Studies on the Murine Chromosomes
I. Cytological Investigations of Mice, Included in the Genus *Mus*[1)*]

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With 12 Tables, 4 Textfigures and 13 Plates (217 Figures)

Contents

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INTRODUCTION

Because of the cytological and genetical importance, the interest of recent cytologists has generally been concentrated on the chromosomes of rodents, particularly of those included in the family Muridae. Thus one can now find a considerable number of works dealing with rats and mice, undertaken with both purely cytological and cytogenetical purposes. With the increasing number of investigations, some problems have been clearly solved by the affirmation of repeated studies by different investigators, but others still remain unsolved as the reported facts and the interpretations of them cannot be brought into complete agreement. In order to make some contribution on this latter aspect, Prof. Oguma suggested to the author to undertake a new study of the chromosomes of the Muridae, under his close guidance and cooperation. The present report constitutes one of a series of studies, and deals with the chromosomes of the three species of mice belonging to the genus *Mus* obtainable in Japan.

The present study is divided into five parts. Part I is devoted to the investigation of the comparative morphology of chromosomes of the following three species; *Mus musculus, Mus molossinus,* and *Mus caroli.* Beginning with the work of Tafani on the chromosomes of the mouse, which appeared as early as 1889, a considerable number of papers have successively been published concerning similar kinds of rodents. The results up to the beginning of 1937 are completely compiled in the list published by Oguma and Makino ('37). The majority of works have been performed on *Mus musculus,* the most common form as a laboratory animal, and only a few

are concerned with some other species, *Mus bactrianus* (often designated as *Mus wagneri*), *Mus molossinus* and *Mus formosanus* (=*caroli*) (Masui '23, Painter '27, Minouchi '28, Oguma '33, Tateishi '35). Due to the tireless efforts of recent cytologists, the morphology of such murine chromosomes has become clear to a great extent. To advance knowledge along this line of investigation a considerable time has been devoted in the present study to discover the morphological differences of chromosomes by a close comparison of corresponding chromosomes in the three species mentioned above, with the further purpose of finding out, if possible, the specific characteristics of the chromosomes, by which these three mouse species may be distinguished. In part II the entire history of the sex chromosomes from their first appearance in the earliest stage of the growing period of auxocytes has been followed until the metaphase of the first maturation division. Many studies attempted with a similar purpose by previous authors are of course familiar to the present writer, but unfortunately the most of the results seem to be far from what were observed in the present study. Darlington and his colleagues (Crew and Koller '32, Koller and Darlington '34, Koller '36, '38) maintain, as is well known, a view that the X-chromosome conjugates partially with the Y, not only in mice and rats but also in some other mammals, and chiasmata are usually found in this part of conjugation. Attention in this study was first directed to the question whether the sex chromosomes would really conjugate in the ordinary way, or whether the interchange of hereditary material actually occurs by means of chiasmata between the X and Y.

Part III describes an investigation of the oocyte chromosomes, the result of which may involve some criticism upon the claim on the sex difference of chiasma frequency in the mouse. Part IV deals with the fertility and chromosomes of the interspecific hybrids, *Mus musculus* × *Mus molossinus*, which have been examined through five generations, and finally Part V gives accounts on the maturation and fertilization of the ovum, with a view of offering the cytological observations as a background for the experimental studies *in vitro* undertaken by recent investigators.

Before going further, the author wishes to acknowledge here his very great indebtedness to his teacher Prof. Kan Oguma, who

suggested the problem and who has lent almost continuous encouragement. The author is grateful for his expert guidance, for many valuable suggestions and for his careful supervision during the course of this work.

MATERIAL AND METHODS

The following three species of mice, including both domesticated and wild forms, comprise the material for the present study:

1. *Mus musculus* L. This species is not indigenous to Japan. The material consists of domesticated albino mice, derived from Carnegie Institution stock, which were introduced in 1932 through the generosity of Prof. J. F. McClendon and Prof. T. Inukai, to whom the author's cordial thanks are due. They have been bred pure since then by inbreeding in this laboratory under the author's care. They carry nothing abnormal in the morphological characters.

2. *Mus molossinus* Temm. et Schleg. This form represents the sole species of the house mouse widely distributed through Hokkaidō, Hondo and Kyūsyū. It is known, according to Dr. Tokuda (unpublished), to distribute also in Formosa and China. They are readily trapped in the warehouse and sometimes in the field. They proved easy to rear in the laboratory but quite difficult to breed under captivity. For this investigation, the specimens obtained in different localities, such as Sapporo, Akita, Tōkyō and Kyōto, furnished the present material.

There has been long known in Japan a remarkable variety of this species under domestication, including the white or spotted forms, being famous as the so-called Japanese waltzing mice. Though they have been occasionally described as a variety of *Mus bactrianus* (or often designated as *Mus wagneri* var. *rotans*), the recent status of taxonomical conception shows, according to Dr. Tokuda, that they are derivatives of *Mus molossinus*. This domesticated species, in addition to the former wild form, was also used for comparison in this study.

3. *Mus caroli* Bonhote.[1] This species is one of the wild mice, closely related to *Mus molossinus*, and confined to Formosa where they are not uncommon in fields. The mice adopted as material were collected through the courtesy of Dr. S. Tateishi and the

1) Synonym of *Mus formosanus* Kuroda (Tokuda, unpublished).

preservation of the testicular material was also kindly carried out by him following the author's directions.

According to the study of Dr. Tokuda, these three above-mentioned species employed in the present study clearly differ from each other in a number of taxonomical characteristics, and there scarcely remains any question among taxonomists as to the specific distinction of these three forms of mice.

For the study of chromosomes in spermatogenesis, the testes preserved with Flemming's strong solution without acetic acid were exclusively employed as the material. In each of the species the testicular material from 10 to 15 specimens was prepared for examination, with the exception of the case of *Mus caroli* in which five specimens were obtainable. For the study of the oocyte chromosomes, the ovaries were fixed with Allen-Bouin's mixture at 37–38°C. The procedure for getting the material for the study of the maturation and fertilization processes in the eggs is described in detail in Part V. Following the usual treatment of the paraffin method, they were cut at varying thicknesses ranging from 10 to 20 micra and stained according to the iron haematoxylin method of Heidenhain with a counterstain of light green, in some cases Newton's gentian violet staining being partly adopted.

With the special purpose of tracing the history of the sex chromosomes in the growing period of auxocytes, other different fixing and staining methods have been tried to give a sharp differentiation of acido- and basophilic elements. The testes fixed with normal Flemming's solution (or some with La Cour's fluid), were stained by (1) Heidenhain's iron haematoxylin method, (2) Flemming's triple staining method, (3) Newton's gentian violet method and (4) Feulgen's basic fuchsin method with light green. By way of comparison to the above cases, some parts of the testicular material were fixed with Gilson's and Allen-Bouin's fluids, and stained with (1) iron haematoxylin, (2) Feulgen's basic fuchsin with light green, and (3) Mann's methyl-blue-eosin.

The figures were drawn with the aid of a Zeiss camera lucida using a 20 × comp. eyepiece and 1.5 mm. apochr. oil immersion objective, at a magnification of 4200 diameters. In making drawings, much effort and special attention have been paid to the careful measurement of the chromosomes and comparison of their shape and size, for the identification of the synaptic mates of the

homologous chromosomes, so that the finished drawings given in this paper are as accurate as it has been possible to make them. The figures were reduced in printing 5/7 of the original (unless otherwise noticed).

Here the author must express his appreciation for the courtesy shown by Dr. M. Tokuda of the Kyōto Imperial University, who has kindly supplied the author with information on his taxonomical conception at several times. The author also wishes to acknowledge his indebtedness to Assist. Prof. S. Tateishi of the Taihoku Imperial University through whose kindness some of the material was made accessible. Further the author's grateful thanks are also offered to Messrs. S. Tarao, H. Niiyama and H. Kichijo for many an act of friendly assistance in the course of this study.

Part I. Comparative morphology of chromosomes in three different species

This section deals with a comparison of the chromosomes of three related but distinct species of mice, *Mus musculus, Mus molossinus* and *Mus caroli.* While it has already been established by previous investigators that the chromosomes of these three forms are identical with each other in number, no one has hitherto attempted a morphological analysis of the chromosomes, with the purpose of finding out, if possible, their specific characteristics.

(1) Accounts and observations regarding *Mus musculus* L.

In spite of the fact that the mouse, *Mus musculus,* has offered the classical and favourite object for cytology (cf. the list published by Oguma and Makino '37), it is quite recently that the morphology of the chromosomes has been fully studied in spermatogenesis. So far as the literature shows, Cox ('26) seems to be the first author who studied the chromosomes in spermatogenesis and reported the exact count of the chromosome number as 40 in diploid and 20 in haploid. This was substantiated by the extensive study of Painter ('28a, b). A little later Cutright ('32) published his observations on spermatogenesis of this species and showed the transformation of the chromatin elements during the growing period of auxocytes. Crew and Koller ('32), on the other hand, investigated the chro-

mosome behaviour during mitosis and meiosis, in which they tried to extend their special idea of chiasma frequency correlated with that of genetical crossing-over. Recently Butarin ('35) and Matthey ('36) respectively have attempted the morphological analysis of the chromosomes of this species to some extent. While no essential difference can be found in the number of chromosomes, one cannot overlook, reviewing their contributions, an important fact that there occurs a marked disagreement among the investigators in respect of the morphology of the sex chromosomes. Cox ('26), Painter ('28), Cutright ('32) and Crew and Koller ('32) are of opinion that the X-chromosome is one of the medium sized elements and the Y is represented as the smallest one or a little larger element than that. Matthey ('36) has expressed, on the contrary, a different view that in the diploid complement "il est beaucoup plus malaisé de dépister le complexe sexuel, XY probablement". Such discrepant conceptions derived from observations on one and the same species should naturally attract one's particular notice and demand a further critical research such as that which the present author attempts.

Spermatogonia. Out of a large number of metaphase plates excellently preserved, fifteen adequate ones were selected and employed for the penetrating study. Five representatives of these plates are shown in Figs. 1 to 5. As reported by the previous investigators, the number of chromosomes was established to be invariably 40. All the chromosomes are of the simple rod-type without exception. In well preserved condition, the individual chromosomes appear as straight or sometimes slightly curved rods of uniform thickness, tapering towards their inner ends, and offer no slight evidence of irregular thickening and bizarre outline which often makes the individuality of chromosomes obscure. They are arranged on the equatorial plate in a radial manner, their tapering ends pointing towards the centre of the equatorial plate. For this reason it is evident that the position of the spindle fibre attachment is practically terminal in every chromosome, though it is still difficult to point out the exact position of the attachment locus. The individual chromosomes possess no particular distinctive feature, beyond that of length. There is no clear-cut distinction of the chromosomes into large and small sets, but only a closely graded seriation occurs. Thus, the present observation is, in respect of the

number and general feature of the chromosomes, in accordance with those by previous authors.

As a means of finding out the sex chromosomes consisting of unequal X- and Y-elements, much effort and time have been devoted to the identification of the synaptic mates of homologous chromosomes. To determine the homologous pairs, the alinemental arrangement of chromosomes by means of comparison of their shape and size being based on the depicted figures of chromosomes, has usually been adopted by many investigators. This method serves, however, only to obtain approximate results, since the microscopical drawings made under the highest magnification are often insufficient to know the real length which may apparently differ according to the angle of inclination against the optical plane. It is necessary, therefore, to consider of some other characteristics than apparent length, shown by chromosomes under observation, whenever one undertakes the determination of real homologous pairs. Having such points in mind, the author adopted the following method of investigation in this study; in the first place, a close microscopical examination was made as accurately as possible, directly upon the individual chromosomes in the metaphase equatorial plate, directing the whole attention towards their real size, shape and position of distribution in the equatorial plate. Then the chromosomes were carefully drawn with the aid of a camera lucida and each of the supposed homologous chromosomes thus identified was numbered in the approximate order of its size. In the second place, the individual chromosomes of a plate thus numbered were copied and placed in the serial alignment.[1] Fortunately the chromosomes are so excellently preserved as to admit a close comparison in respect of their morphology, leaving no shadow of obscurity. The chromosomes placed in the serial arrangement as mentioned above are shown in the annexed figures given in Pl. XIX (Figs. 26–35). In every series there are arranged 19 homologous pairs of autosomes which range in length from *a* to *s* according to the approximate order and an unequal pair placed at the extreme right. It is evident

1) Some investigators raise questions on the identification of the synaptic mates of the homologous chromosomes by means of the serial alinement of chromosomes. But, so far as the measurement of chromosomes is carried on the chromosomes preserved in ideal condition, one will find that it involves only the slightest degree of error.

that of the unequal pair the large chromosome is the X and the small one the Y. The autosome pairs form a closely graded series and there is no pair outstanding in size.

The X-chromosome thus identified is quite prominent in being the largest of all (see *x* in Figs. 1–5, and 26–35). It is markedly longer than the members of the *a*-pair which are the largest of the autosomes, and in the extreme case, as shown in Figs. 1, 3 and 5, it assumes a conspicuous vague contour, apparently being loose in texture. Viewing such a structure, it is highly probable that the extreme elongation of the X is due to unravelling of the inner spiralization of the chromosome. When heavily condensed as seen in Fig. 4, the difference of length between the X and the *a*-elements becomes slight. The X-chromosome is provided sometimes with two transverse constrictions (Fig. 3) and as the result it is divided into three successive segments, showing a definitive tripartite structure which was first pointed out by Oguma ('35) in the X-chromosome of two species of *Rattus* and later found to occur in several forms of mammals. The problem concerning this tripartite structure will be discussed later in detail in comparison with the cases obtained in the other species. At any rate, by this characteristic feature the X-chromosome is readily distinguishable from the others. In the equatorial arrangement the X always takes its position at the periphery of the equatorial plate.

The Y-element, on the other hand, attains a size nearly approximate to the chromosomes of the *s*-pair, but frequently it seems to be slightly more slender than the latter (Figs. 26–35). An exact comparison between them will be made later on the basis of the result of measurements of the length. The Y-chromosome is, therefore, rather difficult to discriminate exactly from the *s*-chromosomes, because of the absence of distinct indicating features. However, the identification of the Y seems not to be absolutely impossible, if its position in the equatorial plate is considered together with its slender appearance (see Figs. 1–5).

On the basis of the above findings, the results reported by previous investigators will next be brought under consideration. As already stated, Cox ('26), Painter ('28), Cutright ('32), and Crew and Koller ('32) reported that the X-chromosome is one of the medium sized elements and the Y is represented by the smallest or a little larger one. The identification of the X- and Y-chromo-

some they made was based merely upon their apparent shape and length seen in the drawings of chromosomes. From this method, however, one can expect an approximate result only in the case when the chromosomes are preserved sufficiently well as to admit a close comparison in respect of their morphological features. The preservation of the chromosomes made by these authors, the present author believes, cannot be considered to be excellent. This will be well understood when the figures given in their papers were compared with the author's own figures presented in this paper. The classical method of fixation adopted by them could not preserve the chromosomes in the natural condition; they bear irregular thickening and obscure individuality that do not allow the accurate examination of shape and length. For this reason the chromosome figures given by these previous authors are satisfactory only for the estimation of the number but cannot be utilized for a further morphological analysis. In striking contrast to the above evidences, Matthey ('36) expressed the view that the unequal pair of the X- and Y-chromosomes cannot be distinguished by means of picking up of the supposed homologous chromosomes and their alignmental arrangement in a serial order. In spite of this fact, he still states that the X-chromosome is likely discernible among the rest by its characteristic features when the preservation of chromosomes was adequately made. It is very important to note that the X-element thus pointed out by him in his figures is represented by an extremely long, probably the largest chromosome and that it knees gently at two points suggesting its tripartite structure. The reason why he failed to pick out the XY pair in the mating up of the spermatogonial chromosomes, is, as it seems to the author, that he failed to notice the delicate but specific characteristics of the X- and Y-chromosomes in his observations.

Primary and Secondary Spermatocytes. The bivalent chromosomes of the primary spermatocyte at metaphase are observed with extreme clearness (Figs. 6–9). There are found without conflict 20 distinct chromosomes. They are arranged in a radial manner distributing well apart at nearly equal distances from one another. The irregular arrangement of tetrads in the equatorial plate as usually shown in the figures given by Cox ('26), Painter ('27, '28) and Cutright ('32) is by no means natural but is evidently induced by the poor method of fixation. Of these 20

bivalents one is represented as the heteromorphic tetrad of the XY-complex and the rest are autosome tetrads of ordinary structure. The latter are found in the majority of cases in the form of a ring, a V, or a horse-shoe, while some individuals assume strange configuration in the case when they lie obliquely in the equatorial plate. This variant configuration is frequently seen in the smaller tetrads. It is very likely that the tetrads are all comprised in the type of the diaschistic tetrads with telomitic fibre attachment (cf. Wilson '25), similar in principle to those generally found in the Orthopteran insects. The tetrads, probably all of them except the XY-bivalent, bear a distinct knob-like swelling, proportionally big in relation to the chromosome body itself, at their inner proximities where the spindle fibres come into contact. Such proximal knobs are specially remarkable when the tetrads are placed in a regular arrangement on the equatorial plate pointing their inner ends towards the centre of the plate. Generally the knobs are seen in every one of four chromatids of which a tetrad is composed, and therefore in the polar view a tetrad generally shows two distinct knobs in close contact at their proximal ends. This structure of the tetrad was pointed out for the first time by Oguma ('35) in *Mus molossinus*, and it seems now to be of general occurrence in every species of *Mus*. The knobs under consideration are undoubtedly produced by the differential condensation of chromatin characteristic of *Mus*, probably in close relation with the specific structure of the attachment locus of the spindle fibres.

Let us next undertake to describe the XY-bivalent more closely. The XY-bivalent appears in sharp distinction to the autosome bivalents on account of its characteristic heteromorphic structure, which is composed of the elongated rod-shaped X connected in its extremity with the smaller one, the Y, in a linear series (Figs. 10–14). It lies in the majority of cases (though not exclusively as noted below) in the most peripheral circle made by larger tetrads in the

TABLE I. *Position of XY-bivalent in the first metaphase plate*

Mus musculus

	Periphery	Interior	Total
Frequency	154	28	182
%	85%	15%	100%

equatorial arrangement at metaphase. But a few cases show its occasional distribution in the interior area of the equatorial plate. The result of a statistical study of this point clearly shows that the peripheral position of the XY-bivalent in the equatorial plate greatly exceeds the cases of the interior position (cf. Table I).

The X- and Y-chromosomes at metaphase always stand, in a linear connection, nearly perpendicular to the equatorial plate in a position approximately parallel to the axis of the spindle. In both the X and the Y the spindle fibres are constantly attached to their free ends opposite the position where they come in contact. They are connected to each other end to end in a linear series by means of fine but distinct fibres. This mode of conjugation of the X and Y is characteristic to the mouse in general, but it is evidently different from that found in *Rattus* (cf. Minouchi '28 and Oguma '35). When observed in the lateral aspect, the X corresponds in its length to one of the chromatids composing the largest autosome tetrad, while the Y to that of the smallest one. The X commonly shows at metaphase a weak affinity for stain acquiring a diffused configuration with vague contour, whereas the Y is always stained as deep as the autosomes with a solid and defined outline. More characteristic and important is the tripartite structure of the X. That is, the X-chromosome exhibits, at least in preparations well preserved and perhaps a little destained, a striking structure provided with two transverse constrictions, which subdivide the entire body of the X into three successive segments. Clear pictures for this point are given in Figs. 10 to 14. It is interesting that the proximal segment with which the Y comes in contact is nearly identical in its relative magnitude and configuration to the Y-element. The most distal segment in which the attachment locus of the spindle fibres is involved, is characterized by rather compact texture, being deeply stained. Such a constant evidence of the tripartite structure of the X cannot be accounted for as a temporary or superficial character but a fundamental one. This is at present not a matter of discussion, because Oguma ('37) who first mentioned this peculiar structure in the X of rats, has shown it to be the general characteristics of the mammalian X-chromosome by his extensive studies (Oguma '35, '37a, b). More detailed treatment of the observed fact, may be given later, together with data obtained in the other species dealt with in this study.

The separation of sister chromosomes at anaphase is quite synchronous, and there is found no element which lags or precedes the others on the way passing to the poles (Figs. 15–16). Each one of 19 autosome tetrads appears, after separation, in the form of two corresponding single V's, each of which is composed of two identical chromatids coming in contact at the inner end where the attachment locus of spindle fibres lies. The XY-bivalent also disjoins into two unequal components, the X and Y, each assuming a single V form respectively. This is seen in absolute clearness by referring to the side view of the anaphasic plate (Figs. 15–16). In going to the pole, the X and the Y as well, neither lag behind nor precede the autosomes. In adequately preserved condition, the X-chromosome retains, after separation, its characteristic tripartite structure in each of the composing two chromatids (Figs. 15–16, *x*), while the Y always assumes a compact appearance assuming a single V shape. Fig. 17 a and b represent the polar view of the anaphasic sister complexes of chromosomes in the first division as observed in a single section in each of which 20 well defined sister chromosomes are observable without any doubt. The identification of the corresponding sister halves is very easy in these figures. One of these complexes contains a distinct X-element which is conspicuous for its enormous size, being probably the largest of all, while in the other the Y-element makes its appearance in the corresponding position of the plate, with a magnitude nearly identical to that of the smallest autosome. At telophase the X as well as the Y, are evidently found situated within their own distinct vesicles, separated from the autosome groups as seen in Fig. 18. Thus the present division produces always two different kinds of secondary spermatocytes, despite the equal number of chromosomes, in respect to the sex chromosomes.

As expected from the mode of the first division, in every equatorial plate of the secondary spermatocyte studied, the number of chromosomes (dyads) was found to be 20 (Figs. 19–24). All the dyads assume the shape of V's, quite identical to the configuration in the anaphase of the previous division. They do not lie with both their arms parallel to the equatorial plate, but with the two arms overlapping each other. They arrange radially with the apices of V's pointed towards the centre of the spindle. Each dyad is so separated at anaphase that it becomes two telomitic rod-shaped

monads resulting in the separation of each arm from its joint (Fig. 25, a–b).

In the chromosome garniture of the secondary spermatocyte metaphase, both the X and Y are rather difficult to identify with certainty. But if the observation includes a sufficient number of clear equatorial plates, one can occasionally come across favourable plates in which the X exhibits its distinguishable feature, as seen in Figs. 19 to 21. In these figures, the X is distinguished for showing a vague outline. Probably due to its loose spiralization of inner chromonemata, it frequently attains an extreme elongation. In the extreme case the X exhibits its characteristic tripartite structure (Fig. 21). It is of great importance that a constant relationship was thus established in the structure of the X-chromosome through three successive generations of germ cells, the spermatogonia, the primary and the secondary spermatocytes. The Y-element, on the other hand, is quite difficult to discriminate from the smaller dyads, due to the failure of the appearance of any distinctive feature.

On the basis of the above observations, some data afforded by previous investigators may be brought under consideration. As already pointed out, the previous workers, such as Cox ('26) Painter ('28), Cutright ('28) and Crew and Koller ('32), have not been successful in preserving the chromosomes of either the primary spermatocytes or the secondary spermatocytes in a natural state. The chromosomes in their figures are not so satisfactory, as they mostly show a too irregular outline with obscure individuality to allow a closer comparison of their shape and structure. It is evident, therefore, that many misconceptions might have been introduced from observation of chromosomes in such an inadequate state. Prior to the present investigation, Matthey ('36) was the only author who had succeeded in preserving the chromosomes of the germ cells of the mouse, so far as *Mus musculus* is concerned. In his figures, the chromosomes are preserved well enough to admit their morphological analysis to some extent. Still, however, he failed in most cases to identify the XY-chromosomes, which, in the present author's material, are rather easy to point out because of their characteristic feature as already mentioned. For example Matthey was able to find the XY-bivalent only in a single case out of six metaphase plates of the first division. In this respect, he

stated that "cette observation, complétement isolée pourrait être, me semble-t-il, un caprice de fixation."

On the other hand, Crew and Koller ('32) expressed a very strange view that the equational segregation of the XY-bivalent occurs in the first division.[1] But, they gave no actual example for the illustration of the post reduction in the second division. The anaphasic figure given by them as the evidence for equational segregation (see Textfig. 11 k, on p. 370 of the paper of Crew and Koller '32), is far from indisputably clear, and involves much of obscurity. Evidence is sufficient to show with all probability that it is no other than the anaphasic figure of an ordinary tetrad of large size. It seems to the author that the ordinary tetrad was misunderstood as the XY-complex by them, on account of its bearing a similar appearance to an XY-figure, the whole error being induced by poor fixation.

A statistical study on the segregation of the X- and Y-chromosomes in the primary spermatocyte division undertaken by the present author, which was carried out on clear anaphasic figures of the first division found in the testes of five different individuals, results in the finding that all of the examined cases showed the reductional segregation of the X- and Y-elements, as given in Table II.

TABLE II. *Segregation of XY in the first anaphase*

Mus musculus

Reductional	Equational
182	0

(2) Accounts and observations regarding *Mus molossinus* Temm. et Schleg.

The morphology of the chromosomes in the wild form of *Mus molossinus* has previously been studied in full detail by Oguma ('35), who has given an accurate account as to the number and shape of chromosomes without any shadow of doubt. By this work our

1) A similar view has been emphasized by Koller and Darlington ('34), and Bryden ('32) in the studies of rats.

knowledge of mammalian chromosomes remarkably advanced along the line of desirable exactness. In the present study the observations have been made with particular attention in comparing with the previous species, *Mus musculus*. In addition, the chromosomes of the domesticated form of this species, and of its famous variety, the so-called Japanese waltzing mouse, are also briefly described. They were formerly considered as derivatives of *Mus bactrianus* or often designated as *Mus wagneri* var. *rotans*, the chromosomes of which were investigated by Masui ('23), Painter ('27) and Minouchi ('28).

(*a*) *Chromosomes of the wild form*

Spermatogonia. The morphological characteristics of the chromosomes of this species are generally identical in essential points to those of the previous form, *Mus musculus* and the number of chromosomes is likewise 40 (in $2n$), all of them being of telomitic nature (Figs. 36–40). The only difference existing between them by which they are distinguishable from one another, is found in the relative length of the individual chromosomes. As a whole, the individual elements of the present species are a little longer than the corresponding ones of the previous form when a close comparison is made. Detailed accounts on this point are to be referred to a later section.

The mating up of the homologous chromosomes was made after the procedure applied in the case of the previous species, and the chromosomes were placed in the serial alinement according to the approximate order of length. Fifteen excellent equatorial plates were examined in this way. Examples are given in Figs. 61 to 70. There are 19 homologous pairs of autosomes ranging, according to relative length, from *a* to *s* and an unequal pair of the X and Y at the extreme right. As readily understood from a glance at these figures, the grade of difference afforded by successive autosome pairs bears much resemblance to the case of *Mus musculus*. Hardly any noticeable differences are discovered between the chromosomes of individuals collected from the different localities, Sapporo, Akita, Tōkyō and Kyōto.

The X-chromosome is characterized by its remarkable configuration, distinguishable from autosomes. Generally it assumes a thinner

outline than the autosomes do and exhibits a diffused contour in more or less degree (Figs. 36–40, *x*). Occasionally a marked constriction is visible near the distal end opposite to that of the spindle attachment, at a position about 1/3 the total length (Figs. 62–63, *x*). In other cases, moreover, two distinct constrictions make their appearance in the chromosome, by the presence of which the entire body of the X-chromosome becomes divided into three segments of apparently similar length (Figs. 65–70, *x*). Considering its absolute length, the X seems to be a little larger than the *a*-chromosomes, the largest autosomes (see *x* in Figs. 61–70). The Y-chromosome, on the other hand, much simulates in magnitude the members of the *s*-pair, the smallest autosomes, and accordingly it is rather difficult to draw a sharp line between them. The Y-element in such a state, therefore, is hardly identifiable with certainty. In the favourable preparations, however, it is often differentiated into a remarkable structure having a slender appearance, though this phenemenon seems not to be absolutely constant. The position of the Y in the equatorial arrangement also serves for its identification to some extent. Briefly, the evidence obtained from a close observation of the sex chromosomes in this species is nearly similar to that of the previous species.

In his study on *Mus molossinus*, Oguma ('35) stated that it is almost impossible to identify the X- and Y-elements with certainty in the diploid group of chromosomes. He kindly gave permission to the author to examine the original preparation from which his figure 19 (Jour. Fac. Sci. Hokkaidō Imp. Univ., Zool. Vol. IV, Plate III) is derived. After a careful examination of this preparation, the author discerned, with the greatest probability, the X-element which is characterized by a rather slender form of the largest length (lying at the position of 10 o'clock in his Fig. 19), and the Y of the smallest size (lying at 5 o'clock).

Primary and Secondary Spermatocytes. In the metaphase plate of the primary spermatocyte there are found twenty chromosomes which include 19 autosome tetrads and a heteromorphic bivalent of the XY-complex (Figs. 41–43). The morphology of the tetrads has been fully studied by Oguma ('35) with extreme accuracy, so that no further detailed description is required here. The tetrads scatter at almost equal distance from each other in the equatorial plate. As is the case in the previous species, the proximal knobs

make their distinct appearance at the inner ends of each chromatid constituting the tetrads. As compared with the former species the area of the equatorial plate within which the bivalents are scattered, is a little narrow in the present species, the diameter of the equatorial plate being evidently small. It is likely, however, that this difference is by no means a fundamental one, but can be explained as an effect of fixation.

As is the case in the former species, the position assumed by the XY-bivalent in the equatorial plate at the first metaphase, is not necessarily confined to the periphery of the plate (Figs. 41–42) but in a few instances it is found in the interior space. (Fig. 43). The frequency of this position, calculated in a total of 284 metaphase figures derived from five different individuals, is given as follows:

TABLE III. *Position of XY-bivalent in the first metaphase plate*

Mus molossinus, wild

	Periphery	Interior	Total
Frequency	213	71	284
%	75%	25%	100%

This record proves evidently that the peripheral distribution of the XY-bivalent is of common occurrence in this species.

The morphological composition of the XY-bivalent is not essentially different from the previous species (see Figs. 45–53). When the material is deeply stained, the X-element assumes the elongated rod form, sometimes gently bent (Fig. 45). It associates by a delicate fibre with the Y-element at one extremity in a linear series and the spindle fibres are attached to their free ends in both elements. The XY-bivalent always stands vertically on the equatorial plate with its long axis parallel to that of the spindle. Noteworthy and important is the segmentary structure displayed by the X which becomes visible in a little destained condition. As shown in Figs. 46 to 53, the X is provided with two transverse constrictions which divide the whole length of the X into three segments of a successive series. It is quite a noticeable fact that these three parts are not equivalent in their nature. The proximal segment to which the Y comes in attachment contrasts strikingly

with the remaining two in possessing a rather stable configuration. This segment is usually stained as deeply as the Y-element and is nearly identical to the latter in either its magnitude or shape. Though not frequently, the Y-element is found also provided with a transverse constriction at its median portion (Figs. 44, 51, 52 and 53, *y*). In such a case, the proximal segment of the X-part also shows a clear constriction at the just identical position. This is sufficient to suggest a possibility that the proximal segment and the Y element would be equivalent in their nature. The annexed figures will illustrate the point more clearly (Figs. 44, 51 and 53). This evidence is significant and important when the origin of the Y-chromosome is taken into consideration. A critical consideration upon this problem which has a broader bearing on the evolution of the sex chromosomes in animals, will be left as a future problem, until the data from the other forms are fully ready.

Of the remaining two segments of the X-part, the extreme distal one involves a terminal condensation of chromatin, probably the so-called polar granule, to which the spindle fibre comes to attach. Though the polar granule itself does not undergo any change, this segment represents a considerable modification of form, probably due to drawing out of the inner spirals. On the other hand, the median segment of the X displays, in the destained condition, a less stained and diffused appearance with vague outline, sometimes elongated to a considerable extent (Figs. 48, 49, 51, 52 and 53). Under such a condition, the demarkation between the distal and median segments becomes apparently indefinite.

At anaphase, the X always disjoins from the Y without exception (Figs. 54–56). In favourable state, the tripartite structure is seen still unaltered in the X-element (see *x* in Figs. 55–56). The anaphasic separation of chromosomes is quite simultaneous in all the elements, and there is neither succession nor precession in any one of them.

The unexceptional segregation of the X- and Y-chromosomes in the first division results constantly in the production of two kinds of secondary spermatocytes with a different complex as regards the X and Y, in spite of the equal number of chromosomes. As a matter of fact, the number of chromosomes was constantly 20 in every equatorial plate of the secondary spermatocytes so far examined (Figs. 57–60). The statements regarding the X- and Y-chromo-

somes of this division made in the former species are applicable likewise to this species too, so that any further description is omitted here.

(*b*) *Chromosomes of the domesticated form*

In this form are included the white or spotted mouse and its famous variety, the so-called waltzing (or dancing) mouse, which have long been known as the tame mouse in Japan. The observations were made on both the original form and the variety, and did not disclose any fundamental difference of chromosomes between them. Therefore, the description is not given here independently for each form.

The investigation on this material reveals that the chromosomes show essentially nothing different in respect of their form, number and other general morphological features, from those observed in the wild form. The detailed description of the data will, therefore, be abbreviated. The number of chromosomes is 40 in diploid, all elements being of telomitic rod-shape (Figs. 71–74). When the individual chromosomes are carefully examined, especially in the alinemental arrangement, one can notice the fact that the X-chromosome possesses probably the greatest length having distinguishable characteristics, while the Y-chromosome is identical in size with the *s*'s, the smallest pair of the autosomes. The X-element appears as a slender and long undulate body in more or less degree, assuming sometimes a vague outline. It is noteworthy that the X presents the tripartite structure, just similar to the case in the foregoing species (see *x* in Figs. 71–74). The Y-chromosome usually has a thinner appearance than the *s*'s. The remaining chromosomes, or the autosomes, constitute 19 homologous pairs which form a fairly well graded series when arranged in the serial alignment (Figs. 82–86). The detailed comparison of chromosomes between the present variety and the wild form will be made in a later section.

The haploid number of chromosomes was 20 in the primary and secondary spermatocytes (Figs. 75–76 and 80–81). As is the case in the foregoing species, a segmentary characteristic of the X-chromosome is strikingly distinct in the primary spermatocyte metaphase (Figs. 75–78) and anaphase (Fig. 79), and even in the secondary metaphase too (Fig. 80). Occasionally, as seen in Figs.

77 to 78, a remnant of the nucleolus was found situated always at the junction of the proximal and median segments of the X. This evidence is of great significance when one refers to the relationship between the X-chromosome and the nucleolus as seen in the meiotic prophase. This is treated in detail under another paragraph (Part II).

Here the author wishes to offer an interesting bit of evidence encountered in this material. It is the position of the XY-bivalent in the metaphase equatorial plate of the primary spermatocyte. So far as the two foregoing cases, *Mus musculus* and *Mus molossinus* (wild), are concerned, the XY-bivalent usually takes position at the periphery of the equatorial plate in the first metaphase, while in occasional cases, it lies in the interior area of the equatorial plate (see Table I and Table III). In the present form the condition is somewhat different. The result of the statistical research for the two different modes of arrangement, peripheral and interior, of the XY-bivalent, based upon a total of 311 equatorial plates of the first metaphase which were derived from six different individuals is as follows:

TABLE IV. *Position of XY-bivalent in the first metaphase plate*

Mus molossinus, domesticated

	Periphery	Interior	Total
Frequency	299	12	311
%	96%	4%	100%

This record is sufficient to show the fact that the interior distribution of the XY-bivalent in the equatorial arrangement is rather *exceptional* in this variety. In this respect, therefore, the domesticated variety is markedly different from its original wild species. It is not uninteresting that the variety show such a slight deviation from the original form in the cytological characteristic.

As to the morphology of the sex chromosomes, the present observations are not in accord with those of Masui ('23) and Painter ('27). As reiterated elsewhere in the foregoing pages, the chromosomes in the figures given by them are not preserved in the natural state; they may be useful for a calculation of the number,

but cannot be utilized for an accurate analysis of the morphological characters. The X-chromosome of the mouse dealt with is not one of the medium sized elements as reported by them, but really it is of the largest as clearly demonstrated in the study of Minouchi ('28) and in this paper too. The work of Minouchi ('28) is highly valued in the fact that it furnished for the first time an accurate knowledge as to the morphology of the chromosomes in the mouse.

Painter ('27), in his work on the Japanese waltzing mouse, stated that in the first division the sex chromosomes show a marked tendency toward segregating early to the poles of the spindle. No such peculiar behaviour of the sex chromosomes has been found in the present material of the waltzing mouse. It is indisputably clear by referring to his figures that the observation of Painter ('27) was based on the abnormally oriented sex chromosomes which were irregularly protruded from the equatorial plate, probably caused by the poor fixation. A similar kind of questionable figures of the sex chromosomes is also encountered frequently in the figures given by English investigators such as Koller and Darlington in the works about *Rattus norvegicus* ('34) and some others. The present author wishes here to call the reader's attention to the fact that it is these erroneous figures from which their dogmatic and hypothetic illustrations have arisen as regards the conjugation and segregation of the sex chromosomes. Though it was already noticed in the study of the carp (Makino '39), the author will repeat again the statement that it is desirable, before making any conclusive statement, for one to bear in mind that such abberations are likely to be caused by the technical procedure.

(3) Accounts and observations regarding *Mus caroli* Bonhote[1]

This species is closely related to *Mus molossinus* and has a distribution confined to Formosa. Tateishi ('35) has given a brief account of the chromosome number of this species. They closely resemble those of the foregoing forms already studied with regard to the main features such as the number of chromosomes and the general morphological characters of their elements, barring a slight difference to be mentioned later.

Spermatogonia. Fifteen excellently preserved metaphase

1) Synonym of *Mus formosanus* Kuroda.

plates were chosen for the detailed morphological study of chromosomes. Forty chromosomes were found arranged radially in the metaphase equatorial plate (Figs. 87–91). As in the previous forms, the complement consists of elongated rod-shaped elements of telomitic nature. The morphological analysis was carried out by the method utilized in the foregoing cases (see p. 312), and the chromosomes placed in pairs were arranged in serial alinement. There are thus produced in each plate 19 homologous pairs of autosomes and the unequal pair of the X- and Y-elements, their examples being given in Figs. 110–119. The autosome pairs, ranging from *a* to *s* in the figures, form a well graded series, with no element outstanding in size. Even under a rough comparison, it may be evident that the individual elements of this species seem to be longer than the corresponding ones of *Mus molossinus*. The details as to the comparison of chromosomes are to be found in a later section.

Similar to the foregoing cases, the X-chromosome is the largest element in the complement, with the characteristic feature readily distinguishable from the rest. In the majority of cases, the X is characterized by its slenderness in form, sometimes being less stained (Figs. 88, 89 and 91). Frequently it is provided with two constrictions in more or less degree, and as the result it shows the tripartite configuration or its modified feature according to the degree of the constrictions. Examples are seen in Figs. 110, 111, and 116.

In striking contrast to the cases found in the previous species, the Y-chromosome of this species is represented by an element slightly smaller than the *s*'s. Though the difference in length between them is very minute, it is clearly and definitely recognizable in the alignmental arrangement of the chromosomes in serial order (see Figs. 110–119). It is noticeable that the size reduction occurs in the Y-chromosome as one of the specific characteristics of the species.

Primary and Secondary Spermatocytes. The metaphase of the primary spermatocyte shows 20 well defined chromosomes which consist of 19 autosome tetrads and an XY-bivalent with heteromorphic structure (Figs. 93–97). The general features of the autosome tetrads are nearly similar to those observed in the other species in their essential nature. They assume the shape of a ring, a V, a horse-shoe or their modified forms, all being of the

telomitic type. The proximal knobs are also distinct in each at their inner ends.

No special finding is to be added on the morphology of the XY-bivalent of this species. As seen in the former cases, at one extremity the X-element contacts with the Y by a delicate fibre in a linear series, holding its long axis parallel to that of the spindle. In the equatorial arrangement, the XY-bivalent occupies a position either at the periphery or in the interior area of the spindle (see *xy* in Figs. 93–97). Table V gives the frequency of the position of the XY-bivalent in the metaphase plate of the primary spermatocyte, as calculated in a total of 87 equatorial plates coming from three different individuals.

TABLE V. *Position of the XY-bivalent in the first metaphase plate*

Mus caroli

	Periphery	Interior	Total
Frequency	54	33	87
%	62%	38%	100%

Though the observed cases are not sufficient, one may be warranted in saying that the XY-bivalent of this species has a tendency to take its position in the interior area of the equatorial plate in higher frequency than in any other species previously recorded.

In the present species the author has also not failed to observe the segmentary structure of the X-chromosome. Just as in *Mus molossinus,* the X displays the tripartite configuration in striking clearness because of the existence of two distinct constrictions (Figs. 98–102, *xy*). The proximal segment seems to be identical with the Y-element in its shape and staining reaction. As to the size, however, these two segments are not considered as homologous with one another. One cannot overlook the fact that occasionally the Y is slightly smaller than the proximal segment. The structure of the other segments also resembles that observed in *Mus molossinus.* The median segment is apparently loose in texture, sometimes considerably elongated. The distal segment contains a terminal condensation of chromatin, the polar granule. This segment frequently undergoes modification of form probably due to elongation of chro-

monema. This may be well recognized by referring to the accompanying figures (Figs. 98–102).

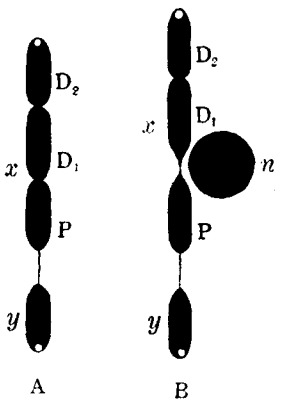
In the ensuing division at anaphase, all the tetrads segregate equally into their constituent halves; the XY-bivalent also does the same without any exception, the X and Y passing to the opposite poles (Figs. 103–105). The separation takes place quite simultaneously in every tetrad. As the result of this division, there arise two sorts of secondary spermatocytes, (a) those having 19 autosome dyads plus the X-element and (b) those possessing an equal number of autosome dyads plus the Y-element. In the fairly fixed state, the X-element is evident in the complement by its distinctive characteristics in shape, size and staining condition, sometimes displaying the remarkable tripartite structure (Figs. 106–107). On the other hand, the Y-element is rather difficult to identify with certainty among the small autosomes of the complement (Figs. 108–109).

(4) The tripartite structure of the X-chromosome

The tripartite structure of the X-chromosome of mammals has been described and discussed for the first time in the study of Oguma ('35) working on some wild rats, and then its occurrence and bearing have been broadened over several other forms of mammals by his extensive studies (Oguma '35, '37a, b). By this valuable and important discovery, the perplexing problems concerning the human sex-chromosome have found their final solution. At present the tripartite structure has been proved to be remarkably characteristic, and of wide occurrence in the X-chromosomes of mammals and some other animals.

As already described in detail, the X-chromosome of the mouse is subdivided into three consecutive segments in a linear series, each segment having nearly equal size, by the presence of two transverse constrictions. The XY-complex of the primary spermatocyte, therefore, is constituted of four distinct parts in a linearly connected series. This difinitive tripartite structure of the X-chromosome was found to occur in each of the three species of mice herein investigated (*Mus musculus, Mus molossinus* and *Mus caroli*). A striking similarity is noticeable among these three species dealt with and no outstanding peculiarity can be observed in any of them, so far as the structural features of the X are concerned.

Oguma ('37a, b), has designated the three segments of the X-chromosome as P, D_1 and D_2 respectively for the proximal, median and distal segments (see Oguma '37a, p. 68). The XY-complex of the primary spermatocyte, therefore, has a constitution of $Y+(P+D_1+D_2)$. In the rodents and likewise in man, the polar granule to which the spindle fibre attaches lies at a point between the P-segment and D_1-segment (Oguma '35, '37a, b). But in the mouse, the condition is somewhat different from the above-mentioned cases. In the case of the mouse, as already mentioned elsewhere in this paper, the spindle fibre attaches at the distal extremity of the X-chromosome, or at the free end of the D_2-segment. Accordingly the X-chromosome of *Mus* always stands perpendicular to the equatorial plate of the primary spermatocyte, with its long axis parallel to the spindle axis, whereas the X of *Rattus* lies horizontally on the metaphase plate with its long axis parallel to a radius (cf. Textfig. 1, A and B).



Textfig. 1. Diagram of the XY-complex in the primary spermatocyte. P, D_1 and D_2 denote P-, D_1- and D_2-segments of the X respectively. *n*, nucleolus.

A, The XY-complex of the first metaphase (side view). B, The same at diakinesis.

Noticeable is the fact that the three segments of the X-chromosome are not necessarily equivalent in their nature. So far as the X-chromosome of the primary spermatocyte is concerned, the proximal or P-segment seems to be rather stable in its nature, since it does not undergo so great a modification of forms as the D_1- and D_2-segments. This segment possesses quite homologous features with the Y-element in its shape, size and staining condition. This seems to be true at least with the cases of *Mus musculus* and *Mus molossinus*. One of the remarkable evidences is the fact that the Y-element and P-segment are provided with a constriction at quite corresponding position in each of them (see Figs. 44, 51, 52, 53 and 138). These evidences strongly suggest a possibility that the P-segment may be equivalent in its nature with the Y-chromosome. The median or D_1-segment is characterized by its loose texture with a vague contour, sometimes being elongated to a considerable extent.

One cannot here overlook the fact that a nucleolus was found situated at the junction of the P- and D_1-segments (see Figs. 77, 78, 138, and Textfig. 1, B). The D_2-segment displays at its tip a chromatin condensation that corresponds to the so-called polar granule to which the spindle fibre comes to attach. This segment represents a considerable modification of form perhaps due to drawing out of the inner spiral chromonemata, though the polar granule itself does not vary.

The tripartite nature of the X-chromosome was found to occur likewise in either the spermatogonia or the secondary spermatocytes. This fact strongly indicates that the tripartite structure of the X is by no means a superficial one but a fundamental character of the latter. It is evident that the inner segment of the spermatogonial X-chromosome containing the attachment locus at its tip, corresponds to the D_2-segment of the X in the primary spermatocyte and the distal segment of the former to the P-segment of the latter. A similar relation is also seen between the secondary spermatocyte and the primary spermatocyte.

(5) Comparative analysis of the chromosomes

In surveying the chromosomes of the three species of mice, *Mus musculus, Mus molossinus* and *Mus caroli,* the author has been early struck by the fact that there occurs a considerable difference among the species as regards the length of the chromosomes. As already noted, the chromosomes of the mouse in general possess no particular distinctive features beyond those of length. The measurement of chromosomes, therefore, has been undertaken with a desire to discover whether any constant difference exists among these species of the mouse in respect of the length of the chromosomes. For this purpose the chromosomes have been employed from the metaphase equatorial plates of the spermatogonia, on which all the chromosomes lie on one horizontal plane along their entire lengths—any plane approximately perpendicular to the microscopic axis—allowing of an exact appreciation of their size. The method of preservation and the subsequent treatment of the tissue have been identical in all cases of the three species (for details see p. 309), since varying methods of fixation and dehydration shrink tissues unequally.

After a survey of a large number of metaphase figures, in

every case fifteen adequate equatorial plates were selected for the mensuration of the chromosomes. In order to make the measurement of the length of the chromosome as accurate as possible and to avoid error so far as possible, every care was taken in the following procedure. In the first place, a close examination was made directly under the microscope upon each chromosome directing attention towards its real size and shape. Then the chromosomes were drawn by the aid of the camera lucida to give the actual length as accurate as possible, at a magnification of 4200 diameters (Zeiss apochr. 1.5 mm × K20). Upon these drawings the measurement was made of the chromosome lengths with the aid of an elastic lead wire and a pair of calipers, and their lengths were expressed in millimeters.

The ratio of length between each member of the largest and smallest pairs of the autosomes comes firstly under consideration for the sake of comparison, since the largest and smallest elements are always conspicuous and readily distinctive in the complement. (In the following descriptions, *a* and *s* are given to indicate the member of the largest and smallest autosome pairs respectively as noticed in the serial alinement of chromosomes. See Pls. XIX, XXI, XXII and XXIV). Generally speaking the size of any chromosome is influenced by the size of the cell in which it is found; that is, the chromosomes contained in a large cell are larger than those in a small cell. But, in such a case the relation of the size of chromosomes varies quite proportionally between the large and small cells. This will be well understood from the following data:

TABLE VI. *Ratio of length between a's and s's in the large (A) and small (B) cells in Mus musculus*

	Length of *a*'s (*mm*)	Length of *s*'s (*mm*)	Ratio of *a*/*s*
A cell (*Fig. 26*)	10.80	4.50	5.40
B cell (*Fig. 35*)	8.50	3.50	2.43

Thus, it is evident that the value of the ratio in length between the largest and smallest chromosomes in the large cell is nearly the same as that in the small cell. On the basis of this fact, it may be justifiable to take the ratio of length of the *a*'s to that of the *s*'s

as the basis of comparison in the different species. In order to increase the degree of reliability of the data, the ratio of length between the *a*'s and *s*'s was first calculated in each of 15 equatorial plates for the three species respectively and then the mean value of the ratio was found from them for each species. Table VII shows the mean value of the ratio of length of the *a*'s to that of the *s*'s by way of comparison in the three species, and in addition, the average lengths of the *a*'s and *s*'s are given as a matter of reference.

TABLE VII. *Average lengths of the a's and s's and the mean value of ratio in length between them in the three different species*

Species	Average length (in *mm*) from 15 cells		Mean value of ratio in length (*a*/*s*)
	a	*s*	
Mus musculus	10.09±0.68*	4.40±0.38*	2.30±0.01*
Mus molossinus	12.04±1.28	4.69±0.51	2.57±0.06
Mus caroli	12.86±1.21	4.55±0.46	2.84±0.30

* *Standard deviation*

By reference to the above table it is evident that the obtained ratio in length between the largest and smallest autosomes (*a*/*s*) seems to furnish the appreciable value characteristic to the different species beyond the range of error in measurement, and that there are differences enough to serve as a basis for specific distinction. The point of special interest and significance lies in the fact that the difference of the chromosome ratio between *Mus musculus* and *Mus molossinus* (2.30~2.57) is approximately equal to that seen between *Mus molossinus* and *Mus caroli* (2.57~2.84).

In this connection the author wishes to record here the result obtained from the Japanese tame and waltzing mice which have been considered as the domesticated race of *Mus molossinus*. The mean value of the ratio of length of the *a*'s to that of the *s*'s, calculated in the same manner as the above, in 10 equatorial plates was found to be 2.63±0.18. This value of the chromosome ratio ranks between those of *Mus molossinus* and *Mus caroli*. This result may be interesting when one recalls the suggestion of Gates ('26)

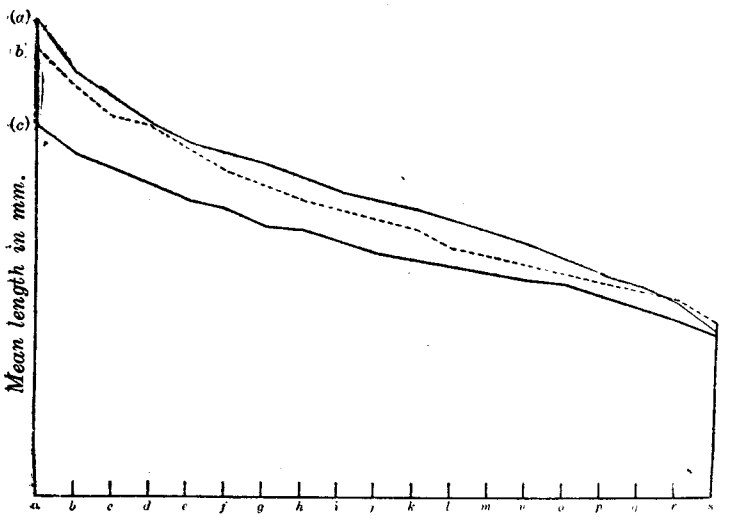
that the Japanese waltzing strain is of hybrid origin.

TABLE VIII. *Comparison of average and relative lengths of autosomes (in the haploid set) calculated in 15 cells*

Chromosome	*Mus musculus*		*Mus molossinus*		*Mus caroli*	
	Average length (in *mm*)	Relative length	Average length (in *mm*)	Relative length	Average length (in *mm*)	Relative length
a	10.09	2.29	12.04	2.57	12.86	2.83
b	9.31	2.12	11.03	2.35	11.41	2.51
c	8.82	2.00	10.26	2.19	10.73	2.36
d	8.47	1.93	10.00	2.13	10.17	2.24
e	8.07	1.83	9.21	1.96	9.66	2.12
f	7.82	1.78	8.74	1.86	9.33	2 05
g	7.34	1.67	8.44	1.80	9.05	1 99
h	7.26	1.65	8.04	1.71	8.68	1.91
i	6.93	1.58	7.77	1.66	8 25	1.81
j	6.60	1.50	7.54	1.61	8.03	1.76
k	6.46	1.47	7.28	1.55	7.80	1.71
l	6.27	1.43	6.89	1.47	7.51	1.65
m	6.09	1.38	6.56	1.40	7.17	1.58
n	5 87	1.33	6.24	1.33	6.82	1.50
o	5.77	1.31	6.06	1.29	6.44	1.42
p	5.46	1.24	5.86	1.25	6.16	1.35
q	5.16	1.17	5.61	1.20	5.74	1.26
r	4.81	1.09	5.33	1.14	5.26	1.16
s	4.40	1	4.69	1	4.55	1

Next an attempt was made to express graphically the differences of length of the chromosomes of the three species. The length of chromosomes cannot be considered as the criterion of comparison in a strict sense, because it varies according to the cell size. But a relative difference may be still expected in the result when the comparison is made on the average length calculated in a sufficient number of cases. In view of this consideration the length of each chromosome, excepting the sex chromosomes, was carefully measured in each of 15 equatorial plate for each species respectively. Then the mean length of each chromosome was found in each species as

given in Table VIII and the data were plotted in a graphical form as shown in Textfig. 2. Along the abscissa the autosomes in the haploid set are arranged in the approximate order, while on the axis of the ordinate is represented the mean length of each chromosome. This graphical representation, though any absolute exactness cannot



Textfig. 2. The curves represent the mean lengths of autosomes in the three species.

——..... *Mus caroli* (*a*)
..... *Mus molossinus* (*b*)
——..... *Mus musculus* (*c*)

Textfig. 3. Total sum of the mean lengths of autosomes (in the haploid set) compared in three species.

A, *Mus caroli.*
B, *Mus molossinus.*
C, *Mus musculus.*

be expected, has the advantage of showing at the same time, the size relation of the individual chromosomes in a single species and the difference of the chromosomes in the three species by way of comparison. When the curves of the three species are compared with one another, it will be conceived that there occur appreciable differences among the species as to the lengths of the chromosomes. On the whole, the differences vary almost proportionally from

species to species, leaving as an exception the remarkable evidence that the mean lengths of the *r*'s and *s*'s are the highest of all in *Mus molossinus*. Generally speaking, so far as the lengths of chromosomes are taken as a criterion for comparison, the three species under consideration rank in the following order: (1) *Mus caroli*, (2) *Mus molossinus*, and (3) *Mus musculus*, even if there are included some possible errors in the measurement and drawing of the chromosomes. A similar relationship is also attained when the total sums of the mean lengths of the chromosomes come under comparison among them, as is clear from the accompanying diagram (cf. Textfig. 3).

Some mention may be made here on the result of mensuration of the X- and Y-chromosomes. Their lengths were measured in the side view of the primary spermatocyte metaphase where they stretch their entire length with their long axes approximately parallel to the spindle axis, on the basis of 15 equatorial plates in each species concerned. Then the mean lengths were found in each. The mean value of the ratio in length between the X- and Y-chromosomes was also calculated through the species. The results are tabulated comparatively in Table IX. It is noteworthy from this data that the

TABLE IX. *Average lengths of the X- and Y-chromosomes and the mean value of ratio in length between them, based on the side views of 15 metaphase plates of the primary spermatocytes*

Chromosome / Species	X-chromosome Average length in *mm*	Y-chromosome Average length in *mm*	Mean value of X/Y in length
Mus musculus	13.45±0.21*	4.30±0.05*	3.13±0.17*
Mus molossinus	13.64±0.22	4.52±0.11	3.12±0.32
Mus caroli	13.68±0.27	4.22±0.12	3.27±0.37

* *Standard deviation*

ratio of length between the X- and Y-chromosomes is almost similar between *Mus musculus* and *Mus molossinus*, while in *Mus caroli* it is a little higher than the above two species.

Besides the above evidence, the position assumed by the XY-bivalent in the equatorial plate of the primary spermatocyte metaphase cannot be overlooked as one of criteria for comparison. As already mentioned in the foregoing sections, the XY-bivalent takes its position either on the peripheral zone of the equatorial plate or in the interior of the latter. The frequency of the position of the XY in two cases above mentioned was calculated and stated earlier in this paper for each species (cf. Tables I, III, IV and V). For convenience of comparison the data are arranged in Table X.

TABLE X. *Comparative table of frequency of the position of the XY-bivalent in the primary spermatocyte*

Species	Frequency in %	
	Periphery	Interior
Mus musculus	85%	15%
Mus molossinus	75%	25%
Mus caroli	62%	38%

Examining the above table one can see that there occurs a specific difference as regards the frequency value, varying with a difference of about 10% between the species. In his study of the hemipteran chromosomes Wilson ('32) pointed out that it is not merely the size or shape of the chromosomes that determine their position, but a less obvious reaction in which their specific quality is involved. In view of that statement, the specific difference in the above data may be of significance.

PART II. SEX CHROMOSOMES IN THE GROWING PERIOD

In this section it is proposed to follow in as detailed manner as possible the behaviour displayed by the sex chromosomes during the meiotic prophase, or the growing period of the primary spermatocytes. Exact cytological knowledge on this point is of great importance from both cytological and genetical standpoints, because of the fact that it bears a close relation with the mode of segregation of the sex-chromosomes in the subsequent division. In the

studies of the rat and some other mammals, Darlington and Koller (Koller and Darlington '34, Koller '36a, b, '38), have emphasized that the X- and Y-chromosomes are associated by means of one or two chiasmata during the meiotic prophase, and that two different modes of segregation of the X and Y in the first division, pre- or post-reduction, are introduced depending on the actual position of chiasmata. The point of interest should rest, therefore, on the question of the chiasma formation, since Minouchi ('28a, b, c, '29) and Oguma ('35, '37a, b) who have followed the history of sex-chromosomes of rats and some other mammals in the minutest detail, entirely deny the conjugation of the X and Y by formation of chiasmata.

It is a well established fact, that through the growing period of the spermatocyte the sex-chromosome of animals, especially in mammals, retains its condensed form as a chromatin-nucleolus or a chromosome-vesicle, which has been reported by earlier investigators under various names, "Kernkörperchen" (Ebner '88), "curious secondary nucleolus" (Moore '93), "Intranuklearkörper" (Lenhossèk '98, Gutherz '22), "corps intranucléaire" (Regaud '01, Duesberg '08), etc. In the mouse the history of the above-noted nucleolar body during the growing period and the various stages of its transformation have been studied in considerable detail by Gutherz ('22), Masui ('23) and Cutright ('32). Gutherz has shown that the chromatin nucleolus (Intranuklearkörper) has nothing to do with the sex-chromosome which is related to the sex determination, but forms a tetrad (Viergruppe). A similar view has been held in the study of Cutright ('32), showing that the chromosome nucleolus is made of the largest pair of autosomes. On the contrary, Masui ('23) has reported that the X and Y elements occur as two separate bodies in the growing period. Such a confusion in studies is sufficient to indicate that knowledge on this account is still in an unsatisfactory status in the mouse.

(1) Behaviour of the X- and Y-chromosomes in the growing period

The observations have been made on material derived from the three species, *Mus musculus, Mus molossinus, Mus caroli* and the data presented have proved to be entirely similar among them. The

main part of the following descriptions has been based on facts observed in *Mus musculus*, the data for the other two forms being partly consulted. In order to trace the complete history of the chromatin nucleolus in the necessary detail up to its transformation into its final shape of the XY-complex, several different staining methods were employed in this study, with the purpose to give a sharp differentiation of acido- and basophilic elements (for details see p. 309). The identification of the X-chromosome was made beyond question in every stage of the growing period, on the basis of its segmentary structure thoroughly established by the foregoing observations.

In the early leptotene stage following the telophase of the last spermatogonial division, there is seen in the nucleus the polar aggregation of chromatin which is probably a pycnotic condensation of the chromatin at the proximal end of each chromosome (Figs. 139–140). This feature closely resembles that reported by Corey ('38) in the corresponding stage of the germ cells of some Orthopteran insects. As to whether the heteropycnotic X- and Y-elements do, or do not, lie at the polar position of the chromatin concentration, cannot be proved with certainty in this observation, because the densely fused chromatin aggregates make a precise study difficult.

After having grown thicker and shorter into the pachytene condition, the chromatin threads lose their polarization and become scattered about in the nucleus. There is found in the nucleus of this stage a chromosomal element which is separated within a distinct vesicle sharply demarkated from other elements and situated at an eccentric position of the nucleus lying near the nuclear wall (Figs. 120 and 141). Remarkable and particularly important is the fact that the chromosome lying in the vesicle displays two distinct constrictions, by the presence of the latter the entire body of the chromosome being subdivided into three segments. Considered from its characteristic tripartite structure and relative magnitude, it is very probable that this element is nothing else than the X-chromosome. That is, in the growing period the X-chromosome constitutes a chromosome vesicle in which to a considerable extent, it retains its individuality. By the time when the next advanced stage, the diplotene, is reached, the nucleus shows a conspicuous spherical nucleolus of large size which appears always lying close

to the X-chromosome vesicle (Figs. 121–123). How this spherical nucleolus is brought about from the preceding stage has not been determined, since no favourable transitional stage between Fig. 120 and Fig. 121 could be obtained. It is, however, a likely consideration that small nucleolar bodies, several in number, which seem to be in connection with the certain chromatin threads in the nucleus of the preceding stage as shown in Fig. 120, fuse together and finally transform into a single large spherical body as seen in the next stage shown in Figs. 121 to 123, because no nucleolar elements could be found in the nucleus of the next stage except the spherical nucleolus just mentioned. The large nucleolus under consideration gives a typical acidophilic reaction with Feulgen's staining method, so that it is no other than the plasmosome or the true nucleolus.

Applying general staining methods such as the use of Heidenhain's iron-haematoxylin, Newton's gentian violet and Feulgen's basic fuchsin, the X-chromosome vesicle is entirely stained into a dense mass without giving any slight differentiation into its structural components, the skeletal matter of chromatin substance and the ground substance inside. Its structural differentiation is only attained to some extent in the properly destained iron-haematoxylin preparation or sometimes in an adequate preparation stained by Flemming's triple method (Figs. 121–123).

A noteworthy and interesting feature found in the diplotene stage is the complete association of the X-chromosome vesicle with the plasmosome. At the beginning of its appearance, the plasmosome lies in close contact to the X-vesicle, while later they become completely associated together into a single huge mass of squarish oblong shape, to which the name 'amphinucleolus' is generally applied. In the preparations stained with iron-haematoxylin and Newton's gentian violet there is no visible demarkation between the X-vesicle and the plasmosome in this amphinucleolus, because both of them are densely stained with the similar dye in a tightly fused state (Figs. 124, 142 and 143). When stained with Feulgen's basic fuchsin accompanying light green and Mann's methyl-blue-eosin, the amphinucleolus shows a sharp differentiation into its components, the X-vesicle and the plasmosome, due to the differences of their affinity for stains. For instance, according to Feulgen's method, the X-vesicle is stained with basic fuchsin while the plasmosome takes light green (Fig. 125). A similar colour differentiation is also

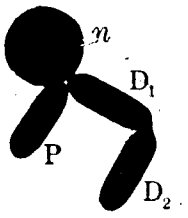
obtained by means of Flemming's triple staining method, though it is not so sharp as by the above methods. The formation of the amphinucleolus has taken place at the rate of about 70% of the observed cases in the stage of diplotene.

Here it may be very desirable and important to make clear in which region of the X-vesicle the plasmosome comes to associate. It is already described that the X-vesicle involves in its ground substance the chromatin skeleton which has maintained its characteristic tripartite structure. As shown in Figs. 121 to 123, the plasmosome comes into association always at the joint part of the median segment and one of the terminal segments. That the terminal segment just mentioned corresponds to the P-segment, is indisputably clear by referring to the fact that the Y-element always comes into contact with this segment as described below (Figs. 133–138; and see also the evidences in Figs. 77 and 78). That is, the plasmosome associates with the X-element at the junction of its P- and D_1-segments as diagramatically shown in Textfig. 4.

As a whole, it is in the later stage of diplotene that the Y-chromosome makes its first appearance in the proximity of the amphinucleolus (Figs. 127–128). The history of the Y-chromosome from this stage backwards has not been traced with complete success in this study, because the existence of some condensed elements simulating the Y in shape and size, strongly disturbs the conclusive identification of the latter. It may be, however, justifiable to state that the Y-element, undergoing heteropycnosis, remains without disintegration during the growing period and it is represented in all probability by one of the pycnotic elements mentioned above.

In the meanwhile, the Y-chromosome moves closer to the amphinucleolus and at last they are brought to the final conjugation (see Figs. 129–132). In conjugation the Y-chromosome always connects at one of its ends with the free end of a certain segment of the X, namely the P-segment as formerly determined, by means of a fine thread. Hence the conjugation of the X and Y is no other than a mere end-to-end connection and there is no evidence of synapsis between the two. Critical stages illustrating this evidence are demonstrated beyond question in the accompanying photomicrographs and drawings with an indisputable clearness (Figs. 129–132 and Figs. 144–147). This observation has thus given a remarkable confirmation to the presumption attained by Oguma ('35) in his

study on the rat, that the X- and Y-chromosomes may develop independently from each other in the growing period. There is no room in this study for doubt with regard to the fact that the X and Y remain as separate entities at least throughout the early stages of the growing period and show absolutely no further relationship to each other. Of course there is neither any sign of side-by-side conjugation between the X and Y in form of chromatin threads, nor any evidence of the formation of chiasmata in any way between them. This result, therefore, is entirely opposed to the recently proposed views of Darlington and his follower, who have established and discussed the genetical and mechanical properties of the sex-chromosomes on the basis of the chiasma formation between them (Koller and Darlington '34, Koller '36a, b, '38).



Textfig. 4. Diagram showing the association of the plasmosome nucleolus (*n*) and the X-chromosome in the growing period. P, D_1 and D_2 indicate P-, D_1- and D_2-segments of the X respectively.

Later on at the commencement of diakinesis, the amphinucleolus undergoes some changes. At first it begins to separate into its constituent parts, the plasmosome and the XY-complex (Figs. 133 and 148), and as diakinesis proceeds, there follows complete separation leaving them as two independent elements. Observations in early diakinesis show that complete separation of the plasmosome from the XY-complex has taken place in about 85% of the cases. After having been separated, the plasmosome and the XY-complex lie rather far apart, and there is no longer seen any association between them (Figs. 134–135 and 149–150). As an exceptional thing, it occasionally happens that the separation of the plasmosome and the X-vesicle takes place prior to the association of the X with the Y (see Figs. 126 and 128). Or in other cases, on the contrary, the plasmosome persists as long in intimate association with the XY-complex as up to late diakinesis (Figs. 136 and 151) or sometimes to the early stage of metaphase (see Figs. 78, 79 and 138).

In parallel with the above changes, the X-chromosome, imbedded heretofore in the ground substance, gradually shows its whole structure of the tripartite nature, assuming a well-defined outline staining deeply (Figs. 133–137). In late diakinesis the ground substance

becomes invisible, and the X-chromosome, associated with the Y, acquires its final form as the XY-bivalent in the metaphase (Figs. 137–138). Thus the XY-complex is exceedingly distinct among the autosome tetrads in the nucleus of late diakinesis, due to its characteristic feature consisting of four segments in a linear connection. At the same time the plasmosome loses its staining capacity and apparently disintegrates.

(2) Critical remarks on the chromatin nucleolus in mammals

Since the direct relation of the sex-chromosome to the basophilic deeply staining body, or the chromatin nucleolus, in the growing period of spermatocytes, has been made clear in Orthoptera and some other insects, the presence of a like structure in mammalian spermatocytes has much attracted the attention of earlier investigators. This body has been described under various names, Kernkörperchen by Ebner ('88), curious secondary nucleolus by Moore ('93), Intranuklearkörper by Lenhossék ('98), corps intranucléaire by Regaud ('01) and by Duesberg ('08), etc., but these authors stated nothing about the destiny of the body and its relation to the sex-chromosome. At that time McClung ('02) made an interesting suggestion that the accessory-like body which he had seen in the mouse is identical with a similar body in insects where it has a direct relation to sex determination.

Considerable discussions have arisen concerning the chromatin nucleolus in mammalian spermatocytes. Allen ('18) seems to be the first to point out in the study on the rat that the sex-chromosome develops from the chromatin nucleolus. A similar conclusion was reached by Federley ('19) in his work on the field mouse. Gutherz ('22), after a careful study of the phenomena in the mouse, concluded that this body does not represent the sex-chromosome component but an autosomal tetrad. This conclusion of Gutherz ('22) was argued by Painter ('24) stating that Gutherz's evidence on the actual division stages is entirely insufficient for such an interpretation. Though the interpretation of Gutherz ('22) is considered to be certainly erroneous on the basis of the present investigation, however, his observation is still of much interest since the formation of the amphinucleolus is suggested from his evidence that the

nucleolar body consists of two elements, the basophilic and acidophilic substances and that in diakinesis they separate into two constituent parts, the basophilic chromosomal substance moving towards the nuclear wall and the acidophilic towards the interior of the nucleus, which later becomes a plasmosome. A little later Painter ('24), in a more extended study of the facts in the opossum, has demonstrated that in this case the chromatin nucleolus of the growing period is made up of the sex-chromosome components. Masui ('23) has reported in the mouse the occurrence of separate bodies in the growing period which later transform into the X and Y. On the basis of the present author's observations upon the same material, however, it is certain that the bodies regarded as the X and Y by him do not represent the real X and Y. Minouchi ('28a, b, c, '29) published valuable works, in which the fate of the chromatin nucleolus was followed in detail in the rat and some other mammals. He arrived at the conclusion that a single chromatin nucleolus (heterokaryosome) appearing in the growing period gives rise later to the XY-complex. He proposed a new conception as regards the mode of synapsis taking place between the X- and Y-chromosomes (Minouchi '28d). These works of Minouchi are highly appreciated for furnishing for the first time a detailed account of the chromatin nucleolus in mammals from the time of its appearance in the growing period until the telophase of the first division. Recently Oguma ('34, '35, '37) has published a different account on the chromatin nucleolus in a series of studies of rats and some allied forms, stating that the X-chromosome appears in the form of a chromosome vesicle in the growing period, out of which the X-chromosome of the final shape develops. This conclusion of Oguma is based on the fact that the chromosome included in the vesicle is provided with a distinct tripartite structure which just corresponds to that of the X-chromosome and there is not contained any element to be taken for the Y. Though not in harmony with the view of Minouchi ('28, '29), this evidence presented by Oguma for rats and allied forms is quite accordant with that established by the author for the mouse in this study. A further critical statement regarding this problem will properly be postponed until the author's own studies extend to the rat and other allied animals.

So far as the present observations go, the following may be

said without fear of contradiction: The X-chromosome of the mouse dealt with assumes the form of a chromosome vesicle during the growing period and the vesicle may be naturally taken for a chromatin nucleolus when it is densely stained with dye. The X- and Y-chromosomes, therefore, remain as separate entities during the early part of the growing period having absolutely no direct connection with each other, and the conjugation takes place between them in late diplotene. Their conjugation is of the most perfunctory character being made by an end-to-end connection, just as the case in the hemipterous *Oncopeltus* (Wilson '12). There is of course neither any slight trace of side-by-side conjugation between the X and Y in form of chromatin threads in the early stage of the growing stage, nor of the formation of any chiasmata between them. The perfunctory connection of the X- and Y-chromosomes in this case, therefore, is highly suggestive of the possibility that crossing-over of genes does not take place between the X and Y of the mouse. In the first maturation division they segregate to opposite poles of the spindle without even one exception. These findings as above summarized, constitute a remarkable confirmation to the views proposed by Oguma ('34, '35, '37a, b) in a series of brilliant papers working on several forms of rodents. Here it becomes necessary to touch the observations made by Crew and Koller ('32) in the mouse, Koller and Darlington ('34) in the rat and Koller ('36a, b, '38) in marsupials and some others, in which the association of the X and Y by chiasmata is maintained. According to Koller and Darlington ('34) for instance, the X and Y have been seen paired at pachytene stage in a form of unequal thick threads and at diplotene stage they are associated by one or two chiasmata. The mode of segregation of the X and Y in the first division differs according to the position of the chiasmata: in a majority of cases they segregate pre-reductionally in the first division, whereas in a minority of cases the post-reductional separation occurs. In reviewing their observations one cannot overlook the fact that they have not at all paid even slight attention to the existence and the bearing of the chromatin nucleolus, which has been proved to be directly converted into the sex chromosomes in the later stage, by the close studies of Minouchi ('28a, b, c), Oguma ('34, '35) and the present author on the same material. It is very surprising to the author that they have neglected the existence of the chromatin nucleolus which is

so conspicuous in the nucleus of the growing period that it cannot escape one's attention in any event. In view of this consideration, there arises a considerable question as to their conclusion that in pachytene the X- and Y-chromosomes conjugate side-by-side in the form of threads forming chiasmata as the autosomes do. If this evidence regarding the chiasma formation of the sex chromosomes is based upon an erroneous finding, it follows that their mechanical and genetical interpretation of the behaviour of the sex chromosomes, emphasized by them on the basis of the chiasma formation, will no longer be valid at all. Further discussion on this point will be made in a paper now in preparation dealing with the rat.

The association between the chromatin nucleolus and the plasmosome in the growing period, which is called by the name of amphinucleolus, has been reported to occur in several groups of insects, such as Hemiptera (Wilson '05a, b), Coleoptera (Stevens '09), Diptera (Stevens '08), Lepidoptera (Kawaguchi '28, '33), and several Orthoptera (Davis '08, Winiwarter '27, Tateishi '35). In mammals clear-cut cases of this phenomenon are quite meagre. Agar ('23) and Greenwood ('23) both described a plasmosome which is attached to the fused X- and Y-chromosomes during the growing stages of the spermatocytes of certain Marsupialia. Painter ('24) working on the opossum also described a process in the course of which the sex-chromosome nucleolus is separated into an oxyphilic and a basophilic portion, but he believed it to be an abnormal case. Recently Tateishi ('35) gave a brief account on the association of the plasmosome with the chromatin nucleolus in late diplotene stage of the rat. So far as the literature shows, the present study seems to offer the first precise account of the amphinucleolus in mammals from its appearance in the early stage of the growing period until the complete separation into its constituent parts, the X-vesicle and the plasmosome in the later stage. As formerly mentioned, the case of Gutherz ('22) in the mouse can be interpreted as evidence of the close association of the basophilic chromatin nucleolus and the acidophilic plasmosome. At the same time it is certain, from the basis of the author's own observation on the same species, that the huge oblong body designated as the chromatin nucleolus by Cutright ('32) in the growing stage of the mouse, is no other than the amphinucleolus which consists of the plasmosome and the real chromatin nucleolus in close association. As regards the significance of the

amphinucleolus, however, nothing is conclusively stated, whereas the secretion or extrusion of a plasmosome has been considered by Gutherz ('22).

PART III. CHROMOSOMES OF THE OOCYTES

The chromosomes in oogenesis of the mouse have been repeatedly reported upon by a number of earlier investigators who mainly directed their observations towards elucidation of the maturation and fertilization phenomena in the ova. The first work in this field seems to have been done as early as 1889 by Tafani, who reported that 20 chromosomes occurred either in the oocytes or in the cleavage. Successively a good many discrepant reports as to the chromosome number of oocytes, ranging from 8 to 30, have been published among the earlier authors, such as Holl ('93), Sobotta ('95, '07), Gerlach ('06), Lams & Doorme ('07), Melissinos ('07), Coe & Kirkham ('07), Long ('08), Long & Mark ('11) and Kingery ('14, '17). These are fully summarized in the new list of chromosome numbers given by Oguma and Makino ('37). Recently Zdenko ('26) has claimed to find 24 chromosomes in the oogonia and 12 in the oocytes, whilst Schachow ('30) has argued that the number of chromosomes in oogenesis of the mouse was 36 in diploid and 18 in haploid. More recently Crew and Koller ('32) have found 40 chromosomes in the oogonium and 20 in the primary oocyte of the mouse[1]. That these disagreements amongst the investigators as to the number of chromosomes are probably not due to their use of different mouse species, is considered nearly certain, judging from the facts established in the recent studies of the male forms extending to various species of mice. From these considerations it is beyond question that the discrepancies thus induced may be accounted for only by the poor fixation under the older technique. The present author has had occasion to observe the oocyte chromosomes in *Mus musculus* and *Mus molossinus*, in the course of the study on the

1) All the accounts above cited seem to have dealt with *Mus musculus*, a common species generally utilized as laboratory animals in Europe and America, though in the majority of cases no definite statement is made in regard to the specific name of the mouse employed by them.

maturation and fertilization phenomena. The results of the observation are presented as follows.

The sections from the ovaries including the oviducts which were fixed in Allen-Bouin's solution and prepared in the usual way, were used as the material for study; for staining Heidenhain's iron-haematoxylin method exclusively was applied. The procedure for getting the material is related in detail in the later chapter, Part V.

(1) Observations on *Mus musculus* L.

The first maturation division of the oocyte is completed prior to ovulation in the ovary. The chromosomes of the primary oocytes were observed only in the normal Graafian follicles advancing regularly in the course of maturation. Several equatorial plates of the polar and lateral aspects were examined, and in every of them the number of chromosomes was invariably 20 (Figs. 152–153). All the chromosomes are of bivalent nature, *i.e.*, ordinary tetrads. This fact becomes more evident when the spindle is viewed laterally, in which all the elements are shown to be composed of equal halves (Figs. 154–155). Compared with the tetrads seen in the primary spermatocytes, those of the oocytes, as seen in the annexed figures (Figs. 152–153), are somewhat deformed in shape, being irregularly thick and condensed. Some of the larger ones appear as rings or thick V's and those of smaller size assume the shape of heavy rods, crosses and like structures. Taking into consideration their structure and also their configurations in lateral aspect, it is beyond doubt that they are all of telomitic nature in their fibre attachment. The chromosomes of the primary oocyte, therefore, are never different as to essential constitution from those of the primary spermatocyte. The abnormal configuration of chromosomes is undoubtedly caused by inadequate preservation.

Now attention is directed towards the sex chromosomes which should be in homogametic condition. As already remarked, every tetrad possesses a symmetrical structure and there is no indication for the occurrence of such a heteromorphic tetrad as the XY-bivalent found in the primary spermatocyte (cf. Figs. 154–155). This evidence implies that the two X-chromosomes conjugate in the usual way in the primary oocyte constituting an ordinary tetrad as the autosomes do. The identification of the X-chromosomes, however,

is practically impossible among the autosomes because of the failure of the characteristic feature distinguishing it from the others. Judging from the relative magnitude of the X-element detected in the spermatogonial cell, it is quite reasonable to say that the XX-bivalent may be represented by one of the larger tetrads in the primary oocyte.

Fig. 156 a, b, shows the sister groups of chromosomes in the anaphase of the first maturation division, in each of which 20 well defined chromosomes (dyads) are found, corresponding in shape and size with each other. After the completion of division, one of the sister chromosome groups at the outer pole is extruded from the egg as the first polar body and the chromosomes left in the egg, without disintegrating in individuality, arrange themselves on the equatorial plate, the metaphase of the secondary oocyte being thus prepared.

In the metaphase equatorial plate of the secondary oocyte 20 distinct chromosomes are demonstrated without any conflict (Figs. 157–158). All the elements assume the shape of a rod in dual nature, tapering at the inner ends, to which the spindle fibres attach, and being arranged radially in the equatorial plate. The homogametic condition of the sex-chromosome gives rise to a single kind of secondary oocyte as regards the chromosome constitution.

(2) Observations on *Mus molossinus* Temm. et Schleg.

So far as the number of chromosomes is concerned, the present species shows nothing different from the former one, there being found 20 chromosomes with extreme distinctness either for the primary (Figs. 159–160) or for the secondary oocytes (Fig. 161). Furthermore, in respect of the other morphological characteristics, the chromosomes of these two species do not admit any visible distinction, so far as the configuration of chromosomes of the oocytes is dealt with. The account on the sex-chromosome given for the preceding species is also applicable for the present species without any change.

From the foregoing observations the inference is warranted that the chromosomes of the oocytes are not essentially different in general characteristics such as number, shape and behaviour, from those of the spermatocytes, and that the cytological proof for the

homogametic condition of the sex-chromosome is established, the two X-chromosomes constituting an ordinary bivalent after conjugation.

(3) Remarks on the sexual difference of chiasma formation

Some mention is to be made here on the sexual difference in respect of chiasma formation. Certain animals are known which show some difference in the sex incidence of chiasma formation. According to the partial chiasmatype hypothesis emphasized by Darlington and his colleagues, predicating that chiasma formation is conditioned by crossing-over, the difference in sex incidence of chiasma frequency is closely correlated with the difference in sex incidence of genetical crossing-over. Strong evidence for this statement is found in several species of *Drosophila* and *Bombyx mori*. Recent study made by Maeda ('39) has revealed that in the silkworm the chiasma is formed normally in the male but is entirely absent in the female. Such is not the case in the mouse, since the chiasma formation normally occurs in both sexes. The investigation made by Crew and Koller ('32), however, has shown that in the mouse there exists a difference in sex incidence of chiasma frequency which corresponds very closely to the difference in sex incidence of genetical crossing-over. That is, in the mouse the chiasma frequency is higher in the females than in the males. Though the present author is not in a position fully to criticize the results presented by Crew and Koller ('32) since no special observations have been made regarding chiasma frequency in this study, it does, however, seem apparent to the author that any conclusive result cannot be expected from the study of Crew and Koller ('32) above cited; it is quite impossible to calculate accurately the exact number of chiasmata in such bivalents of indefinite configurations with obscure individuality as seen in the figures presented by Crew and Koller ('32). The author's own observations upon the oocyte chromosomes of *Mus musculus* and *Mus molossinus* are sufficient to indicate that the differences between the sexes seemingly found in the configurations of metaphase bivalents are not beyond the differences of configurations mechanically induced by the fixing method employed, and further that the heavy, condensed features as seen in the metaphase bivalents of the oocytes, which are probably caused by inadequate fixation, lead to the misconception that they contain many

chiasmata. Genetically considered, in the mouse the sexual difference in crossing-over seems to be as yet a matter of controversy. According to Dunn ('20), Castle & Wachter ('24), Snell ('31) and Roberts and Quisenberry ('35), it has been shown that crossing-over is more frequent in the females than in the males, while Detlefsen ('25) is of opinion that there is no difference of the crossing-over value between the two sexes.

PART IV. FERTILITY AND THE CHROMOSOMES OF THE HYBRIDS BETWEEN *Mus musculus* AND *M. molossinus*

In animals, especially in mammals at least, species crosses are not usually fully fertile. A famous example is found in the mule, the hybrid between the horse (*Equus caballus*) and the ass (*E. asinus*). In spite of the fact that the study of hybridization in mammals is of great importance, not only from the economical standpoint but also from the pure scientific point of view, no particular progress has been attained in this direction during many centuries, owing probably to much difficulty of rearing up the progenies. The work of Dice ('33), whose experiments have succeeded in mating a number of species and subspecies of *Peromyscus* (deer mouse), is probably the most extensive investigation in this field.

Some attempts at a crossing experiment were made by the author in the present study to test the fertility in the species crosses between the related species, *Mus musculus* L. and *Mus molossinus* Temm. et Schleg., on which chromosome studies have been fully carried on as already described. The author's interest is also concerned with the behaviour of chromosomes in the hybrid forms, since Swezy ('28) reported, in the case of a hybrid rat colony, existence of a quite unusual condition of chromosomes which previously has not been known.

Mus musculus L. used in this cross is the pure bred albino mouse derived from the Carnegie Institution strain. The other species *Mus molossinus* Temm. et Schleg. is the wild form which is the sole representative of the house mouse indigenous to Japan. The mice used were trapped in the warehouse as well as in the fields of the suburbs of Sapporo. They are reared under captivity without

difficulty, but failed to breed pure in the laboratory[1]. They differ from *Mus musculus* in a number of morphological properties, among which the smaller size seems to be the most conspicuous.

The method of mating is absolutely simple, as whenever two individuals of different sexes belonging to different species are enclosed together in a cage. Under conditions strange to both they are less likely to fight but they readily mate. Inbreeding of their descendants has also been easily made with fertile offsprings.

(1) Fertility

Attempted crosses carried out between the two species above mentioned have proved to be fully successful with fertile offsprings in every test. Crossing of *musculus* female with *molossinus* male and the reciprocal crosses between the two likewise produced offsprings with a similar result. Their descendants were also inbred through five generations. The table gives a summary of the crossing made in the course of this study (Table XI)[2].

TABLE XI. *The results of crossing between musculus and molossinus*

Generation	No. of litters	No. of hybrids	No. of males	No. of females	No. of ♂'s to 100 ♀'s
F_1	14	81	41	40	102.50
F_2	11	66	33	33	100.00
F_3	7	30	16	14	114.29
F_4	4	18	9	9	100.00
F_5	2	12	6	6	100.00
Back-cross	13	69	36	33	109.19
Total	51	276	141	135	104.44

The hybrid individuals belonging to the F_1 generation possess without exception the coat colour of the normal wild type entirely indistinguishable from the parent *Mus molossinus*. The segregation

1) Among several matings, only one pair produced a litter giving two young.

2) Besides these experiments the following crosses, $F_1 \times F_2$, $F_1 \times F_3$, $F_2 \times F_3$, and $FR_1 \times FR_1$, were attempted and, in every case, obtained several offsprings that were completely fertile.

in the FR_1 generation was in the ratio of 21 normal wild to 20 albino, so far as the coat colour is concerned.

The number of young per litter is 5 to 6 in the majority of cases examined (Table XII). There was found no indication of increasing infertility among individuals belonging to succeeding generations of hybrids so far as these studies go, and likewise inbreeding seemed to exert no influence upon the viability of the animals.

TABLE XII. *Average number of young per litter in five and back-cross generations*

Generation	Average no. of young per litter
F_1	5.79
F_2	6.00
F_3	4.29
F_4	4.50
F_5	6.00
Back-cross	5.30

It is known that one phenomenon attending hybridization is a distortion in the sex ratio. The sex ratio in mammals is said to be altered by hybridizing. King ('11) pointed out this fact in interracial crosses of rats, concluding that hybridizing alters the sex ratio by producing a marked increase in the relative proportion of males. Green ('30), working on the species cross of mice, informed that the sex ratio shows a great excess of males among the individuals belonging to the back-cross generation. The data regarding the sex ratio obtained in the present experiments are shown in Table XI. According to this record the sexes exist in the ratio of 104.44 males to 100 females in the total of 276 hybrid offsprings herein examined. The number of the males is slightly greater than that of the females. This sex ratio is not considered to be higher than that which is normal for either of the parent species (cf. Green '30). There was thus found no unusual excess of males in this record. At the same time, it becomes evident upon referring to the table, that inbreeding has little influence on the sex ratio of the hybrids which had been inbred for five generations, so far as the present data are concerned.

(2) The chromosomes

Details on the chromosomes of the parent species, *Mus musculus* and *Mus molossinus,* have been fully stated in the foregoing pages (Part I). The number of chromosomes is the same in the two species: viz., 40 in diploid and 20 in haploid. The shape of the

chromosomes is also identical in both species, all the elements being of the simple rod type. That the difference found to exist between these two species lies alone in the length of chromosomes, has been also detailed in the previous section.

The testes of hybrids belonging to generations from F_1 to F_5 including those of back-cross hybrids, which were obtained from at least three individuals in each, comprise the material for this study. The number and behaviour of chromosomes were carefully studied in each. The testes showed no sign of histological degeneration so far as examined, and there has never been detected any structural change different from the normal conditions in each case. The ovaries of the F_1 and F_2 hybrids which were also histologically examined, proved to be entirely normal.

So far as the scope of these observations is concerned, the number and behaviour of the chromosomes of the hybrid forms did not show any trace of abnormality in any generation of the hybrids, normal spermatozoa being always yielded. The spermatogonia contained without exception 40 chromosomes including X- and Y-elements in every case of the hybrids (see Figs. 162, 163, 171, 172, 179, 182, 187, 192, 193). The conjugation of chromosomes took place quite regularly at meiosis of hybrids and 20 bivalent chromosomes were always observable in the primary spermatocyte metaphase (Figs. 164, 165, 173, 174, 180, 183, 188, 194). The segmentary characteristic is also evident in the X-chromosome of the primary spermatocyte metaphase, as seen in Figs. 166, 175, 176, 181, 184, 189 and 190. There has never been encountered any slight evidence for the existence of univalent chromosomes and the formation of multivalent chromosomes in the primary spermatocyte. The bivalent autosomes of the hybrids exhibit in appearance, specially in the conditions of chiasma formation, nothing different from those of the parent species. The separation of bivalents is quite regular in the first division and the X- and Y-elements also disjoin normally migrating to the opposite poles (Figs. 167, 168, 190, 191). In the F_1 and F_2 hybrids were observed a few exceptional cases in which the X and Y segregate somewhat early to the poles (Fig. 167). The formation of bridges and any other like structures which disturb the anaphase separation of bivalent chromosomes, are entirely absent. As expected from these results in the first division, the number and behaviour of chromosomes of the secondary spermato-

cytes are entirely regular: in each case observed the number of chromosomes was determined to be 20 (Figs. 169, 170, 177, 178, 185, 186). The points thus far noted may be understood more clearly by reference to the accompanying figures (Figs. 162–194 given in Pl. XXVIII).

As already mentioned, the difference in the chromosome between the parent species lies in the length of the chromosomes. Having this in mind, some attempt was made to analyse morphologically the chromosomes of the F_1 hybrids with a hope of finding the parental sets of chromosomes. But this trial was found to be difficult, because the difference of length in the chromosomes of the parent species is not so conspicuous that one can pick them out with certainty in the mixed condition of the hybrid. Only the *a*-chromosome originated from *Mus molossinus* was evidently detected out in the F_1 hybrid on account of its prominent large size (see Figs. 162–163).

(3) Some remarks on fertility and chromosomes of the hybrids in mammals

The cytological studies of the hybrids are generally less extensive in animals than in plants in which latter a considerable amount of work has been done along these lines. The works on the species hybrids of the Lepidoptera by Federley ('13, '14, '15, '16, '31) and those of *Drosophila* by Sturtevant ('20, '21, '29), Kerkis ('33) and Dobzhansky and Tan ('36), are probably the most extensive investigations in this line. Limited to mammals, the literature dealing with this subject is quite scanty. As the classical example of this is found the work of Wodsedalek ('16) on the mule which is the sterile hybrid between the horse (*Equus caballus*) and the ass (*E. asinus*). He showed, as the cause of sterility, many kinds of abnormalities and the complete failure of the sperm formation occurring in the testes of the mule, which was caused, according to his interpretation, by the difference of the chromosome complex in the parent species. This study on the mule, however, seems to contain many challengeable points under the present state of knowledge, especially in respect of the chromosome morphology, since the studies of Painter ('24) on the horse and of Meladze ('37) on the ass, showed the existence of a similar karyotype in the above two forms. On the cause of sterility in the mule, therefore, a

reinvestigation is required, which may offer an important clue for the perplexing problems attending the hybrid sterility in mammals generally. Apparently a similar condition to the mule is encountered in the hybrid male between the domestic cow (*Bos taurus*) and the yak (*Poephagus grunniens*), which is proved to be completely sterile. According to Krallinger ('31) the chromosome number of the domestic cattle is certainly 60 and recently Zuitin ('38) reported the same chromosome complex, quite similar in number, to exist in the yak. These facts suggest a possibility that sterile hybrids may be produced even in the cross between two related forms in which the chromosomes are *morphologically* identical: that is, the sterility of the hybrid between allied forms does not necessarily depend upon the karyological disharmony (morphologically speaking) between them, and perhaps it is due essentially to the difference of the genetic constitution between the two, being in genic causes. Many suggestions related to this problem may be found by reference to the works performed on *Drosophila* which is advantageous in having connection with the gene analysis. *Drosophila melanogaster* and *D. simulans* possess metaphase chromosomes that appear quite identical under the microscope, whereas the hybrids between them are completely sterile. Kerkis ('33, '36) showed that in the hybrids between *D. melanogaster* and *D. simulans* the gonads are rudimentary, and spermatogenesis and oogenesis do not advance beyond spermatogonia and oogonia. From the comparative genetical work on *D. melanogaster* and *D. simulans*, it has been demonstrated that the arrangement of genes is different in these species (Sturtevant '29). Hybrid sterility is also established in the cross between *Drosophila pseudoobscura* and *D. miranda* in which the chromosomes of the females seem to be identical when observed at metaphase being morphologically indistinguishable from each other, but in gene arrangement they greatly differ (Dobzhansky & Tan '36). Furthermore, cases are known in *Drosophila* where the interracial crosses also give rise to sterile hybrids (Dobzhansky '34). These findings in *Drosophila* are very significant and important in showing that the similarity (or dissimilarity) of the chromosomes as seen at the metaphase stage is not at all necessarily proportional to the similarity of their gene arrangements, even between the forms taxonomically closely related.

Recent studies on the hybrids of sheep carried on by Russian

authors may contribute to some extent towards our knowledge on cytology of the hybrid in mammals. Butarin ('35), dealing with the species cross between arkhar (*Ovis pollii karelini*) and kurdiuchny ram (*Ov. steato pyga*), showed that the chromosome numbers in the hybrid and the original forms are identical, being 60 in diploid and 30 in haploid, and further that spermatogenesis in the hybrid proceeds normally. From this result, he suggests a possibility that the hybrids under consideration must be fertile. Similar evidence is also revealed to exist in the interracial hybrid of the sheep (*Ovis aries*) by Novikov ('35), indicating that during the process of spermatogenesis of hybrids no abnormality was observed to occur. In the hybrids between the sheep (*Ovis aries*) and the goat (*Capra hircus*), on the other hand, the condition seems to be different, since it is known that in about 45% of the matings between the goat (♀) and the sheep (♂), the females became pregnant but aborted before the time for normal parturition (cf. Berry '38). According to the study of Berry ('38) which was made on the amniotic tissue of the embryo, the number of chromosomes is 60, 54 and 57 for the goat, the sheep, and their hybrid respectively. On the basis of his finding he concluded that the chromosome complex of the goat differs sufficiently from that of the sheep to prevent the complete development of a normal hybrid from the two forms. Repeated investigations which have been since made by several workers such as Sokolov ('30), Krallinger ('31), Novikov ('35), Butarin ('35), Bruce ('35) and Pchakadze ('36), seem to be sufficient to indicate that the chromosomes of the sheep and the goat are nearly identical not only in the number but also in the complex. The cause of the early fetal death of the sheep-goat hybrids is thus a matter still remaining for future investigation.

Although no karyological investigation has been made, it has been shown by Pchakadze ('32) who made the histological study of testes, that the hybrids of Bacterian camel (*Gamelus bactrianus*) and Arabian camel (*C. dromedarius*) must be considered fertile as their testes contain many spermatozoa.

Here the observations made by Swezy ('28) on the hybrid rats come under notice. In a hybrid colony of rats derived from interracial crosses between the albino rats (*Rattus norvegicus* var. *albinus*) and the wild gray rats (*R. norvegicus*), Swezy ('28) found two kinds of individuals, one possessing a diploid chromosome

number of 42 and haploid number of 21 and 31 and the other having 62 diploid and 31 and 21 haploid chromosomes. Matings made between members of the colony, according to her, produced litters some of which had 62 and some 42 chromosomes. Such an unusual condition as this, had never been reported previously either in animals or in plants so far as the author is aware, and even up to the present time, he has not found reference to any one such like case in the literature. The explanation for this information is not sufficiently made by Swezy ('28), who partly finds the possibility of its interpretation in the method of fragmentation of chromosomes. There is a possibility to consider that hybridization never produces such a result, because the chromosome constitution of rats is essentially identical either in the albino form or in the wild form as definitely proved by recent investigators (Minouchi '28, Oguma '35, Tateishi '35), and further matings made between these two forms always produce fertile offsprings in the normal manner through successive generations as shown by King ('11) and others. Judging from the figures presented in the paper of Swezy ('28) on which her conclusion has been based, it seems probable to the author that the chromosome counts given by her are wholly questionable. Firstly, she observed the spermatogonial chromosomes in the prophase nucleus. As is well known, the chromosomes at the prophase stage generally assume an undulatory elongated appearance and the individual elements are not exactly distinguishable from each other, accurate counting is thus quite impossible. Under such circumstances one cannot expect any correct result from Swezy's observations on the spermatogonia. Secondly, Swezy's observations of the primary and secondary spermatocyte chromosomes seem to be made always on the anaphasic equatorial plates in which the chromosomes advance a little in their separation, because in her figures some chromosomes are always placed overlapped. And further, her statement that the number of small chromosomes present in the cells with 31 chromosomes is much greater than that present in the cells with 21 chromosomes in either the primary or secondary spermatocytes, is sufficient to suggest the fact that the anaphasic separation of the smaller chromosomes is generally earlier than in the larger ones. Furthermore, none of the chromosomes show any characteristic configurations, due to inadequate preservation, being irregularly scattered in the equatorial

plate. In view of these considerations, and also on the basis of the author's own observations upon the rat, the observations made by Swezy and her conclusion therefrom ('28) cannot be considered as valid.

To the best of the author's knowledge, the only chromosome study on the mouse hybrids is found in the work of Painter ('27) on Gates' "non-disjunction" (*v–o*) mice (interracial hybrids) which are descendants from the cross between pure normal and Japanese waltzing mice. According to his descriptions, the number and the behaviour of chromosomes are always normal in these interracial hybrids, except for the one fact that a large portion of one of the small autosomes *i. e.*, the q chromosomes, was missing in this stock, giving a parallel condition to the genetic evidence. Though the species hybrids of mice have been considered completely fertile from the experiment of Green ('30, '35) in the cross betwen *Mus musculus* and *Mus bactrianus*, the chromosomes of the hybrids have not yet been investigated. As already described in the foregoing pages, the present investigation has clearly established that the crosses between *Mus musculus* (the domesticated European form) and *Mus molossinus* (the wild Asiatic form) always give rise to fertile offsprings in a quite normal manner through successive generations, and further that the chromosomes of the hybrids are completely normal both in their morphological constitutions and in the behaviour, the course of spermatogenesis and perhaps of oogenesis proceeding regularly. Furthermore, the sex ratio of the hybrids nearly approaches that which exists in the normal. Thus the situation in this study is sufficient to show that there is found in the results of crossing no essential difference in the interspecific cross from those which occur in the varietal cross. Further, the findings of the present investigation suggest a possibility that *Mus musculus* and *Mus molossinus* under consideration are quite homologous in their genomes.

PART V. MATURATION AND FERTILIZATION IN THE EGG *of Mus musculus* L.

On account of the difficulties encountered in procuring the material, due to the less frequent periods of oestrus, the smaller number of eggs discharged at ovulation, and the minute size of the

eggs associated with the occurrence of internal fertilization, the progress of cytological studies of maturation and fertilization of the mammalian ovum has been very slow. At present knowledge on this subject is confined to that published in some classical works such as those made by Gerlach ('90), Sobotta ('95, '07), Rubaschkin ('05), Kirkham ('07), Sobotta and Burckhard ('10), Longley ('11), Lams ('13), *etc.*, which deal chiefly with the rodents. It is granted that there remain important facts of doubtful import for geneticists. Recently, on the other hand, experimental analysis has been made by Yamane ('30, '35, '37) and Pincus and Enzmann ('35) using rabbit ova towards the elucidation of the mechanism of fertilization in mammals. Under such circumstances it seems absolutely necessary to renew investigations guided by the improved cytological technique.

As the material of the study, the purely bred white mice, *Mus musculus* L., derived from a strain coming from the Carnegie Institution were employed. Along with the study of chromosomes in the male germ cells, the material for this investigation has been accumulated since 1936.[1)]

The mice used were killed during the period of most active breeding, namely, April, May, June and July. It is stated that in mice, ovulation occurs soon after parturition independent of copulation (Sobotta '95). When found to be pregnant, the females were separated and mated with males.

There is little difficulty in deciding when copulation took place; it is usually verified by such a fact that after copulation the vaginal opening has been closed with fluid. Occasionally, however, one finds the animals in the act of copulation. Aided by such ways, the animals during oestrus and those after pairing were selected for the study.

At intervals ranging from a few hours to about 24 hours or more after copulation, the female mice were killed and their ovaries together with the oviducts were dissected out and fixed, some in dilute Flemming's solution and some in Bouin's (B-15) fluid at

1) In addition to this species, observations were partially made for comparison on the wild species, *Mus molossinus* and also on the hybrids between these two species. No essential differences of phenomena among them having been found, the data from *molossinus* and also from the hybrids, were not included in this description.

37–38°C. In addition, some of the material was furnished by animals in which the sequence of events during the period of oestrus was incompletely known. Thus a series of eggs in different stages of maturation and fertilization was obtained. They were subjected to the dioxane method for dehydration and clearing, and embedded in paraffin. Heidenhain's iron-haematoxylin method with light green was employed for staining sections.

(1) The full grown ovum

The full grown ovarian egg containing the resting nucleus is approximately spherical in shape and lies in an eccentric position in the Graafian follicle (Fig. 195). Such a mature follicle is found occupying a position at the periphery of the ovary. The ovum is surrounded by the zona pellucida and the latter again by the corona radiata. Surrounding the corona there are several layers of cells, stratum granulosum, which are arranged lining the inner surface of the follicle and enclosing a huge cavity filled up with the fluid, liquor folliculi.

The egg has a diameter ranging from 0.065 to 0.070 mm under the fixed condition. The size of the mouse egg therefore nearly approximates that of the rat[1] (0.060–0.065 mm, after Sobotta and Burckhard '10) and of the guinea-pig (0.055–0.060 mm, after Rubaschkin '05). In the rabbit, it is considerably larger, nearly twice as large as the mouse egg, since, according to Yamane ('37), the rabbit egg measures 0.110–0.120 mm in diameter.

In the nucleus of the resting stage, which is somewhat eccentrically placed in the egg, one finds chromatin elements with vague outline together with two kinds of distinct, round nucleoli (Fig. 196). One of the latter is smaller in size and characterized by staining deeply with haematoxylin, while the other one, apparently larger than the former, shows intense affinity for light green.

(2) Formation of the first polar body

Since the changes in the nucleus advancing towards the first maturation division seem to proceed successively during a very

1) Gilchrist and Pincus ('32), who based their measurements on living eggs, state that the average size of the fertilized egg of the rat is about 0.071 × 0.076 mm.

short duration, it is not easy to trace serially the complete course of changes in the material. It seems probable, however, that during these preparatory stages for the first division, the smaller nucleolus which is stained with haematoxylin, is divided into a number of minute granular elements (Figs. 197 and 198) and they finally disintegrate. At the same time the larger acidophilic one staining green appears to be given off outside the nucleus in the deutoplasm as seen in Fig. 199. At the farther advanced stage of diakinesis, immediately preceding the formation of the first polar spindle, well defined bivalent chromosomes make their appearance being scattered along the inner wall of the nucleus as shown in Fig. 200. The nucleus in this state now lies in close proximity to the periphery of the egg, in anticipation of the coming stage.

After the disappearance of the nuclear membrane has successively taken place, the chromosomes make their arrangement on the equatorial plate and thus the first polar spindle is formed (Figs. 201–202). The spindle when first formed lies close to the periphery of the egg with its axis approximately perpendicular or somewhat oblique to the radius of the egg and later one of the poles seems to swing a little toward the centre of the egg. A similar event is said by Rubaschkin ('05) to occur in the egg of the guinea-pig and by Kirkham ('07) in the mouse egg. This is, however, not the case with the cat, because Longley ('11) reported that the first polar spindle of the cat is originally formed perpendicular to the surface of the egg.

The number of chromosomes observed at metaphase is decided to be 20 as already described in detail (Fig. 201). After the separation of chromosomes the outer pole of the spindle which upheaves on the egg surface, gets extruded as the first polar body. This body is usually oval in form and lies in the perivitelline space of the egg (Fig. 203). It does not readily disintegrate after being extruded, but it is usually demonstrable even when the egg is discharged from the ovary into the oviduct and commences its development after insemination. The chromatin contained in the polar body may exist as a number of threads or may be gathered together in a few threads or in a single compact mass.

It is of some interest to note here that the structural changes of the follicle which take place upon maturation are more complicated in the rabbit where ovulation depends on mating, than in the mouse which is a spontaneously ovulating animal. According to Pincus

and Enzmann ('37), the follicle of the rabbit shows, prior to the first maturation division, a characteristic structure named the 'spider-web' type which is characterized by the spider-web like arrangement of follicle cells, holding the egg surrounded by its corona in their center. That such a remarkable and particular feature associated with follicle maturity exists in the rabbit seems to correlate to the fact that the follicles of the rabbit do not undergo maturation without the mating stimulus.

(3) Formation of the second polar spindle and ovulation

After the expulsion of the first polar body, the aggregated daughter chromosomes belonging to the egg organize very rapidly, recovering their individuality, and soon they are drawn into the equator of a new spindle, which is the second polar spindle (Fig. 203). The number of chromosomes observed at the metaphase was constantly 20 without any slight doubt (Fig. 204). The second polar spindle quite agrees with the first polar spindle in the fact that the spindle is prepared with its long axis parallel to the surface of the egg (see Figs. 203, 205, 210 and 211). As a rule, the second polar spindle appears near the position where the first polar body has already emerged (Figs. 203 and 210).

As thus far noted, the egg undergoes the first maturation division, prior to ovulation, and still farther advances in its maturation process up to the metaphase stage of the second maturation division. The maturation process of the egg seems to be obliged to stop at this state, until insemination occurs, even if it is discharged into the oviduct.

The Graafian follicle, as it appears just before its rupture, is provided with a thin layer of granulosa cells stiffly adhering to the membrana propria, and the egg, as already stated, contains the second polar spindle of the metaphase and is accompanied by the first polar body. It lies practically free from the granulosa cells in the follicle suspended in the liquor folliculi and is surrounded by the zona pellucida and the corona radiata, cells of the latter presenting a characteristic radiating arrangement in intimate adhesion with one another (Fig. 205). Another noteworthy feature of this follicle is the enormous expansion of the antral cavity which holds the liquor folliculi under pressure. According to Brambell ('28) it

has been shown that the consistency of the liquor folliculi becomes more viscous at this time than in the foregoing stages.

Though the present study failed to observe the eggs in process of leaving the ovary, it is obvious by referring to the feature of the egg after ovulation, that following the rupture of the follicle, the egg is discharged together with the liquor folliculi into the oviduct, surrounded by the zona pellucida and the corona radiata and also accompanied by a number of granulosa cells.

(4) Insemination of the spermatozoon and formation of the second polar body

In the sections through the upper portion of the oviduct obtained from the animal killed at about ten or more hours after copulation, there are some eggs in which insemination has already occurred (Figs. 206–207). The number of eggs discharged at ovulation was in an individual six and seven respectively in the two sides of oviducts, while in the other individual it was two and six respectively in the two sides.

A single spermatozoon enters an egg, only the head of the former penetrating the egg. In Figs. 208 and 209 is distinctly shown the head of the spermatozoon just after penetration into the egg. Having made a close examination upon 21 fertilized eggs, the conclusion arrived at was that there was no indication to show that polyspermy had actually occurred. Recently Yamane ('37) experimentally proved also that the rabbit egg is always monospermic in the normal insemination.

After insemination, the consistency of the follicular cells as well as of those of the corona radiata surrounding the egg, seems to be changed remarkably; they appear to be adhesive no longer and get entirely detached from the egg, lying irregularly dispersed around it (see Figs. 206 to 217). The fertilized eggs, therefore, appear in the oviduct practically free from these cells; only the zona pellucida directly surrounds each egg. This observation is of significance because of the fact that the experimental studies by Yamane ('30, '35) and by Gilchrist and Pincus ('32), performed upon the rabbit and the rat respectively *in vitro*, also showed that there is a rapid dispersion of the follicle cells of eggs placed with live sperm.

At the time when sperm penetration has just occurred the egg possesses, as mentioned above, the second polar spindle which persists in the stage of metaphase, and is accompanied in the perivitelline space by one polar body, the first polar body[1] (Figs. 210–211). Shortly after the entrance of the spermatozoon into the egg, the arrested second division commences to advance gradually in its course, and as a result the formation of the second polar body is brought about (Fig. 212). From this evidence it is obvious that ovulation occurs prior to the completion of the second maturation division, and that during ovulation the process leading to the second division is arrested, lying quiescent, as it were, in the metaphase condition. It will be easy to see from these observations that stages subsequent to the metaphase, and the process leading to and culminating in the second division, take place only after the entry of the spermatozoon into the egg. Insemination is therefore a necessary antecedent to the second maturation division. This observation is of considerable significance in giving a cytological proof to the result experimentally attained by Yamane ('30, '37) in the rabbit egg *in vitro*. Upon referring to the earlier works which were performed on some rodents and carnivora (Sobotta '95, Rubaschkin '05, Kirkham '07, Sobotta and Burckhard '10, Lams '13, Longley '11, etc.), the above-mentioned relation existing between maturation and insemination, seems to be general in mammals and is also known to occur in the lower vertebrates such as the amphibians and fishes (cf. Makino '34).

(5) Formation of the pronuclei and their conjugation

Following the completion of the second maturation division, the chromosomes left in the egg apparently lose their individuality and tend to fuse together forming a compact mass of irregular outline (Fig. 213). Soon thereafter a nuclear membrane appears which encloses the chromatin mass. Inside the nucleus thus organized

1) There is found no instance in which a later division actually occurs in the first polar body so far studied in this material, though Sobotta ('95) in the mouse egg and Rubaschkin ('05) in the egg of the guinea-pig described its occasional occurrence. According to Yamane ('30, '37) it was found that the rabbit egg after normal insemination shows in the majority of cases three polar bodies, two of which are large, representing the first and second polar body while the remaining small one is a result of the division of the first polar body.

the chromatin begins to disintegrate into irregular outline and gradually loses much of its affinity for stain. Then the nucleus takes the appearance of a spherical vesicle with smooth circumference, inside which dissolved chromatin elements with vague contour are scattered about. The nucleus thus formed is the completed female pronucleus, and it lies free in the deutoplasm (Figs. 214–215). The female pronucleus then begins to move towards the deeper part of the egg and seems to undergo a slight growth in volume (Fig. 216). Meanwhile, the head of the spermatozoon inseminated is converted into the male pronucleus and also migrates along with the female pronucleus towards the interior of the egg where they ultimately conjugate (Fig. 217). The position where the two pronuclei actually meet is not at the geometric centre of the egg but is somewhat eccentric. At the time of meeting the female and male pronuclei are alike in appearance but distinguishable only by their size.

SUMMARY

The study is divided into five parts. Part I is devoted to the investigation of the comparative morphology of chromosomes of the following three species of mice; (1) *Mus musculus* L. (the pure albino form), (2) *Mus molossinus* Temm. et Schleg. (both wild and domesticated forms), and (3) *Mus caroli* Bonh. (a wild form). The number of chromosomes was found to be 40 in diploid and 20 in haploid in the above three species. The diploid complements consist of elongated rod-shaped elements of telomitic type in every species. In the paired arrangement they constitute 19 homologous pairs of autosomes and an unequal pair of the X- and Y-chromosomes. There is no clear distinction of autosomes into large and small sets but a closely graded seriation occurs.

The X-chromosome is in all probability the largest of all and usually prominently displays distinguishable peculiarities, such as a loose texture, a less affinity to stain and the existence of distinct constrictions. The Y-element attains a size nearly approximate to, or a little smaller than, the smallest members of the autosome.

The X-chromosome is provided with two clear transverse constrictions which subdivide the whole length of the X into three consecutive segments, namely P-, D_1- and D_2-segments. This

remarkable tripartite nature of the X was established through the spermatogonium, the primary and secondary spermatocytes. In the growing period, the plasmosome or true nucleolus is found associated with the X at the junction of its P- and D_1-segments. In the primary spermatocyte the Y-chromosome connects in its one end with the free end of the P-segment of the X by means of a fine fibre. In both the X and Y the spindle fibres are constantly attached to their free ends opposite the position where they come in contact. For this reason, the XY-complex always stands at the primary spermatocyte metaphase, in a linear connection, nearly perpendicular to the equatorial plate approximately parallel to the axis of the spindle.

The chromosomes of the three species herein dealt with are morphologically analysed and compared on the basis of the following points; (1) the mean value of the ratio of length between the largest and the smallest autosomes, (2) the mean lengths of each member of autosomes, (3) the mean value of the ratio of length between the X- and Y-chromosomes, and (4) the frequency of the position of the XY-complex in the equatorial plate of the primary spermatocyte metaphase. The results of comparison are summarized in Tables VII, VIII, IX and X, and in Textfigs. 2 and 3.

In Part II the history of the sex chromosomes has been followed from their appearance in the early stage of the growing period of the spermatocyte until the metaphase of the first maturation division. The X-chromosome assumes the form of a chromosome vesicle during the growing period. From the late pachytene through to the commencement of diakinesis the X-vesicle completely associates with the plasmosome (the true nucleolus) into a single huge mass of squarish oblong shape, thus forming the so-called amphinucleolus. In the later part of the diplotene stage the Y-chromosome comes to lie in the proximity of the X-chromosome vesicle. The X- and Y-chromosomes remain as separate entities during the early part of the growing period having absolutely no relationship to each other. The conjugation of the X and Y takes place in the late diplotene stage. Their conjugation is of the most perfunctory character and made by an end-to-end connection. There exists of course not even a slightly trace of either side-by-side synaptic conjugation between the X and Y in the form of chromatin threads, or of the formation of chiasmata between them. In the first maturation division they

completely segregate, leaving no exceptional case, to opposite poles of the spindle.

Part III deals with the investigation of the chromosomes of the primary and secondary oocytes in *Mus musculus* L. and *Mus molossinus* Temm. et Schleg. The chromosomes of the oocytes are not essentially different in respect of general morphological characteristics such as the number, shape and behaviour, from those of the spermatocytes. The two homologous X-chromosomes constitute an ordinary tetrad after conjugation as the autosomes do. The observations made in this investigation seem to show that there exists no sexual difference as regard to chiasma formation.

Part IV deals with the fertility and chromosomes of the interspecific hybrids between *Mus musculus* L. and *Mus molossinus* Temm. et Schleg., which have been examined through five generations and in the back-crosses. The crosses between these two forms always give rise to fertile offsprings in a quite normal manner through successive generations. The sex ratio of the hybrids nearly approaches that which exists in the normal. The results are summarized in Table XI. The chromosomes of the hybrids are entirely normal both in their morphological constitutions and in the behaviour, showing 40 diploid and 20 haploid chromosome numbers in each generation. The course of spermatogenesis and perhaps of oogenesis proceeds regularly.

Part V describes cytological observations of the phenomena of the maturation and fertilization in the ovum. In this study the following subjects chiefly were investigated; (1) the processes of the first and second maturation divisions in the ovarian ova, (2) the causal relationship between the insemination and the second maturation division, and (3) the formation of the male and female pronuclei and their conjugation. The results of these observations were compared with those obtained in the experimental studies made *in vitro* on the similar problem by other investigators.

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Explanation of Plate XVIII

Figs. 1–25. Chromosomes of *Mus musculus* L. *x*: X-chromosome. *y*: Y-chromosome.
1– 5. Spermatogonial chromosomes (polar view).
6– 9. Chromosomes of the primary spermatocytes (metaphase polar view).
10–14. Chromosomes of the primary spermatocytes in side view, showing the XY-complex. Note the tripartite nature of the X.
15–16. Shromosomes in the anaphase of the primary spermatocyte division, showing the segregation of the X and Y.
17*a*–*b*. Sister complexes of chromosomes at anaphase of the primary spermatocyte.
18. Telophase of the primary spermatocyte division. Note the X and Y lying in separate vesicles.
19–21. Chromosomes of the secondary spermatocyte metaphases, X-class.
22–24. The same, Y-class.
25. Sister chromosome complexes at anaphase of the secondary spermatocyte.

Explanation of Plate XIX

Figs. 26–35. Chromosomes of *Mus musculus* L. Paired alignments of homologous mates from spermatogonial chromosomes in a descending order. Fig. 26 corresponds to Fig. 1, Fig. 27 to Fig. 2, Fig. 28 to Fig. 5, Fig. 31 to Fig. 3, and Fig. 33 to Fig. 4.

Explanation of Plate XX

Figs. 36–60. Chromosomes of *Mus molossinus* Temm. et Schleg. *x*: X-chromosome. *y*: Y-chromosome.
36–40. Spermatogonial chromosomes (polar view).
41–43. Chromosomes of the primary spermatocyte metaphases.
44. Pro-metaphase, showing the XY-complex.
45–53. Side views of the primary spermatocyte metaphases showing the XY-complex.
54–56. Chromosomes of the primary spermatocytes at anaphases, showing the segregation of the X and Y.
57–58. Chromosomes of the secondary spermatocyte metaphases, X-class.
59–60. The same, Y-class.

Explanation of Plate XXI

Figs. 61–70. Chromosomes of *Mus molossinus* Temm. et Schleg. Paired arrangements of homologous mates from spermatogonial chromosomes in a descending order. Fig. 62 corresponds to Fig. 37, Fig. 66 to Fig. 36, Fig. 68 to Fig. 39, Fig. 69 to Fig. 38, and Fig. 70 to Fig. 40.

Explanation of Plate XXII

Figs. 71–86. Chromosomes of the domesticated race of *Mus molossinus*. *x*: X-chromosome. *y*: Y-chromosome. *n*: Plasmosome nucleolus.

71–74. Spermatogonial chromosomes.

75–76. Chromosomes of the primary spermatocyte metaphases.

77–78. Side views of the primary spermatocyte metaphases, showing the XY-complex.

79. Primary spermatocyte anaphase, showing the segregation of the X and Y.

80. Chromosomes of the secondary spermatocyte, X-class.

81. The same, Y-class.

82–86. Serial alignments of paired chromosomes from the spermatogonial metaphase. Fig. 82 corresponds to Fig. 71, Fig. 83 to Fig. 74, Fig. 85 to Fig. 73, and Fig. 86 to Fig. 72.

Explanation of Plate XXIII

Figs. 87–109. Chromosomes of *Mus caroli* Bonhote. *x*: X-chromosome. *y*: Y-chromosome. *n*: Plasmosome nucleolus.

87– 91. Spermatogonial chromosomes, polar view.

92. Pro-metaphase of the primary spermatocyte, showing the XY-configuration.

93– 97. Chromosomes of the primary spermatocyte metaphases, showing the XY-complex.

98–102. Side views of the primary spermatocyte metaphases showing the XY-complex.

103–105. Primary spermatocyte anaphases, showing the separation of the X and Y.

106–107. Chromosomes of the secondary spermatocyte metaphases, X-class.

108–109. The same, Y-class.

Explanation of Plate XXIV

Figs. 110–119. Chromosomes of *Mus caroli* Bonhote. Paired alignments of homologous elements from the spermatogonial chromosomes in a serial order. Fig. 110 corresponds to Fig. 87, Fig. 111 to Fig. 88, Fig. 112 to Fig. 89, Fig. 113 to Fig. 90, and Fig. 116 to Fig. 91.

Explanation of Plate XXV

Figs. 120–138. Camera lucida drawings of the nuclei in the growing period of the primary spermatocyte, from *Mus musculus*. The plasmosome nucleolus and chromatin nucleolus are shown in detail and the autosome elements in rough sketch in each. *n*: Plasmosome nucleolus. *x*: X-chromosome. *y*: Y-chromosome.

120. Nucleus at the pachytene stage, showing the X-vesicle situated at an eccentric position of the nucleus. Note the tripartite structure of the X (Fe-hematoxylin).

121. A portion of a nucleus at early diplotene stage, showing the plasmosome nucleolus and the X-vesicle in close contact (Fe-hematoxylin).

122–123. Plasmosome nucleolus and the X-vesicle in close contact at the diplotene (Flemming's triple staining).

124. Nucleus at the diplotene stage, showing the complete association of the X-vesicle with the plasmosome nucleolus (amphinucleolus). Fe-hematoxylin preparation.

125. A portion of a nucleus at the diplotene showing the amphinucleolus. Feulgen's basic fuchsin and light-green. Plasmosome nucleolus is stained with light-green and the X-vesicle with basic fuchsin.

126. A portion of a nucleus at late diplotene, showing the separation of the X-vesicle from the plasmosome nucleolus.

127. Nucleus at late diplotene stage, showing the X-vesicle, plasmosome and Y-chromosome.

128. Nucleus at late diplotene stage. Note the X-vesicle and the Y-chromosome lying in close proximity. (Fe-hemat.).

129. Nucleus at late diplotene stage, showing the X-vesicle and the Y-chromosome in conjugation. (Fe-hemat.).

130–132. A portion of a nucleus at late diplotene, showing the X-vesicle and the Y-chromosome in conjugation. (Fe-hemat.).

133. Nucleus at early diakinesis, showing the configuration of the XY-complex after conjugation. (Flem. triple).

134–135. Nucleus at diakinesis, showing the separation of the plasmosome nucleolus from the XY-complex. (Fe-hemat.).

136. A portion of a nucleus at late diakinesis, showing the XY-complex and the plasmosome which begins to disintegrate. (Fe-hemat.).

137. A portion of a nucleus at late diakinesis, showing the configuration of the XY-complex. (Newton's gentian violet).

138. Stage just preceding the metaphase (prometaphase), showing the XY-complex and the remnant of the plasmosome nucleolus. (Fe-hemat.).

Explanation of Plate XXVI

Figs. 139–151. All are photomicrographs of nuclei in the growing period of the primary spermatocyte, at a magnification of about 2000 diameters (from *Mus musculus*). For detailed explanation, refer to Pl. XXV.

139–140. Nucleus at early leptotene stage, showing the polar aggregation of chromatin.

141. Nucleus at the pachytene stage; the same as Fig. 120.

142. Nucleus at the diplotene stage; the same as Fig. 124.

143. Nucleus at the diplotene stage, showing the amphinucleolus.

144. Nucleus at late diplotene; the same as Fig. 129.

145. Nucleus at late diplotene, showing the conjugation of the Y with the X.

146. Nucleus at late diplotene; the same as Fig. 130.

147. Nucleus at late diplotene; the same as Fig. 131.

148. Nucleus at early diakinesis; the same as Fig. 133.

149–150. Nucleus at diakinesis: Fig. 149 corresponds to Fig. 134 and Fig. 150 to Fig. 135.

151. Nucleus at late diakinesis: the same as Fig. 136.

Explanation of Plate XXVII

Figs. 152–158. Chromosomes of the oocytes of *Mus musculus*.

152–153. Chromosomes of the primary oocytes in the metaphase polar view.

154–155. The same, in side view; 20 elements are seen in each.

156 *a*, *b*. Sister complexes of chromosomes at anaphase of the primary oocyte division: 20 elements are seen in each plate.

157–158. Chromosomes of the secondary oocytes in the metaphase polar view.

Figs. 159–161. Chromosomes of the oocytes of *Mus molossinus*.

159–160. Chromosomes of the primary oocytes in the metaphase side view: 20 elements are seen in each.

161. Chromosomes of the secondary oocyte metaphase.

Explanation of Plate XXVIII

Figs. 162–194. Chromosomes of the hybrids, *Mus musculus*×*Mus molossinus*. *a*: a-chromosome. *x*: X-chromosome. *y*: Y-chromosome.

Figs. 162–170. Chromosomes of F_1. 162–163. Spermatogonia. 164–165. Primary spermatocytes. 166–167. Primary spermatocytes, side views. 168. Anaphase of the primary spermatocyte. 169–170. Secondary spermatocytes.

Figs. 171–178. Chromosomes of F_2. 171–172. Spermatogonia. 173–174. Primary spermatocytes. 175–176. Side views of the primary spermatocytes. 177–178. Secondary spermatocytes.

Figs. 179–181. Chromosomes of F_3. 179. Spermatogonium. 180. Primary spermatocyte. 181. Side view of the same.

Figs. 182–186. Chromosomes of F_4. 182. Spermatogonium. 183. Primary spermatocyte. 184. Side view of the same. 185–186. Secondary spermatocytes.

Figs. 187–191. Chromosomes of F_5. 187. Spermatogonium. 188. Primary spermatocyte. 189. Side view of the same. 190. Anaphase of the primary spermatocyte. 191 *a*, *b*. Sister complexes of chromosomes at anaphase of the primary spermatocyte.

Figs. 192–194. Chromosomes of FR_1. 192–193. Spermatogonia. 194. Primary spermatocyte.

Explanation of Plate XXIX

Figs. 195–205. Photomicrographs of ovarian eggs showing the maturation process.

195. Graafian follicle containing a full grown egg nearing maturity. From the section of ovary. ×100.

196. Enlarged view of the egg cell shown in Fig. 195. Diameter 0.067 mm. In the nucleus are seen two distinct spherical nucleoli of large and small size. ×400.

197–198. Two successive sections, showing the disintegration of nucleoli. In Fig. 197 there are several minute chromatic spheres which have resulted from the disintegration of the small nucleolus stained with haematoxylin. Fig. 198 shows the large nucleolus which is stained with light-green. ×400.

199. Showing the acidophilic nucleolus which is cast off outside the nucleus. Chromatin elements appear as the form of thick threads with bivalent nature. ×600.

200. Stage immediately before the first maturation division. In the nucleus which lies close to the periphery of the egg, there are visible well-defined bivalent chromosomes. ×600.

201. Polar view of the first maturation division metaphase in the egg. ×600.

202. Egg showing the first polar spindle at metaphase side view. ×400.

203. Egg showing the second polar spindle at metaphase, accompanied by the first polar body. ×400.

204. Polar view of the second maturation division metaphase in the ovarian egg. ×200.

205. Graafian follicle just before rupture (preovulatory stage). Note the radiating cells of the corona and the zona pellucida surrounding the egg. The egg surrounded by them is suspended in the liquor folliculi and shows the second polar spindle at metaphase, accompanied by the first polar body which is found in the following section. ×100.

Explanation of Plate XXX

Figs. 206–217. All are photomicrographs of eggs in fertilization stages, from the sections of oviducts.

206–207. Longitudinal sections of the upper portions of oviducts, showing two fertilized eggs together with a number of follicular cells in each. ×100.

208. Section of an egg showing the head of the spermatozoon just after entry into the egg (indicated by an arrow). ×600.

209. Sperm head after penetration within the egg. ×400.

210. Egg just after the entrance of the spermatozoon. A piece of the sperm head is seen at right. The egg shows the metaphase spindle of the second maturation division (at left) and is accompanied by the first polar body (above the spindle). ×400.

211. Second polar spindle at metaphase in the egg at the same stage as the previous one. ×400.

212. The next stage to Figs. 210–211, showing the second polar body about to constrict off. ×400.

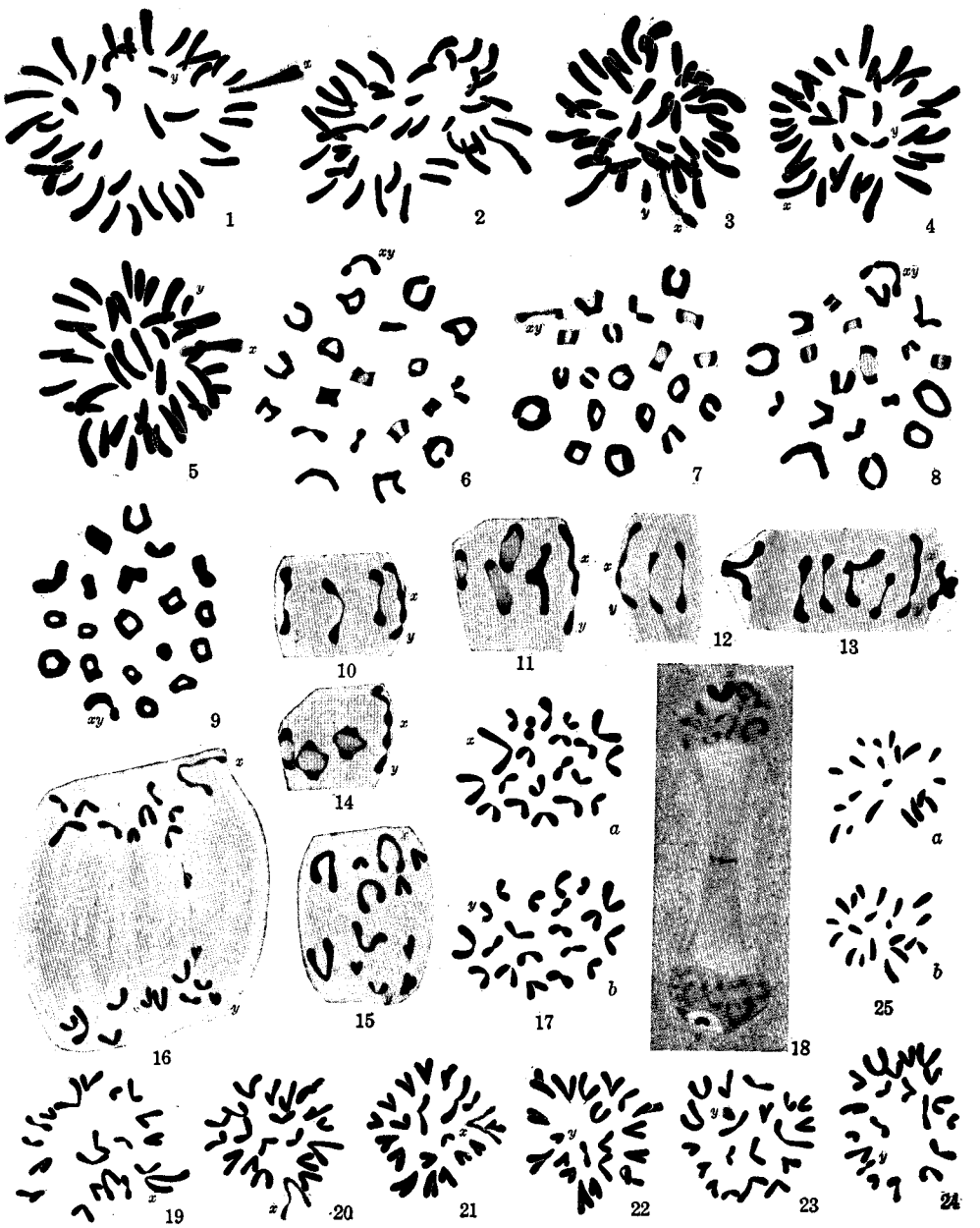
213. The next stage to Fig. 212, showing the extruded second polar body. The chromosomes left in the egg are found fused together into a mass. ×400.

214. At upper right in the egg is seen the organized female pronucleus. Note the first and second polar bodies which are found overlapping one another. ×400.

215. Egg showing female (upper) and male (lower) pronuclei before conjugation. The second polar body is seen at top. ×400.

216. Showing the growth in size of the female pronucleus. ×400.

217. The female (larger) and male (smaller) pronuclei in close contact within the egg. ×600.



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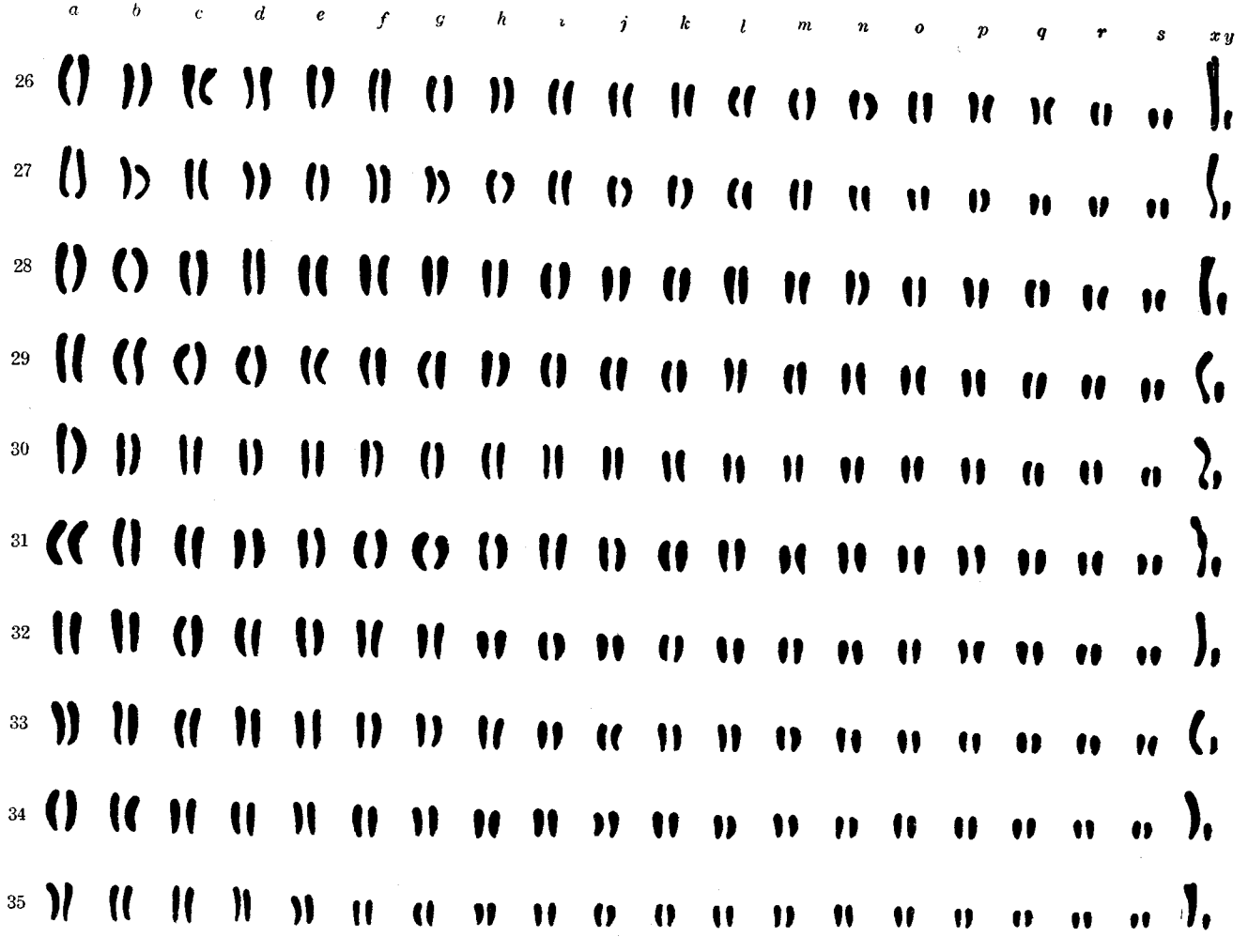

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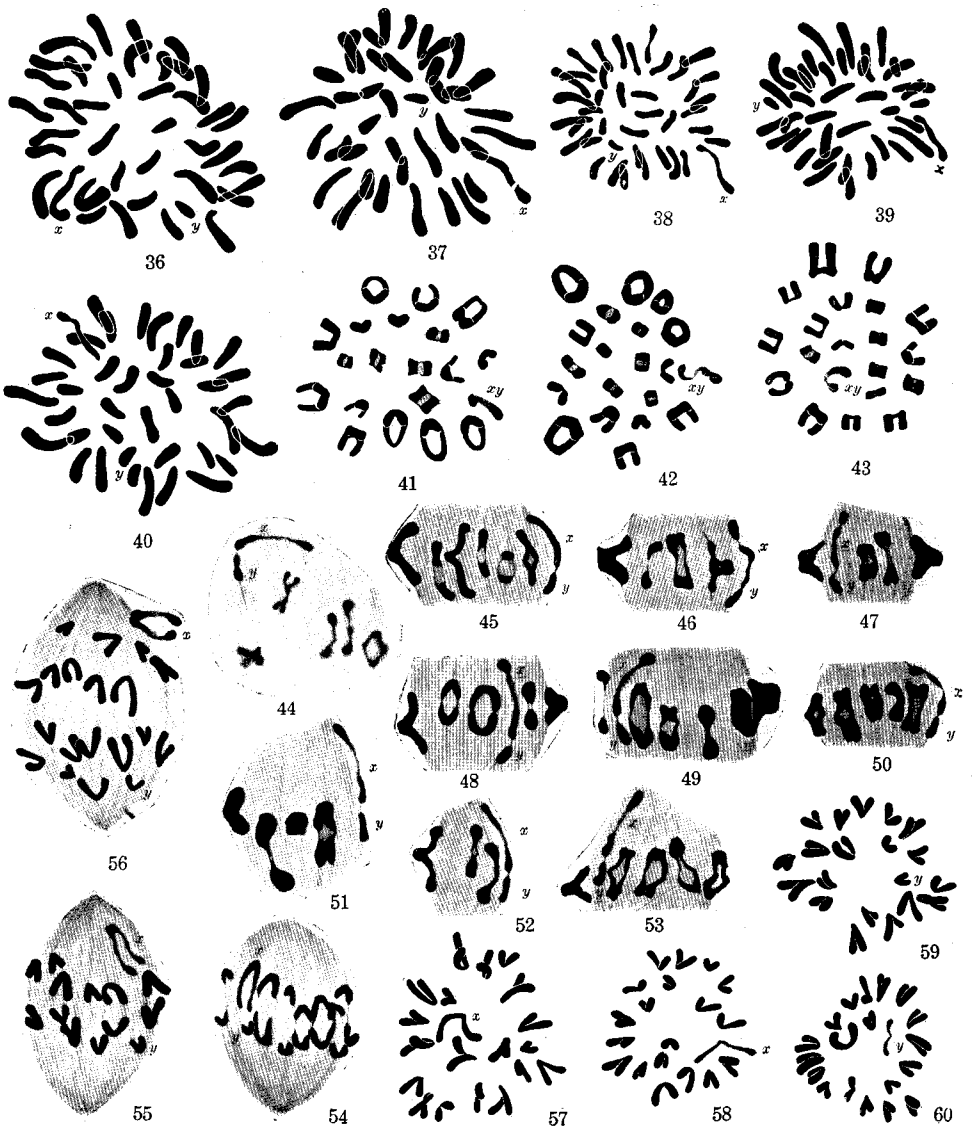

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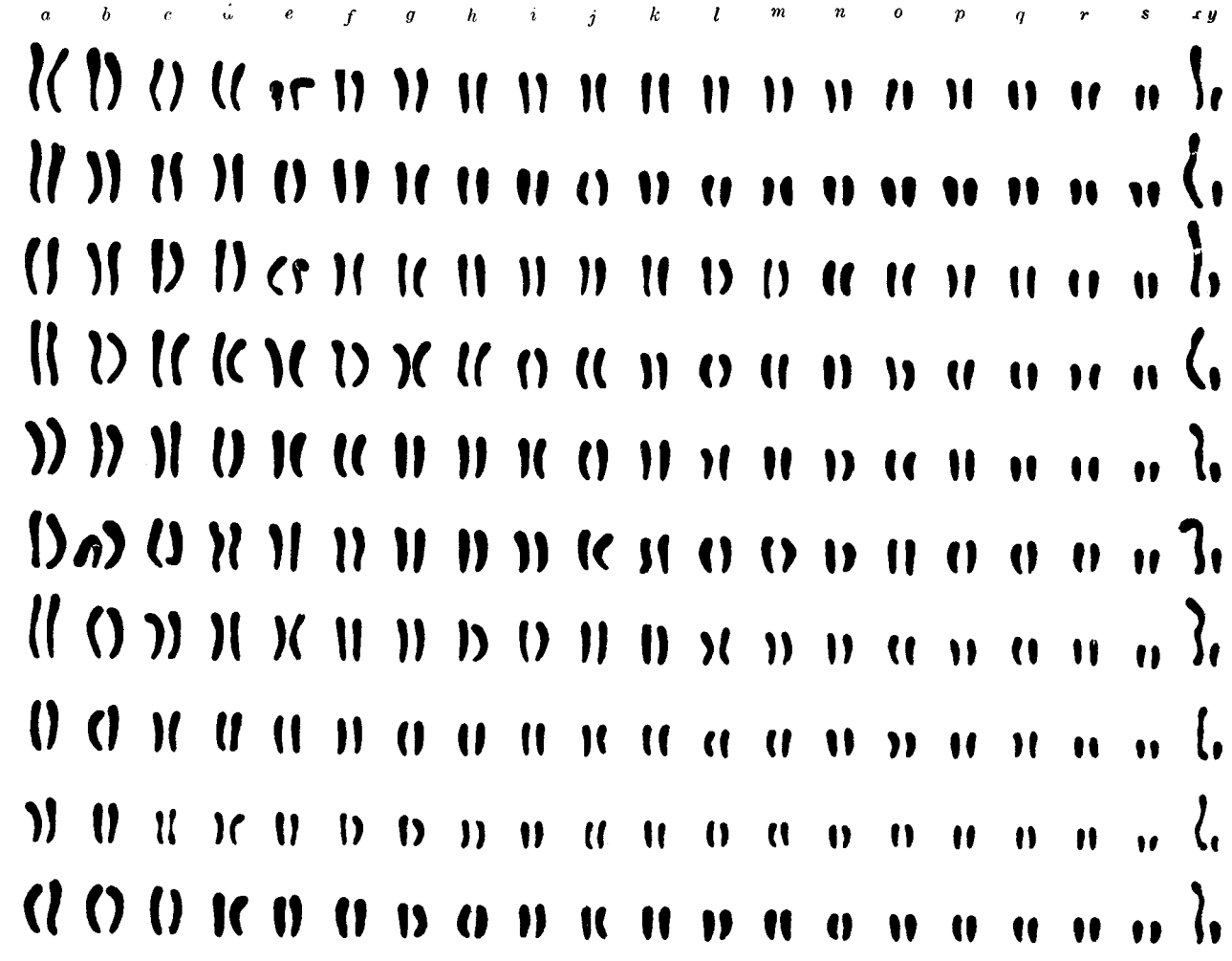

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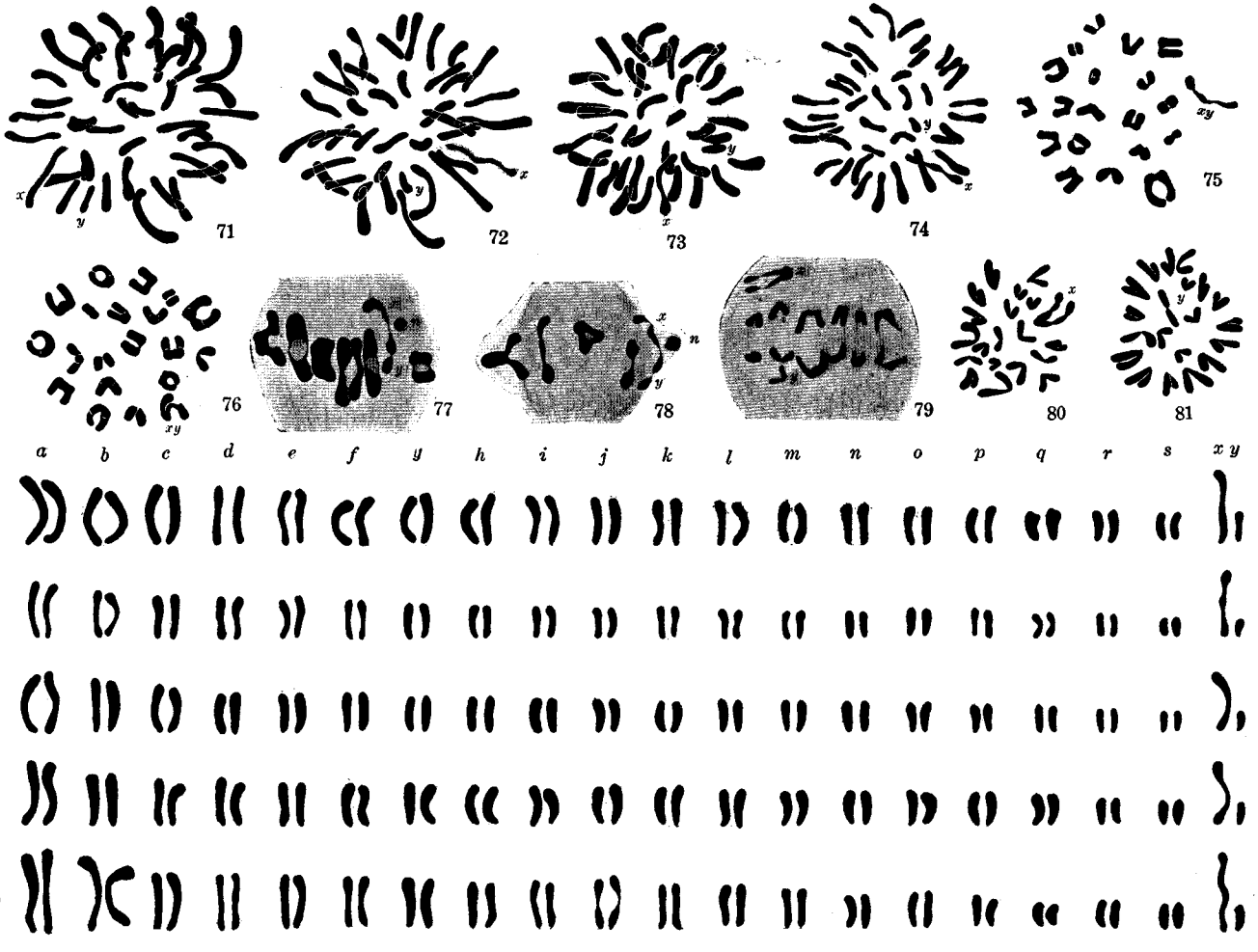

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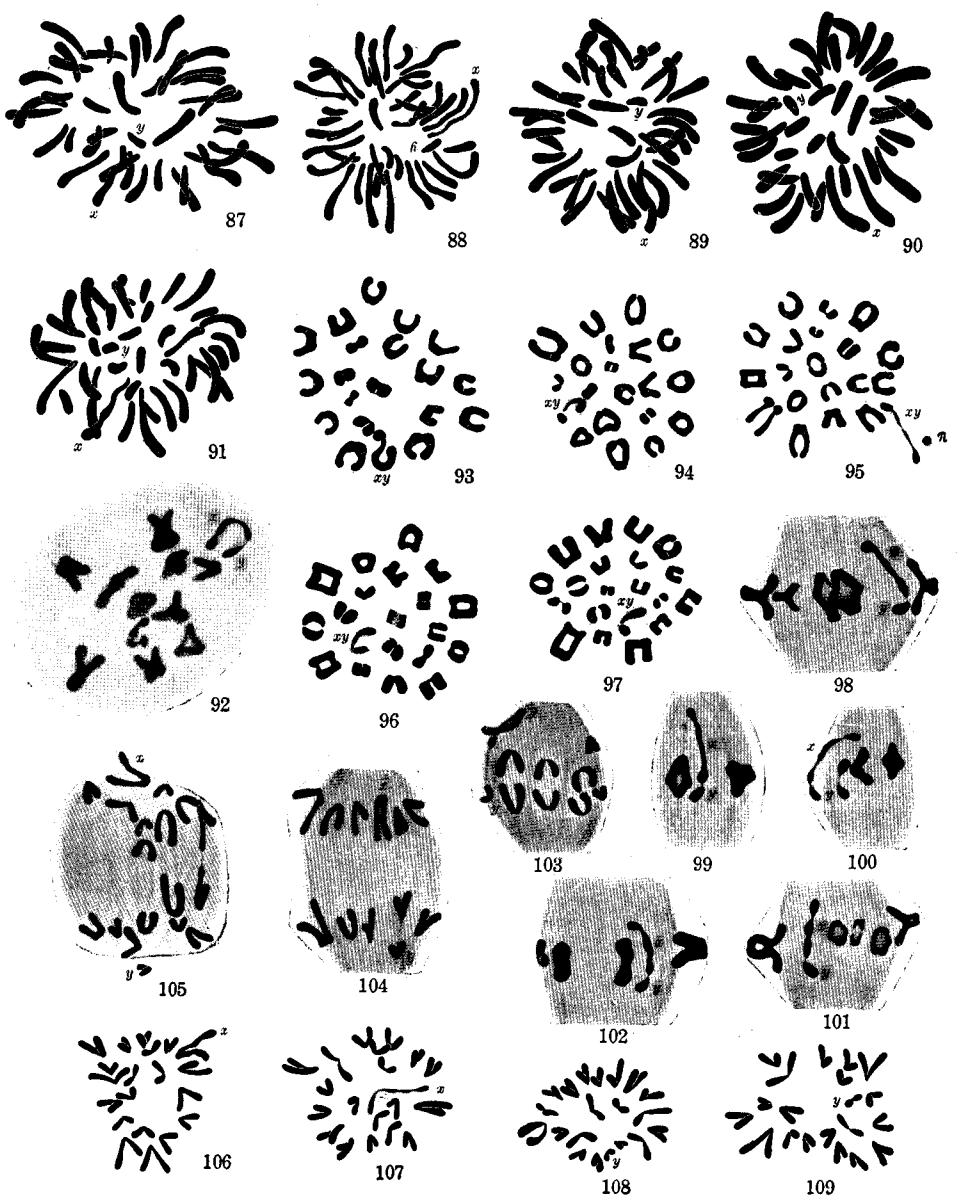

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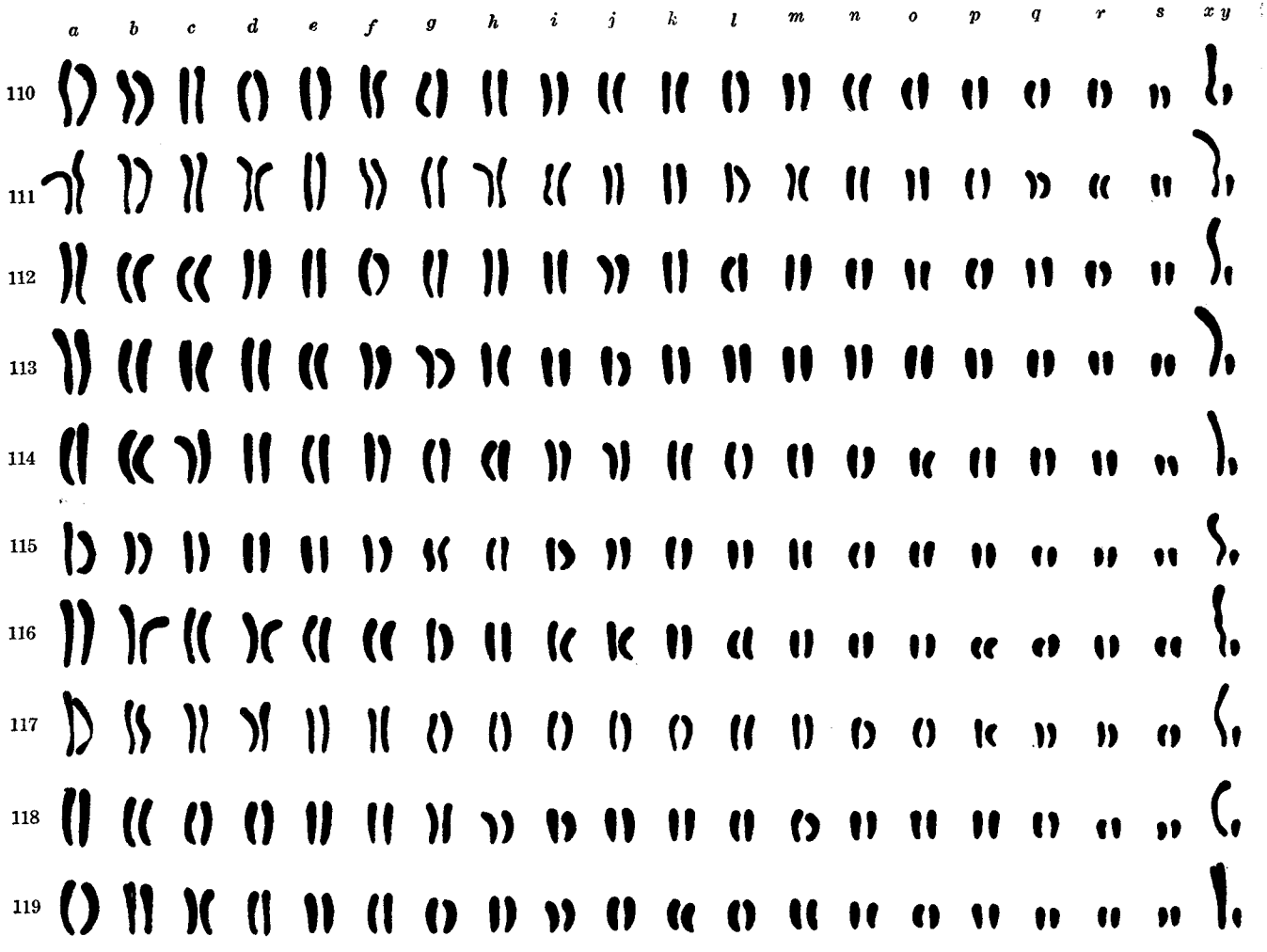

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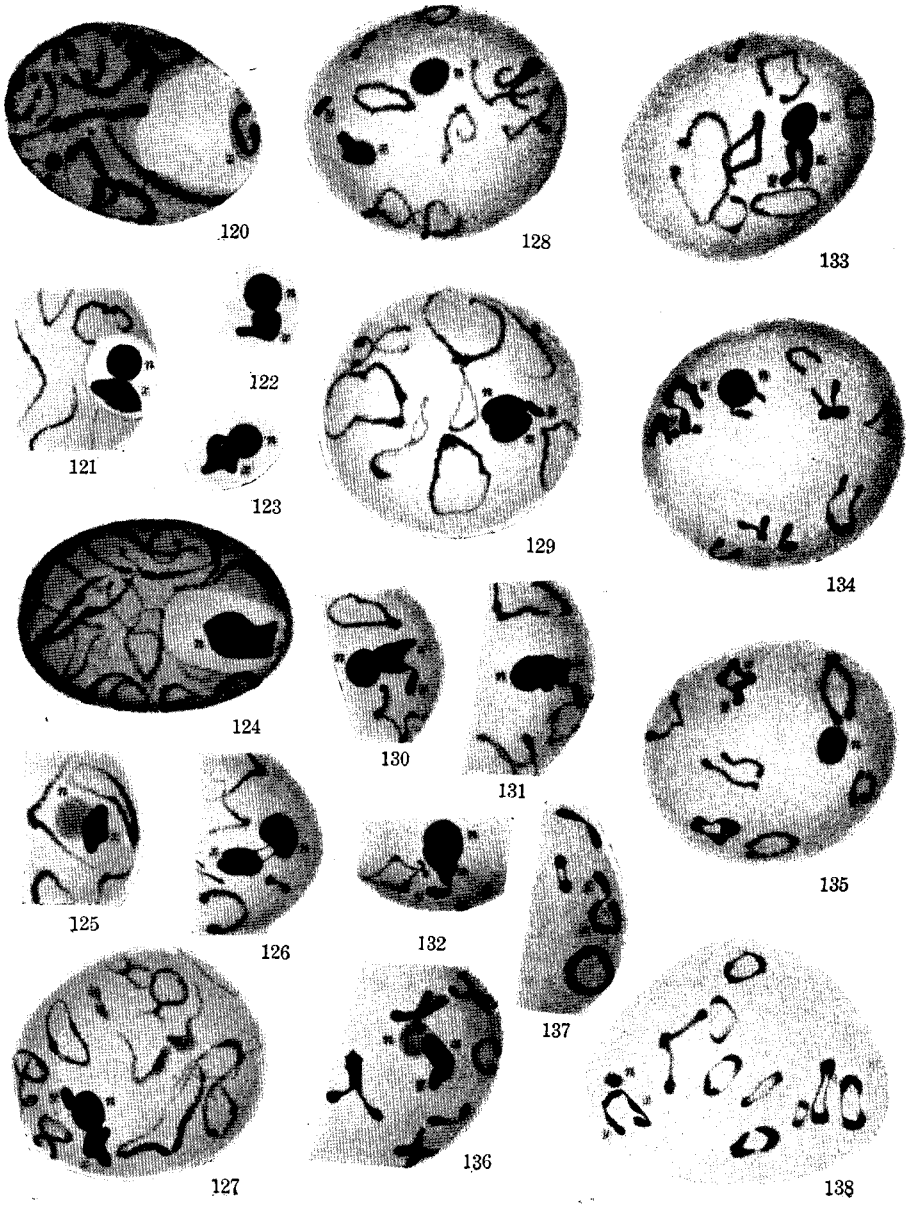

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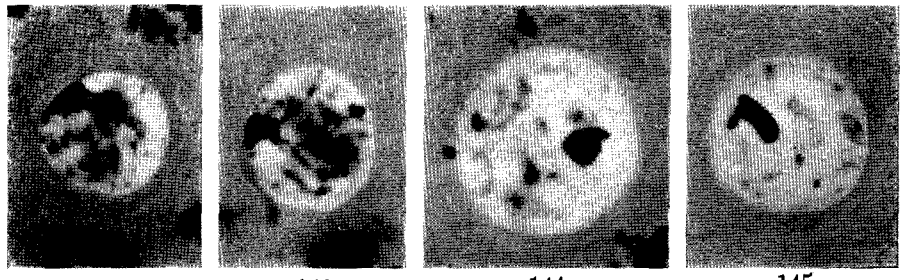

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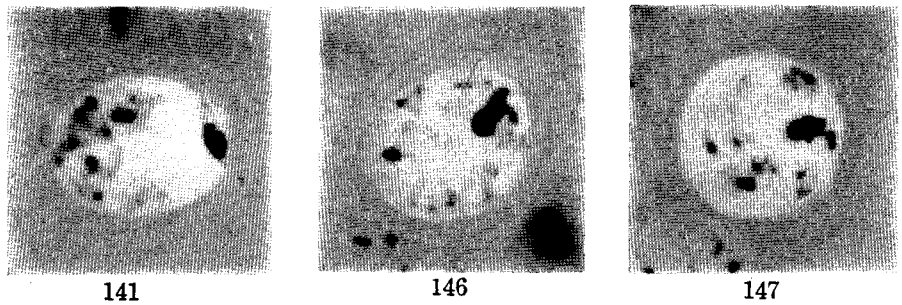

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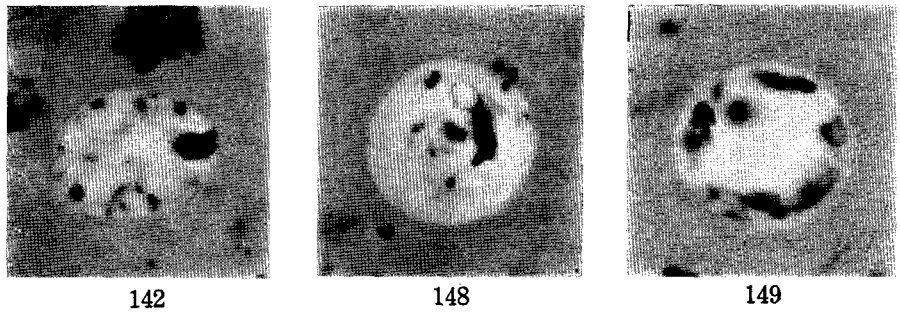

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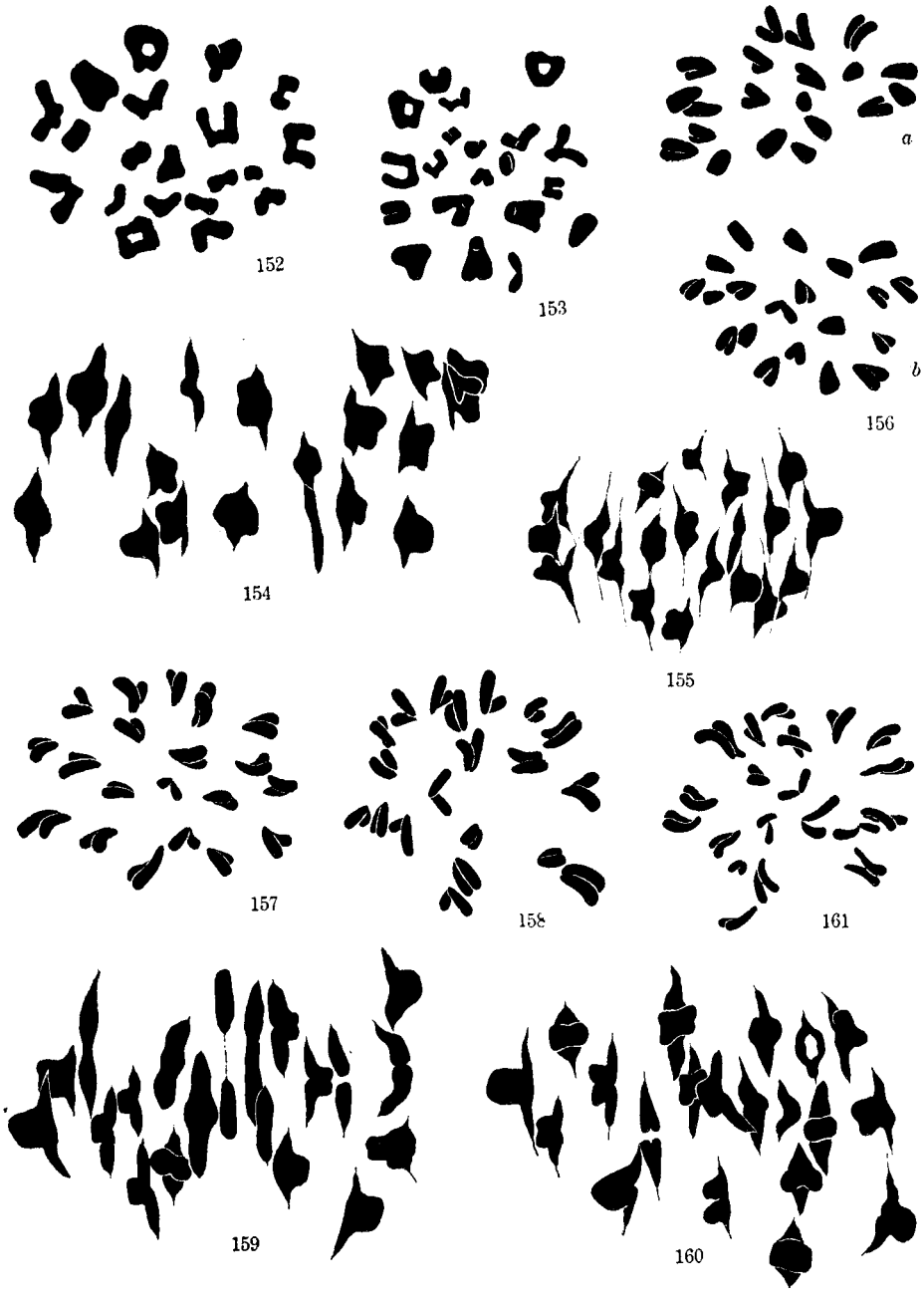

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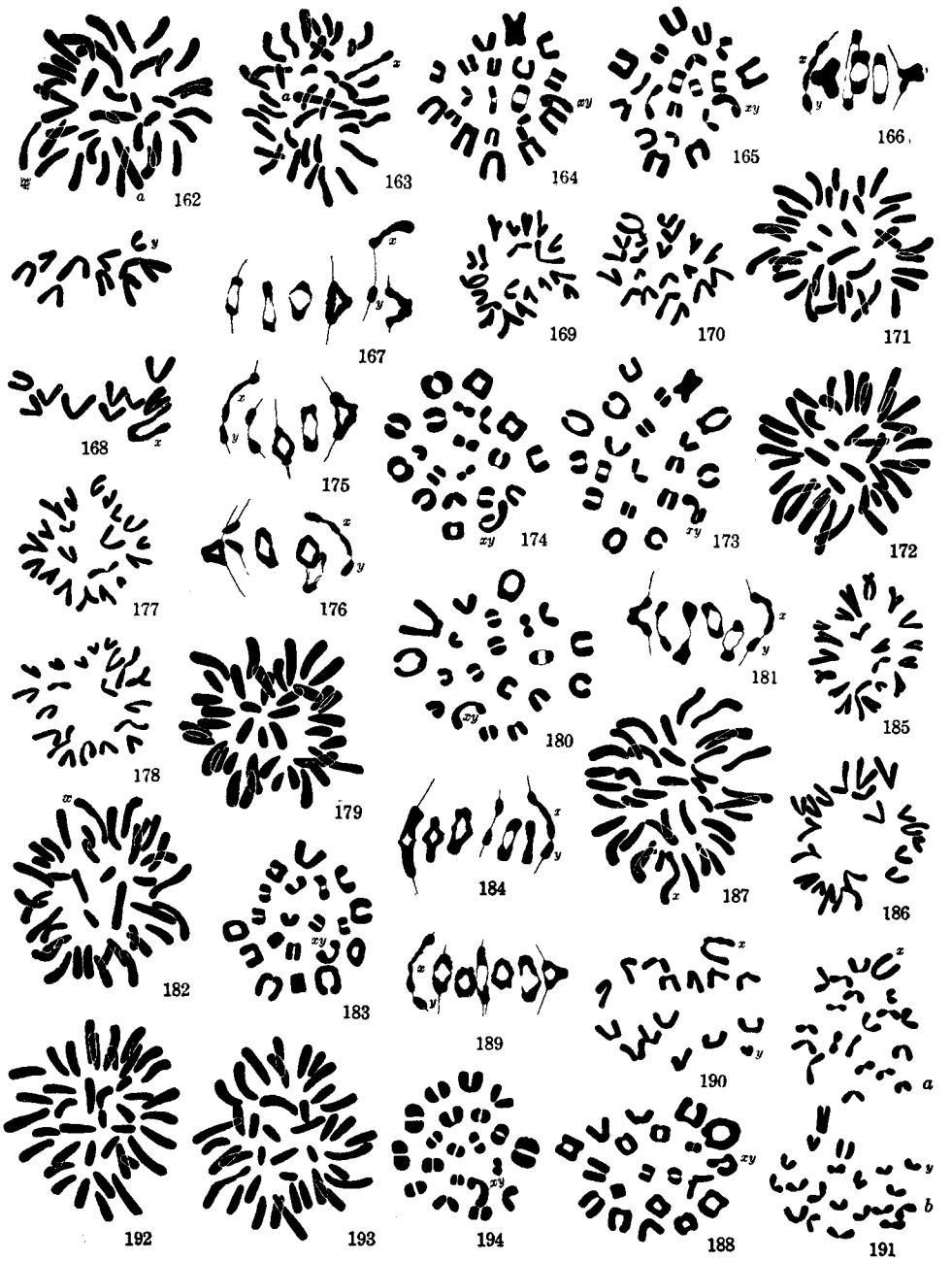

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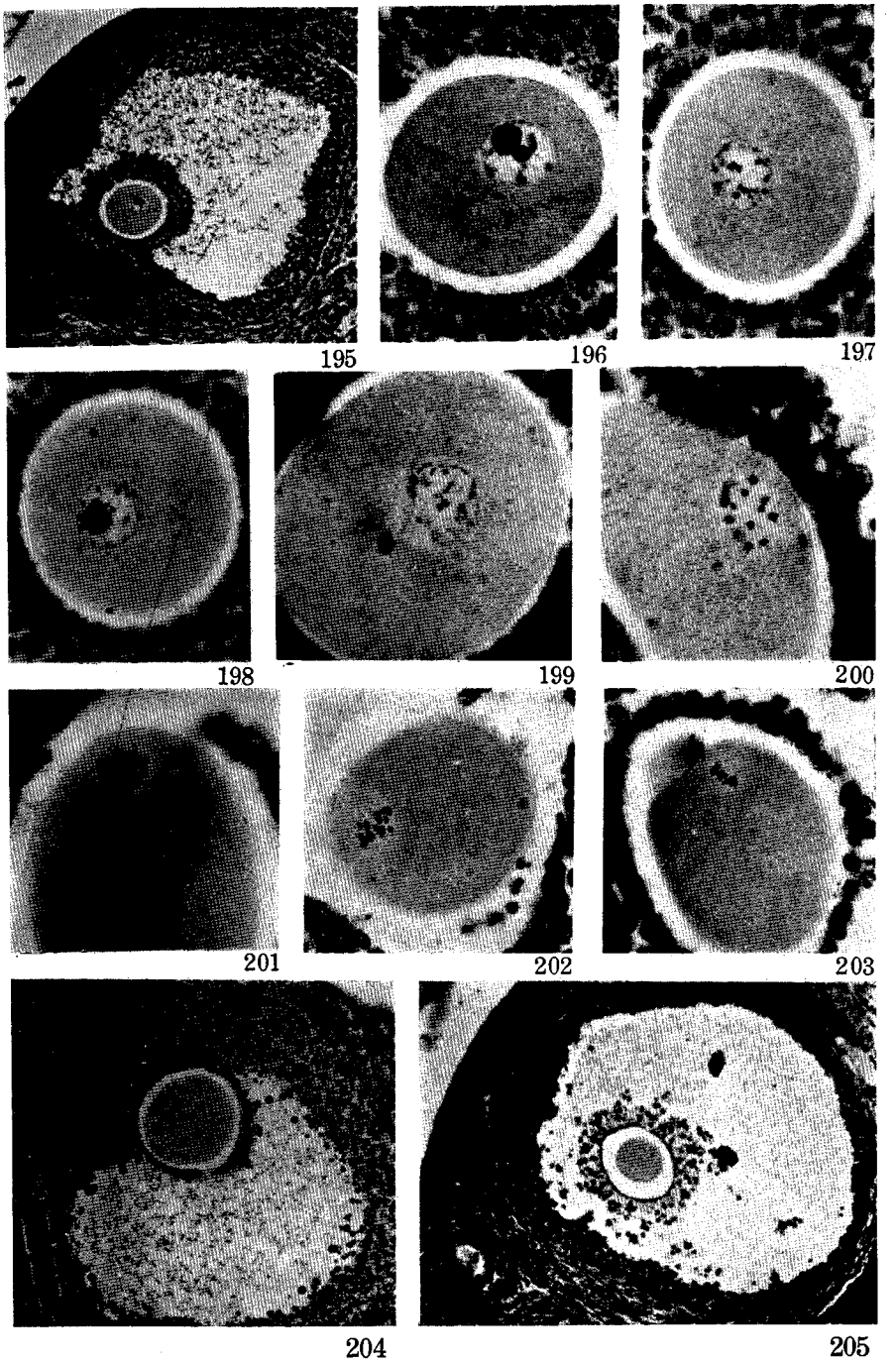

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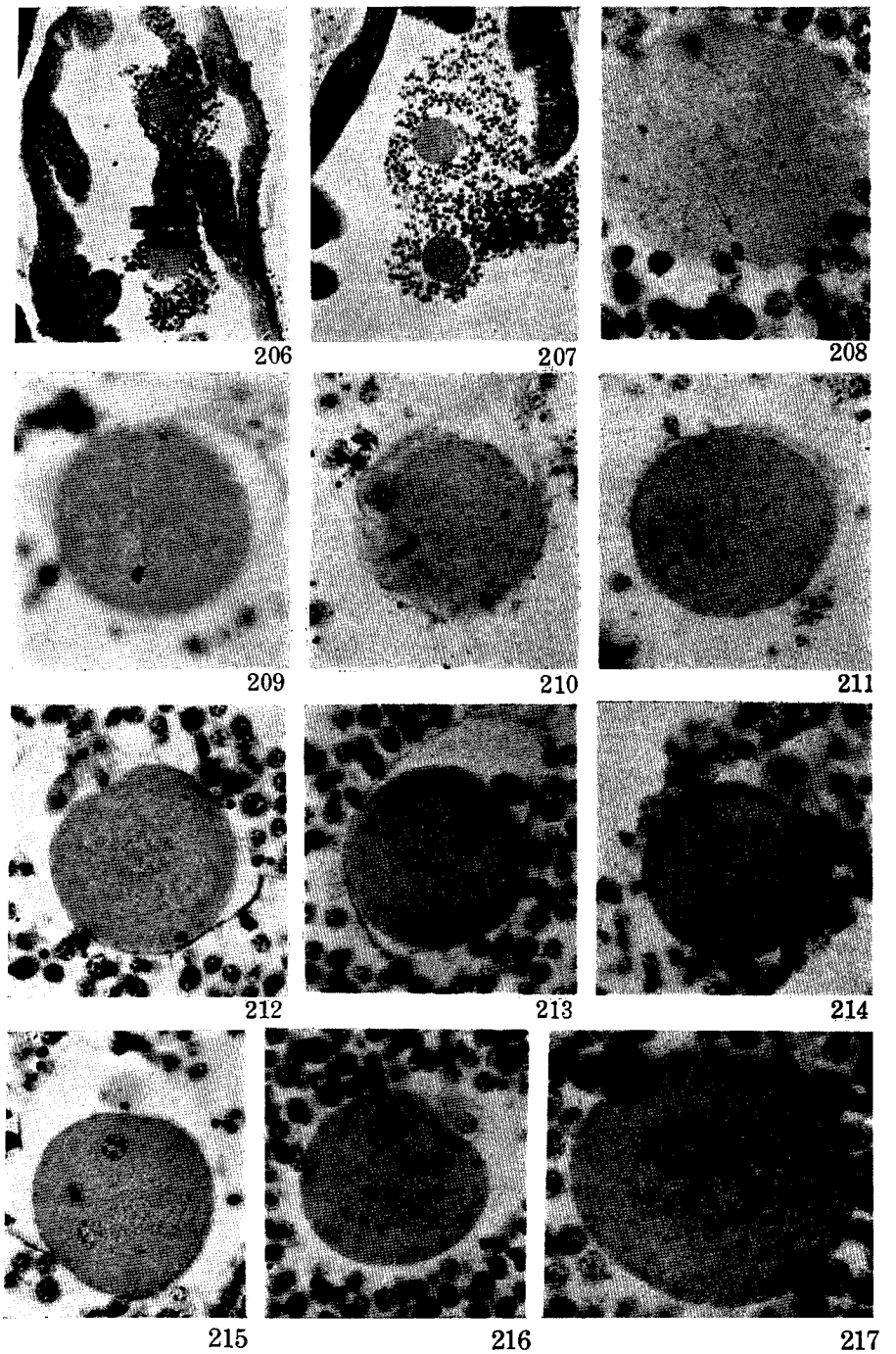

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