation with microscope. The result of this research indicates that the diploid complement consists of in each 22 autosomal pairs of homologous chromosomes ranging from *a* to *v*, and an unequal pair of the X and Y elements. The evidence may be clearly understood by reference to Figs. 113-117. From these figures the fact becomes evident that the autosomal pairs form a closely graded series and there is no pair outstanding in size. This feature may be appreciable as the karyological characteristic peculiar to this species, since in the foregoing forms, as already mentioned, one or two pairs of chromosomes are outstandingly large in size.

The X chromosome is very distinctive from the rest in the diploid group, on account of its prominently large size, being probably the largest of all, with a rather slender outline (see *x* in Figs. 65-68 and 113-117). In the majority of cases observed, the X is provided with two transverse constrictions, by the presence of which it displays a definitive tripartite configuration being subdivided into three distinct segments, showing a light undulation. Sometimes the X gently bends at a point where its proximal segment comes to join with the successive median segment (see *x* in Figs. 65-66). The Y element was found to be represented by a chromosome a little

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1) In this report Tateishi mentioned the variation of the chromosome number to occur in this species, but he corrected his early accounts in his recent private letter.

longer than the members of the smallest pair (*v*'s). The comparison of size and shape seems to show that the Y corresponds to the members forming the *t*-pair in relative magnitude, as was the case with *R. norvegicus* and *R. rattus* already investigated.



Figs. 65-83. Chromosomes of *Rattus fulvescens coxinga*.  $\times 3400$ . *x*: X-chrom. *y*: Y-chrom. Figs. 65-68, Spermatogonial chromosomes. Figs. 69-71, Primary spermatocyte chromosomes. Figs. 72-80, Side views of the primary spermatocyte metaphases, showing the XY complex. Fig. 81, Anaphase of the first division showing the segregation of the X and Y. Note the configuration of the X. Fig. 82, Secondary spermatocyte chromosomes, X-class. Fig. 83. The same, Y-class.

*Spermatocyte chromosomes.* The metaphase plates of the primary spermatocytes invariably show 23 bivalents of which 22 are autosomal bivalents and the one is the XY complex (Figs. 69-71). There has been no variation in the number of chromosomes so far as the present material is concerned. The larger tetrads lying on the periphery usually take the form of a horizontal ring or a thick V, surrounding the smaller ones with the shape of a dumbbell and a cross. The XY complex is very distinct due to its heteromorphic nature and lies always on the periphery.

The configuration of the X as seen in the metaphase plate of the first division is quite interesting and noticeable in this species. As readily observable in the lateral aspect of the spindle, the X exhibits a conspicuous knee shape in appearance (Figs. 72-80 and 126-127). Strictly speaking, the X displays a definitive tripartite configuration composed of three distinct segments, proximal, median and distal, each having nearly equal size; it bends at about a right angle at or near the point where the proximal segment comes to join with the median segment. The proximal segment, therefore, stands, under this condition, vertical to the equatorial plate, while the remaining piece of the X which involves the median and distal segments, lies in the most cases parallel to the equatorial plate giving a somewhat undulating appearance (see Figs. 73, 74, 75, 77). But sometimes a modified configuration was found in which the distal segment bends again inwards, as seen in Figs. 72, 76, 78, 79 and 80. Occasionally the proximal segment of the X elongates in a considerable degree as shown in Figs. 76 and 80, showing a similar appearance to the X chromosome of man, given in the paper of Oguma ('37). Thus the various modifications occur in the manner, degree and direction of bending of the segments. The Y element comes to attach with the X by a thin thread at its free end, standing always perpendicularly to the equatorial plate. As thus noted, the configuration of the XY complex is very peculiar in this species and there has been found no comparable case in the rats formerly investigated.

Some remarks should be made here on the spindle attachment of the X chromosome. There are two probable cases to be considered relating to this point. One of the cases coming first under consideration concerns with the fact that in the X the spindle fibres attach to its knee-point where the proximal segment joins to the

median segment, and the other deals with the fact that the attachment locus lies at the free end of the proximal segment of the X, where the latter conjugates with the Y element, as was the case with the foregoing species already described. The structural configuration expressed by the X of this species, as above mentioned, shows the former view to be most probable, being in favour of the interpretation that the spindle fibres attach to the knee-point at the joint of the proximal and median segments, since the X always holds its bent apex directed towards the centre of the equatorial plate in metaphase arrangement of the first division (cf. Figs. 72-80). The structural condition of the X in the spermatogonial division seems to tend to support this view, on account of the evidence that the X shows a gentle curve at the joint part of the proximal and median segments as already noticed. Furthermore, the configuration shown by the X in anaphase of the first division seems to be in much favour of this view, since the X expresses a subtelomitic<sup>1)</sup> appearance, being composed of the distinct, long and short, arms, as clearly demonstrated in Figs. 81 and 128. If the above conditions be natural, the X chromosome of this species would be syntelomitic in structure, having subtelomitic attachment. To make a conclusive statement on this point, however, further confirmation in various phases of divisions is, as it seems to the author, necessary on a more comprehensive material.

There are distinguishable, as usual, two sorts of secondary spermatocytes, resulted in the distribution of the X and Y chromosomes in the first division. Both of them contain the equal number of chromosomes, each showing 23, but the one possesses an X element in its complex (Fig. 82) and the other the Y (Fig. 83). The X is rather distinctive among the rest on account of its characteristic shape and size.

##### 5. *Rattus confucianus culturatus* (Thomas)

This is a remarkable upland form found throughout the whole island of Taiwan and extends generally above 5000 ft. (Aoki & Tanaka '41). This rat is a close relative of the former *R. fulvescens coxinga* and bears a close resemblance to the latter in several of

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1) Oguma ('42a) applied a new term "subtelomitic" to "subterminal" usually used.



superficial characters. In parallel to this bodily characters, there exists a pronounced agreement between the chromosomes of these two forms, either in the number or in the general morphological features, as described below.

The chromosomes of the present species have previously been briefly reported by Tateishi ('35) and the present author (Makino '42 b). The material employed for this study consists of a pair of testes derived from an individual caught in Arisan.

*Spermatogonial chromosomes.* The number of chromosomes established in this species exhibits a complete coincidence with that occurring in the previous form *R. f. coxinga*, in having 46 chromosomes in diploid (Figs. 84-86). Morphologically considered, the chromosomes are also much alike between these two species and no appreciable difference is visible. All the members constituting the diploid complement take the form of a rod-shape and arrange with their inner ends pointing towards the centre of the equatorial plate. The evidence thus presented may be sufficient to show that all the elements have an orthotelomitic fibre attachment.

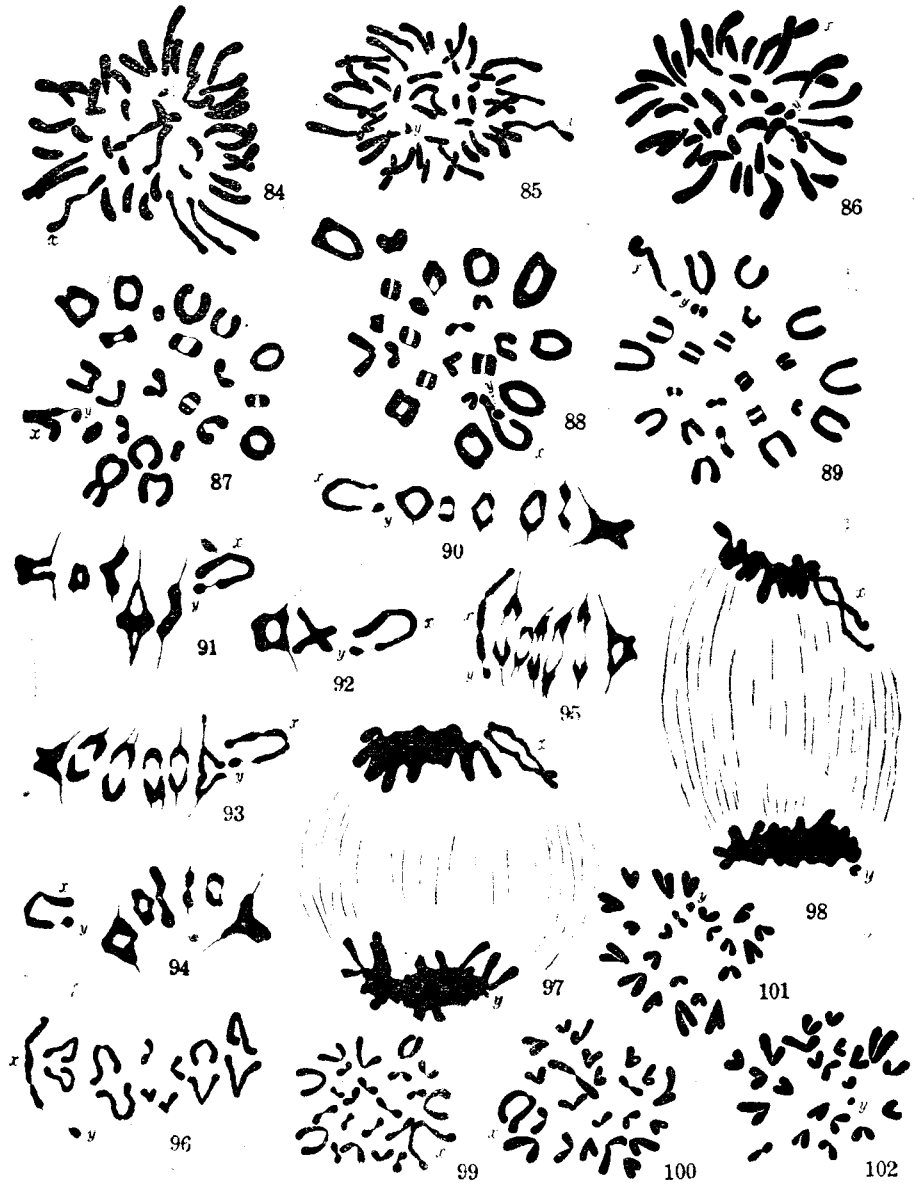
The morphological analysis was carried out by means of the procedure usually utilized in the foregoing cases and the chromosomes placed in pairs were arranged in the serial alignment. There are thus produced in every complement 22 autosomal pairs with identical size and shape, and an asymmetrical pair composed of the X and Y (Figs. 118-122). The autosomal pairs which range from *a* to *v* in the alignments, form a well graded series without any element remarkably outstanding in length. In this character, therefore, it can be stated that the present species shows nothing dissimilar from the previous rat, *R. f. coxinga*. A close comparison of chromosomes, however, makes it evident that the individual elements of this species are a little longer than the corresponding ones of the previous form (compare Figs 118-122 with Figs. 113-117).

Similar to the previous case, the X chromosome of this species is the largest element in the complement having the characteristic feature readily distinctive among the others. The X is characterized by its slenderness in appearance, sometimes being less stained, and in the majority of cases it exhibits two constrictions resulting in the tripartite segmentary structure. By this structure the X always shows an undulation in greater or less degree.

The present species offers a striking contrast to the former species in the feature of the Y chromosome, since the latter is extremely minute in size, being prominent among the rest. It is markedly smaller than the members of the smallest pair (*v*'s), being nearly one-half the size of the latter. Based on this remarkable minute size of the Y, the distinction between the present and the previous forms is very evident.

*Spermatocyte chromosomes.* In general morphological feature of the spermatocyte chromosomes there is a close resemblance between the present and former species. The metaphase plate of the primary spermatocyte shows likewise 23 well defined chromosomes of bivalent nature, and of them 22 are autosomal tetrads of symmetrical structure and the remaining one is the XY complex having a heteromorphic configuration (Figs. 87-89). As usual in the others, the larger tetrads assume the shape of a ring or a thick V and are distributed on the periphery of the equatorial plate surrounding the smaller ones of varying shapes.

A feature which is characteristic to this species and requires special attention, is found in the XY complex. In fact the XY complex is very particular in its configuration and behaviour, being wholly different from any one of the former species. The X element always shows very clearly the tripartite structure, being subdivided into three segments, of which the proximal segment conjugates with the Y on its free end. The X invariably bends inwardly at the two joints of each segment, as to form an elliptical configuration. Under this condition the XY complex disposes itself in the periphery of the equatorial plate, always holding two free ends of the Y and the X as to point towards the centre of the equatorial plate. The feature mentioned here may be better understood by reference to the accompanying figures (*xy* in Figs. 90-94 and 129-130) than from any detailed description. This morphological peculiarity displayed by the XY complex may be, as it seems to the author, favourably accounted for by following the explanation of Oguma ('35) that the X structurally shows the vesicular construction which has been brought about *in situ* up to metaphase of the first division through the meiotic growing period. In other words, the X has the configuration just emerged out from the vesicle inside which it had heretofore been imbedded during the growing period, without showing any alteration. That the X assumes the form of a chro-



Figs. 84-102. Chromosomes of *Rattus confucianus culturatus*.  $\times 3400$ . x: X-chrom. y: Y-chrom. Figs. 84-86, Spermatogonial chromosomes. Figs. 87-89, Primary spermatocyte chromosomes. Figs. 90-94, Side views of the primary spermatocyte metaphases, showing the XY complex. Figs. 95-96, Anaphases of the first division, showing the configuration of the XY complex. Figs. 97-98, Telophases of the first division, showing the segregation of the X and Y. Figs. 99-100, Secondary spermatocyte chromosomes, X-class. Figs. 101-102. The same, Y-class.

mosome vesicle during the growing period and develops from the chromatic skeleton imbedded in the ground substance of the vesicle, has been actually demonstrated in *Mus* by the present author, who followed the whole history of the sex chromosome throughout the growing period (Makino '41, pp. 338-343). Relating to this phenomenon Oguma ('35) has formerly expressed the superior view that the X chromosome generally possesses a vesicular nature in which the chromatin condensation takes place in various manners, grades and times in the course of meiosis, and further that in *Apodemus* and *Evotomys* the duration of the vesicular construction extends up to metaphase of the first division, an evidence quite comparable to the present case.

Later on at the commencement of anaphase the XY complex expresses a striking change in appearance. As shown in Figs. 95 and 96, at anaphase the X entirely casts off its previous configuration; it now stretches itself into a linear chain of segments with the companion of the Y at one end, showing somewhat undulation. Under this condition the entire body of the XY complex always stands vertical to the equator. Then, following the separation of autosomal elements each into their component halves, the X also disjoins from the Y and they migrate to the opposite poles with their distal ends pointing towards the poles, showing a distinct dual nature (Figs. 97-98). This anaphasic configuration of the XY chromosomes in this species shows nothing different from that found in *Mus* neither in the appearance nor in the behaviour (Makino '41), and this result naturally leads to the conclusion that in the X the spindle fibres come in contact with its distal free extremity opposite the end where the Y comes in connection, similarly to the cases established in mice (Makino '41). This is quite an unique example specially characteristic to the present species, and has never been encountered in the other species of rats, in every of which the attachment locus lies in the proximal segment of the X, though there occurs a slight variation. In this respect, therefore, a connection between *Mus* and *Rattus* seems to be indicated.

There are discernible two kinds of secondary spermatocytes, though each have an equal number of chromosomes, resulting in the reductional segregation of the X and Y in the first division. The one consists of 22 autosome dyads plus the X element (Figs. 99-100) and the other contains the same complex of the autosome dyads plus

the Y (Figs. 101-102). The general morphological feature of the dyads is entirely identical to that observed in the foregoing species of rats. The X, as well as the Y, are rather distinctive among the others on account of their remarkable shape and size.

## PART II. COMPARATIVE MORPHOLOGY OF THE CHROMOSOMES IN THE SPECIES INVESTIGATED

Concerning the chromosomal relationship existing among the studies species, the status seems to be premature to make any discussion at present, since the data now obtained are too poor to deal with this subject, the number of species observed also being too small. In this chapter, therefore, the morphological research of chromosomes will be carried out with the purpose to find out what kinds of characters, available for the distinction of species, constantly occur in the species of rats herein concerned.

The chromosomes of the five species of rats dealt with are morphologically analysed and compared on the basis of the following three points, (1) the number of chromosomes, (2) the relative magnitude of the autosomal elements, and (3) the behaviour and some morphological characters of the sex chromosomes.

(1) *Comparison of the chromosome numbers.* The five species of rats under study can be provisionally divided into two distinct groups of closely related species with regard to their morphological, as well as oecological characters. The one is a group of the house- and field-rats, all being lowland dwellers, and *Rattus norvegicus*, *R. rattus* and *R. losea* belong to this group. The other is a group including the two mountainous forms, *R. fulvescens coxinga* and *R. confucianus culturatus* which are upland inhabitants. Being very interesting, the number of chromosomes of these rats shows a condition which is quite parallel to the above evidence. That is, in the lowland forms to which *R. norvegicus*, *R. rattus* and *R. losea* belong, the number of chromosomes is constantly 42 in diploid, which consists of 40 autosomes of orthotelomitic nature and the XY elements, whereas the upland dwellers, *R. f. coxinga* and *R. c. culturatus*, uniformly possess the diploid number of 46, comprising 44 orthotelomitic autosomes and the XY elements. This constant evidence is very interesting in showing that the two groups of animals having been in different morphological and oecological

conditions show a different chromosome number respectively, in parallel to their bodily characters. Setting aside the trivial hypothesis of fragmentation, there are no data favourable to interpret the cause of the variation of the chromosome number in this case, but the above fact may offer something significant when the consideration will be made in connection with the evolution and specialization of the rats under investigation.

(2) *Comparison of the autosomes.* In surveying the chromosomes, the author has been early struck by the fact that there occurs a considerable difference among the species under study as regards the length of certain chromosomes. As already noted, the chromosomes of the rats under consideration generally show no remarkable demarkation from one another beyond length, since all the elements uniformly assume the form of a simple rod. The mensural observations of chromosomes, therefore, may be available for the purpose to discover the characteristic difference existing among the species in respect of the length of the chromosomes. For this purpose the chromosomes of the spermatogonial complex arranged in the serial alignment may be most favourable as a matter of reference.

In the first place, the comparison was made regarding the three lowland forms, *R. norvegicus*, *R. rattus* and *R. losea*, in which there is a complete agreement in respect of the number of chromosomes, being uniformly 42 in diploid. As shown in the serial alignments (Figs. 19-28), the autosomal series of *R. norvegicus* consists of 20 pairs of homologous elements ranging from *a* to *t*, of which the first two pairs are comprised of prominently large chromosomes outstanding in size (*a*'s and *b*'s), and the third largest pair (*c*'s) downwards the elements form a well graded series in diminution of length. Therefore a sharp demarkation in length can be drawn between the *b*'s and *c*'s. The X element is the third largest in size, and the Y is identical in size to the smallest autosomes. A quite similar condition is also found to occur in *R. rattus* (refer to p. 30 and Figs. 103-107). Closer examination, however, offers appreciable differences between these two related species as regards the relative length of chromosomes. One of them is found to lie in the relative magnitude between the second and third largest elements (*b*'s and *c*'s). To express the comparison numerically, the ratio in length between them was calculated. As proved in the author's previous study (Makino '41), the size of the chromosomes varies in a quite propor-

tional relation between the large and small cells, so that the ratio of length between certain chromosomes may be applicable as the basis of comparison in different cells. The ratio obtained in length between the *b*'s and *c*'s is on the average 1.34 in *R. norvegicus*, while in *R. rattus* it is shown to be 1.18 in the average value (cf. Table III). This result indicates the fact that the third largest chromosomes (*c*'s) are on the whole longer in *R. rattus* than in *R. norvegicus*. The significant difference is also found when the ratio of length between the largest (*a*'s) and smallest elements (*t*'s) is compared (cf. Table III); in *R. norvegicus* the mean value of the ratio under consideration is found to be 3.66, whereas in *R. rattus* it is 4.44. On the strength of these facts, therefore, it can be stated that the obtained ratios may be valuable as a useful criterion for the specific distinction when the chromosomes of these two allied species of rats are brought under comparison.

TABLE III. Mean value of ratio in length

Species	Mean value of ratio in <i>b/c</i>	Mean value of ratio in <i>a/t</i>	Number of cells observed
<i>Rattus norvegicus</i>	1.34 ±0.03	3.66 ±0.18	42
<i>Rattus rattus</i>	1.18 ±0.02	4.44 ±0.13	10

In the case of *R. losea*, however, the condition is considerably different, though the number of chromosomes is similarly 42 in diploid. As obvious from the serial alignments of chromosomes (Figs. 108–112), of the autosomal pairs, the first largest pair (*a*'s) is represented by extremely large elements, quite prominent among the rest. From the second largest pair (*b*'s) to the fourth largest (*d*'s) the elements exhibit no remarkable variation in length, and from the fifth largest (*e*'s) downwards the members shows a closely graded series. Therefore, the demarkation lines in length may be drawn between the *a*'s and *b*'s and again between the *d*'s and *e*'s. Particularly the difference of length between the *a*'s and *b*'s is very noticeable in this species, and it is remarkably larger than that in *R. rattus* and *R. norvegicus*. Table IV shows for reference the mean value of the ratio of length calculated between the *a*'s and *b*'s, by way of comparison between *R. losea* and its close relative, *R. rattus*. Based on these tangible differences, therefore, the chromosomes of *R. losea* are clearly distinguishable from those of *R. norvegicus* and

*R. rattus*, irrespective of the equal number of chromosomes existing among them.

TABLE IV. Mean value of ratio in length

Species	Mean value of ratio in $a/b$		Number of cells observed
<i>R. losea</i>	1.49	$\pm 0.06$	10
<i>R. rattus</i>	1.22	$\pm 0.03$	10

In addition to this, the specific feature of the X and Y chromosomes in *R. losea*, considerably different from those in the above two species, is also very serviceable for the distinction of the species. That is, the X chromosome of *R. losea* is represented by the largest element of all, showing a slight bending near its tip, and the Y is very minute sized, being approximately one-half the size of the smallest autosomes.

In the second place, the chromosomes of the upland forms, *R. fulvescens coxinga* and *R. confucianus culturatus*, come under consideration. These two species, closely allied with each other, are clearly distinguishable from the three lowland species above concerned, with regard to the number of chromosomes. These two forms possess uniformly 46 diploid chromosomes, in striking contrast to the lowland forms having 42 chromosomes. The diploid complement consists of 22 homologous pairs of autosomal elements having an orthotelomitic fibre attachment and an unequal pair of the X and Y elements. Along with the chromosome number, an uniformity is also found between these two species in the fact that the autosomal pairs form a fairly well graded series in the alignment arrangement and there is no element outstanding in size (Figs. 113-122). But a comparison of chromosomes in the serial alignment seems to show that the individual elements of *R. c. culturatus* are a little larger in relative length than the corresponding ones of *R. f. coxinga*. To see this evidence more clearly, the ratio of length was calculated between the largest and smallest elements ( $a/v$ ) in the two species respectively, and the result was given in Table V. The data of this table seem to point out that the larger chromosomes are relatively much longer in *R. c. culturatus* than in *R. f. coxinga*.



TABLE V. Mean value of ratio in length

Species	Mean value of ratio in a/v		Number of cells observed
<i>R. fulvescens coxinga</i>	3.35	±0.20	8
<i>R. confucianus culturatus</i>	4.33	±0.15	9

As above mentioned, the morphological difference existing in the autosomal elements is not very large and apparent between *R. f. coxinga* and *R. c. culturatus*, but further investigation reveals that a clear-cut difference between these two species lies in the nature of the sex chromosomes, by which the two forms are distinguishable beyond doubt. With regard to this point the detailed accounts will be given in the following section.

(3) *Comparison of the sex chromosomes.* Throughout the whole cases herein concerned, the sex chromosomes uniformly show the constitution of a simple XY-type, made up of a very large X element and a conspicuously small Y. The X is characterized by the tripartite segmentary structure in every species studied. The X and Y conjugate in meiosis as usual and segregate in the first maturation division without exception. No unusual and special behaviour has been observed as regards the X and Y in any species studied. On the other hand, however, the X, as well as the Y, show the species-specific characteristics in some morphological features, which are available as a basis for specific distinction to some extent. This section deals with this point.

The comparison was firstly made with the lowland species which have uniformly 42 diploid chromosomes. In *R. norvegicus*, the X is represented by one of the larger elements which ranks third in the relative magnitude, being a little longer than the c-elements, while the Y is nearly identical in size to the members of the smallest autosomal pair (Figs. 19-28). A quite agreeable condition was found to occur in *R. rattus* (Figs. 103-107), either in the morphological feature or in the behaviour in meiosis. In these cases, therefore, the nature of the X and Y elements cannot be utilized at all as a basis of specific distinction.

Adverse to the above case, *R. losea* possesses very characteristic sex chromosomes which clearly differ in several of morphological characters from those of *R. norvegicus* and *R. rattus*. The X element

of this species is extremely large sized, probably corresponding in length to the largest autosomes and shows always a gentle bending at a point a little distant from its extreme tip. The Y element is the smallest of all and attains the size approximately one-half the members of the smallest autosomal pair (Figs. 108–112). The ratio in length obtained between the X and Y, is approximately 3.5:1 in *R. rattus*, whereas in *R. losea* it is found to be about 5.1:1. Due to such a morphological peculiarity of the sex chromosomes, therefore, *R. losea* can clearly be distinguishable from *R. norvegicus* and *R. rattus*. And further, it is of special interest that the following two species, *R. rattus* and *R. losea*, which are regarded to be closely allied from a number of taxonomical characters, show in each well specialized characteristics of the chromosomes, not only in the sex chromosomes but also in the autosomes as already mentioned.

Next the accounts concerning the highland forms, *R. fulvescens coxinga* and *R. confucianus culturatus*, come under notice. As formerly noted, the autosomal elements showed no appreciable difference when these two forms were compared, but further observations indicate that a significant difference between them lies in the nature of the sex chromosomes, not only in their morphological characters but also in their behaviour during meiosis, as mentioned below. The X chromosome of *R. f. coxinga* is characterized by the extremely large size, being probably the largest of all, and the Y is represented by an element which is a little longer than the smallest autosomes (*v*'s), and rather corresponds to the *t*-chromosomes in size (Figs. 113–117). In the relative size the X chromosome of *R. c. culturatus* is in a harmonious condition with that of *R. f. coxinga*, since it is the largest element in the complement. But the Y, contrary to the former case, is of extremely minute size in this species (Figs. 118–122). It is markedly smaller than the smallest autosomes, being nearly one-half the size of the latter. The ratio of length between the X and Y is approximately 5:1 in *R. f. coxinga*, while in *R. c. culturatus* it is nearly 7:1. This tangible size difference occurring in the Y chromosome is thus available for the distinction of species when the chromosomes of these two forms are compared (Figs. 113–122).

In addition to the above evidence, the configuration and behaviour of the XY complex in the meiotic division are also very characteristic of the two species respectively. In metaphase of the first

meiotic division, the X chromosome of *R. f. coxinga* always takes a peculiar knee-shape, accompanying the Y connected at one end, as demonstrated in Figs. 72-80. As described in detail in the foregoing descriptions, the configurations of the X in metaphase and in anaphase seem to be in favour of the explanation that the X possesses a subtelomitic fibre attachment, different from the other forms herein studied (Figs. 72-81).

The evidence found in *R. c. culturatus* is wholly different from the above. As already given in detail in the previous chapter, the X of this species, being in conjugation with the Y, represents very complicated configuration in the first metaphase holding the vesicular construction of the growing period (see Figs. 90-94). But in anaphase the X stands vertical to the equatorial plate with its long axis, having the fibre attachment at its free terminal opposite the end where the Y comes in contact (Figs. 95-96). Throughout all the cases herein dealt with, there has never been found such an example as the present one. Due to this peculiar nature of the sex chromosomes, therefore, *R. c. culturatus* is clearly distinguishable, not only from the allied species, *R. f. coxinga*, but also from the other forms of Muridae.

Looking over all the cases coming under study, an interesting evidence which requires special attention, comes into notice in connection with the evolution of the sex chromosomes. It is the fact showing that there occurs a remarkable variation of size in the Y chromosome among the species observed. In other words, the Y is reduced in size from species to species. So far as the scope of the present study is concerned, *R. norvegicus* and *R. rattus* seem to have the Y element which is the largest among the studied species. The Y chromosome of *R. fulvescens coxinga* ranks next in size. And, *R. losea* comes third in the relative magnitude of the Y compared in all. The smallest Y chromosome in all cases compared is found to occur in *R. confucianus culturatus*. Such are the cases in which the size of the Y varies by species among the related forms of animals, while another case has been known in which the size-diminution of the Y occurs within one and the same species. The remarkable instances of the latter has been recorded in *Oecanthus* by the present author (Makino '32) and in *Mus* by Oguma ('37). A series of these evidences may be sufficient to support the view recently emphasized by Oguma ('42 b) regarding the evolution of the sex

chromosome, that there occurs the final disappearance of the Y chromosome through gradual and progressive reduction, as a process of transformation of XY-type into XO.

### Summary

The chromosomes of five species of rats belonging to the genus *Rattus* were morphologically investigated in male germ cells, from the comparative viewpoints. The material employed was mostly obtained in Taiwan.

(1) The species under study and their chromosome numbers established are as follows:

Species	2n	n	Sex-chrom.
(1) <i>Rattus norvegicus norvegicus</i>	42 spg	21 (I, II)	XY
(2) <i>R. rattus rattus</i>	42 spg	21 (I, II)	XY
(3) <i>R. losea</i>	42 spg	21 (I, II)	XY
(4) <i>R. fulvescens coxinga</i>	46 spg	23 (I, II)	XY
(5) <i>R. confucianus culturatus</i>	46 spg	23 (I, II)	XY

Interesting is the fact that, *R. norvegicus*, *R. rattus* and *R. losea* which are nearly related in taxonomical and oecological characters, all being lowland dwellers, uniformly possess 42 diploid chromosomes, while the closely allied, mountainous forms, *R. f. coxinga* and *R. c. culturatus*, show a diploid number of 46. As regards the chromosome number, therefore, the rats under investigation can be sorted into two groups, in a parallel condition to the taxonomical and oecological characters.

(2) In the former lowland forms, the diploid complement consists of 20 autosomal pairs, all being of orthotelomitic type and an unequal X-Y pair, whereas in the latter mountainous forms, it is composed of 22 autosomal pairs of similar orthotelomitic nature and an X-Y pair. The autosomes show in every case no distinctive characters beyond those of length. With a desire to see whether any constant difference exists among the species as regards the length of the chromosomes, some mensural observations of chromosomes were undertaken. *R. norvegicus* is clearly distinguishable from *R. rattus* in the relative ratio of length between the second and third largest chromosomes (*b*'s/*c*'s) and also in that between

the first largest and smallest chromosomes ( $a's/t's$ ), as shown in Table III. *R. losea* is remarkable in containing a pair of outstandingly large sized elements in the complex, so that this species shows a tangible difference from the above two species in the ratio of length between the first and second largest chromosomes ( $a's/b's$ ), as seen in Table IV.

Between the highland forms, *R. f. coxinga* and *R. c. culturatus*, there is present no marked distinctive feature when their autosomes come under comparison, beyond the rather trifle fact that the larger chromosomes are relatively longer in *R. c. culturatus* than in *R. f. coxinga* (cf. Table V).

(3) All the species studied contain the sex chromosomes of a simple XY-type without exception. In every species the X is characterized by the tripartite structure made up of three consecutive segments. The X and Y conjugate in meiosis as usual and segregate without exception in the first division, running to the opposite poles (cf. Table I and Table II). No aberrant behaviour of the sex chromosomes during meiosis has been observed in any case.

In morphological nature, the X, as well as the Y, possess distinctive characteristics, particular to each species. *R. norvegicus* and *R. rattus* are in an agreeable condition in the nature of the sex chromosomes; the X is represented by one of the larger elements which ranks third in the relative magnitude, while the Y approximates in size the members of the smallest autosomal pair. *R. losea* is quite distinctive from the above two species in having very characteristic sex chromosomes, which consist of the X of extremely large size being probably the largest of all, and the Y which is the smallest in the complement, being about one-half the size of the smallest autosomes.

As regards the highland forms, there lies also tangible difference in the sex chromosomes between the two species. The X of *R. f. coxinga* is represented by the largest element of all. It is the same with the case of *R. c. culturatus*. But a remarkable difference between these two species is found in the magnitude of the Y chromosome. The Y element of *R. f. coxinga* is considerably longer than the smallest autosomes, whereas in *R. c. culturatus* it is of extremely minute size, being approximately one-half the size of the smallest autosomes. In addition, the XY complex displays in the course of the first meiotic division very characteristic features,

peculiar to the species respectively, not only in its configuration but also in its behaviour.

It is not uninteresting that the size of the Y chromosome varies among the species studied, showing a gradual reduction by species, when the consideration is made in connection with the evolution of the sex chromosomes.

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### Explanation of Pl. IV

Paired alignments of homologous elements from spermatogonial chromosomes in an approximate order of size.

Figs. 103-107, from *Rattus rattus rattus*.

Figs. 108-112, from *Rattus losea*.

### Explanation of Pl. V

Serial alignments of paired chromosomes from the spermatogonial metaphases in a descending order.

Figs. 113-117, from *Rattus fulvescens coxinga*.

Figs. 118-122, from *Rattus confucianus culturatus*.

### Explanation of Pl. VI

All are photomicrographs, ca.  $\times 2000$ . (S. Makino Photo.).

Fig. 123. Spermatogonial metaphase of *Rattus losea*. Note the X element. The same as Fig. 46.

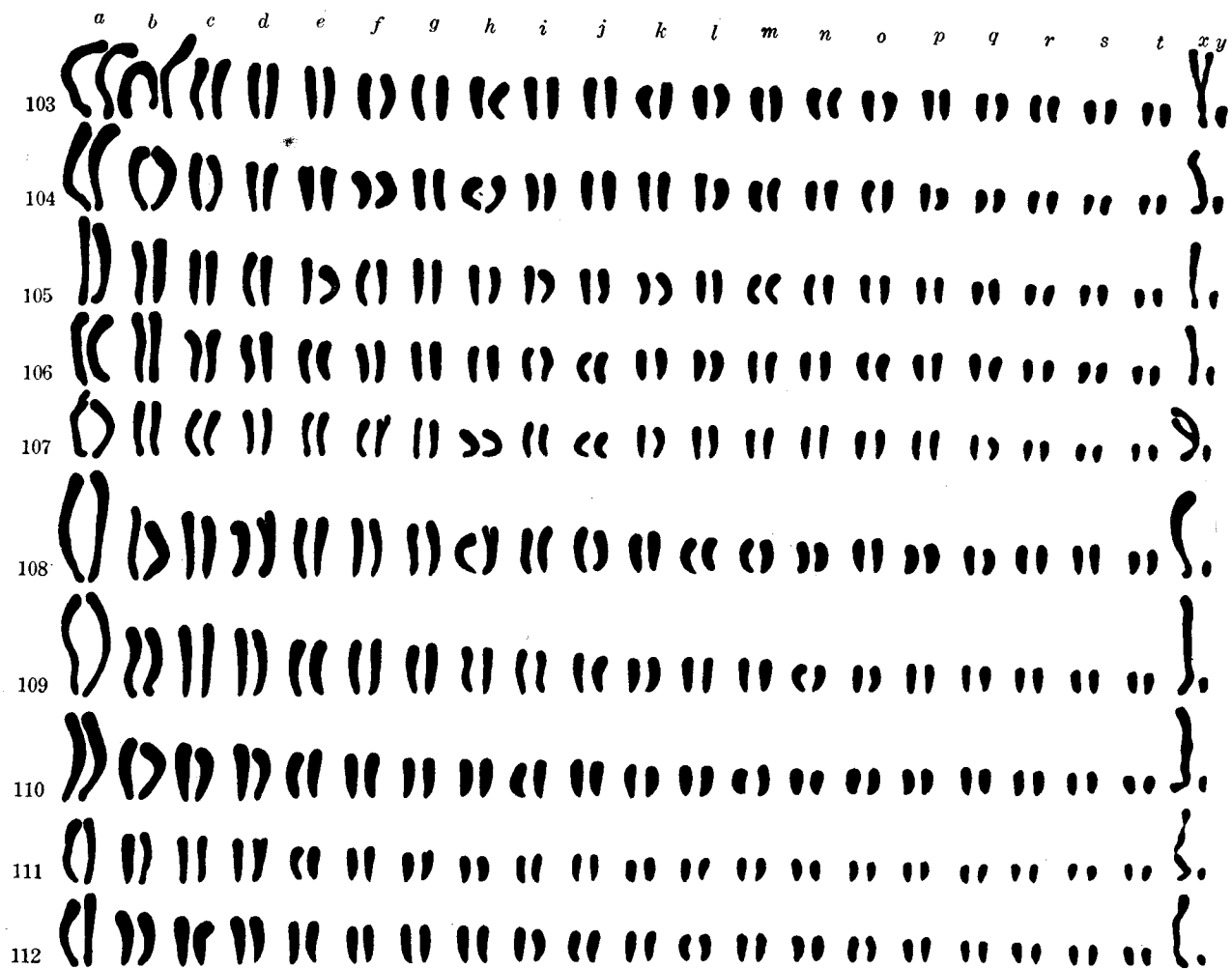
Figs. 124-125. Side views of the primary spermatocyte metaphases of *R. losea*, showing the XY complex.

Figs. 126-127. Side views of the primary spermatocyte metaphases of *Rattus fulvescens coxinga*, showing the XY complex. Fig. 126 corresponds to Fig. 73 and Fig. 127 to Fig. 80.

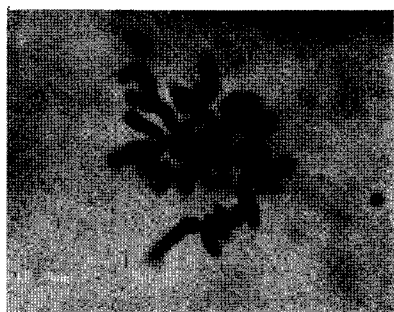
Fig. 128. Anaphase of the first division in *R. f. coxinga*, showing the segregation of the X and Y. The same as Fig. 81.

Figs. 129-130. Side views of the primary spermatocyte metaphases of *Rattus confucianus culturatus*, showing the XY complex. Fig. 129 corresponds to Fig. 90, and Fig. 130 to Fig. 91.





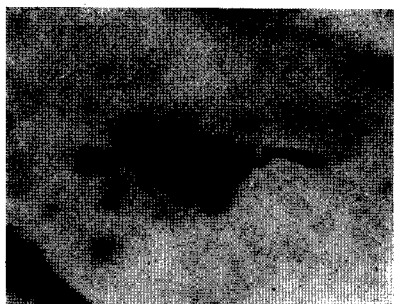
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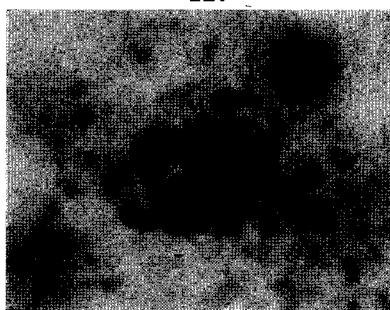
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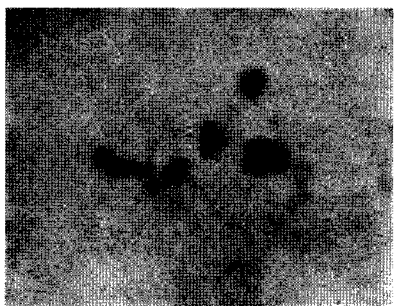
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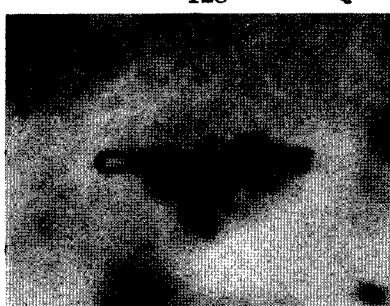
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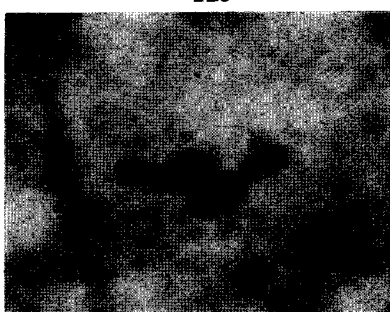
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