PHILOSOPHICAL TRANSACTIONS.

I. *A Metrical Analysis of Chromosome Complexes, showing Correlation of Evolutionary Development and Chromatin Thread-Width throughout the Animal Kingdom.*

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(Received January 22,—Read February 8, 1912.)

[Plates 1–5.]

Contents.

Introduction.

The phenomena connected with development of the germ-cells have been studied in many animal types, and each year papers are published upon Spermatogenesis and Oogenesis in new species, thereby adding to the knowledge that we already possess. It is impossible, through lack of space, to enumerate here the work done upon the subject, but reference to any comprehensive bibliography will show that organisms representative of the various phyla of the animal kingdom, and in many cases representative of the classes, orders, families, and genera of these phyla have been carefully studied.

Certain species, moreover, have proved themselves particularly suited to cytological investigation, and have accordingly been studied independently by several workers; such examples are seen in the papers of van Beneden, Boveri, Brauer, Edwards, Marcus, and Tretjakoff, upon the Nematode, Ascaris; and in those of Flemming, Janssens, Meves, Montgomery, vom Rath, and the Schreiners, upon the Amphibian, Salamandra. The class that has been most thoroughly studied is the Insecta. The Orthoptera have been dealt with by Baumgartner, Carnoy, Davis, Gérard, McClung, Montgomery, Moore, Nowlin, Otte, Robertson, vom Rath, de Sinety, Sutton, Wilcox, Zweiger, and myself; the Hemiptera have been

studied by HENKING, MONTGOMERY, PAULMIER, STEVENS, and WILSON; and the Coleoptera by AUERBACH, DEBAISIEUX, HENDERSON, and HOLMGREN. DONCASTER, HENKING, and MARK have written upon Hymenoptera; GRÜNBERG, MEVES, MUNSON, and SPICHARDT upon Lepidoptera; and CHOLODKOVSKY and STEVENS upon the Diptera. The Orthoptera are particularly good material, and we now possess detailed accounts of development of the sex-cells in Forficulidæ, Blattidæ, Phasmidæ, Acridiidæ, Locustidæ, and Gryllidæ. The study of these numerous types has led to the discovery of many important facts, and to the enunciation of several hypotheses.

The results appear similar throughout the Metazoa, and the process of development in the male organism may be summarised as follows: The spermatogonial resting stages show the chromatin disposed in granules upon linin threads, while the nucleus usually appears as a complete reticulum. In polar views of the metaphase, the somatic number of chromosomes is seen in the equatorial plane; this number varies, but is constant for the species. In many organisms the chromosomes have been observed to be paired, and to possess definite size-relationships, which persist in the later spermatocytes; this was discovered by SUTTON in *Brachystola magna*, and has since been noted by many cytologists. In certain groups, moreover, the number of chromosomes is uneven, and the presence of an odd or unpaired chromosome has led to speculation upon the problem of sex-determination; this chromosome is the "heterotropic" or X chromosome of WILSON, the "accessory" chromosome of MCCLUNG, and the "monosome" of DAVIS.

After a definite number of cell generations the spermatogonia divide, and form daughter primary spermatocytes, and a long growth period intervenes between the telophase of the former and the prophase of the latter; throughout this stage the chromatin is again seen in granules upon a linin basis, being arranged in a reticulum or many filaments: the odd chromosome, when present, takes no part in the general dissociation, but remains as a compact body apposed to the nuclear membrane. At the close of this period the cells have considerably increased in volume, and enter the prophase of the first maturation mitosis. In polar views of this metaphase the number of chromosomes is seen to be half that of the preceding divisions, and, since the same size-relationships reappear whenever identification is possible, it has been assumed that an association of members of the spermatogonial pairs has occurred during the intervening period. This view, originally put forward by MONTGOMERY is now accepted by most cytologists, but is denied by DUESBERG, FICK, GÉRARD, and MEVES. It has, moreover, led to the belief that the members of each pair have respectively a paternal and maternal origin, and that their conjugation unites like elements derived from the two parents; there is, however, difference of opinion as to the manner in which this conjugation takes place, for some writers have described a side-to-side union, whereas others affirm that it is end to end. If this association of chromosomes is universal, and if there is complete fusion, as BONNEVIE, SAINMONT, VON WINIWARTER, and WILSON believe, we may find in this phenomenon a solution

of the problem of Mendelian inheritance, for this period of lateral juxtaposition may supply the opportunity for the exchange and segregation of the character factors.

The primary spermatocyte cell divides to form two daughter secondary spermatocytes, and these in turn divide to form daughter spermatids. There is no true resting stage between these two mitoses, but the chromosomes of the spermatid become dissociated in fine granules, which are again coloured only faintly by the iron hæmatoxylin. Both cell and nucleus then elongate, and eventually a characteristic spermatozoon is formed, consisting of a strongly-staining chromatin head, a middle-piece, and a fine, thread-like tail. The cleavage of the chromosomes in the first maturation mitosis has been described as transverse by some writers and as longitudinal by others, and the same difference of opinion exists concerning the second maturation division; upon this hangs the whole controversy of pre- and post-reduction. In the eumitotic type of maturation, neither mitosis is supposed to separate associated chromosomes: in the pseudomitotic type, however, one is said to be reductional, although cytologists are not agreed as to the division at which this occurs. Bouin, Collin, Davis, Farmer, Henking, King, Korschelt, Lerat, Maréchal, McGill, Montgomery, Moore, Nichols, Schockaert, the Schreiners, Stevens, and Wilson believe that this separation takes place at the first, whereas McClung, Robertson, Sutton, and others believe that the second mitosis is responsible.

In organisms whose spermatogonia possess an odd chromosome, dimorphism of spermatozoa results, for this chromosome passes entire to one daughter cell at either the first or second maturation division, and, since the oogonia are said to possess a pair instead of a single chromosome, it has been inferred that only spermatozoa containing the odd chromosome can produce females on amphimixis. The heterotropic chromosome is thus regarded as a possible sex-determinant. In certain other organisms the spermatogonial number of chromosomes is even, but the members of one pair are unlike; dimorphism of spermatozoa therefore again results. The two corresponding oogonial chromosomes are said to be equal in size to the larger member of the spermatogonial pair; consequently, spermatozoa possessing the smaller must produce males, and those possessing the larger females. It must, however, be remembered that this interpretation is not universally accepted, for Hertwig, Paulmier, Wassilieff, Zweiger, and others regard the odd chromosome as a degenerating element, which will eventually become extinct.

I have thought for some time that an exhaustive study of germ-cell mitoses must yield information concerning many of the phenomena now under discussion. We possess accounts of development in a very large number of species, but these enable us to compare only consecutive phases and the number of chromosomes appearing on the various spindles; no attempt seems to have been made to measure dimensions and volumes of members composing an individual complex, or to construct to scale a model representing accurately a metaphase figure. Without such measurements no

comparison can be made between chromosomes of various species, genera, families, orders, and classes, nor can we determine the process by which the total volume of chromatin is rendered constant in any organism; it is obvious that chromosomes cannot undergo repeated division, longitudinal or transverse, without diminution in volume, and, if this volume is not increased at different periods, the total mass must eventually become infinitesimally small. I have, moreover, seen no paper dealing with the relative positions of the chromosomes on the spindle or with the dimensions and curves of the ellipses constituting mitotic figures.

I have therefore selected material representative of the various subdivisions of the animal kingdom, and have endeavoured to carry out a thorough analysis of the chromatin complexes of these cells. Great care has been exercised to eliminate all source of error; by levelling the microscope platform and drawing-table with a spirit level, and by making many camera lucida drawings of each complex and individual chromosome studied, reproductions have been obtained that must represent truly the objects drawn. We know that the spermatogonial and spermatocyte chromosomes have been observed to possess definite size-relationships by which they can be identified, and certain groupings as regards size have, moreover, been noted in several species of a genus. The dimensions of individuals of a complex, however, have not been shown to be constant for all members of the species at corresponding stages, and consequently we do not know what relationships exist between dimensions of chromosomes in successive cell generations of any species, or of members of allied or widely separated groups. If the dimensions are constant, we must ask ourselves whether differences in volume are arbitrary or can be brought under one general scheme.

In the following pages will be found measurements of chromosomes forming the complex of organisms representing the various phyla and classes of the animal kingdom, and in certain cases representing allied orders, families, and genera: these dimensions with the volumes deduced from them appear in tables at intervals throughout the text. We shall, therefore, be able to compare not only individual chromosomes but the total volume of chromatin in successive mitoses of the same and different organisms: such comparison will be found to throw new light upon the theory of chromatin function and the phenomena connected with the resting stages and growth period, and in the latter case will furnish fresh data in the controversy of pre- and post-reduction. For the sake of convenience this paper has been divided into five parts, each dealing with a different stage in the course of these investigations.

We know from the work of Altmann, Halliburton, Hoppe-Seyler, Kössel, Liebermann, Mathews, and Miescher, corroborated by the more recent researches of other writers, that the formula of chromatin varies according to the amount of albumin associated with the nucleinic acid, and that the percentage of albumin may vary in the chromatin of the same nucleus as a result of physiological conditions.

The chemical composition of these substances is at present imperfectly understood, but there is strong reason for supposing that the lack of affinity for the iron hæmatoxylin found in stages other than those of actual mitosis is due to the absorption of albumin: the intense staining power of the chromosomes on the mitotic spindle may consequently be accepted as proof of a composition containing a maximum percentage of nucleinic acid, as has been pointed out by LILIENFELD, RÜCKERT, WILSON, and others. In the prophase of division, particularly of the primary spermatocyte mitosis, the ragged filaments stain only slightly with the iron hæmatoxylin; as these contract to form the chromosomes characteristic of the metaphase figure their affinity for the stain increases, and we find distinct correlation between the size and compactness on the one hand and the degree of staining on the other. We must therefore assume, in the present state of our knowledge, that the intensity of colour and closeness of particles constitute an index to the percentage of albumin present; and, since in the various metaphases the chromosomes appear equally dark, must conclude that their chemical composition is approximately the same. If, moreover, we find that a particular chromosome possesses invariable dimensions in a certain metaphase, and that these bear a fixed relationship to those of corresponding chromosomes in succeeding metaphases, we need not concern ourselves with speculation upon chemical composition.

GENERAL METHODS.

All material dealt with in this paper was preserved in Perenyi's chromo-nitric acid fluid, the strong chromo-aceto-osmic acid fluid of Flemming, Hermann's platino-aceto-osmic acid solution, or a mixture of equal volumes of corrosive sublimate and acetic acid. The first named has not been used by many writers upon this subject, but has proved excellent for preserving the various stages of cell development in the Insecta, and I have accordingly used it extensively for material belonging to this class; the last named has been used only for Protozoa.

When fixing with Perenyi's fluid I allowed the material to remain in the fixative for two hours, and then transferred it for one hour to a 50-per-cent. aqueous solution of alcohol; after remaining for twelve hours in a 70-per-cent. solution it was stored in a solution of 80-per-cent. The material to be fixed in the fluids of Flemming and Hermann was placed in the fixative for 24 to 48 hours; it was then washed in running water for 24 hours, and passed successively through 30-per-cent., 50-per-cent., and 70-per-cent. aqueous solutions of alcohol, remaining four hours in each of the two first named and eight hours in the last. It was then stored in a solution of 80-per-cent. alcohol. All Protozoa were fixed in corrosive sublimate and acetic acid for 10 or 15 minutes; they were then washed for 10 minutes in several changes of 50-per-cent. alcohol, and were stored in 70 per cent.

In certain cases the testes and ovaries were dissected out before fixation; in others the specimens were immersed whole, the appendages being first removed and

the integument of the abdomen slit open to ensure immediate access to the fluid. When required for embedding they were placed for 24 hours in a 90-per-cent. solution of alcohol, and then passed successively through a 95-per-cent. solution, absolute alcohol, cedar wood oil, and xylol. They were then embedded in paraffin having a melting-point of 52° C. Sections were cut with an ordinary Cambridge rocking microtome to a thickness of 8 μ or 10 μ, and were stained on the slide.

Only nuclear stains were used, viz., Heidenhain's iron hæmatoxylin, Grenacher's hæmatoxylin, iron brazilin, and safranin: I have found none equalling the iron hæmatoxylin for Metazoa, and with two exceptions have made all drawings for this paper from material so stained. Whenever Protozoa were mounted whole, Grenacher's hæmatoxylin was used, for the iron hæmatoxylin and brazilin are unsuited to material that is not cut in thin sections. In certain cases the same material was fixed with different fluids and coloured with different stains in order to discover if the apparent dimensions and volumes of the chromosomes are in any way dependent upon chemical reaction; if this is so, the measurements obtained cannot represent truly those of the living cell.

When staining with the iron hæmatoxylin I used as a mordant an aqueous solution of iron alum, in which the sections remained for six hours; they were then stained for 15 hours, and the excess of colour later washed out with a very weak solution of iron alum. When a plasma stain was used in conjunction with the hæmatoxylin, the slides were first counterstained for 10 minutes in eosin or picro-carmine, but, since these seriously diminish the definition of the chromosomes, this method was not adopted when camera lucida drawings were required. In the iron brazilin method, first described by Hickson,* no second stain is necessary, for both cytoplasm and chromatin are affected. The slides were placed for two or three hours in a mordant consisting of a solution of iron alum in 70-per-cent. alcohol, and were then stained for 24 hours; a series of Triton chromosomes thus stained is shown in the plates of this paper. The safranin used was a solution in 50-per-cent. alcohol, in which the slides remained for 12 to 24 hours; the excess of colour was later washed out with a 95-per-cent. solution of alcohol. The definition obtained with this stain is bad, and renders it useless where camera lucida drawings must be made.

When staining Protozoa in Grenacher's hæmatoxylin the specimens were taken down through successive strengths to 30-per-cent. alcohol; they remained in the stain for 15 minutes and were thoroughly washed in 30 per cent. The excess of stain was removed with a slightly acid solution of 70-per-cent. alcohol after the material had been passed for a few minutes through 50 per cent.

The preparations were studied by means of a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1·30, in conjunction with compensating oculars Nos. 6, 12, and 18. I have used throughout these investigations the holoscopic oil-immersion sub-stage condenser made by Messrs. Watson and Sons, of High Holborn,

* Hickson, S. J., 'Quart. Jour. Micr. Sci.,' 1901, vol. 44.

London ; this has a numerical aperture equal to that of the objective described above, and is the best that I have found for this work. The source of illumination was an inverted incandescent gas lamp, placed invariably at a distance of 215·9 mm. from the microscope, this measurement being made from the near edge of the mantle to the axis of the mirror. When difficulty was experienced in resolving chromosomes of a complex a Gifford screen was interposed. All original drawings were made with a large Abbé camera lucida at a magnification of 3048 diameters, and are reproduced in the plates of this paper at 2800 diameters. The drawing-table was invariably placed at a vertical distance of 251·46 mm. below the upper surface of the ocular, and the draw-tube of the microscope was pulled out to read 145 mm. Whenever a drawing was about to be made both microscope platform and drawing-table were carefully levelled with a clinometer to avoid the possibility of error due to foreshortening.

The magnification was estimated by means of a Zeiss stage-micrometer graduated to $\frac{1}{100}$ mm., and may be accepted as accurate ; since, moreover, all drawings have been made under precisely similar conditions as regards relative positions of ocular, drawing surface, etc., any slight error will not invalidate the reproductions for purposes of comparison. All figures have been drawn to the same scale so that the reader may be able to compare dimensions of complexes and individual chromosomes directly by means of an ordinary pair of compass dividers ; and, in the case of the individual chromosomes dealt with in Part I, lengths obtained from many drawings of each have been plotted out in the plates to facilitate this comparison.

Although no effort has been spared to avoid error, it is impossible to eliminate entirely the personal element from work of this nature ; and, where measurements are made of bodies that are sometimes only 1/120,000 inch in diameter and 1/30,000 inch long, corroboration by independent workers is most necessary. Since the results obtained from this analysis are important, and since a new hypothesis must depend entirely upon their accuracy, I hope these measurements will be supported by those of other cytologists derived from the same or different material.

Part I.—The Spermatogenesis of Stenobothrus, with a Comparison between the Chromosome Complexes of several Species of the Genus.

Material and Methods.

The specimens of *Stenobothrus bicolor* (Charp) and *S. parallelus* (Zett) were caught in Monmouthshire at the end of July, and those of *S. viridulus* and *S. bicolor* var. *nigrina* (Fieb) in Flintshire at the end of August. The grasshoppers were killed within a few hours of capture, and were fixed immediately in Perenyi's fluid ; in the case of the two first named the sexual organs were dissected out before immersion in the fixative, but in the others were not removed until required for embedding. The sections were stained with Heidenhain's iron hæmatoxylin, iron brazilin, and safranin. A detailed

account of the procedure employed in fixing and staining with the reagents mentioned above has already been given under the heading "General Methods."

Contrary to the usual procedure in papers upon spermatogenesis, I have begun with a study of the secondary spermatocyte cells; this has been considered advisable because these cells are numerous and overlapping of chromosomes seldom occurs. The greater number of chromosomes in the spermatogonia, and the twisted figures presented by the primary spermatocyte tetrads render measurement more difficult in these earlier stages, and their analysis has consequently been deferred.

The Secondary Spermatocyte Metaphase of *Stenobothrus bicolor* var. *nigrina*.

Dimensions of the Chromosomes.

A study of the 12 secondary spermatocyte metaphases shown on Plate 1 leads to certain important conclusions. Firstly, the number of chromosomes in the equatorial plane is invariably eight or nine, the difference depending on the absence or presence of a particular chromosome, easily recognisable on account of its great breadth; this is the heterotropic or accessory chromosome, and is found in only 50 per cent. of these cells. Secondly, the chromosomes in each cell can be divided into two length-groups, there being invariably three long bent chromosomes, and five shorter chromosomes appearing as straight rods. The investigation of many cells at this stage has satisfied me that this number and grouping may be accepted as constant for all members of the species—possibly for all members of the genus.

Each chromosome is composed of two equal rods, lying one above the other. The component rods of the three long chromosomes are respectively apposed to one another at the point at which they are bent, their ends being usually turned outwards from the equatorial plane; the component rods of the shorter chromosomes are in contact at one extremity.

Let us first consider the three long chromosomes. Since they can be easily distinguished from the other chromosomes, we are led to ask if they can be individually recognised; their lengths must be constant or, within certain limits, arbitrary, and in the former case must be equal or unequal.

I have tried to measure accurately the lengths and diameters of these chromosomes by making a very large number of camera lucida drawings, selecting only those whose major axis lay exactly at right angles to the microscopic line of vision irrespective of the cells in which they were found. In all such cases every point throughout the entire length of the rod can be focussed simultaneously, and the true length must be found, for no foreshortening can have occurred. By drawing each chromosome many times and comparing the lengths obtained, the possibility of error in draughtsmanship has been reduced to a minimum.

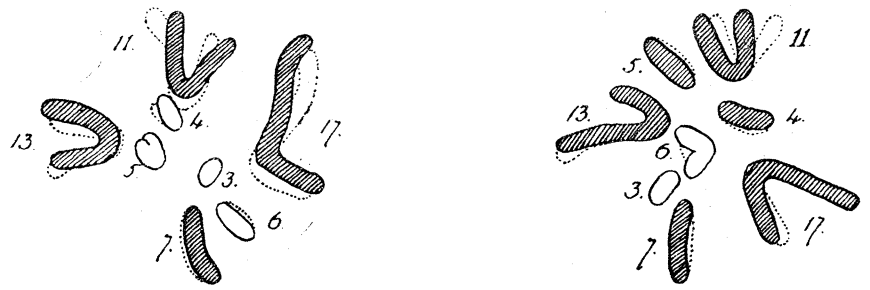
Figs. 13–52, Plate 2, are examples of drawings made from the three long chromo-

somes. The corresponding lengths, measured through the centre of each and represented by vertical lines, are shown plotted out on Plate 2, and are seen to fall into three distinct groups. Figs. 13–21 are 22·86 mm. long, with the exception of fig. 16, which is 22·23 mm.; thus, of the 10 chromosomes independently drawn and measured, nine are of the same length and the error in the case of one is only 0·63 mm. Figs. 22–41 show lengths of chromosomes constituting the second group; 18 are seen to be 17·78 mm. long, and two 18·25 mm., *i.e.* a difference from the normal of 0·47 mm. The chromosomes forming the third long group are represented by figs. 42–52, and their lengths without exception are 15·24 mm.

This proves that the three long chromosomes have three distinct lengths. Forty chromosomes have been measured, and 37 have been found to be 22·86, 17·78, or 15·24 mm. long, the maximum error in the case of the three remaining chromosomes being only 0·63 mm.; moreover, since the differences in length between the groups are respectively 5·08 and 2·54 mm., it is impossible that a chromosome belonging to one group can have been mistaken for one belonging to another.

The chromosomes are cylindrical rods with rounded ends, and their diameter is invariable throughout their entire length. This diameter is constant for the three long chromosomes and is 2·54 mm. It now remains to be seen whether each cell contains one member of each group, three members of one group, or members arbitrarily belonging to the three groups; an investigation of the complexes shown on Plate 1 will enable us to answer this question.

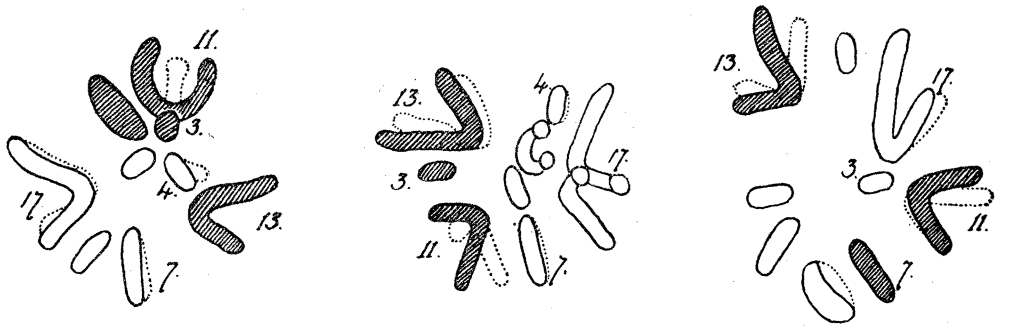
In figs. 4 and 12, Plate 1, the three long chromosomes are unforeshortened, and their lengths are respectively 22·86, 17·78, and 15·24 mm. These measurements agree exactly with those found for the three groups on Plate 2, and prove that



DIAGRAMS 1 and 2 (corresponding with figs. 4 and 12, Plate 1).—In these and the following diagrams the shaded chromosomes are lying at right angles to the microscopic line of vision throughout their entire length; all others are foreshortened.

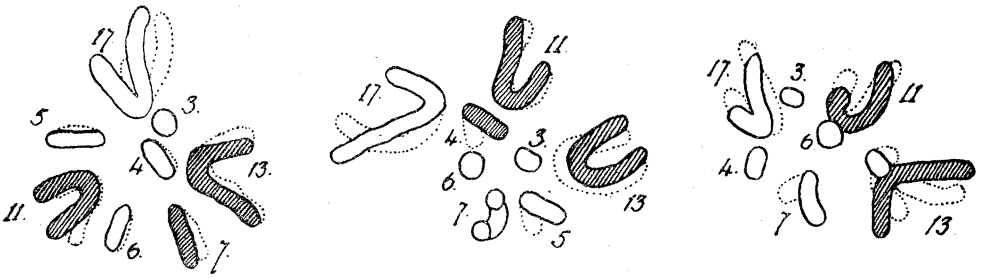
each cell does not contain three chromosomes of the same length, but one representative of each group. I have been unable to find another cell in which these three chromosomes are unforeshortened, but corroboration can be got from those in which two of the three can be accurately measured; if our hypothesis is to be supported, these must be shown to belong to different groups, and the length, as seen, of the third must be compatible with that found for members of the remaining group.

The accompanying diagrams of figs. 2, 6, and 9 show Chromosomes 13 and 11 to be lying in a manner such that all points in their respective lengths can



DIAGRAMS 3, 4, and 5 (corresponding with figs. 2, 6, and 9 of Plate 1).

be focused simultaneously. Their lengths are respectively 17·78 and 15·24 mm.; consequently they must belong to the medium and small groups. Moreover, the apparent length of the third and foreshortened chromosome is less than 22·86 mm., but exceeds the lengths found for the groups mentioned above; its identity is therefore established. Further corroboration can be obtained from figs. 1, 3, and 8, for the diagrams show the same two long chromosomes to be lying suitably for measurement,

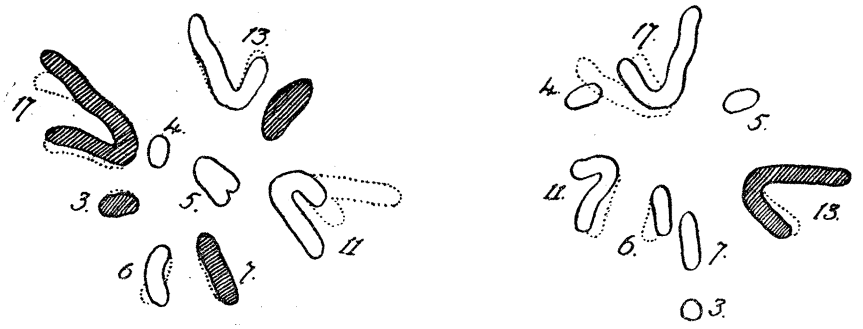


DIAGRAMS 6, 7, and 8 (corresponding with figs. 1, 3, and 8, Plate 1).

and their lengths are once more found to be 17·78 and 15·24 mm. In fig. 1 the third chromosome must represent the longest group, for its length, as seen, is 18·52 mm.; the same conclusion must be drawn in figs. 3 and 8, for the length of this foreshortened chromosome exceeds 15·24 mm., and the representative of the medium group has already been identified.

Diagrams 9 and 10 show only one long chromosome that can be focused simultaneously throughout its entire length. Its length is 22·86 mm. in fig. 7 and 17·78 mm. in fig. 5; consequently it represents the long and medium group respectively in these two complexes. In fig. 7, the two remaining long chromosomes obviously belong to the medium and small groups, for their apparent lengths are 15·88 and 13·33 mm. In fig. 5, the lengths of the two foreshortened chromosomes are 21·08 and 12·95 mm.; the former, therefore, must be the longest of the three, and, since the medium chromosome has already been recognised, the latter must be the shortest.

We have accordingly seen that, whenever a long chromosome is placed so that all points in its length can be focused simultaneously, the length found falls into one of three groups, viz., 22·86, 17·78, and 15·24 mm. In the two complexes in which all long chromosomes can be measured accurately, one has been found representing each group; and in all other cases the lengths of the foreshortened chromosomes are in complete accord with this supposition. Moreover, if the lengths found for these groups on Plate 2 were incorrect, it is inconceivable that we should not find at least one cell in which the measurements of the long chromosomes could not be reconciled with this hypothesis; consequently these lengths must be assumed to be correct.



DIAGRAMS 9 and 10 (corresponding with figs. 7 and 5, Plate 1).

Having satisfied ourselves regarding the long chromosomes, we can consider the remainder of the complex. The same system of measurement has been adopted, for many camera lucida drawings have been made of each chromosome; examples of these are shown on Plate 2, and their lengths will be found plotted out as before on Plate 3.

It will be seen that the lengths of these shorter chromosomes fall into five distinct groups; the heterotropic and fourth longest ordinary chromosome are equal, but the former can always be recognised by its great breadth. Examples of these two chromosomes are given in figs. 53–77; and, of the 25 represented, 19 are 10·16 mm. long, the maximum error in the case of figs. 55, 63, 66, 67, 71, and 72 being 0·28 mm.

The two next longest chromosomes are represented by figs. 78–91 and 92–105 respectively, and the lengths, without exception, are 8·89 and 7·62 mm. In the case of Chromosome 5, shown in figs. 106–120, twelve are 6·35 mm. long, and the error in figs. 108, 111, and 117 is 0·25 mm. The smallest chromosome of the complex is shown in figs. 121–135 inclusive; 15 have been drawn, and show an uniform length of 5·10 mm.

We have therefore found that, of the 83 short chromosomes depicted on Plate 2, 74 possess a length that falls precisely into one of five groups. The difference between any two consecutive groups is 1·25 mm., and, since the maximum error in

the nine remaining chromosomes does not exceed 0·28 mm., a mistake in identity is impossible.

By a process similar to that described for the long chromosomes, I have satisfied myself that every cell contains one representative of each short group. The identity of each chromosome is denoted in the diagrams by a numeral, which has sometimes been omitted, for the identification of chromosomes that are much foreshortened cannot always be proved deductively. The numerals used are not invariably consecutive, and the reason for this will be given later.

I have been unable to find a cell in which all chromosomes are lying exactly at right angles to the microscopic line of vision, and such a cell probably does not exist, for the ends of the long chromosomes appear to be turned outwards from the equatorial plane; consequently drawings of a complex cannot represent the true lengths of all chromosomes, nor can they prove identity in every case. Several writers have included in their papers plates showing the individual members of numerous complexes, arranged in their relative order as regards size; unless the chromosomes in their material lie in a manner such that all points in their respective lengths can be focussed simultaneously—and I have strong reason for doubting this—the drawings cannot identify every chromosome, and the order is therefore not established.

I have already pointed out that all camera lucida drawings have been made at a magnification of 3048 diameters and are reproduced in this paper at 2800 diameters; the actual lengths of the eight groups constituting the secondary spermatocyte complex must therefore be 7·5, 5·8, 5, 3·3, 2·9, 2·5, 2·1, and 1·7 μ.

A detailed account has been given of the procedure employed in measuring chromosomes, and checking the measurements by a subsequent identification of individuals in a complex, because I propose to draw certain important conclusions from these results. Firstly, the morphological individuality of the chromosomes, hitherto postulated as a result of mere visual examination, has now been proved, for members of each group have been shown to possess constant dimensions. Secondly, we have found that the diameter of each ordinary chromosome is the same throughout its length, and this diameter is constant for all members of the complex with the exception of the heterotropic chromosome; it is, therefore, possible to determine the volume of any chromosome, and the total volume of chromatin on the spindle. Thirdly, a study of the lengths of the ordinary chromosome rods leads to the discovery that they constitute a series in arithmetical progression, the short chromosomes being consecutive members. I shall deal later with this phenomenon when we have examined the secondary spermatocyte cells of another species of Stenobothrus.

Relative Positions of the Chromosomes.

In order to discover if the chromosomes occupy constant positions on the spindle, we must turn to the preceding diagrams. These show Chromosome 7 to be lying

between Chromosomes 13 and 17 in figs. 2, 3, 4, 8, and 12; in figs. 1, 5, and 9 it lies between Chromosomes 11 and 13; and in figs. 6 and 7 between 11 and 17. The equatorial plane of each complex is not exactly at right angles to our line of vision, consequently the chromosomes appear at slightly different vertical distances; but no possible turning of the complex about the spindle axis can make Chromosome 7 move to a position between the long chromosomes other than those shown in the diagrams. Further proof can be obtained from the odd chromosome. In fig. 2 it lies between Chromosomes 11 and 17, and in figs. 7 and 9 between Chromosomes 11 and 13; moreover, in figs. 2 and 7 it is on the side of the complex opposite to Chromosome 7, whereas in fig. 9 both chromosomes are close together. The study of others affords similar results, and we must accordingly assume that the individual chromosomes do not occupy definite relative positions in the equatorial plane.

If we measure distances between long chromosomes when such distances are unforeshortened, we find no agreement between the measurements obtained. In fig. 4, the points in Chromosomes 13 and 17 nearest to one another can be focussed simultaneously, and the length between them is 13·33 mm. The unforeshortened distance between these chromosomes in fig. 5 is 10·67 mm. In fig. 3 the distance between Chromosomes 11 and 17 is 3·81 mm.; in fig. 5, 6·35 mm.; and in fig. 11, 11·10 mm. The measurement of distances in a complex is always difficult, for only certain chromosomes lie in one horizontal plane, and in all other cases foreshortening occurs. The number of divisions on the milled screw of the fine adjustment allows relative heights to be estimated only approximately; in the 12 complexes on Plate 1, the total number of divisions through which this screw was turned varied from 1·16 to 3.

These results prove that the distances between chromosomes are not constant. This, however, must be expected, for the cells are unlikely to be at precisely similar stages of the metaphase; we have no means of determining at what moment in the process of division and separation of component rods the cells have been fixed, and, since the process is continuous, a selection of complexes must afford examples of cells preserved at minutely varying stages. Moreover, we do not know that every chromosome appears in the equatorial plane at the same instant; in certain metaphases the odd chromosome has been observed to lag; and, if this is obvious in one case, we have no reason for supposing that other chromosomes may not likewise take up their positions and divide at intervals too small to be perceived.

Volumes of the Chromosomes.

Since the chromosomes consist of cylindrical rods with rounded ends and of an uniform diameter, their volumes can be determined. By assuming the ends to be half-spheres of a diameter equal to that of the rod, and by multiplying the length of the intermediate portion by the sectional area, the volume of each rod can be accurately measured; each chromosome is composed of two rods, and its volume is therefore twice that found by the above method.

TABLE showing Measurements of Chromosomes composing Secondary Spermatocyte Complex of *Stenobothrus bicolor*.

	Length of rod.	Diameter of rod.	Volume of chromosome, *i.e.* of two rods.
	μ.	μ.	Cub. μ.
Chromosome 17	7·5	0·83	7·9
„ 13	5·8	0·83	6·1
„ 11	5·0	0·83	5·2
„ 7	3·3	0·83	3·3
„ 6	2·9	0·83	2·9
„ 5	2·5	0·83	2·4
„ 4	2·1	0·83	2·0
„ 3	1·7	0·83	1·5
„ X	3·3	?	?
Total volume of chromatin on spindle, exclusive of the heterotropic chromosome			31·3

THE SECONDARY SPERMATOCYTE METAPHASE OF *Stenobothrus viridulus*.

The chromosomes have been drawn and measured as described in the case of *S. bicolor*. Plate 2 shows examples of this metaphase, and the cells closely resemble those on Plate 1. The complex is again composed of eight or nine members, according as the heterotropic chromosome is absent or present: the three long and five short chromosomes lie on the spindle without definite arrangement, and I have been unable to find cells in which the distance between any two appears constant. Each ordinary chromosome consists of two rods, whose diameter is invariable throughout their length, and equal to that found in *S. bicolor*; these rods lie one above the other, and one passes to each pole in the subsequent anaphase.

Plates 2 and 3 show drawings of 124 chromosomes, and their respective lengths will be found on Plate 3. These again constitute eight groups, viz., 22·86, 20·32, 17·78, 10·16, 8·89, 7·62, 6·35, and 6·10 mm., the heterotropic chromosome as before being equal to the fourth longest. The error in the case of a few chromosomes is small, and may be ignored.

With the exception, therefore, of one long chromosome, the lengths are identical with those found in *S. bicolor*, and, since the diameter of the rods is the same in both species, the complexes are similar in every respect. The third long chromosome in *S. bicolor* is absent in *S. viridulus*, whereas the second long chromosome in the latter is absent in the former; the difference cannot be due to error in draughtsmanship, for the lengths are such that mistake in identity is impossible. Moreover, the second long chromosome in *S. viridulus* differs in length from the shortest of the three by an amount exactly equal to that between the medium and shortest

of the long groups in *S. bicolor*. This will be made obvious by a direct comparison of the two series.

Relative Lengths of the Eight Ordinary Chromosomes.

S. bicolor . . . . 1 : $1\frac{1}{2}$: 2 : $2\frac{1}{2}$: 3 : . . . . : 5 : 6 : – : 8.
S. viridulus . . . 1 : $1\frac{1}{2}$: 2 : $2\frac{1}{2}$: 3 : . . . . : – : 6 : 7 : 8.

From these figures it will be seen that the short chromosomes in both species are consecutive members of the same series—a series in strict arithmetical progression. A gap occurs between the short and long chromosomes, which are likewise members of the series, although not consecutive. We are consequently led to make a startling proposition: is it not possible that a genus contains a series of chromosome rods in arithmetical progression, and that the species of that genus differ only in the possession of members of that series? Such a proposition carries with it further propositions, for the question must arise, how and when these differences occurred: has natural selection caused a certain number of these elements to evolve or disappear, while purely local conditions determined which members should arise or be eliminated? These problems must prove of vital importance in the great question of the function of chromosomes, and their solution will probably throw fresh light upon many points connected with evolutionary development and the classification of individual organisms; I shall accordingly deal with them later, when we have more data upon which to base our hypotheses.

TABLE showing Measurements of Chromosomes composing Secondary Spermatocyte Complex of *Stenobothrus viridulus*.

	Length of rod.	Diameter of rod.	Volume of chromosome, *i.e.* of two rods.
	μ.	μ.	Cub. μ.
Chromosome 17	7·5	0·83	7·9
„ 15	6·7	0·83	6·9
„ 13	5·8	0·83	6·1
„ 7	3·3	0·83	3·3
„ 6	2·9	0·83	2·9
„ 5	2·5	0·83	2·4
„ 4	2·1	0·83	2·0
„ 3	1·7	0·83	1·5
„ X	3·3	?	?
Total volume of chromatin on spindle, exclusive of the heterotropic chromosome			33·0

THE SPERMATOGONIAL METAPHASE OF *Stenobothrus bicolor*.

The spermatogonial cells lie at the extreme anterior end of the follicle, and their metaphases show the somatic number of chromosomes. Although this number is

double that of the secondary spermatocyte complex, it is possible to find cells in which overlapping does not prevent identification and measurement of the chromosomes; camera lucida drawings have, therefore, been made as before.

These give dimensions corresponding exactly with those on Plate 2. The three long bent chromosomes of the secondary spermatocyte complex are represented in this case by six, which fall into three length-groups, viz., 22·86, 17·78, and 15·24 mm.; the shorter chromosomes are likewise paired, and are respectively 10·16, 8·89, 7·62, 6·35, and 6·10 mm. long. The heterotropic chromosome is equal to the fourth longest, but can be recognised by its greater breadth; it occurs unpaired in all cells, and thereby renders the somatic number uneven. The chromosomes exhibit no definite arrangement in the equatorial plane, and, with the exception of the increased spindle diameter due to the greater number of chromosomes present, the complex resembles that of the secondary spermatocyte.

Fig. 266, Plate 3, is an example of this metaphase. Many chromosomes are lying at right angles to the microscopic line of vision, and the correspondence between their lengths and those of the cells already studied can accordingly be verified by means of compass dividers. The existence of pairs representing each length-group is particularly evident in this figure.

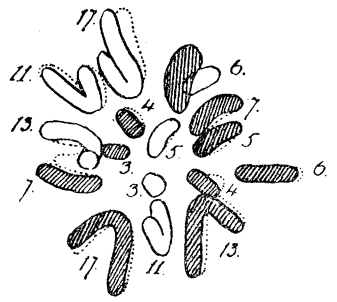


DIAGRAM 11 (corresponding with fig. 266, Plate 3).

The ordinary chromosomes are again seen to be composed of two cylindrical rods, whose diameter is constant and equal to that of the previous examples. The volume of each chromosome is consequently equal to that of the corresponding chromosome in the secondary spermatocyte; and, since the only difference between the complexes of these two stages is that each length-group is represented by one chromosome in the former and by two in the latter, the total volume of ordinary chromatin in the spermatogonium must be double that in the secondary spermatocyte cell. This is important, for it concerns the intermediate growth period.

In the anaphase of the spermatogonial mitosis the two rods composing each chromosome separate and pass to opposite poles; the cytoplasm then constricts, and two daughter primary spermatocytes are formed, each possessing 17 single rods divisible into eight length-groups. Whether we estimate the total volume of chromatin, or the volumes of individual rods, the result is the same, the daughter cells possessing one half of the chromatin that appeared on the spermatogonial spindle.

The volume of chromatin passing to each primary spermatocyte is therefore exactly equal to that seen in the equatorial plane of the secondary spermatocyte cell. A complex similar in every way to that of the latter can be produced if the single rods of like members are apposed to one another. This would not be remarkable if the secondary spermatocyte mitosis immediately followed the

spermatogonial, but the primary spermatocyte mitosis intervenes, and we are accordingly confronted by a problem. The total volume of chromatin passing to each primary spermatocyte has been found equal to that passing to each secondary spermatocyte; consequently the amount of chromatin appearing on the spindle of the former must be double that originally received from the parent spermatogonium. At some period, therefore, between the formation of the primary spermatocyte and its subsequent division, the total volume of the chromatin is doubled.

This can occur only during the growth period; and we now understand the reason for this long intervening period, in which the cells increase in diameter and the chromatin is disposed in granules upon linin threads. Moreover, the behaviour of the heterotropic chromosome during this period is in complete accord with that of the ordinary chromosomes. Since it does not divide at the primary spermatocyte mitosis, but passes entire to one daughter cell, no loss is incurred; consequently there is no necessity for an increase in its volume. It therefore takes no part in the general dissociation of chromosomes, and remains throughout the growth period as a compact and darkly staining body apposed to the nuclear membrane. This phenomenon has been observed in every case where the spermatogonial number of chromosomes is uneven and results in dimorphism of spermatozoa; and affords conclusive proof that the growth period is concerned not only with an increase in the cell volume, but with an increase in the chromatin itself.

The spermatids, arising from the secondary spermatocyte division, must possess eight ordinary chromosomes, each consisting of one rod; the total volume of chromatin is accordingly one-fourth of that found in the spermatogonial metaphase, if the heterotropic chromosome is excepted. If the amount of chromatin is not doubled before transformation into ripe spermatozoa, and if the mature ovum contains an equal volume, the oosperm will possess an amount equal to half that of the spermatogonium, viz., 16 single rods divisible into eight pairs. But the spermatogonium has been derived from the oosperm, and we must, therefore, assume that the dissociation of chromosomes in the spermatid is accompanied by an increase in their volumes, and that each spermatozoon contains an amount equal to that of the secondary spermatocyte metaphase. A corresponding increase must likewise take place in the ovum after the second maturation division, and the union of these two cells will accordingly result in the formation of a nucleus containing 16 pairs of rods.

Moreover, the same volume of chromatin appears in successive spermatogonial metaphases, and, since the chromosomes split longitudinally in these mitoses, the loss must be counteracted by growth during the intervening periods. The number of spermatogonial generations has been found constant for the species, and each division is followed by a resting stage in which the chromosomes break up into fine granules disposed on linin threads. We have seen that the volume of chromatin in the secondary spermatocyte metaphase is half that of the earlier mitoses, and have

noted that no resting stage precedes this division; we have found that the volume is the same in both spermatogonial and primary spermatocyte metaphases, and that the increase in the latter occurs during the growth period. Coincidence cannot be responsible for the fact that the normal amount of chromatin is found to be halved in the only mitosis that is not preceded by a resting stage; and we must accordingly realise that the total volume remains constant as a result of growth occurring during the intervals of division. The dissociation of chromosomes witnessed in these stages obviously facilitates this growth, and the phenomena of the resting stages are therefore explained.

Drawings representing members of the eight spermatogonial length-groups are given in figs. 267–275, Plate 3. Each chromosome is composed of two of these rods, and, since in this case there are two ordinary chromosomes of each length, the total volume is got by multiplying those of the individual rods by four.

TABLE showing Measurements of Chromosomes Composing Spermatogonial Complex of *Stenobothrus bicolor*.

	Length of rod.	Diameter of rod.	Volume of chromosome, *i.e.* of two rods.
	μ.	μ.	Cub. μ.
Chromosome 17	7·5	0·83	7·9
„ 13	5·8	0·83	6·1
„ 11	5·0	0·83	5·2
„ 7	3·3	0·83	3·3
„ 6	2·9	0·83	2·9
„ 5	2·5	0·83	2·4
„ 4	2·1	0·83	2·0
„ 3	1·7	0·83	1·5
„ X	3·3	?	?
Total volume of chromatin on spindle, exclusive of the heterotropic chromosome			62·6

THE SPERMATOGONIAL METAPHASE OF *Stenobothrus viridulus*.

As in the case of the species already investigated, the complex is composed of one heterotropic and 16 ordinary chromosomes. The former is equal in length to the fourth longest, and the latter are divisible into eight length-groups, corresponding exactly with those of the secondary spermatocyte cell. No definite arrangement of chromosomes on the spindle has been observed, and the equatorial plane appears similar to that shown in fig. 266, Plate 3.

Each chromosome is composed of two rods, and since the complex possesses two representatives of each length-group, the total volume of ordinary chromosomes is again obtained by multiplying the volumes of the individual rods by four.

TABLE showing Measurements of Chromosomes composing Spermatogonial Complex of *Stenobothrus viridulus*.

	Length of rod.	Diameter of rod.	Volume of chromosome, *i.e.* of two rods.
	μ.	μ.	Cub. μ.
Chromosome 17	7·5	0·83	7·9
„ 15	6·7	0·83	6·9
„ 13	5·8	0·83	6·1
„ 7	3·3	0·83	3·3
„ 6	2·9	0·83	2·9
„ 5	2·5	0·83	2·4
„ 4	2·1	0·83	2·0
„ 3	1·7	0·83	1·5
„ X	3·3	?	?
Total volume of chromatin on spindle, exclusive of the heterotropic chromosome			66·0

THE PRIMARY SPERMATOCYTE METAPHASE OF *Stenobothrus bicolor* AND *S. viridulus*.

Before dealing with this metaphase we must study the preceding growth period and prophase, for their phenomena will enable us to determine the constitution of the chromosomes that appear later on the spindle.

The growth period has been shown by many investigators to be concerned with a considerable increase in the cell volume, and we have seen from the foregoing pages that this increase is not confined to the cytoplasm, but is shared by the chromatin itself. During this stage the nucleus contains an apparent reticulum of chromatin granules on linin threads, the individuality of the ordinary chromosomes being lost in the general network. The reticulum is evenly disposed throughout the nucleus, and the granules are invariably seen in single rows. I have found no trace of separate vesicles as described by SUTTON in *Brachystola magna*; DAVIS has shown one such vesicle in *Stenobothrus curtipennis*, but I believe it to be a direct result of fixation.

The reticulum breaks later into numerous convoluted filaments, and the granules are seen to be split. It has already been suggested that the chromosomes dissociate in order to facilitate growth by offering a larger surface for absorption; and this is now confirmed, for the granules are seen here to divide when their individual volumes have been doubled. This moment, therefore, marks the end of the growth period so far as the chromatin is concerned.

The spiremes shorten and condense, and are found to be associated in pairs. The shapes assumed are either crosses or boomerangs, and, since in the former two filaments are evidently lying across one another, we must conclude that in the latter

two have joined end to end. The number of filaments is 16, and the somatic number of chromosomes is thus halved. As condensation continues the granules become more closely associated on the linin threads, and the staining capacity is increased; still later we recognise the outlines characteristic of the chromosomes, which are ragged and stain grey with the iron hæmatoxylin. We cannot compare dimensions at this period, for there is no means of determining whether two chromosomes are at precisely the same stage or not; but a study of form enables us to understand their constitution in the subsequent metaphase, when they are so twisted that visual resolution is extremely difficult.

Drawings of these condensing chromosomes are given in figs. 276–301, Plate 3. Each chromosome is seen to be composed of two arms, which are joined together sometimes at one extremity, sometimes at both extremities, and sometimes at neither extremity; each arm has evolved from the condensation of a single filament—a filament that originally consisted of a row of split granules on a linin thread. The longitudinal split caused by the cleavage of these granules can still be perceived, and is shown by a black line in figs. 277, 278, 281, 282, 284, 285, 287, 288, 289, and 297. Moreover, the varying width of the arms shows that their section is not a circle, but an ovoid figure, whose major axis is approximately double the minor; each arm is consequently seen to be composed of two rods, formed by the condensation of the split halves of a single row of granules, which doubled their volumes prior to cleavage.

This leads to an important conclusion. If the primary spermatocyte mitosis separates the two arms of each chromosome, it must be reductional, for each daughter cell will receive eight pairs of rods, representing one member only of each spermatogonial pair; if, on the other hand, this division separates the rods composing each arm, and not the arms themselves, it must be equational, for each daughter cell will receive a split half of both members constituting the spermatogonial pair. Moreover, if the division is equational the subsequent secondary spermatocyte mitosis must separate these split halves, and must therefore be reductional.

In every spermatogonial and secondary spermatocyte division, the plane of cleavage of the chromosomes is parallel to the length of the component rods, which are never intersected, but pass intact to opposite poles. In the spermatogonial metaphase we have counted 16 ordinary chromosomes, each composed of two rods, and, in the secondary spermatocyte, eight chromosomes, similarly constituted; the measurements given in the preceding tables, moreover, show the rods to be of invariable dimensions, and they must accordingly be regarded as indivisible units so far as mitosis is concerned. We have no reason for supposing that the primary spermatocyte division is an exception to this rule: unless it is an exception, in that the rods are intersected transversely or longitudinally, one mitosis must be reductional, and the eumitotic type of maturation is disproved.

In a recent paper upon the spermatogenesis of *S. biguttulus*, GÉRARD has denied a

conjugation of chromosomes, and has postulated instead an association of granules upon parallel linin threads. I have failed to observe this in my material, and believe that he has mistaken the split halves of individual granules for independently associating chromomeres; I committed a similar error in an earlier paper upon *S. viridulus*, not having then realised the significance of the paired granules on the spireme threads.

Plate 3 shows camera lucida drawings of these complexes in *S. viridulus*. The drawings of condensing chromosomes were made from cells of *S. bicolor*, but no confusion need result, for these stages are the same in both species. The metaphases represented by figs. 303–305 show nine chromosomes, the heterotropic again being recognisable. This chromosome has remained more or less unchanged throughout the growth period, at which stage it is represented by fig. 301. The remainder again exhibit definite relative sizes, there being three large and five small chromosomes in each complex. No constant arrangement exists in the equatorial plane, nor are relative distances in any two cells found to be the same.

The following tables show measurements of volumes of these complexes. We must, however, remember that these have been obtained not directly, but by deduction; we have shown that the volumes should have certain values, and since, although immeasurable, they apparently agree with our assumptions, we may accept the computation in these tables as correct.

TABLE showing Deduced Measurements of Chromosomes composing Primary Spermatocyte Complex of *Stenobothrus bicolor*.

	Length of rod.	Diameter of rod.	Volume of chromosome, *i.e.* of four rods.
	μ.	μ.	Cub. μ.
Chromosome 17	7·5	0·83	15·8
„ 13	5·8	0·83	12·2
„ 11	5·0	0·83	10·4
„ 7	3·3	0·83	6·6
„ 6	2·9	0·83	5·8
„ 5	2·5	0·83	4·8
„ 4	2·1	0·83	4·0
„ 3	1·7	0·83	3·0
„ X	3·3	?	?
Total volume of chromatin on spindle, exclusive of the heterotropic chromosome			62·6

TABLE showing Deduced Measurements of Chromosomes composing Primary Spermatocyte Complex of *Stenobothrus viridulus*.

	Length of rod.	Diameter of rod.	Volume of chromosome, *i.e.* of four rods.
	μ.	μ.	Cub. μ.
Chromosome 17	7·5	0·83	15·8
„ 15	6·7	0·83	13·8
„ 13	5·8	0·83	12·2
„ 7	3·3	0·83	6·6
„ 6	2·9	0·83	5·8
„ 5	2·5	0·83	4·8
„ 4	2·1	0·83	4·0
„ 3	1·7	0·83	3·0
„ X	3·3	?	?
Total volume of chromatin on spindle, exclusive of the heterotropic chromosome			66·0

THE CHROMOSOME COMPLEXES OF OTHER SPECIES OF STENOBOTHRUS.

We have found that the ordinary chromosomes of *S. bicolor* and *S. viridulus* are composed of rods of an uniform and constant diameter, and differ from one another only in length; we have further found that these lengths constitute members of a series in arithmetical progression, the difference between consecutive members being equal to half the diameter of the rods. It will therefore be interesting to compare these complexes with those of a third species, for it is possible that this series exists throughout the genus. Moreover, the two species already studied differ from one another in the possession of one long chromosome, the series being represented in *S. bicolor* by Chromosomes 3, 4, 5, 6, 7, 11, 13, 17, and in *S. viridulus* by Chromosomes 3, 4, 5, 6, 7, 13, 15, and 17, and we are likely to find in this manner a means of corroborating or checking our present classification.

THE CHROMOSOME COMPLEX OF *Stenobothrus parallelus*, ZETT.

The chromosomes have been measured as already described, and the rod-lengths again fall into eight groups. These are respectively 1·7, 2·1, 2·5, 2·9, 3·3, 6·3, 6·7, and 7·5, and are consequently members of the series mentioned above; the possession of Chromosome 14 distinguishes this species from the other two, the longest and five shortest chromosomes being the same in all three cases. The heterotropic chromosome is again equal in length to the fourth longest, and its diameter varies throughout its length, and exceeds that of the ordinary rods.

The spermatogonial complex consists of a heterotropic chromosome and 16 pairs of rods, representing the eight length-groups; the primary spermatocyte complex is similarly composed, but the ordinary rods are arranged in tetrads or groups of four;

no two tetrads are of the same size, and the eight lengths are consequently again represented. The secondary spermatocyte metaphase shows the heterotropic chromosome in 50 per cent. of the cells, and the ordinary chromosomes are composed of pairs of rods representing the length-groups.

No definite arrangement exists in the equatorial plane of any metaphase, and the diameter of the rods is the same as that found in the species previously studied. Examples of the nine members representing the eight length-groups, taken from the secondary spermatocyte metaphase, are shown in figs. 306–314, Plate 4, and may be compared with those on Plates 2 and 3.

TABLE showing Measurements of Chromosomes composing Complex of *Stenobothrus parallelus.*

	Length of rod.	Diameter of rod.	Volume of dyad, *i.e.* of two rods.	Volume of tetrad, *i.e.* of four rods.
	μ.	μ.	Cub. μ.	Cub. μ.
Chromosome 17	7·5	0·83	7·9	15·8
„ 15	6·7	0·83	6·9	13·8
„ 14	6·3	0·83	6·5	13·0
„ 7	3·3	0·83	3·3	6·6
„ 6	2·9	0·83	2·9	5·8
„ 5	2·5	0·83	2·4	4·8
„ 4	2·1	0·83	2·0	4·0
„ 3	1·7	0·83	1·5	3·0
„ X	3·3	?	?	?
Total volume of chromatin on spindle of spermatogonial and primary spermatocyte cell, exclusive of the heterotropic chromosome . . .				66·8
Total volume of chromatin on spindle of secondary spermatocyte cell, exclusive of the heterotropic chromosome				33·4

THE CHROMOSOME COMPLEX OF *Stenobothrus bicolor.*

Three varieties of *S. bicolor* are recognised, viz., *nigrina* (Fieb), *mollis* (Charp), and *purpurascens* (Fieb); I have accordingly thought it advisable to study the species itself in order to discover if the complex differs in any way from that of the variety, *nigrina.*

Examples of the chromosome rods are given in figs. 315–323, Plate 4, and will be found to correspond exactly with those drawn on Plate 2. Since the same eight length-groups are represented, variation is evidently unaccompanied by differences in the complex, or differences exist that are too small to be recognised with the means now at our disposal; this is important, for it may enable us in future to determine whether a particular organism represents a distinct species or merely a variety of another species.

TABLE showing Series of the Chromosome Rods found in Stenobothrus, and the Complexes to which they individually belong.

	Length.	*S. bicolor.*	*S. bicolor*, var. *nigrina.*	*S. viridulus.*	*S. parallelus.*
	μ.				
Chromosome 17 . . .	7·5	1	1	1	1
„ 16 . . .	7·1	—	—	—	—
„ 15 . . .	6·7	—	—	1	1
„ 14 . . .	6·3	—	—	—	1
„ 13 . . .	5·8	1	1	1	—
„ 12 . . .	5·4	—	—	—	—
„ 11 . . .	5·0	1	1	—	—
„ 10 . . .	4·6	—	—	—	—
„ 9 . . .	4·2	—	—	—	—
„ 8 . . .	3·7	—	—	—	—
„ 7 . . .	3·3	1	1	1	1
„ 6 . . .	2·9	1	1	1	1
„ 5 . . .	2·5	1	1	1	1
„ 4 . . .	2·1	1	1	1	1
„ 3 . . .	1·7	1	1	1	1
„ 2 . . .	1·2	—	—	—	—
„ 1 . . .	0·8	—	—	—	—
„ X . . .	3·3	1	1	1	1

CHROMOSOMES OF THE SOMATIC CELLS.

These appear to be identical with those of the germ cells, as can be seen from figs. 324–331, Plate 4, where chromosome rods from a somatic cell of the ovary of *S. parallelus* are depicted. Fig. 333 shows a late prophase of this division—a stage immediately followed by segmentation of the spireme into chromosomes. Fig. 334 shows a telophase of another and similar cell, and the thread-width is here plainly shown to be the same as found for germ-cell rods of the genus. Fig. 335 represents an abnormal condition of the chromosomes in an ovum of *S. parallelus*, due doubtless to the flaking of yolk in the process of section cutting; the chromosomes appear to be easily resolved into spheres of a diameter equal to that of the normal rods.

SUMMARY OF PART I.

1. In all metaphases the relative positions of the chromosomes in the equatorial plane appear to be arbitrary.

2. The rods composing all ordinary chromosomes are cylindrical with rounded ends, and of an uniform and constant diameter, viz., 0·83 μ. In each species eight lengths have been found, and these constitute members of a series in arithmetical progression, of which the difference between consecutive terms is equal to the radius of the rod. The heterotropic chromosome does not belong to this general series, for, although equal in length to the fourth longest rod, its diameter varies at different points and exceeds 0·83 μ.

3. The rods are indivisible units, and, since each spermatogonial and secondary spermatocyte chromosome is composed of two, and each primary spermatocyte chromosome of four, their morphological individuality is metrically proved.

4. The eight rod-lengths are not the same in any two species; the longest and five short chromosomes occur in all, but identity is always established by the two remaining chromosome rods.

5. The complexes of a species and its variety appear to be identical; differences, if existing, are too small to be recognised.

6. The somatic chromosomes are identical with those of the germ cells.

7. The total volume of ordinary chromosomes is the same in spermatogonial and primary spermatocyte metaphases, whereas only half this amount appears in that of the secondary spermatocyte.

8. The volume of chromatin received by each daughter primary spermatocyte is doubled during the succeeding growth period, and the same phenomenon occurs in all resting stages between mitoses. The dissociation of chromosomes into minute granules during resting stages is presumably for the purpose of offering a larger surface for nutrition, for each granule splits when its volume has been increased, and condensation on both sides of this longitudinal split results in the formation of two rods in the place of one. The growth period ends with the splitting of the granules so far as the chromatin is concerned, but growth of cytoplasm continues until the chromatin filaments have condensed sufficiently to be counted.

9. No resting stage intervenes between maturation divisions, and the loss of chromatin occasioned by the first is consequently not counteracted by subsequent growth. Reduction in the total volume is thus effected, and the original amount is restored only on amphimixis. One maturation division must be reductional in Weismann's sense of the word.

Part II.—The Chromosome Complex of Allied Tribes, Families, Orders, and Classes, showing Morphological Correlation of Chromosome Rods throughout the Order Orthoptera.

Section A.—The Chromosome Complex of Allied Tribes.

We have found that in Stenobothrus the chromosomes are composed of rods, whose diameter is invariably 0·83 μ, and whose lengths constitute members of a series in arithmetical progression. It is possible that this series is not confined to the genus, and we must consequently study the complexes of organisms belonging to allied tribes.

The Acridiidæ are divided into several tribes or sub-families, of which the Tryxalides are represented by Stenobothrus. I now propose to deal with the chromosomes of five species of Melanoplus, belonging to the Acridides, and one species of Arphia and Dissosteira, belonging to the Œdipodides; if the series in

these is the same as in Stenobothrus, we shall have reason for supposing that it exists throughout the family.

I am indebted for this material to Dr. GORDON HEWITT, of the Central Experimental Farm, Ottawa; these grasshoppers are not found in the British Isles, and he very kindly arranged for a supply of male specimens caught in August and fixed in Perenyi's fluid. I also take this opportunity of thanking Mr. NORMAN CRIDDLE, of Treesbank, Man., for his kindness in collecting the material and for his readiness to help me in this matter. The grasshoppers were sent to me from Canada in a storage solution of 80-per-cent. alcohol, and, on arrival, sections were cut 8 μ thick and stained with Heidenhain's iron hæmatoxylin.

Sub-Family Acridides.

The Chromosome Complex of Melanoplus angustipennis, *Dodge.*

The ordinary chromosomes again appear to be rods of a constant diameter, which is the same as that observed in Stenobothrus, viz., 0·83 μ. Figs. 336–343, Plate 4, are examples of the rod-lengths drawn from chromosomes of the secondary spermatocyte complex, and are seen in certain cases to be identical with those of Stenobothrus. The longest corresponds with Chromosome 8, and the shortest, being spherical, is identical with Chromosome 1 of our series; the intermediate lengths are respectively equal to those of Chromosomes 2, 3, 4, 5, 6, and 7. Thus, the eleven ordinary chromosomes are of eight distinct lengths, of which the short are in certain cases represented by more than one. The heterotropic chromosome is equal in length to Chromosome 5, but is easily distinguishable on account of its great breadth; an example is given in fig. 344. It will be remembered that the odd chromosome in Stenobothrus was found to be equal to Chromosome 7.

The cytoplasm of this and the following species of Melanoplus is more opaque than that of Stenobothrus, and the checking of lengths has consequently been more difficult. The total volume of chromatin on the spermatogonial and primary spermatocyte spindles, exclusive of the heterotropic chromosome, is approximately 44 cu. μ, and that of the secondary spermatocyte 22 cu. μ; any slight inaccuracy in the estimation of individuals will not seriously affect these totals, which are approximately two-thirds of the corresponding values found for Stenobothrus

The Chromosome Complex of Melanoplus atlansis, *Riley.*

The ordinary rods have the same diameter as in all previously studied material, and the complex is again divisible into eight lengths, which are identical with those observed in *M. angustipennis.* Certain of these are represented in the secondary spermatocyte cell by more than one chromosome, and the total number, eleven, is accordingly accounted for. Figs. 345–352 are examples of these lengths, and will

be seen to correspond exactly with Chromosomes 1, 2, 3, 4, 5, 6, 7, and 8 of the general series.

The heterotropic chromosome is again equal in length to Chromosome 5, and distinguishable on account of its excessive breadth. The total volume of chromatin on the various spindles differs slightly from that of *M. angustipennis*, since the rod lengths represented by more than one chromosome are not the same in both species.

The Chromosome Complex of Melanoplus bivittatus, *Say.*

The same thread-width is again observed, and is constant throughout the lengths of the ordinary chromosomes. Figs. 354–361 represent the ordinary rods, which are identical with those of the two species already dealt with. Certain lengths are again represented by more than one chromosome, and the individuality of the species is established.

The heterotropic chromosome is similar in every respect to those described for the genus, and an example is given in fig. 362.

This species has been investigated by NOWLIN, whose resolution of the complex into a regularly graded series, of which the longest member is approximately four times the length of the shortest, accords with these results.

The Chromosome Complex of Melanoplus dawsoni, *Scudder.*

I have, unfortunately, found very few cells dividing in the slides that I have made of this species, and consequently have been unable to check lengths thoroughly. The dimensions, however, appear to be precisely similar to those of the Melanopli already described, and it is unlikely that the thread-width can have been inaccurately drawn. This is invariably 0·83 μ, and may now be regarded as constant for this genus, as it has been shown constant for Stenobothrus.

Figs. 379–386 are examples of the eight rod-lengths taken from secondary spermatocyte cells.

The Chromosome Complex of Melanoplus packardii, *Scudder.*

The chromosomes are again seen to be rods of a constant diameter, which is 0·83 μ. The heterotropic chromosome alone exceeds this thread-width, and is once more equal in length to Chromosome 5. Figs. 363–370 show the eight ordinary rod-lengths, which correspond exactly with those already drawn; certain are represented by two chromosomes. The heterotropic chromosome is shown in fig. 371, and is indistinguishable from those of the other species.

Fig. 371A is a drawing of a secondary spermatocyte metaphase seen from the polar view; the heterotropic and 11 ordinary chromosomes are apparent, but certain of the latter are not lying with their major axes at right angles to the microscopic line of vision, and are accordingly foreshortened.

Sub-Family Œdipodides.

The Chromosome Complex of Arphia pseudonietana, *Thomas.*

Apparently, the thread-width, 0·83 μ, is common also to this sub-family, for all rods, with the exception of the heterotropic chromosome, possess this diameter. We have found the same thread-width in the Tryxalides and Acridides, and its existence in this third sub-family suggests that it is constant for the family.

Figs. 372–377 are examples of the ordinary rod-lengths composing the complex, and are identical with those previously drawn and studied; in this organism there are apparently only six lengths, of which the long chromosomes are represented by more than one. The heterotropic chromosome, shown in fig. 378, seems to be similar to that of the Melanopli, being equal in length to Chromosome 5 of the general series.

Fig. 387 is an example of a secondary spermatocyte metaphase, and shows the heterotropic and 10 ordinary chromosomes. It must, however, be remembered that certain of the latter are foreshortened, and that their respective lengths consequently exceed those shown. Fig. 388 shows a lateral view of a primary spermatocyte metaphase; the tetrads are seen on comparison to be considerably larger than the dyads of the previous figure, and this agrees with the deduction made from the study of Stenobothrus in Part I of this paper, viz., that the total volume of chromatin on the secondary spermatocyte spindle is half that of the spermatogonial and primary spermatocyte cells.

The Chromosome Complex of Dissosteira carolina, *Linn.*

The diameter of the component rods is again seen to be 0·83 μ, and the heterotropic chromosome is distinguishable on account of its greater breadth. Figs. 411–2, Plate 4, show two ordinary chromosome rods taken from the secondary spermatocyte metaphase; fig. 412A is an example of the heterotropic chromosome.

Section B.—The Chromosome Complex of Allied Families.

Family Locustidæ.

The Chromosome Complex of Steiroxys trilineata.

Dr. H. S. Davis, of the University of Florida, Gainesville, has very kindly sent me preparations of this locustid, and I take this opportunity of again thanking him for them and for other material, which he has forwarded to me embedded in paraffin. The testes were fixed in Hermann's fluid, and the sections, cut 7 μ thick, were stained with Heidenhain's iron hæmatoxylin and Bordeaux red.

The spermatogonial metaphase shows 29 chromosomes, of which the majority are spherical or short rods; the largest is the heterotropic chromosome, recognisable on account of its breadth, which is at least one and a half times that of the ordinary rods. The diameter of the latter is invariably 0·83 μ, and this proves that the thread-

width previously observed is not confined to the Acridiidæ. Fig. 408, Plate 4, shows a spermatogonial metaphase, the heterotropic chromosome being marked X.

The spermatogonial and secondary spermatocyte chromosomes are individually composed of two equal rods, whose diameter is invariable throughout their length; the primary spermatocyte complex shows 14 tetrads, and, in 50 per cent. of the cells, the large heterotropic chromosome. Fig. 409 is an example of this metaphase. I have not measured the lengths of the rods, for most are spherical or very short, and an attempt at individual identification may lead to error; there is, however, no doubt that the short ordinary chromosomes represent Nos. 1, 2, 3, and 4. Fig. 410 shows the secondary spermatocyte metaphase of this species, and can be compared with the spermatogonial in which the complex is composed of pairs.

Family Gryllidæ.

The Chromosome Complex of Gryllus domesticus, Linn.

The crickets were caught in July, and the testes fixed in Perenyi's solution; sections were cut 8 μ thick and stained with Heidenhain's iron hæmatoxylin.

Twenty-one chromosomes are found in the spermatogonial, and 10 and 11 in the spermatocyte complexes, and these numbers agree with those given by BAUMGARTNER for the species. The ordinary chromosomes are composed of rods, whose diameter is invariably 0·83 μ, and this suggests that the thread-width is common to all Orthoptera. Moreover, the 10 rod-lengths are members of the general series, for they correspond respectively with Chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 14, and 15, and, since six of these lengths have already been observed in various species of Stenobothrus, similarity exists between complexes of the two families. BAUMGARTNER has remarked that the difference between certain spermatogonial pairs is so slight that grouping in threes or fours seems preferable, and visual examination of the eight short chromosomes unaccompanied by actual measurements undoubtedly suggests this. The heterotropic chromosome is equal in length to Chromosome 5, as in the case of Melanoplus, and can again be recognised by its great breadth, which is not constant throughout its length; it remains intact during the growth period and passes in this state to one pole during the first maturation mitosis. In the prophase of this division the condensing filaments appear in the characteristic form of rings and crosses, which represent spermatogonial pairs in conjugation; I have, however, failed to discover whether reduction is effected at the first or second mitosis.

Figs. 397–407, Plate 4, are examples of the heterotropic and 10 ordinary chromosome rods composing this complex, and the following table shows the dimensions and volumes as in previous cases:—

TABLE showing Measurements of Chromosomes composing Complex of *Gryllus domesticus*.

	Length of rod.	Diameter of rod.	Volume of dyad, *i.e.* of two rods.	Volume of tetrad, *i.e.* of four rods.
	μ.	μ.	Cub. μ.	Cub. μ.
Chromosome 15	6·7	0·83	6·9	13·8
„ 14	6·3	0·83	6·5	13·0
„ 9	4·2	0·83	4·2	8·4
„ 8	3·7	0·83	3·8	7·5
„ 7	3·3	0·83	3·3	6·6
„ 6	2·9	0·83	2·9	5·8
„ 5	2·5	0·83	2·4	4·8
„ 4	2·1	0·83	2·0	4·0
„ 2	1·2	0·83	1·0	2·0
„ 1	0·8	0·83	0·6	1·2
„ X	2·5	?	?	?
Total volume of chromatin on spindle of spermatogonial and primary spermatocyte cell, exclusive of the heterotropic chromosome . . .				67·1
Total volume of chromatin on spindle of secondary spermatocyte cell, exclusive of the heterotropic chromosome				33·6

Family Forficulidæ.

The Chromosome Complex of Forficula auricularia, *Linn.*

The material was collected in July, and preserved in the fixatives of Flemming, Hermann, and Perenyi; ZWEIGER recommends the first, but I have obtained best results with the second. The sections were cut 8 μ thick, and stained with Heidenhain's iron hæmatoxylin.

The spermatogonial complex is composed of 24 chromosomes, each consisting of two rods, whose diameter is invariably 0·83 μ. The length of the rods is 1·2 in 18, and 0·8 μ in 6 chromosomes. In the anaphase the rods of each pair pass to opposite poles. At the end of the growth period the granules on the linin threads split, and condensation proceeds until 12 compact tetrads appear on the spindle; these are of two sizes, but so small that I have failed to determine whether this mitosis separates rods on either side of the original longitudinal split, or on either side of the space between conjugating chromosomes. ZWEIGER sees here an instance of pre-reduction, but the question is unimportant, for this or the next mitosis must be reductional. In the interval between the maturation divisions the chromosomes remain as compact bodies scattered throughout the nucleus, but no increase of chromatin occurs, and consequently there is no true resting stage. The secondary spermatocyte metaphase shows 12 chromosomes, each composed of two rods, whose dimensions are identical with those of the spermatogonia, the length of nine being 1·2, and of three 0·8 μ.

ZWEIGER estimates the normal number of chromosomes as 26, but professes to have found cysts in which all spermatogonia had either 24 or 28, the corresponding spermatocytes having 13, 12, and 14 respectively; DE SINETY, however, counted only 24 and 12. In lateral views of metaphases I have noted chromosomes whose rods have separated and are passing to opposite poles while those of the remainder are still in juxtaposition in the equatorial plane; in polar views this would make the complex appear to be composed of an increased number if the equatorial plane was inclined slightly to the horizontal. Whenever I have counted 24 or 12, the chromosomes are lying close together and the complex is circular; when, however, a greater number is seen, the chromosomes are always more widely separated and the irregular complex outline suggests that the view is not strictly polar. If, as I believe, the number estimated originally by DE SINETY is correct, trimorphism does not exist, and ZWEIGER's refutation of the sex-determination theory is invalid.

ZWEIGER further states that two or three chromosomes do not become dissociated during the growth period. I have not observed this in all cells, and such bodies, when seen, appear to be nucleoli or chromosomes whose dissociation has been delayed; in the former the dimensions always exceed those of any chromosome seen on the spindle. His division of the chromosomes into 16 large, 2 medium, and 6 small, agrees with my results, but I have failed to distinguish an intermediate size, and have consequently classified them as 18 long and 6 short. The dimensions already given prove that they belong to the general series.

TABLE showing Measurements of Chromosomes composing Complex of *Forficula auricularia*.

	Length of rod.	Diameter of rod.	Volume of dyad, *i.e.* of two rods.	Volume of tetrad, *i.e.* of four rods.
	μ.	μ.	Cub. μ.	Cub. μ.
Chromosome 2	1·2	0·83	1·0	2·0
„ 1	0·8	0·83	0·6	1·2
Total volume of chromatin on spindle of spermatogonial and primary spermatocyte cell				21·6
Total volume of chromatin on spindle of secondary spermatocyte cell . .				10·8

Examples of metaphases are given on Plate 4. Figs. 389 and 390 represent the spermatogonial cell, and figs. 391–2 and 393–4 the primary and secondary spermatocytes respectively. Figs. 395 and 396 show two secondary spermatocyte complexes in which 14 chromosomes appear, but the irregular complex outline and widely separated chromosomes suggest that the equatorial plane is inclined slightly

and that rods of precociously split chromosomes are thus being mistaken for the chromosomes themselves. These drawings have been made from material fixed in the three solutions already mentioned, and prove that the fixative does not affect chromosome dimensions.

Summary of Part II.

The diameter of the ordinary chromosome rods of Stenobothrus is the same as that observed in organisms representing the allied sub-families, Acrididæ and Œdipodides, and the allied families, Locustidæ, Gryllidæ, and Forficulidæ; and, since all rod lengths in these organisms constitute members of the same series in arithmetical progression, we have strong reason for supposing that strict uniformity exists throughout the order.

Part III.—The Chromosome Complex of Organisms Belonging to the Remaining Phyla, Showing Morphological Correlation of Chromosomes Throughout the Metazoa.

Phylum Vertebrata.

Class Primates.

The Chromosome Complex of Homo.

I have not studied the human germ cells but have used sections of the embryo taken at the end of the third week. The material was preserved with Perenyi's fixative, and the sections were cut 8 μ thick and stained with Grenacher's hæmatoxylin.

The same chromosome diameter is again observed, and the component rods are similar in every respect to those of the Arthropoda already dealt with. Fig. 494, Plate 5, shows drawings of chromosome rods taken from several dividing cells of the embryo, and these are indistinguishable from many chromosomes already drawn; this is important, for it proves that the thread-width found in the Arthropoda is not confined to the phylum, and suggests its existence in Mollusca, Annelida, etc. We shall, however, have opportunity for testing this proposition when other types have been investigated, and in the meantime must be content with the establishment of a thread-width common to man and the Arthropods.

Class Amphibia.

The Chromosome Complex of Triton cristatus.

The material was collected in May and June, and fixed in the solutions of Flemming and Hermann, recommended by Janssens in his paper upon this subject. The sections were cut 8 μ thick, and stained either with Heidenhain's iron hæmatoxylin or iron brazilin.

The spermatogonial complex shows 24 and the primary and secondary spermatocytes 12 chromosomes. Each spermatogonial and secondary spermatocyte chromosome is composed of two and each primary spermatocyte of four rods, which again appear to be indivisible units, possessing the same dimensions in all metaphases. The diameter is invariably 0·83 μ, but I have experienced considerable difficulty in measuring lengths, for, where many are long, overlapping occurs in the equatorial plane, and few can be found lying at right angles to the microscopic line of vision throughout their entire length; although I have made 30 slides from this material, I have failed to find a cell in which all chromosomes can be identified, and the checking of measurements has consequently not been facilitated. The measurements, however, have been made as described for Stenobothrus in Part I, and the dimensions shown in the following table must represent as nearly as possible the true lengths. My results, moreover, agree with those of JANSSENS, who counted 12 pairs of chromosomes in the spermatogonia and found no two pairs to be of the same size. Members of a pair are often on opposite sides of the complex and appear to have no connection.

Examples of the 12 rod-lengths are given on Plate 5, where figs. 413–448 show 36 drawings; rods of the same length are placed one below the other, there being two stained with iron hæmatoxylin and one stained with brazilin, representing each length-group. We have already shown that the fixative has no effect upon chromosome dimensions, and these reproductions prove that the same can be said of the stain.

TABLE showing Measurements of Chromosomes composing Complex of *Triton cristatus*.

	Length of rod.	Diameter of rod.	Volume of dyad, *i.e.* of two rods.	Volume of tetrad, *i.e.* of four rods.
	μ.	μ.	Cub. μ.	Cub. μ.
Chromosome 24	10·4	0·83	11·1	22·2
„ 20	8·8	0·83	9·2	18·4
„ 18	7·9	0·83	8·4	16·8
„ 17	7·5	0·83	7·9	15·8
„ 15	6·7	0·83	6·9	13·8
„ 13	5·8	0·83	6·1	12·2
„ 12	5·4	0·83	5·6	11·2
„ 11	5·0	0·83	5·2	10·4
„ 9	4·2	0·83	4·2	8·4
„ 7	3·3	0·83	3·3	6·6
„ 4	2·1	0·83	2·0	4·0
„ 3	1·7	0·83	1·5	3·0
Total volume of chromatin on spindle of spermatogonial and primary spermatocyte cell				142·8
Total volume of chromatin on spindle of secondary spermatocyte cell .				71·4

The complex is seen not only to be composed of rods belonging, without exception, to the general series, but to possess certain chromosomes in common with types already studied. Chromosomes 11, 12, and 13 appear sometimes bent and sometimes as straight rods; in Stenobothrus, 11 and 13 were invariably bent.

Phylum Mollusca.

Class Gastropoda.

The Chromosome Complex of Helix.

Preparations of the hermaphrodite gland were kindly lent to me by Prof. Hickson from the zoological laboratories of Victoria University; the sections were cut 4 μ thick and stained with Heidenhain's iron hæmatoxylin.

I have unfortunately been able to find very few cells undergoing mitosis, and consequently cannot give measurements of all individuals composing the complex. Fig. 449, Plate 5, however, shows a polar view of the spermatogonial metaphase, in which 16 chromosomes appear; the same thread-width is again observable, and the chromosomes are composed of short rods. Fig. 450 is a drawing made from one pole of a telophase seen from the lateral aspect.

The chromosome rods appear to belong to the general series, and we have now observed the thread-width, 0·83 μ, in Vertebrata, Mollusca, and Arthropoda; this is important, for it suggests a common diameter throughout the animal kingdom.

Phylum Annelida.

Class Chætopoda.

The Chromosome Complex of Lumbricus.

The material was fixed in Perenyi's fluid and stained with Heidenhain's iron hæmatoxylin; Calkins found that the tissues shrank when embedded in paraffin, and consequently mounted teased portions entire, but I have not observed this in my sections.

There is no definite arrangement in zones, and cells of all stages are found together. The spermatogonia produced by repeated division form a multinucleate mass, which only later constricts into individual cells attached to a central protoplasmic body called the blastophore; an example of this early stage is shown in fig. 525, Plate 5, where the small size of the nuclei is particularly noticeable. Thirty-two chromosomes appear on the spermatogonial spindle, each composed of two equal rods or spheres; they appear to be of the same size, but may possess differences too small to be recognised. Their diameter is 0·83 μ, and they are accordingly of the general thread-width. Fig. 451, Plate 5, shows a spermatogonial metaphase; the constitution of each chromosome is not here evident,

for the upper rods or spheres cover those lying below. The last spermatogonial mitosis is followed by a normal growth period, in which the reticulum of the nucleus is re-established.

The prophase of the first maturation division is marked by the appearance of an apparently continuous spireme, which is longitudinally split, and breaks later into 32 chromosomes consisting, as in all previous mitoses, of two equal spheres. These unite in pairs, and are the best example of tetrad-formation that I have seen, for chromosomes here conjugate instead of condensing filaments as in other types.

The secondary spermatocyte spindle shows 16 chromosomes, similar in every respect to those of the spermatogonium. No resting stage has intervened, and the total amount of chromatin has accordingly been halved; an example of this metaphase is given in fig. 452. CALKINS states that maturation is post-reductional, but I have failed to discover whether the first mitosis separates halves of associating chromosomes or halves on either side of the original longitudinal split; the rods, however, are not individually bisected, so one division must be reductional.

Figs 453–455 represent a single rod, a dyad, and a tetrad respectively, and a comparison with those of other organisms proves that they belong to the general series.

TABLE showing Measurements of Chromosomes composing Complex of Lumbricus.

	Length of rod.	Diameter of rod.	Volume of dyad, *i.e.* of two rods.	Volume of tetrad, *i.e.* of four rods.
Chromosome 1	μ. 0·8	μ. 0·83	Cub. μ. 0·6	Cub. μ. 1·2
Total volume of chromatin on spindle of spermatogonial and primary spermatocyte cell				19·2
Total volume of chromatin on spindle of secondary spermatocyte cell . .				9·6

PHYLUM NEMATHELMINTHIA.

Class Nematoda.

The Chromosome Complex of Ascaris megalocephala.

The independent study of this species by several cytologists renders superfluous any allusion to the ordinary phenomena of maturation; there are, however, two features affecting our present research. Firstly, BOVERI has remarked that two varieties exist, differing only in the number of chromosomes; these have been called respectively "univalens" and "bivalens," according as their mature germ-cells have been found to contain one or two. WILSON has suggested as an explanation of this

dimorphism that the chromosomes of HERTWIG's "variety univalens" are bivalent, and equivalent to two such chromosomes as appear in "variety bivalens"; and further suggests that the latter in their turn are plurivalent and represent an aggregate of lower units. Secondly, he alludes to BOVERI's discovery that the germ cells can be traced back to the two-cell stage of segmentation by saying: "From the outset the progenitor of the germ cells differs from the somatic cells not only in the greater size and richness of chromatin of its nuclei, but also in its mode of mitosis; for in all those blastomeres destined to produce somatic cells a portion of the chromatin is cast out into the cytoplasm, where it degenerates, and only in the germ cells is the sum total of the chromatin retained." It will be remembered that HÄCKER observed that in Cyclops, although no chromatin is extruded, the germ nuclei are richer in chromatin, but contain fewer chromosomes than the somatic nuclei, and WILSON has again explained this by assuming the chromosomes to be bivalent.

The material that I have studied consisted of the ovary of "bivalens"; this was dissected out and preserved in Flemming's strong solution, the sections being subsequently cut 8 μ thick, and stained with Heidenhain's iron hæmatoxylin.

The chromosomes are again seen to be composed of cylindrical rods of an uniform diameter, which is the same as that found in the organisms already investigated, viz., 0·83 μ. The four chromosomes of the oogonia can be arranged in pairs as regards length, and these lengths reappear in the later secondary oocytes, in which only two chromosomes are found. Figs. 456–7, Plate 5, are examples taken from numerous camera lucida drawings made from these cells, and represent the two rods, whose lengths exceed those of any yet measured; the oogonia and primary oocytes possess two of each and the secondary oocytes one.

Let us now consider WILSON's explanation of the apparent dimorphism of this species. If, as he suggests, the two chromosomes in the secondary oocytes are equivalent to the single chromosome in the corresponding stage of "univalens," the volume of the latter must be 27·1 cu. μ, as can be seen from the following table; and, since secondary oocyte chromosomes are composed of two equal rods, the volume of each of the latter must be 13·55 cu. μ. The volumes of the rods in "bivalens" are respectively 7·55 and 6·00 cu. μ, and the difference must accordingly result from an increased diameter, an increased length, or both. But we have found that in various genera, tribes, families, orders, and classes of Arthropoda, and in classes representing Vertebrata, Mollusca, Annelida, and the phylum now under consideration the chromatin thread-width is constant and is 0·83 μ. If this thread-width is invariable throughout the Nemathelminthia—and we have strong reason for supposing that it is invariable—the increase in volume must be due to additional length only; and, since the combined lengths of the rods of "bivalens" are 25·4 μ, the single rods of "univalens" must be of this length. Having no material, I cannot say if this is so, but it seems improbable that a rod thus exists, whose length is approximately double that of the longest found in the

phyla mentioned above; moreover, the drawings of cells undergoing mitosis in "univalens" do not appear to warrant this assumption, and without this assumption we cannot accept WILSON'S interpretation of bivalency. There is, however, a possible solution of the problem. If the surplus chromatin cast out into the cytoplasm in segmentation of "bivalens" represents waste material, the chromatin that remains may possess a volume approximately equal to that seen in the germ cells of "univalens," and if eventually the germ cells of the former arise from a cell devoid of the surplus chromatin, the chromosomes will be the same in both varieties. It is thus possible that "univalens" has lost the waste chromatin, whereas "bivalens" is passing through a stage when extrusion is not complete.

In speaking of the Nemathelminthia SHIPLEY has said, "Their structure is unusually monotonous, and, owing perhaps to their largely parasitic mode of life, they show practically none of those external features which are so useful to the systematist in other groups."* It is, therefore, possible that we are dealing here with two distinct species, or even with distinct genera. This, however, is unimportant so far as this paper is concerned.

WILSON'S suggestion of plurivalency of chromosomes is possible so long as the latter are regarded as combinations of units end to end. In the somatic cells the long chromosomes are actually seen to break into shorter bodies equivalent to the spheres or short rods found in the allied *A. lumbricoides*, and, if this segmentation is later shared by the germ-cell chromosomes, complete similarity will be established between these apparently different complexes.

TABLE showing Measurements of Chromosomes composing Complex of *Ascaris megalocephala*, var. "*bivalens*."

	Length of rod.	Diameter of rod.	Volume of dyad, *i.e.* of two rods.	Volume of tetrad, *i.e.* of four rods.
	μ.	μ.	Cub. μ.	Cub. μ.
Chromosome 33	14·2	0·83	15·1	30·2
„ 26	11·2	0·83	12·0	24·0
Total volume of chromatin on spindle of oogonial and primary oocyte cell . .				54·2
Total volume of chromatin on spindle of secondary oocyte cell				27·1

PHYLUM NEMERTINEA.

The Chromosome Complex of Lineus lacteus, *Mont.*

For this study sections were cut of maturing ova and early blastula stages, the stain used being Heidenhain's iron hæmatoxylin.

* 'Cambridge Nat. Hist.,' 1896, vol. 2.

I have been unable to count the chromosomes composing the complex owing to their smallness, but the number probably agrees with that found by Coë in the allied Cerebratulus and Micrura, viz., 32 in the somatic, and 16 in the mature germ cells. Figs. 458–471, Plate 5, represent chromosomes of ova and blastomeres of the four- and eight-cell stages, and show that we have at last reached the limit of the general thread-width ; as in all previous cases the diameter is uniform throughout the length, but is considerably less than 0·83 μ. The lengths appear to be members of the series already enumerated, and I have reason for supposing that certain are respectively equivalent to Chromosomes 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, and 16 ; these measurements must not, however, be accepted without further corroboration. The diameter is invariably 0·42 μ, *i.e.* half that of all previously studied material. I shall deal with this phenomenon later, when we have investigated the remaining phyla of the animal kingdom.

Phylum Echinodermata.

Class Echinoidea.

The Chromosome Complex of Echinus esculentus.

The material consisted of preparations showing ova passing through the various phases of maturation, and the four-, eight-, and sixteen-cell stages of segmentation following amphimixis. Sections were cut 8 μ thick, and stained with Heidenhain's iron hæmatoxylin.

Boveri has counted 18 chromosomes in this genus, and the number of rod-lengths cannot therefore exceed nine. Drawings representing these are given in figs. 475–493, Plate 5, and show that, as in the Nemertinea, the general thread-width has been reduced by one half. In the figures, two rods of each length are placed one above the other, and the nine lengths appear to correspond exactly with those of Chromosomes 1, 3, 4, 5, 6, 7, 8, 11, and 12 of the general series ; I have found them difficult to measure, and the lengths shown may be slightly inaccurate in one or two cases, but no such difficulty has been experienced in determining the diameter, which is invariably 0·42 μ.

The following table shows the dimensions and approximate volumes of the rods ; whatever inaccuracy may exist in the lengths measured will not appreciably affect the validity of the total for purposes of comparison with other organisms. Since the thread-width is the same as in the Nemertinea, and half that of the other phyla studied, Roman numerals have been used to designate members of the series, in order that confusion may not arise between chromosomes of the two diameters.

TABLE showing Measurements of Chromosomes composing Complex of *Echinus esculentus*.

	Length of rod.	Diameter of rod.	Volume of rod.
	μ.	μ.	Cub. μ.
Chromosome XII	5·4	0·42	0·72
„ XI	5·0	0·42	0·66
„ VIII	3·7	0·42	0·49
„ VII	3·3	0·42	0·43
„ VI	2·9	0·42	0·38
„ V	2·5	0·42	0·32
„ IV	2·1	0·42	0·27
„ III	1·7	0·42	0·21
„ I	0·8	0·42	0·10
Total volume of chromatin on spindle of oogonia			14·32
Total volume of chromatin on spindle of secondary oocyte			7·16

Class Asteroidea.

The Chromosome Complex of Asterias glacialis.

The material used consisted of ova undergoing maturation, and the methods were the same as in Echinus.

I have not measured the lengths of all members of the complex, but figs. 472–474, Plate 5, represent rods taken from the oogonia, and the lengths are seen to be identical with certain of the general series. The diameter is again constant, and is the same as in Echinus, viz., 0·42 μ, and this presupposes uniformity throughout the phylum.

PHYLUM CŒLENTERATA.

Class Anthozoa.

The Chromosome Complex of Alcyonium digitatum.

I am indebted for my material to Prof. HICKSON, who kindly lent me preparations from his collection, cut 6 μ thick and stained with Delafield's hæmatoxylin; I also wish to thank him for valuable advice concerning the study of these cells and the systematic arrangement of organisms dealt with in this paper.

Apparently, chromosomes can be found only in certain stages, and are difficult to observe, even in segmenting ova. Fig. 495, Plate 5, is an example of a telophase taken from the latter, and shows the characteristic spindle and chromosomes. The thread-width is seen to be identical with that shown previously for Lineus, Echinus, and Asterias, and consequently appears to be common to all Metazoa below Nemathelminthia. Moreover, the chromosomes are shorter than those of the two

first named, and may be shorter than those of the last, which is represented on Plate 5 by only three rods.

Summary of Part III.

The diameter of the chromosome rods found in Arthropoda is the same as that in various organisms representing Vertebrata, Mollusca, Annelida, and Nemathelminthia. In the lower Metazoan phyla, viz., Nemertinea, Echinodermata, and Cœlenterata, this thread-width is seen to be halved, for all chromosome rods have a diameter of 0·42 μ. Certain lengths, moreover, appear to be common to Vertebrates, Arthropods, etc., and the general series is again established. It is, however, possible that intermediate lengths exist in this series, and that the difference between terms is less than we can at present determine with the means at our disposal.

Part IV.—The Chromatin Elements of the Protozoa.

We have found that the diameter of rods composing chromosomes of Arthropoda is invariable, and the respective complexes are combinations of lengths cut from one general thread; the results of further study have, moreover, suggested that this diameter will be found throughout the Vertebrata, Mollusca, Annelida, and Nemathelminthia, whereas in the Nemertinea, Echinodermata, and Cœlenterata it has been reduced by one-half. The Metazoa, therefore, appear divisible into two groups according as the rod-width is 0·83 or 0·42 μ. I now propose to compare these results with others obtained from the Protozoa, for we may thus be able to elucidate the problems of the origin of chromosomes and their later differentiation into the two groups mentioned above.

In what appears to be the most primitive type of Protozoa no nucleus exists, and the chromatin granules are scattered throughout the entire mass of protoplasm; this has been observed in certain Rhizopoda and Flagellata, *e.g.* Achromatium and Tetramitus, which have respectively been shown by Schewiakoff and Calkins to undergo a fission of granules prior to cell division. In remarking upon this Wilson says "the arrangement of chromatin granules to form chromosomes appears to be of a secondary importance as compared with higher forms," and refers again to it as "sustaining Brauer's conclusion that the essential fact in the history of the chromatin in mitosis is the fission of individual granules."

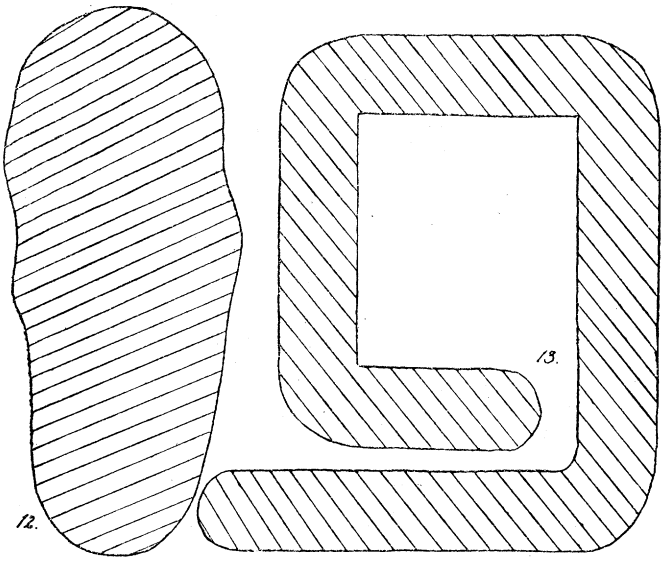
Chromosomes have, however, been observed in many forms, included in the Heliozoa, Rhizopoda, Flagellata, and Infusoria, and may or may not be preceded by a spireme stage. They appear to arise, as Wilson has shown, through the linear arrangement of granules, which often result from fragmentation of one or more large chromatin masses.

In the Infusoria, upon which exhaustive work has been done by Hickson and others, two kinds of nuclei exist, differing fundamentally and called respectively the mega-

nucleus and micronucleus. The former varies greatly in shape, being an ovoid mass in Dendrocometes and Paramœcium, and a semicircular rod in Carchesium, Epistylis, and Vorticella; in Dendrosoma it is a straight rod extending throughout the stolon and branching into the bases of the arms. Its division is invariably amitotic, and may be followed by fragmentation. HICKSON has shown that in the last named it is composed of granules varying in size, and this agrees with the observations of COLLIN and ENTZ upon Ephelota and Acineta respectively; he has, moreover, pointed out that its division is unequal in these organisms, for the gemmule receives only a small portion of the meganuclear substance of the parent cell. The micronuclei, on the other hand, always divide mitotically, and with certain unimportant modifications a spindle and chromosomes are formed similar to those observed in the Metazoa. The number of micronuclei is not invariably constant for the species, and varies considerably in different types; *Paramœcium caudatum* and Vorticella have one, *Paramœcium aurelia* two, Spirochona three, Dendrocometes two to five, and Stentor more than twenty. When, however, they are multiple, one only takes part in conjugation, while the remainder degenerate and disappear.

HICKSON believes that the meganucleus is purely somatic in function and that the micronucleus alone corresponds with the sexual nucleus of the Metazoa; LILLIE affirms that detached portions devoid of meganucleus invariably perish, and LE DANTEC insists that the micronucleus is not indispensable in regeneration of lost parts. We need not concern ourselves, however, with these problems beyond determining how far they affect the substance of our present research.

Throughout the Metazoa reproduction may be said to depend upon certain



DIAGRAMS 12 and 13, representing meganuclei of Paramœcium and Vorticella respectively. (× 1524.)

phenomena occurring within the single nucleus of the germ cell. These nuclei vary in size according to their stage of development, but no marked difference exists

between representatives of the various phyla, as can be seen from Plate 5, where figs. 525–531 depict resting nuclei of a vertebrate, arthropod, annelid, nemathelminth, nemertine, and echinoderm. If now we consider the Protozoa, we find that in all possessing a nucleus one only takes part in conjugation and is similar in structure to those of the Metazoa; figs. 523–4 show resting stages of such nuclei and can be compared with those already mentioned. The meganucleus of the Infusoria, however, is a body differing in every respect from any observed in organisms above or below this group; no chromosomes have been found in it, and its division is not mitotic. Its size, moreover, is greatly in excess of all sexual or somatic nuclei, as can be seen from diagrams 12 and 13, whose scale is one-half that of the figures of the plates. Whatever function is eventually assigned to it seems unlikely to be connected with the normal process of reproduction accompanied by chromosome-formation, and we may accordingly ignore it in this paper.

Class Sporozoa.

The Chromosome Complex of Monocystis agilis.

This Protozoon is found in the seminal vesicles of Lumbricus, and the methods of fixation and staining were the same as those described in the case of the latter.

According to recent researches (see Brasil and Hoffmann) two adult individuals of Monocystis come together and become enclosed in a common cyst wall. The nucleus of each individual in the cyst undergoes several successive mitoses and the resultant nuclei become the nuclei of the gametes. The zygotes formed by the conjugation of the gametes give rise to the sporoblasts, and each of these divides to form the eight sporozoites of the pseudonavicellæ.

All the chromosomes drawn are taken from the mitoses found in the formation of the sporozoites. I have been unable to count these or measure lengths accurately, but the diameter of the thread is invariably 0·21 μ. Examples of chromosomes are given in figs. 519–522, Plate 5.

Class Flagellata.

The Chromosome Complex of Euglena viridis.

The specimens, which were mounted whole, were fixed in corrosive sublimate and acetic acid, and stained with Grenacher's hæmatoxylin.

The chromosomes appearing on the spindle are numerous, and I have been unable to count them or determine lengths of all; from the measurements made the latter seem to belong to the general series, for I have identified chromosomes corresponding with Nos. 2, 4, 5, 6, and 9. The diameter of all chromosomes studied is 0·21 μ. Figs. 496–507, Plate 5, show 12 rods drawn from a polar aspect of the equatorial plane.

Class Infusoria.

The Chromosome Complex of Paramœcium caudatum.

The material was cut in sections, and the fixation and staining were the same as those adopted for Euglena.

The micronucleus increases considerably in size before the formation of the mitotic spindle, upon which many small chromosomes appear. Figs. 508–518 are examples of these, and apparently correspond with Chromosomes 5, 6, 7, and 8 in certain cases. Lengths are difficult to measure accurately, but the diameter is invariably 0·21 μ.

Summary of Part IV.

In all Protozoa studied the thread-width of chromosomes is found to be 0·21 μ, *i.e.*, half that of all phyla including and below Nemertinea, and one quarter of all phyla above the latter. Rods are seen, moreover, to correspond in length with certain of the general series already drawn, but intermediate lengths probably exist; if such occur, the difference between terms of the series is smaller than our present estimate.

Part V.—Disquisition, Hypothesis, General Summary, Bibliography, and Explanation of Plates.

Disquisition.

Resolution of the Spermatogonial Complex into Pairs of Chromosomes.

All writers believe that the chromosomes of a complex possess definite size-relationships, which are constant for the species, and this has been proved by exact measurements in the first part of this paper. The phenomenon has been dealt with by many investigators, particularly in America, where McClung pointed out in 1905 that a genus is marked by the characteristic arrangement of a series of chromosomes, and that a species exhibits this grouping but is distinguished on account of size differences in chromosomes, spindles, and other cell-parts.

The existence of pairs of chromosomes was first noticed and described by Montgomery in the spermatogonial cells of the Hemiptera, and was shortly afterwards observed by Sutton in the corresponding stage of the Orthopteron, *Brachystola magna;* the work of these two writers has led to further research and to the belief, now held by most cytologists, that the members of each spermatogonial pair are derived respectively from the male and female parent. In every organism studied, the somatic or spermatogonial number of chromosomes has been found to be halved in the primary spermatocyte metaphase, and, since the same size-relationships persist in the latter, we have been forced to conclude that an association of chromosomes occurs during the growth period or primary spermatocyte prophase.

The pairing of the spermatogonial chromosomes has been observed by MONTGOMERY in Amphibia, and by the SCHREINERS in Pisces. It has been found in the Arachnids, Epeira and Lycosa, by BERRY and MONTGOMERY; the latter has, moreover, described it in the Nemathelminth, Ascaris, and the SCHREINERS have found it in Tomopteris. It appears to be a feature common to the germ cells of all insects. WILSON'S researches upon the Hemiptera have corroborated the original work of MONTGOMERY upon that group, and further support has been given by the investigations of STEVENS upon Aphis. In the Orthoptera it has been noted by BAUMGARTNER in Gryllus, DE SINETY and ZWEIGER in Forficula, and by DAVIS, GÉRARD, MCCLUNG, MONTGOMERY, NOWLIN, OTTE, and ROBERTSON in many genera of the Acridiidæ and Locustidæ; moreover, many of these organisms have been dealt with in this paper, and the measurements given prove the widespread occurrence of this phenomenon. I know of no evidence that it does not occur, and, until this is found, we may assume it to be universal.

MONTGOMERY originally stated that the members of each pair differ slightly, the member derived from the female parent being the larger; this has not been corroborated by later investigations, and I have seen no trace of it in my material. He further stated that the members of each pair are intimately connected and lie close to one another in the equatorial plane. This may have been observed in certain organisms but is not found in all, for we have seen that members of a pair may lie on opposite sides of the spindle; in the drawing of a spermatogonial cell on Plate 3, the two chromosomes representing Nos. 5, 7, 11, 13, and 17 lie either on sides of the spindle opposite to one another or far apart.

It must be remembered that the doctrine of subsequent conjugation of like members is still opposed by certain cytologists, but, whether this explanation of numerical reduction is eventually proved or disproved, the phenomenon of the pairs must remain.

The Growth Period.

This name has been given to the resting stage preceding the maturation mitoses because the cytoplasm increases considerably in volume at this period. Certain writers, however, have remarked that the loss of chromatin resulting from repeated division must be counteracted by a corresponding growth at some stage, for otherwise the total amount in the cell will eventually become infinitesimally small; consequently, it has been suggested that the increase seen is not confined to the cytoplasm, but is shared by the chromatin itself, and that the total volume of the latter thus remains more or less constant. This view has, moreover, led to the further hypothesis that all resting stages between successive mitoses are concerned with an increase in chromatin volume, and that the loss is not compensated only in the growth period.

This doctrine was put forward by JANSSENS in 1901, for he says, in a paper upon Tritons: "Le filament nucléinien s'est nourri pendant tout le stade qui sépare les

couronnes polaires d'une division et le stade peloton de la division suivante, et on peut affirmer qu'il a pour le moins doublé sa masse . . . . à chaque étape de repos entre deux divisions successives, un nouvel élément nucléinien s'élabore à l'intérieur de l'ancien." In speaking of the actual growth period, he further says : " Il s'agit ici de cellules qui sont dans une période de puissante nutrition et surtout d'abondante production de substance nucléinienne." The same view was expressed by McClung, seven years later, in Volume 4 of the 'Kansas University Science Bulletin,' for he there suggests that the chromosomes increase in size after each division by taking up material from the cytoplasm ; he seems, however, to emphasise increase in the latter during the growth period, and affirms that at this stage reproduction is replaced by constructive metabolism, and the chromosomes, after exhausting their metabolic resources, unite their common energies to build up a new cytoplasm.

We have found in Stenobothrus that the volumes of individual chromosomes are constant, and that the total volume of chromatin is the same in spermatogonial and primary spermatocyte cells, whereas only half this amount is observed in the secondary spermatocytes. Since half of the spermatogonial volume of chromatin passes to each daughter primary spermatocyte, the volume must be exactly doubled at some stage before the first maturation mitosis ; this can occur only during the intervening growth period, when the ordinary chromosomes are dissociated in granules, and the reticulum persists until these split upon the linin threads. The fission of granules must accordingly be regarded as the end of the growth period so far as the chromatin is concerned, for it cannot occur before their volumes have been doubled by absorption from the cytoplasm. Further proof is furnished by the behaviour of the heterotropic chromosome, which takes no part in the general dissociation, but remains throughout this period as a darkly staining and more or less compact body apposed to the nuclear membrane. All writers are agreed that this chromosome passes entire to one daughter cell at a subsequent mitosis ; and, since no loss in volume is thereby entailed, an increase prior to division is unnecessary.

Gérard has denied a conjugation of chromosomes, but affirms that the granules become associated in pairs : I believe that he has mistaken the fission of granules for this association. In an earlier paper upon the spermatogenesis of *S. viridulus* I likewise failed to realise the significance of this cleavage, but have since studied my preparations again and am convinced that the chromosomes conjugate in pairs.

The growth of the cytoplasm does not end with the splitting of the granules, but continues until the polar loops have evolved from the reticulum ; in Stenobothrus it attains its maximum size when the chromatin filaments have condensed sufficiently to be counted. It therefore seems impossible that this subsequent growth can be due to chromatin influence. The volume of chromatin has been shown to remain constant, whereas that of the cytoplasm varies considerably in successive cell generations ; consequently the chromatin must maintain its volume at the expense of the cytoplasm. The observations of numerous investigators upon the absorption

and extrusion of nucleinic acid by the former are in accord with this view, for the condensing filaments have been shown to become richer in their percentage of this acid as they approach the characteristic shapes of the metaphase figure. It is difficult to believe that the chromatin exists at the expense of the cytoplasm, and that the latter conversely exists at the expense of the former, and, since the chromatin remains constant in volume, whereas the cytoplasm varies, we must assume that the first is the important substance, and that the second is purely nutritive. It may be argued that the cytoplasm is not a homogeneous compound, and that it contains substances that furnish reserve material both for itself and the chromatin, for we know that a close relationship exists between the two and that an exchange of material constantly occurs between them; this, however, cannot affect McClung's suggestion that the chromosomes put forward their common energies to construct a new cytoplasm, and I find it impossible to reconcile this view with the facts now before us.

The explanation of the growth period as a means of counteracting the loss of chromatin entailed by mitosis must therefore be accepted as true, and the view of Janssens is proved in entirety. If the dissociation of chromosomes into minute granules occurs for the purpose of offering a greater surface for nutrition, we must conclude that the same reason governs dissociation in all resting stages. The secondary spermatocyte mitosis is the only division not preceded by a resting stage, and consequently no increase of chromatin volume takes place; it is at this division that reduction occurs by which the somatic amount is halved, and the mature spermatozoon thus carries a volume that combines with the chromatin of the ovum to form once more the amount constant for the species.

The Maturation Mitoses.

Two types of maturation have been postulated, viz., eumitotic, in which both mitoses are supposed to divide the chromosomes equationally, and pseudomitotic, in which one is said to separate morphologically univalent elements; the latter, moreover, has been divided into pre- and post-reductional, for some writers affirm that this separation occurs at the first division, and others that it occurs at the second.

The whole controversy concerning reduction in this sense of the word has arisen from the great difficulties experienced in determining the constitution and plane of cleavage of the tetrads or their equivalents. Two processes of tetrad-formation have been described, and are the same in principle. In the first type they arise from a continuous spireme thread, which segments transversely into rods representing half the somatic number of chromosomes; each rod, considered as morphologically equivalent to two chromosomes, joined end to end, splits longitudinally, and a quadrivalent element is thus formed. Where true tetrads are not found, the chromosome is composed of two arms, formed by the longitudinal cleavage of a single primary rod; these rods open out to form a ring, their ends remaining in

contact. In the second type the tetrads are formed by a conjugation of chromosomes during synapsis, and, since each conjugating chromosome is longitudinally split, a quadrivalent body is again produced. In this case reduction in the number of chromosomes is not effected by the segmentation of a spireme thread into double lengths, but by association of members of the spermatogonial pairs.

The first type of tetrad was described by BOVERI in 1887 in a paper upon Ascaris; both divisions were said to be longitudinal and equational, consequently no reduction in WEISMANN'S sense occurred. This was corroborated in 1893 by the work of BRAUER upon the same material, but the more recent researches of MARCUS and TRETJAKOFF have shown one division to be transverse and reductional. Other writers, moreover, have found this method of tetrad-formation to be invariably accompanied by one longitudinal and one transverse division, as may be seen from the papers of HÄCKER, HENKING, PAULMIER, RÜCKERT, TOYAMA, and VOM RATH; and it is probable that pseudomitosis will eventually be established for all tetrads of this type.

The second type has been observed by many writers, but great difference of opinion exists as to the manner in which the chromosomes conjugate; some affirm that the union is end to end, others affirm that it is strictly lateral. MONTGOMERY believes that the members of the spermatogonial pairs become attached to one another, end to end, after the last spermatogonial division, and, since these rods split longitudinally, a quadrivalent body is formed similar to that already described. This end-to-end union is accepted by BLACKMAN for Myriapoda, DAVIS, NOWLIN, ROBERTSON, and SUTTON for Acridiidæ and Locustidæ, DUBLIN for Pedicellina, KING for Bufo, FARMER and MOORE for Selachians, and MOTTIER and WASSILIEFF for other organisms. On the other hand, a side-to-side conjugation has been noted by VON WINIWARTER in Mammalia, JANSSENS in Amphibia, MARÉCHAL and the SCHREINERS in Pisces, BONNEVIE in Gastropoda, MARCUS and TRETJAKOFF in Ascaris, the SCHREINERS in Annelida, and LERAT in Crustacea; the SCHREINERS even affirm that lateral conjugation takes place granule by granule, and a similar phenomenon has recently been described by GÉRARD in *Stenobothrus biguttulus*.

MEVES has denied that true tetrads occur in Salamandra, and has shown that at the first maturation division the rings break at the points of junction of the ends; both divisions are said to be equational, for the second separates halves on either side of a primary longitudinal split. This example of eumitosis has not been corroborated by the later work of MONTGOMERY and the SCHREINERS upon the same and allied material; JANSSENS upheld the views of MEVES in a paper upon Tritons, but has since shown a reducing division in Batrachoseps. DE SINETY attempted in 1901 to prove two equational divisions in the Phasmidæ, but his arguments are inconclusive. In the circumstances, we have reason for supposing that pseudomitosis will eventually be established for tetrads belonging to the second type.

Most cytologists now accept the doctrine of pre-reduction, and among these may be

mentioned Bouin, Collin, Davis, Farmer, Grégoire, Henking, Holmgren, Janssens, King, Korschelt, Lerat, Maréchal, McGill, Montgomery, Moore, Nichols, Nowlin, Paulmier, Schockaert, the Schreiners, Stevens, Wallace, Wilson, and Zweiger. The principal supporters of post-reduction are Blackman, Griffin, Gross, Henderson, Linville, McClung, Robertson, Sutton, Tretjakoff, Voinov, and vom Rath.

I have shown in my measurements of chromosomes in Stenobothrus that the rods are indivisible units, possessing invariable dimensions in all metaphases of the species. In the spermatogonial figure there are 16 pairs of these rods representing the eight length-groups; in the primary spermatocyte cell there are eight groups of four rods, and in the secondary spermatocyte eight groups of two rods. It is impossible to measure accurately the primary spermatocyte chromosomes, but a study of the shortening and condensing filaments in the prophase of this mitosis conclusively proves that the tetrads are formed by the association of two longitudinally-split chromosomes, which later become twisted one upon the other; these associating chromosomes are undoubtedly the members of the spermatogonial pairs, and assume the form of rings, crosses, and figures of eight. The method of association varies, for certain chromosomes unite end to end, whereas others lie across one another at a point in their length. The free ends eventually come in contact, and the result in every case is a figure that can be represented diagrammatically by four parallel rods, of which two have arisen from the longitudinal cleavage of one associating chromosome, and two from a similar cleavage of the other. We thus have a quadrivalent chromosome morphologically identical with that described by Boveri and others in the first type of tetrad-formation.

I am convinced that the space enclosed by rings and figures of eight separates two longitudinally split chromosomes. De Sinety's proof of two equational divisions was based upon the belief that this space arises from the opening out of halves of a longitudinally split rod—a belief that is contrary to the interpretation of most cytologists.

The plane of cleavage in both maturation divisions of Stenobothrus is parallel to the length of the rods, which are never divided into smaller units; both mitoses are therefore longitudinal, as de Sinety has pointed out. If the first maturation division separates halves on either side of the ring space, a reduction must occur; each daughter secondary spermatocyte will receive two rods, formed by the cleavage of one member of each spermatogonial pair. The second maturation division must therefore be equational. If on the other hand the first mitosis separates halves of each longitudinally split chromosome—halves that have arisen as a direct result of growth and cleavage of granules during the preceding growth period—the division must be equational, and each daughter cell will receive two rods representing respectively one half of a paternal and maternal chromosome. The subsequent mitosis must separate these rods, and will consequently be reductional.

If the two methods of conjugation inevitably result in tetrads similarly constituted, the controversy regarding end-to-end and side-to-side union must be meaningless. Moreover, it seems probable that the question of pre- and post-reduction is also unimportant, for, according to these results, one maturation division must separate paternal and maternal elements. I am inclined to think that pre-reduction will eventually be found to be universal, but it is possible that both methods exist in the animal kingdom.

The Heterotropic Chromosome.

An odd or unpaired chromosome in the spermatogonial complex was discovered by HENKING, who in 1891 showed that in Pyrrhocoris dimorphism of spermatozoa is caused by the passage of this chromosome entire to a daughter cell during one maturation mitosis; this phenomenon has since been observed in many organisms, as can be seen from the papers of BAUMGARTNER, BERRY, BLACKMAN, DAVIS, GÉRARD, GROSS, GUTHERZ, MCCLUNG, MONTGOMERY, NOWLIN, OTTE, PAULMIER, ROBERTSON, DE SINETY, STEVENS, SUTTON, WALLACE, WILSON, ZWEIGER, and myself. WILSON, moreover, showed that in insects allied to Pyrrhocoris, viz., Anasa, Alydus, Harmostes, and Protenor, the complex of the female contains a pair instead of an odd chromosome, and this has been corroborated by the more recent work of MORRILL upon Anasa, Chelinidea, Protenor, etc. The existence of two chromosomes in the female germ-cell corresponding to one in the male immediately suggested that only spermatozoa possessing the odd chromosome can take part in the production of females on amphimixis, whereas only those devoid of it can take part in the production of males; thus the odd or heterotropic chromosome was regarded as a possible sex-determinant, and the theory formulated by MCCLUNG in 1892 is now held by many cytologists. Furthermore, the recent work of BALTZER (1909) has shown that dimorphism of mature germ-cells is not confined to the male organism, for in the sea urchins, Sphærechinus and Echinus, 50 per cent. of the mature ova contain a chromosome not found in the spermatozoa; the male is therefore homogametic and the female digametic.

In 1905 WILSON discovered a second type of dimorphism in the Hemiptera, Brochymena, Cœnus, Euchistus, and Lygæus, and a similar phenomenon was observed by STEVENS in the Coleopteron, Tenebrio. In this case the males have two unpaired chromosomes in the spermatogonia, one being large and the other small; the female complex has a pair corresponding with the larger of the two. This second type has been found in other Hemiptera by BORING, MORRILL, PAYNE, and STEVENS, in Coleoptera and Diptera by STEVENS, in Orthoptera by WASSILIEFF, DAVIS, GUTHERZ, JORDAN, and MORSE, and in Nemathelminthia by BORING, BOVERI, EDWARDS, and GULICK. These idiochromosomes have been designated X and Y by WILSON, who says: "If Y is supposed to disappear, the second type becomes identical with the first. It is almost certain that such has been the actual origin of the first type; for I was able to show that in different species of Hemiptera a series

of gradations exists between forms in which Y is nearly as large as X (Mineus, *Nezara hilaris*), and those in which it is very small (Lygæus, *Nezara viridula*, etc.). Its final disappearance would leave X without a synaptic mate as an odd or unpaired chromosome." In this manner the phenomenon of the second type is regarded as a modification of that of the first; spermatozoa possessing the large idiochromosome must apparently produce females, and those possessing the small idiochromosome males.

In certain organisms, *e.g.*, Agalena, *Ascaris lumbricoides*, Conorhinus, Fitchia, Homo, Phylloxera, Syromastes, etc., the X chromosome has been observed to be multiple, consisting of two or more equal or unequal components; these are separate in all ordinary mitoses, but in the maturation divisions of the male combine to form a group, which passes entire to one pole. The female complex contains two such groups, and the number of chromosomes in the two sexes may therefore differ by two, three, four, or five. Moreover, recent work has shown that in aphids, females are produced from all fertilised ova, and functional spermatozoa are apparently descended only from spermatids possessing the odd or X chromosome.

This interpretation is not accepted by all writers. PAULMIER and ZWEIGER have independently affirmed that such chromosomes are not concerned with sex-determination, but are degenerating and will eventually become extinct; FOOT and STROBELL have denied the existence of a heterotropic chromosome in Anasa, believing that the body in question is merely a plasmosome; and the same opinion is held by MOORE and ROBINSON regarding Periplaneta. There seems, however, little doubt that such chromosomes exist, although they may not be recognisable in all organisms, and, if this is true, they must be regarded as an accompaniment if not an actual determinant of sex. WILSON considers them as a link in a chain of factors by which sex is determined and inherited, and has formulated a physiologically quantitative conception of sex-determination reconciling the latter with the plus and minus principle underlying Mendelian inheritance; and a similar interpretation has now been adopted by BOVERI and GOLDSCHMIDT.

Consideration of the measurements given in this paper will, I think, show that the remarkable feature of the heterotropic chromosome is not its behaviour in the growth period and first maturation division, but its diameter. In all organisms belonging to the five highest phyla of the animal kingdom the chromosome rods have been found to possess a common diameter, and to differ from one another only in length; the diameter of the odd chromosome, however, exceeds the thread-width of all ordinary rods and is not constant throughout its entire length. This does not seem to have been noted previously, but is most significant; for, unless the abnormal diameter is due to conjugation and fusion of ordinary rods, we must be dealing here with a body differing from the latter in every respect. This explanation of plurivalency may, however, be true, for gradations have been observed between the apparently homogeneous heterotropic chromosome and corresponding bodies composed of several

more elementary units, such as are seen in the germ cells of Acholla ; the one may be a later stage of the other. In the present state of our knowledge, speculation regarding the particular function of this chromosome seems useless, but I am inclined to think that it is undergoing some process of evolutionary development or degeneration, resulting eventually in the formation of a chromosome of normal thread-width or in complete extinction ; and the former suggestion appears to be the more probable. That it accompanies sex-determination cannot be denied, but this can be no reason for assuming that it is the controlling agent in this matter ; we must accordingly be careful not to transform into a dogma an assumption that may or may not ultimately be proved.

The Number of the Chromosomes.

The following list compiled from papers of numerous writers shows the number of chromosomes in complexes throughout the Metazoa.

Class.	Order.	Organism.	Number.	Author.
		PHYLUM VERTEBRATA.		
Mammalia	Primates	Man	16	Bardeleben.
	Rodentia	Rat	16	Moore.
		Guinea-pig	16	Bardeleben.
		Mouse	24	Sobotta.
	Ungulata	Ox	16	Bardeleben.
Amphibia	Urodela	*Triton cristatus*	24	Meek.
		,,	24	Janssens.
		Salamandra maculosa	24	Flemming.
	Anura	*Rana*	24	Vom Rath.
Pisces	Cyclostomi	*Myxine glutinosa*	54 ?	Schreiners.
	Teleostei	*Salmo*	24	Bohm.
	Selachii	*Scyllium*	24	Moore.
		PHYLUM MOLLUSCA.		
Gastropoda	Pulmonata	*Limax agrestis*	32	Linville.
		,, *maximus*	32	,,
		Limnæa elodes	32	,,
		Helix	24	Vom Rath.
		Carinaria	32	Boveri.
		Phyllirhoe	32	,,
		Pterotrachea	32	,,
		Crepidula	60	Conklin.
		Enteroxenos ostergreni	34	Bonnevie.
		PHYLUM ARTHROPODA.		
Crustacea	Copepoda	*Cyclops strenuus*	24	Rückert.
		,, ,,	24	Häcker.
		Anomalocera	32	Vom Rath.
		Diaptomus	32	Rückert.
		Euchæta	32	Vom Rath.
		Heterocope	32	Rückert.
	Isopoda	*Oniscus asellus*	32	Nichols.
Myriapoda	Schizotarsia	*Scutigera forceps*	37	Medes.
	Symphyla	*Scolopendra heros*	33	Blackman.

Class.	Order.	Organism.	Number.	Author.
		PHYLUM ARTHROPODA—*continued.*		
Insecta	Orthoptera . . .	*Mermiria*	23	McClung.
		Syrbula admirabilis . . .	23	Robertson.
		,, *fusca-vittatus* . . .	23	,,
		,, *acuticornis* . . .	20	Montgomery.
		Stenobothrus bicolor . . .	17	Meek.
		,, *viridulus* . . .	17	,,
		,, *parallelus* . .	17	,,
		,, *biguttulus* . .	17	Gérard.
		,, *curtipennis* . .	17	Davis.
		,, *vagans* . . .	17	De Sinety.
		Dissosteira carolina . . .	23	Davis.
		Arphia tenebrosa	23	,,
		,, *pseudonietana* . .	23	Meek.
		Hippiscus tuberculatus . .	23	Davis.
		Chortophaga viridifasciata .	23	McClung.
		Caloptenus femur-rubrum .	24	Wilcox.
		Melanoplus atlansis . . .	23	Meek.
		,, *angustipennis* .	23	,,
		,, *dawsoni* . . .	23	,,
		,, *packardii* . . .	23	,,
		,, *bivittatus* . . .	23	,,
		,, ,, . . .	23	Nowlin.
		,, *femoratus* . . .	23	Davis.
		Brachystola magna . . .	23	Sutton.
		Steiroxys trilineata . . .	29	Davis.
		,, ,, . . .	29	Meek.
		Xiphidium fasciatum . . .	33	McClung.
		Locusta viridissima . . .	33	Otte.
		Orphania denticauda . . .	31	De Sinety.
		Forficula auricularia . . .	24	Meek.
		,, ,, . . .	24	De Sinety.
		,, ,, . . .	24—28	Zweiger.
		Labidura riparia	24	De Sinety.
		Leptynia attenuata . . .	36	,,
		Gryllus assimilis	29	Baumgartner.
		,, *domesticus* . . .	21	,,
		,, ,, ...	21	De Sinety.
		,, ,, ...	21	Meek.
		Gryllotalpa vulgaris . . .	12	Vom Rath.
	Lepidoptera . .	*Papilio rutulus*	28	Munson.
		Pieris brassicæ	28	Henking.
		Bombyx mori	28	Toyama.
		Liparis	20—24	Mayzel.
	Coleoptera . . .	*Dytiscus marginalis* . . .	40 ?	Henderson.
		,, ,, . . .	40	Giardina.
		Silpha carinata	32	Holmgren.
		Hydrophilus	16	Vom Rath.
	Hymenoptera . .	*Lasius niger*	20	Henking.
	Hemiptera . . .	*Pentatoma*	14	Montgomery.
		Pyrrhocoris apterus . . .	24	Henking.
		Syromastes marginatus . .	22	Gross.
		Agelastica alni	24	Henking.
		Aphis rosæ	—	Stevens.
		,, *œnothera*	—	,,
Arachnida . . .	. . . . .	*Agalena nœvia*	40 ?	Wallace.
		Lycosa insopita	28	Montgomery.
		Epeira	—	Berry.

Class.	Order.	Organism.	Number.	Author.
		PHYLUM ANNELIDA.		
Chætopoda . . .	Oligochæta . . .	*Allolobophora*	22	Foot.
		Lumbricus	32	Calkins.
		,, ...	32	Meek.
	Polychæta . . .	*Ophryotrocha puerilis* . . .	4	Korschelt.
		Chætopterus	18	Mead.
		Myzostoma gabrum . . .	24	Wheeler.
		Tomopteris onisciformis . .	28	Schreiners.
	Gephyrea . . .	*Thalassema*	24	Griffin.
Onychophora . .	. . . . .	*Peripatus balfouri*	28	Montgomery.
		PHYLUM NEMATHELMINTHIA.		
Nematoda . . .	. . . . .	*Ascaris megalocephala* . .	2, 4	Boveri.
		,, ,, . .	2, 4	Vom Rath.
		,, *lumbricoides* . . .	24	Carnoy.
		,, *clavata*	24	,,
		Strongylus filaria	12	Struckman.
		Spiroptera	12	Carnoy.
		Filaroides	16	,,
		Coronilla	8	,,
Chætognatha . .	. . . . .	*Sagitta bipunctata*	18	Stevens.
		,, *elegans*	18	,,
		PHYLUM NEMERTINEA.		
Turbellaria . . .	Polycladidæ . .	*Prosthercerœus vittatus* . .	12	Francotte.
		Prosthiostomum siphunculus .	16	,,
		Leptoplana tremellaris . .	16	,,
		Cycloporus papillosus . . .	16	,,
		Planaria simplissima . . .	8	Stevens.
		Thysanozoon	18	Van der Stricht.
Trematoda . . .	Polystomeæ . .	*Polystomum integerrimum* .	8	Goldschmidt.
	Schizonemertea .	*Cerebratulus*	32	Coë.
		Micrura	32	,,
		PHYLUM ECHINODERMATA.		
Echinoidea . . .	Regularia . . .	*Echinus*	18	Boveri.
		Toxopneustes	36—38	Wilson.
		PHYLUM CŒLENTERATA.		
Hydrozoa . . .	Anthomedusæ . .	*Tiara*	28	Boveri.
	Leptomedusæ . .	*Æquorea forskalea* . . .	12	Häcker.

This table is incomplete, but suffices to prove the following propositions:

(1) The number of chromosomes usually varies from 16 to 32; 24 is most often found.

(2) The number is often the same in widely separated organisms.

(3) The number sometimes varies considerably in closely allied organisms.

(4) The number neither increases nor decreases as we pass from low to higher phyla, and no correlation exists between it and our present classification.

Dimensions of the Chromosomes.

I am aware of no previous attempt to measure all chromosomes of a complex, and writers appear to have been content with division of the latter into large, medium, and small when such size-differences were evident; moreover, the chromosomes have sometimes been observed to constitute an approximately graded series, and the reappearance of these relationships in subsequent cell generations is regarded as proof of morphological individuality. Furthermore, similarity between complexes of certain allied groups has suggested correlation of individual chromosomes and definite somatic characters, and the establishment of this correlation has been, and still is, the aim of McClung and other American cytologists.

The theory of morphological individuality has been confirmed by the comparative study dealt with in this paper, for we have proved that chromosomes possess constant dimensions and can be identified in successive metaphases. The diameter of the component rods is 0·21 μ in Protozoa, and 0·42 and 0·83 μ in low and higher Metazoan phyla respectively, and no difficulty has been experienced in determining these widths, for all phases of mitosis are suited to such measurements and foreshortening does not occur. The existence of intermediate widths is possible but unlikely, since many organisms have been studied and each has been shown to belong to one of the three categories.

The rod-lengths throughout the animal kingdom constitute members of a series in arithmetical progression, and the difference between consecutive terms has been estimated in the early part of this paper to be half the greatest thread-width. This difference, however, is probably smaller, and rods that we regard as consecutive members may be individually separated by several intermediate lengths. When chromosomes are composed of short straight rods placed so that overlapping does not occur accurate measurements can be made, but long bent rods crowded on the spindle are extremely difficult, if not impossible, to measure exactly. In the circumstances we must regard the lengths shown as approximations, and must be prepared to substitute a smaller difference between terms in our series when greater precision can be obtained; this is, however, unimportant, for the series must in any case remain. I have adopted the method of numerical classification of rod-lengths in order to facilitate identification; this may prove useful in that it allows comparison to be readily made between the individuals of complexes in allied or separated organisms, and enables a mental picture to be made of the members of any complex. Chromosome 1 is a sphere whose diameter is equal to that of the greatest thread, Chromosome 2 is a rod whose length is one and a half times this diameter, Chromosome 3 a rod whose length is twice the diameter, and so on.

In Part I of this paper we found that various species of Stenobothrus can be distinguished individually by rod-lengths of the long chromosomes, and I suggested that general conditions have determined the total number of these, while more local

conditions determined which should arise or be eliminated by natural selection. This explanation seemed to be confirmed by the similarity found between these complexes and those of the allied genera, Arphia and Melanoplus, and further by that of the allied family, Gryllidæ; it was, however, shown to be inconclusive when we considered the Forficulidæ, for the chromosomes of *F. auricularia* are rods of two lengths, and neither is found in the complex of Stenobothrus; thus complete dissimilarity exists between the complexes of allied families. Moreover, Chromosomes 3, 4, 7, 11, 13, 15, and 17 are found on the spindle of both Triton and Stenobothrus, and the short rods of *F. auricularia* are identical with those of Lumbricus, whose chromosomes are of one length; thus similarity exists between the complexes of a vertebrate and an arthropod, and between those of an arthropod and an annelid, whereas the chromosomes of the two arthropods are unlike. These are not isolated instances, for the complex of *Labidura riparia* has been shown by DE SINETY to be almost identical with that of Forficula, and similar complexes are seen in the allied orders, Coleoptera, Hemiptera, Hymenoptera, and Lepidoptera. We cannot, therefore, assume that chromosomes of certain lengths have disappeared or evolved in a complex, for no correlation exists between rod-lengths and the somatic characters upon which our classification is based; widely separated organisms may possess certain lengths in common, whereas closely allied organisms may not afford evidence of this phenomenon.

If, moreover, we suggest that short chromosomes are found in highly differentiated animals and longer chromosomes in those of less complexity, contradictory evidence is at once forthcoming; the chromosomes of vertebrates are longer than those of arthropods, annelids, and molluscs. The converse is similarly refuted, for the longest chromosomes that I have seen occur in the Nemathelminth, *Ascaris megalocephala;* and in either case we must assume a relationship between Forficula and Lumbricus closer than that between Forficula and Stenobothrus. We have already shown that no correlation exists between our present classification and the number of chromosomes in a complex, and, if no correlation exists between lengths, the attempts of MCCLUNG and his followers to establish relationships between dimensions of rods and somatic characters must prove abortive if carried beyond the limits of an individual family.

HYPOTHESIS DEALING WITH THE ORIGIN AND FUNCTION OF CHROMOSOMES.

Since no correlation apparently exists between number and length of chromosomes and our classification of the animal kingdom, and, since current theories seem to be contradicted by the facts enunciated in the preceding pages, I have constructed a working hypothesis, which may assist us in the search for an explanation of these phenomena.

If we assume that the substance of the primordial cell has become differentiated, and if we define evolution as a transition of matter from an indefinite incoherent

homogeneity to a definite coherent heterogeneity a stage must be reached when the aggregation of ultimate units becomes evident; such a stage is found in the akaryote Protozoon, Achromatium, for chromatin granules are seen scattered throughout the cytoplasm. These when formed must be functionally identical, for differentiation of complex follows that of simple units, and reproduction is consequently accompanied by approximately equal division of the total number; moreover, the loss caused by such numerical reduction must be compensated by subsequent growth or the number will become infinitesimally small. Such processes are found in Achromatium, for the granules are seen to grow and divide in the intervals of cell-division, and constriction of the parent in the latter ensures separation of approximate halves of the total number.

As differentiation proceeds, a more accurate division becomes necessary, and modification of this simplest amitotic method must accordingly be anticipated; this is observed in certain Flagellata, *e.g.* Chilomonas and Tetramitus, for the granules are collected before cell-division near a permanent central sphere, which separates approximate halves. There seems little doubt that we are dealing here with the simplest organisms, and WILSON has said, "such forms probably give us the most primitive condition of the nuclear substance, which only in higher forms is collected into a distinct mass enclosed by a membrane; and the scattered granules are comparable to those forming the chromatin reticulum and chromosomes in the higher types."

Differentiation and aggregation of particles must be followed by differentiation of granules, and when these become individually specialised amitosis is no longer adequate, for the halves of all such granules must pass to daughter cells in successive generations. A mechanism must, therefore, be evolved, and, if we turn to other Protozoa, we find a mitotic spindle, which is essentially an arrangement for distributing halves of individual bodies to daughter cells; the central sphere of Tetramitus is probably the prototype of this spindle. Moreover, if transition from indefinite incoherent homogeneity to definite coherent heterogeneity continues, further differentiation accompanying evolution must result in growth of the granules, and this growth will not be uniform throughout the complex; the existence of chromosomes of various lengths in organisms more complex than those already mentioned accords with this supposition, and, since the diameter appears to be constant throughout the Protozoa, we must assume that increase is effected by purely linear growth.

Moreover, if greater complexity of the organism is thus accompanied by greater volume of chromatin, a stage must eventually be reached when chromosomes have attained a considerable length; it is not unreasonable to suppose that such length is limited by spindle mechanism or facilities for further growth, and the limit imposed must vary slightly in different organisms as a result of differences in physical conditions of which we know nothing. We must, therefore, anticipate a rearrangement of chromatin bodies at some period, and, if linear growth continues to accompany

evolutionary development, such rearrangement must entail reduction in length compensated by increase in diameter. This undoubtedly occurs, for if we turn to the animal kingdom we find that the diameter of chromosomes in low Metazoa is constant but is twice that seen in Protozoa.

Now increase in thread-width must take place as a result of conjugation of pre-existing bodies, and we must ask in what manner this is effected. Two alternatives are suggested; the first postulates union of entire chromosomes in groups of four; the second postulates union of smaller units. Let us consider the former. The chromosomes of complexes possessing the somatic number can always be arranged in pairs, but before conjugation is possible must be capable of arrangement in fours; if, however, the length of individual chromosomes depends upon evolution and is the result of growth, what reason have we for supposing that at any moment the complex will be composed of groups of four? Certain chromosomes grow faster than others, and if the members of a short pair overtake those of a longer pair further growth must result in inequality before other groups of four can be established. If, moreover, we assume that chromosomes become grouped in fours whenever two pairs exist of the same length, various thread-widths must be found in a complex, and we have not observed this. Furthermore, to suppose that they grow according to a rule depending upon future conjugation contradicts the theory that growth is a direct accompaniment of evolution, and, if this is contradicted, why should they grow at all? The suggestion that chromosomes become united end to end and that short chromosomes thus become equal to longer chromosomes removes the difficulty of regulated growth only one step, for, if they are continuously growing, a combination at one moment must be delayed until other combinations can be established, and in the meantime the first has been rendered impossible by further growth. We must, therefore, discard this theory and turn to the alternative, which postulates conjugation between granules. If this occurs, the occasions when a greater thread-width can be evolved must be very numerous, and we have, therefore, disposed of the difficulties presented by an assumed conjugation of entire chromosomes; in the present state of our knowledge speculation as to the manner in which this union is effected must prove abortive, but we have reasons for believing that chromosomes can be resolved into component granules of a like diameter, and conjugation of such smaller units is the only explanation that appears possible.

Moreover, if evolution is continuous and development accompanied by further increase in chromosome lengths, a stage must occur when rods of the greater diameter have reached limits imposed by physical conditions; a second increase in thread-width then becomes necessary, and we must accordingly anticipate recurrence of such increase at regular periods as evolution proceeds. This supposition is supported by actual facts, for reference to the animal kingdom shows that transition to a double thread-width again occurs as we pass from Nemertinea to Nemathelminthia, and, since all phyla including and above the latter possess chromosomes of

this increased diameter, we must assume that rod-lengths have not yet reached the stage that necessitates further doubling of the thread.

Now, if increased diameter is the result of conjugation of pre-existing bodies, a numerical reduction of chromosomes must occur, for the total volume of chromatin cannot increase perceptibly during this transition, and, since the newly formed thread-width is double that previously seen, the number is probably reduced to one-fourth. Moreover, if increased diameter occurs in order to facilitate further growth, the resulting chromosomes must be short, otherwise the limit will again be reached before evolution has proceeded far. We must, therefore, expect to find in organisms whose thread-width has recently been increased a reduced number of long chromosomes, followed immediately by segmentation into shorter lengths; and this segmentation will approximately re-establish the number reduced in the process of conjugation. Let us now turn to the animal kingdom and seek examples of these supposed phenomena. The lowest phylum possessing the greatest chromosome diameter is the Nemathelminthia, and the complex of *Ascaris megalocephala* shows either two or four chromosomes; this number is considerably smaller than that usually found, and consequently appears to corroborate our suggestion regarding numerical reduction. Moreover, these chromosomes are the longest that I have seen, and this accords with our theory of conjugation. The chromosomes of the allied species, *A. clavata* and *A. lumbricoides*, are in every way different, for they number twenty-four, and are all spherical; is it therefore unreasonable to suppose that the complexes of the two latter represent the slightly later stage when the long chromosomes have segmented into short chromosomes, whose number corresponds with that normally found in organisms, and whose shape is suited to a new course of linear growth? This seems even more probable when we remember that the long chromosomes of *A. megalocephala* have been observed actually to segment into spheres in the blastomere destined to produce somatic cells, and, if this segmentation is eventually shared by the germ cells, the links in our chain will be complete. Moreover, if the objection is raised that the total volume of chromatin on the spindle of *A. megalocephala* exceeds that of the spheres in allied species, an explanation is at once forthcoming, for a considerable portion of this chromatin is extruded from the nucleus at the moment when segmentation occurs.

Further corroboration seems to be afforded by other Nemathelminthia, for the chromosomes of Strongylus are either spheres or very short rods. Moreover, short or spherical chromosomes compose the complexes of Sagitta and of the Annelida, Allolobophora, Lumbricus, Myzostoma, and Thalassema; those of Nereis and Peripatus are slightly longer, and represent early stages of linear growth. The long chromosomes of Ophryotrocha in no way contradict the theory, for the reduced number suggests that this species is undergoing transition as in the case of *A. megalocephala.*

Turning now to the Arthropoda, we find spherical chromosomes composing the

complexes of Agelastica, Aphis, Pentatoma, Pyrrhocoris, and Syromastes among Hemiptera, of Lasius among Hymenoptera, of Dytiscus, Hydrophilus, Silpha, and Tenebrio among Coleoptera, and of Bombyx, Leucoma, Liparis, Papilio, and Pieris among Lepidoptera. In Orthoptera similar chromosomes are found in the Forficulidæ, Forficula and Labidura, and in the Phasmid, Leptynia, whereas the longer chromosomes seen in the Acridiidæ, Locustidæ, and Gryllidæ are examples of the later stages of linear growth. Spherical chromosomes occur in the Myriapoda, Scolopendra and Scutigera, and short rods in the Arachnida, Agalena, Epeira and Lycosa; the chromosomes of Copepoda are comparable with those of Acridiidæ, etc., being usually of medium length. Further confirmation is supplied by the Mollusca and Vertebrata, for, in the former, the chromosomes of Helix, Limax, Lymnœa, Crepidula, Diaulula, Enteroxenos, and Pterotrachea are rods of short and medium length, whereas in the latter these lengths have been considerably increased, as can be proved by reference to plates depicting metaphases of cells in Homo, Mus, Triton, Salamandra, Amblyostoma, Rana, Salmo, etc. Thus the drawings of chromosomes of many types made by other investigators in no way contradict the theory of linear growth preparatory to transition to a greater thread-width.

Similar phenomena are found in phyla of the smaller chromosome diameter; the chromosomes of Coelenterata are shorter than those of Echinodermata, which in their turn are shorter than those of Nemertinea. Thus a complete cycle seems to have been established, and examples have been found of all stages of growth and transition to a greater thread-width; these data cannot constitute proof, but suffice to show that the hypothesis, which we have built up synthetically, accords with actual phenomena.

We can now consider the function of chromosomes; are they bearers of hereditary characters or nutritive substances? Most cytologists believe the former. We have seen that throughout the animal kingdom reproduction is carried on by cells, which are divisible into two substances, cytoplasm and chromatin: the latter has been proved in this paper to be constant in volume, whereas the former varies according to the stage of development. Moreover, an elaborate mechanism has been evolved to ensure exact division of the chromatin bodies, which are arranged and bisected according to a definite plan; the cytoplasm, on the other hand, divides amitotically, and only approximate halves are separated by constriction of the parent cell. We know that intimate relationship exists between chromatin and cytoplasm, and that one lives at the expense of the other; if one is constant in volume while the other varies, surely the former must live at the expense of the latter. Moreover, if the chromatin contains the bearers of hereditary characters, the precise division of its bodies and their constant volumes are at once explicable, but, if the cytoplasm alone possesses this function, what reason can be offered for its varying volume? We cannot suggest that its volume is periodically decreased as a result of nourishing a substance that is itself regarded as nutritive. I shall therefore assume that the chromosomes

are directly concerned with the transmission of hereditary characters, and, since their chemical composition is apparently identical with that of the Protozoan granules, shall assume a like function for the latter. Moreover, these granules are comparable with the spheres resulting from segmentation, for they likewise appear to undergo linear growth and become rods.

Furthermore, it is difficult to suppose that differentiation occurred simultaneously throughout the cell; if granules are a visible expression of differentiation of particles, the process must be gradual, and at all periods particles must exist as distinct units that are not aggregated. Moreover, those granules that later became individually specialised were divided on the spindle, while the more elementary units were separated by mere constriction of the cytoplasm. This probably still occurs in highly organised Protozoa—possibly in certain Metazoa—and may be the explanation of the long period of juxtaposition seen in conjugation of the former, when the cytoplasm flows for hours from one zygote to the other, whereas mitosis is completed in a few minutes; HICKSON has drawn attention to this and insisted that in many Protozoa the essential portion of the conjugation process is the fusion of the cytoplasms. If this is so, the present controversy regarding chromatin function becomes meaningless, for the chromosomes can be nothing more than aggregates of specialised units, and, being the direct result of evolutionary processes, may or may not represent the total bearers of heredity in the cell. Moreover, such explanation accords with the apparently contradictory evidence furnished by DELAGE, MORGAN, SEELIGER, and VERWORN in experiments upon the fertilisation of enucleated Echinoderm ova. In these organisms differentiation cannot have proceeded far, and we must accordingly expect to find many characters transmitted by the cytoplasm; the proportion of these becomes smaller as evolutionary development continues, and their apparent absence in organisms of greater complexity has doubtless led to the belief that the chromatin alone is concerned with heredity throughout the animal kingdom.

Let us now consider the significance of these interpretations. We have shown in this paper that no correlation exists between our present classification and the number or length of chromosomes, but, if we divide organisms into three groups according to their chromatin thread-width, correlation is at once evident. Strict uniformity, however, cannot be anticipated, for it is improbable that apparently equal complexity in two different organisms has entailed a precisely similar rate of linear growth. Moreover, we must remember that the low organisms of to-day cannot be identical with those from which the higher organisms have evolved; the animal kingdom is moving on parallel lines, and our systematic grouping must not be mistaken for the representation of a procession in hypothetical time.

Furthermore, the rearrangements of chromatin occurring periodically in transition to a greater thread-width show that different combinations of character factors exist in an organism at different stages of its phylogeny, and these rearrangements may be likened to the shuffling of a pack of cards. This is most important, for, if proved,

the similarity of dimensions among chromosomes can represent nothing more than the morphological equivalency of the actual measurements. All chromosomes evolved on transition to an increased thread-width are spheres, and the complexes of all animals possessing the same number of such chromosomes must therefore appear identical; all organisms of a certain thread-width must have passed or be passing through this stage, and only when linear growth has converted these spheres into rods of various lengths can individual chromosomes be recognised and species identified by their complexes. Moreover, on the unproved and highly improbable assumption that in the sphere stage certain chromosomes in the complex of one organism corresponded functionally with certain in the complex of another and different organism, the rate of linear growth is unlikely to have been the same in both, and we have consequently no reason for inferring that a similarity in length found in existing chromosomes of these organisms is proof of identity, and therefore of functional equivalency.

In the first part of this paper we observed that the complexes of various species of Stenobothrus can be distinguished by the presence or absence of certain rod-lengths, and I suggested that in the course of evolution certain chromosomes had appeared or been eliminated. The study of allied organisms corroborated this suggestion, which became elaborated into a fascinating hypothesis; all chromosomes of the same length were considered as functionally identical, and all animals were the result of various combinations of these fixed lengths, which constituted members of a series in arithmetical progression. This hypothesis was, however, completely contradicted in the course of later study carried out upon other groups, and we must now realise that Chromosome 13 of *Stenobothrus viridulus* does not correspond with Chromosome 13 of *S. bicolor*. In the former it is the shortest of the three long chromosomes, but in the latter the medium, and I therefore believe that Chromosome 11 of *S. bicolor* is the equivalent of Chromosome 13 of *S. viridulus*, and that the difference in length is due to an increased growth in the latter accompanying evolutionary development. For the same reason we must suppose that no relationship exists between the chromosomes of a certain length occurring in the complexes of both Stenobothrus and other animals, *e.g.*, Triton, and, if this is true, the possibility of eventually establishing correlation of somatic characters and chromosomes vanishes simultaneously with the consideration of organisms belonging to any but the most closely allied groups. If the chromosomes are continuously growing at varying rates, existing similarity between dimensions of certain chromosomes in different complexes is unlikely to have occurred previously, nor will it be found at a future date when other chromosomes may exhibit similarity likewise destined to disappear.

In conclusion, I must again insist that the hypothesis of linear growth preparatory to and following transition to an increased thread-width is based upon data insufficient for purposes of proof; investigations of a thorough nature are necessary before such an hypothesis can be accepted. It is, however, the only explanation

that appears to accord with these phenomena, and as such may be of value, whether eventually proved or disproved, in that it suggests a line of thought different from those hitherto adopted.

General Summary.

1. In metaphases the relative positions of chromosomes in the equatorial plane are arbitrary.

2. Ordinary chromosomes are composed of cylindrical rods with rounded ends; each spermatogonial and secondary spermatocyte chromosome consists of two such rods and each primary spermatocyte of four, and the same composition occurs in the corresponding stages in the female.

3. The somatic chromosomes are identical with those of the germ-cells.

4. The rods appear to be indivisible units, which are separated in the various mitoses but never individually bisected.

5. The loss in chromatin volume caused by mitosis is compensated by growth in the intervening resting stages, when granules double their volume and split upon the linin threads; later condensation results in the formation of two rods in the place of one.

6. Reduction in number of chromosomes occurs in the prophase of the first maturation division when members of each pair conjugate; reduction in chromatin volume occurs in the interval between the maturation mitoses, for no true resting stage is seen and the loss caused by the preceding division is therefore not compensated.

7. One maturation mitosis is reductional in Weismann's sense of the word.

8. Whenever chromosomes have been measured the diameter of the component rods has been found to be $0{\cdot}83\,\mu$ in phyla including and above Nemathelminthia, $0{\cdot}42\,\mu$ in phyla below the latter, and $0{\cdot}21\,\mu$ in Protozoa; these thread-widths are the same in all mitoses, for rod dimensions are constants.

9. The rod-lengths of chromosomes throughout the animal kingdom constitute members of a series in arithmetical progression, of which the difference between terms does not exceed half the greatest thread-width, *i.e.* $0{\cdot}42\,\mu$. It is probable that this difference is small, and that rods now considered as consecutive members of the series will eventually be found to be separated by several intermediate lengths.

10. Complexes of closely allied organisms usually show chromosomes of similar dimensions, but widely separated organisms may possess certain in common, whereas sister families may be entirely unlike in this respect. No correlation exists of rod-lengths of chromosomes and our classification of the animal kingdom, and the same absence of correlation is seen if we consider the number of chromosomes.

11. The heterotropic chromosome, when present, possesses a diameter greater than that of the ordinary chromosomes, and this diameter is not always constant throughout its length; it cannot therefore belong to the general series. Throughout

the growth-period it remains as a compact and darkly staining body apposed to the nuclear membrane, and passes entire to one daughter cell at the first or second maturation division.

12. In organisms whose complexes are composed of chromosome rods of various lengths, species appear to be distinguishable in this respect, for no two have been found in which complexes are identical; the absence or presence of a certain rod-length may possibly enable us in future to determine whether an organism belongs to a distinct species or merely represents a variety.

13. The chromosome rods of Vertebrata are usually long, those of Mollusca medium or short, and those of Arthropoda medium, short, or spherical; in Annelida and Nemathelminthia, with one or two exceptions, the rods are spherical. In Nemertinea, which is the highest phylum possessing the smaller chromatin thread-width, they appear to be longer than those of Echinodermata, which in turn have longer chromosomes than occur in Cœlenterata. The lowest Protozoa show spherical granules, whereas normal chromosomes of various lengths are observed in others of greater complexity. The volume of chromatin remains constant in all metaphases of the species, with the exception of the secondary spermatocyte or oocyte mitosis, in which it is halved; the volume of individual chromosomes likewise remains constant, except in the first maturation division, where members of pairs are observed to have conjugated. There seems, however, a general tendency for the total volume to increase as we ascend the animal kingdom, and consideration of each sub-division possessing a definite chromatin thread-width shows an increase in rod-lengths in the higher organisms; on the other hand, the lowest animals in each sub-division appear to possess complexes composed of chromosomes whose rods are spherical, and of a diameter equal to that of the thread-width common to the sub-division.

14. In consideration of the facts enumerated above, I have constructed the following working hypothesis :—

The chromatin granules of the simplest Protozoa are a visible expression of differentiation and aggregation of specialised particles concerned with the transmission of hereditary characters, and such granules probably do not represent the sole bearers of heredity in the cell. The granules become converted into rods by purely linear growth, accompanying evolutionary development and greater complexity of the organism; this rate of growth is not the same in all chromosomes, and rods of various lengths are therefore evolved. A stage is later reached in phylogeny when rods have attained a maximum length, the limit depending upon physical conditions, possibly connected with spindle mechanism; when this occurs, chromatin units conjugate in fours, and the resulting rods have a diameter equal to twice that previously seen. These segment later into spherical chromosomes of the new thread-width, and such chromosomes are prepared to enter a new course of linear growth, accompanying further evolutionary development. Thus the chromosomes of all phyla below Nemathelminthia have evolved.

When the length limit has again been reached, conjugation once more takes place, and rods are formed having a diameter equal to that observed in Nemathelminthia and higher phyla; these rods later segment into spherical chromosomes of the new thread-width, and further evolutionary development results in conversion of the latter to rods of various lengths, such as are seen in complexes of Vertebrata. Thus increased complexity of the organism is accompanied by increased chromatin volume in the nucleus due to linear growth of granules or spherical chromosomes, and the animal kingdom can be divided into three groups, each representing a complete cycle of this process.

The heterotropic or odd chromosome alone does not belong to the general series, for its diameter exceeds the normal thread-width; unless it can be shown to be multiple, *i.e.* composed of several normal rods, such as are found in association in certain organisms, we must assume that it differs in every respect from the normal chromosomes In any case, it appears to be undergoing some process of development or disintegration—probably the former—and may or may not be the determining factor of sex.

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EXPLANATION OF THE PLATES.

(All drawings have originally been made with a camera lucida at 3048 diameters, and are reproduced here at a magnification of 2800. Full details concerning methods of preparation and study are given at the beginning of this paper.)

Plate 1.

Figs. 1–12.—Polar views of secondary spermatocyte metaphases of *Stenobothrus bicolor*. The heterotropic chromosome is seen in figs. 2, 7, 9, and 10, Chromosome 7 being absent from the last-named complex.

Plate 2.

Figs. 13–21.—Examples of Chromosome 17, the longest of the complex.

Figs. 22–41.—Examples of Chromosome 13, the medium long chromosome.

Figs. 42–52.—Examples of Chromosome 11, the shortest of the three long chromosomes.

Figs. 53–63.—Examples of the heterotropic chromosome, found in 50 per cent. of these cells, and recognisable by its breadth.

Figs. 64–77.—Examples of Chromosome 7, the longest of the short chromosomes; its length is equal to that of the heterotropic chromosome.

Figs. 78–91.—Examples of Chromosome 6.

Figs. 92–105.—Examples of Chromosome 5.

Figs. 106–120.—Examples of Chromosome 4.

Figs. 121–135.—Examples of Chromosome 3, the shortest of the complex.

(N.B.—All the above drawings represent rods taken from secondary spermatocyte cells of *S. bicolor;* only those have been drawn that were lying at right angles to the microscopic line of vision. The lengths of the first 39 chromosomes are plotted out in vertical lines at the foot of this plate.)

Figs. 40–135.—Lengths of the corresponding chromosome rods; the lengths are represented by vertical lines, and prove that the complex can be divided into eight groups.

Figs. 136–141.—Polar views of secondary spermatocyte metaphases of *S. viridulus;* the heterotropic chromosome is seen in figs. 136, 137, 140, and 141.

Figs. 142–150.—Examples of Chromosome 17, the longest of the complex.

Plate 3.

Figs. 151–160.—Examples of Chromosome 15, the medium long chromosome.

Figs. 161–168.—Examples of Chromosome 13, the shortest of the three long chromosomes.

Figs. 169–179.—Examples of the heterotropic chromosome, found in 50 per cent. of these cells, and recognisable by its breadth.

Figs. 180–197.—Examples of Chromosome 7, the longest of the short chromosomes; its length is equal to that of the heterotropic chromosome.

Figs. 198–214.—Examples of Chromosome 6.

Figs. 215–231.—Examples of Chromosome 5.

Figs. 232–248.—Examples of Chromosome 4.

Figs. 249–265.—Examples of Chromosome 3, the shortest of the complex.

Figs. 142–240.—Lengths of chromosome rods shown above; the lengths are represented by vertical lines, and prove that this complex also can be divided into eight groups.

(N.B.—All drawings have been made from secondary spermatocyte metaphases of *S. viridulus*; and, as in the case of *S. bicolor*, only those rods have been drawn that were lying at right angles to the microscopic line of vision.)

Figs. 241–265.—Lengths plotted out of the corresponding chromosome rods.

Fig. 266.—Polar view of spermatogonial metaphase of *S. bicolor*. Sixteen ordinary chromosomes are here seen, divisible into eight groups; the heterotropic chromosome can be recognised by its great breadth. A diagram showing the identity of the chromosomes is given on page 16.

Figs. 267–275.—Chromosome rods constituting the spermatogonial complex of *S. bicolor*. Fig. 270 is the heterotropic chromosome; the remainder show the eight length-groups, which are identical with those plotted out on Plate 2. Each spermatogonial complex possesses four rods of each length, *i.e.* two chromosomes.

Figs. 276–301.—Drawings of condensing chromosome filaments taken from the primary spermatocyte prophase of *S. bicolor*. The longitudinal split in component arms caused by the original cleavage of a single row of primary granules is shown by a black line in figs. 277, 278, 281, 282, 284, 285, 288, 289, and 297.

Fig. 302.—The heterotropic chromosome seen during this prophase; it has remained throughout the growth-period as a compact body, taking no part in the general dissociation.

Figs. 303–305.—Polar views of the primary spermatocyte metaphase of *S. viridulus*. Each shows three large and five small tetrads; the heterotropic chromosome is absent in fig. 304.

Plate 4.

Figs. 306–314.—Chromosome rods constituting the secondary spermatocyte complex of *S. parallelus*. Fig. 314 is the heterotropic chromosome, which is found in 50 per cent. of these cells; the remainder show eight length-groups as in all previous cases.

Figs. 315–323.—Chromosome rods constituting the secondary spermatocyte complex of *S. bicolor* var. *nigrina*. Fig. 323 is the heterotropic chromosome, which is found in 50 per cent. of these cells; the remainder show the eight length-groups already found for members of the species itself.

Figs. 324–332.—Chromosome rods constituting the somatic complex of *S. parallelus*; they are seen to possess dimensions identical with those of the germ nuclei.

Fig. 333.—Condensing filaments of somatic cell taken from ovary of *S. parallelus*. This stage is immediately followed by segmentation into chromosomes, and shows the characteristic thread-width.

Fig. 334.—Lateral view of late telophase of somatic cell of *S. parallelus*. The thread-width is seen to be 0·83 μ.

Fig. 335.—Abnormal condition of chromosomes in ovum of *S. parallelus*, presumably due to flaking of yolk in the process of section cutting. The chromosomes are resolved into spheres of a diameter equal to that normally found in the genus.

Figs. 336–344.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus angustipennis*. The last is the heterotropic chromosome, recognisable on account of its great breadth; the remainder show eight lengths to which the 11 ordinary chromosomes belong.

Figs. 345–353.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus atlansis*. The last is the heterotropic chromosome, and the remainder show eight lengths.

Figs. 354–362.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus bivittatus*. The last is the heterotropic chromosome, and the remainder show eight lengths.

Figs. 363–371.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus packardii*. The last is the heterotropic chromosome, and the remainder again show eight lengths.

Fig. 371A.—Secondary spermatocyte complex of *Melanoplus packardii* seen from polar aspect. Ten ordinary chromosomes are present, and the heterotropic chromosome. It must be remembered that certain of the former are foreshortened.

Figs. 372–378.—Chromosome rod-lengths in secondary spermatocyte complex of *Arphia pseudonietana*; the last is the heterotropic chromosome, and the remainder show six lengths to which the 11 ordinary chromosomes belong.

Figs. 379–386.—Chromosome rod-lengths of secondary spermatocyte complex of *Melanoplus dawsoni*, showing eight lengths to which the 11 ordinary chromosomes belong.

Fig. 387.—Polar view of secondary spermatocyte metaphase of *Arphia pseudonietana*, showing one heterotropic and ten ordinary chromosomes; certain of the latter are foreshortened.

Fig. 388.—Lateral view of primary spermatocyte metaphase of *Arphia pseudonietana*. The tetrads are seen to be considerably larger than the chromosomes of the preceding figure.

Fig. 389.—Polar view of spermatogonial complex of *Forficula auricularia*, showing 24 chromosomes. This cell was fixed in Flemming's fluid.

Fig. 390.—Ditto.

Fig. 391.—Polar view of primary spermatocyte metaphase of *Forficula auricularia*, showing 12 tetrads. This cell was fixed in Flemming's fluid.

Fig. 392.—Ditto.

Fig. 393.—Polar view of secondary spermatocyte metaphase of *Forficula auricularia*, showing 12 chromosomes. This cell was fixed in Hermann's fluid.

Fig. 394.—Ditto. Fixed with Perenyi's solution.

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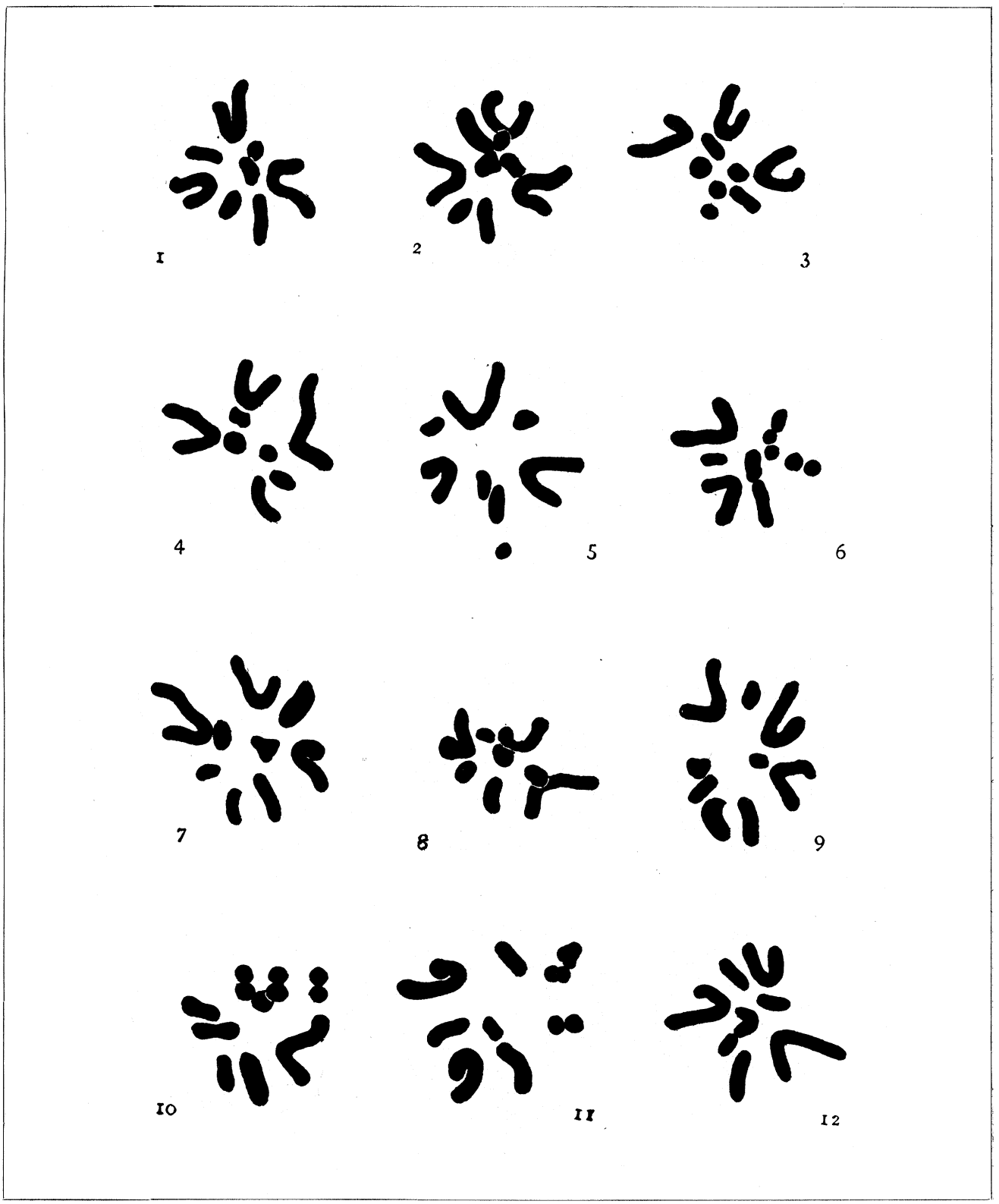
(N.B.—In all drawings in the above-mentioned plates the representation of the cytoplasm, where shown, is purely diagrammatic.)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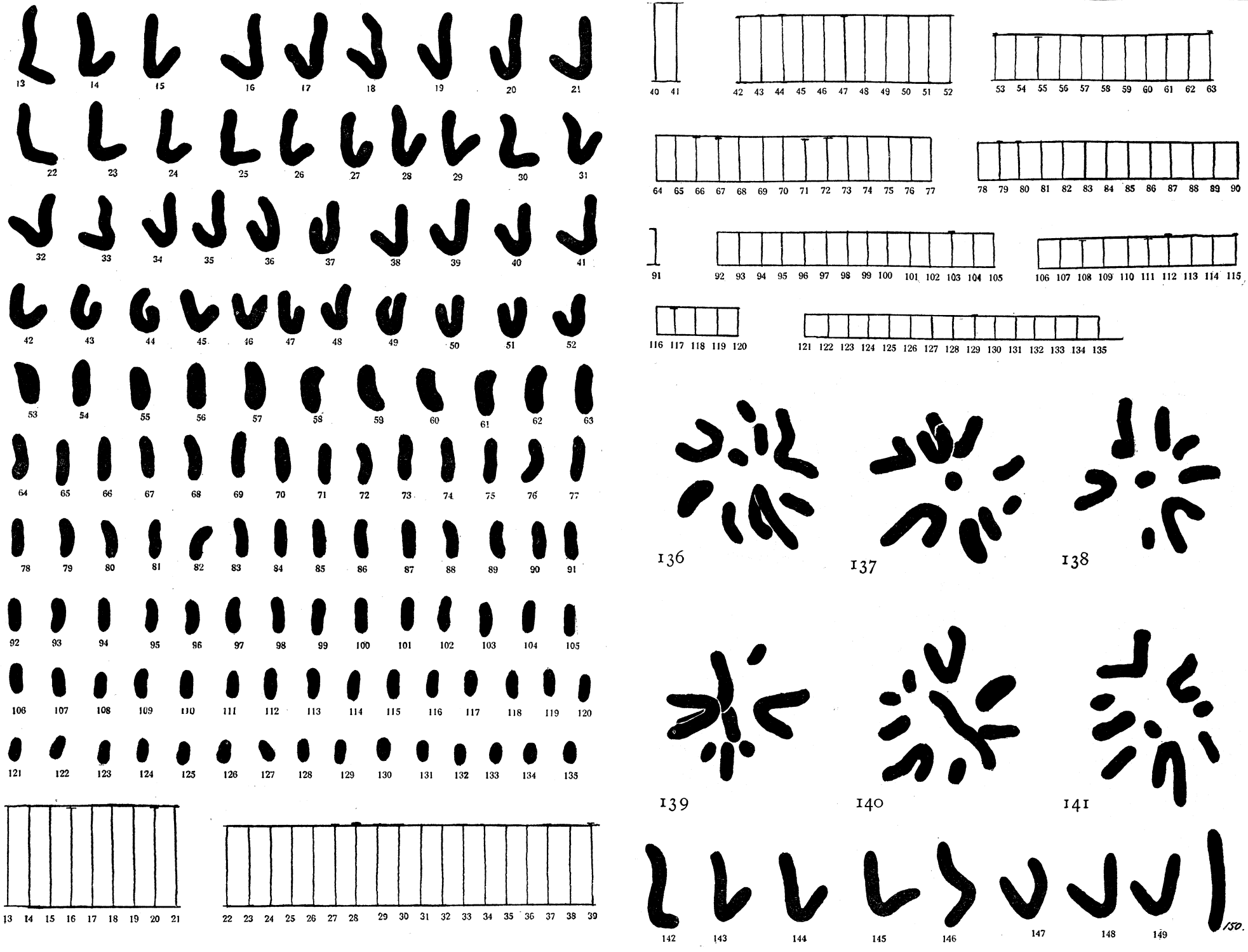
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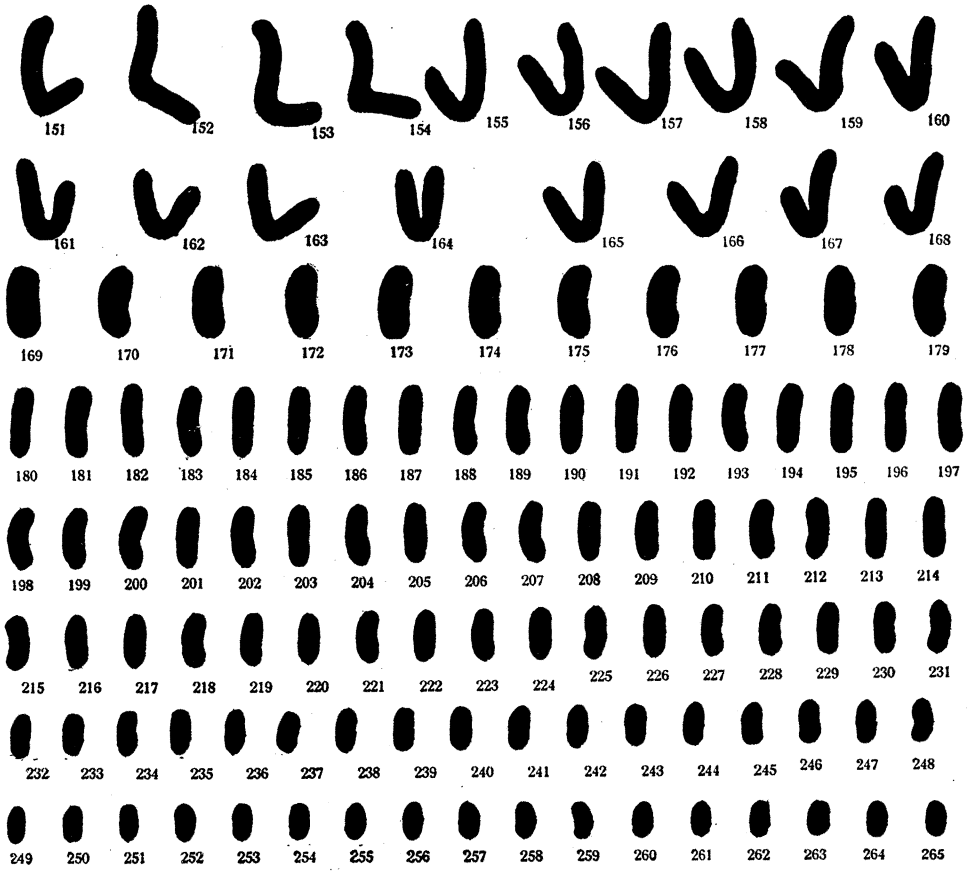
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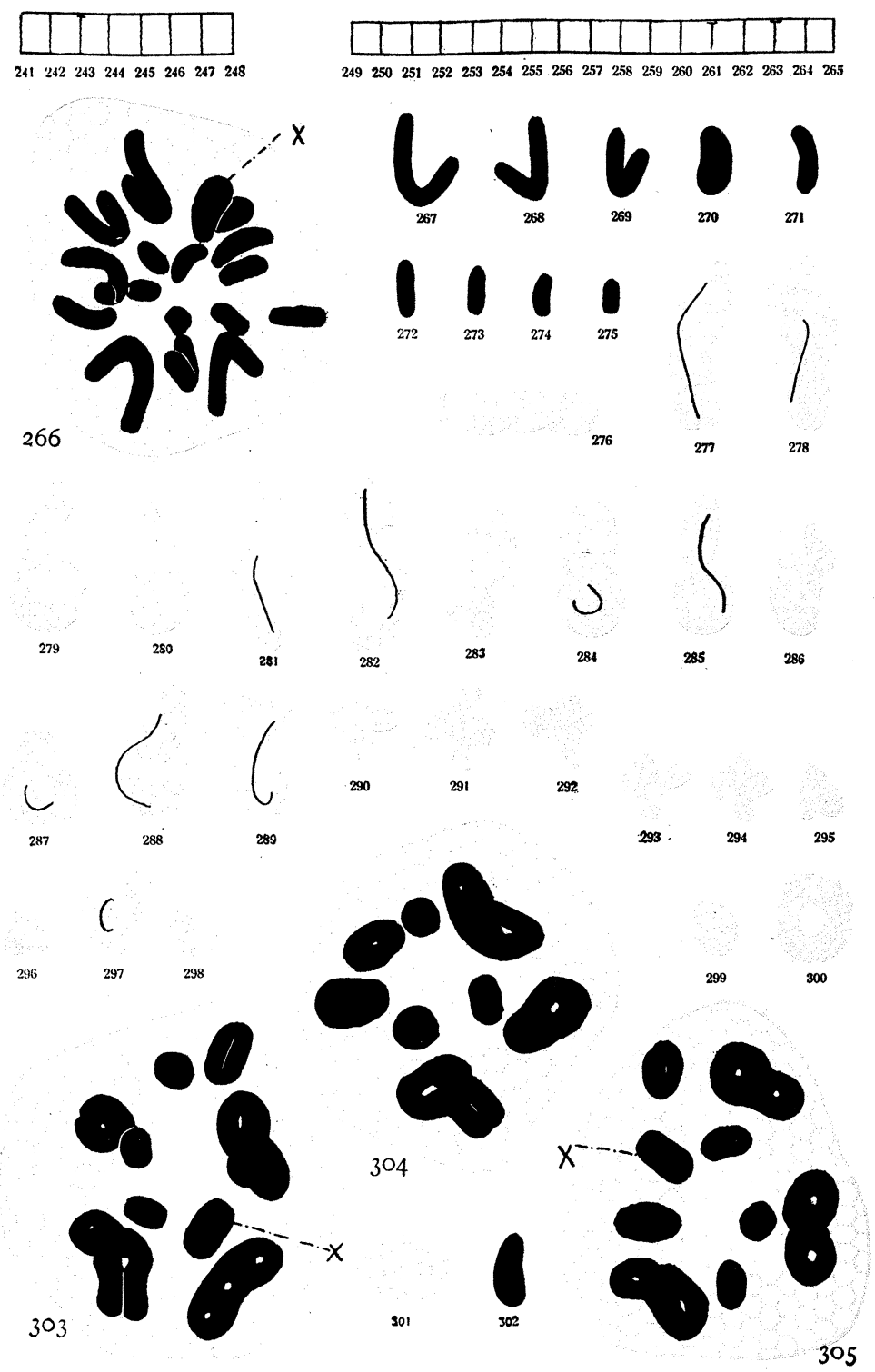
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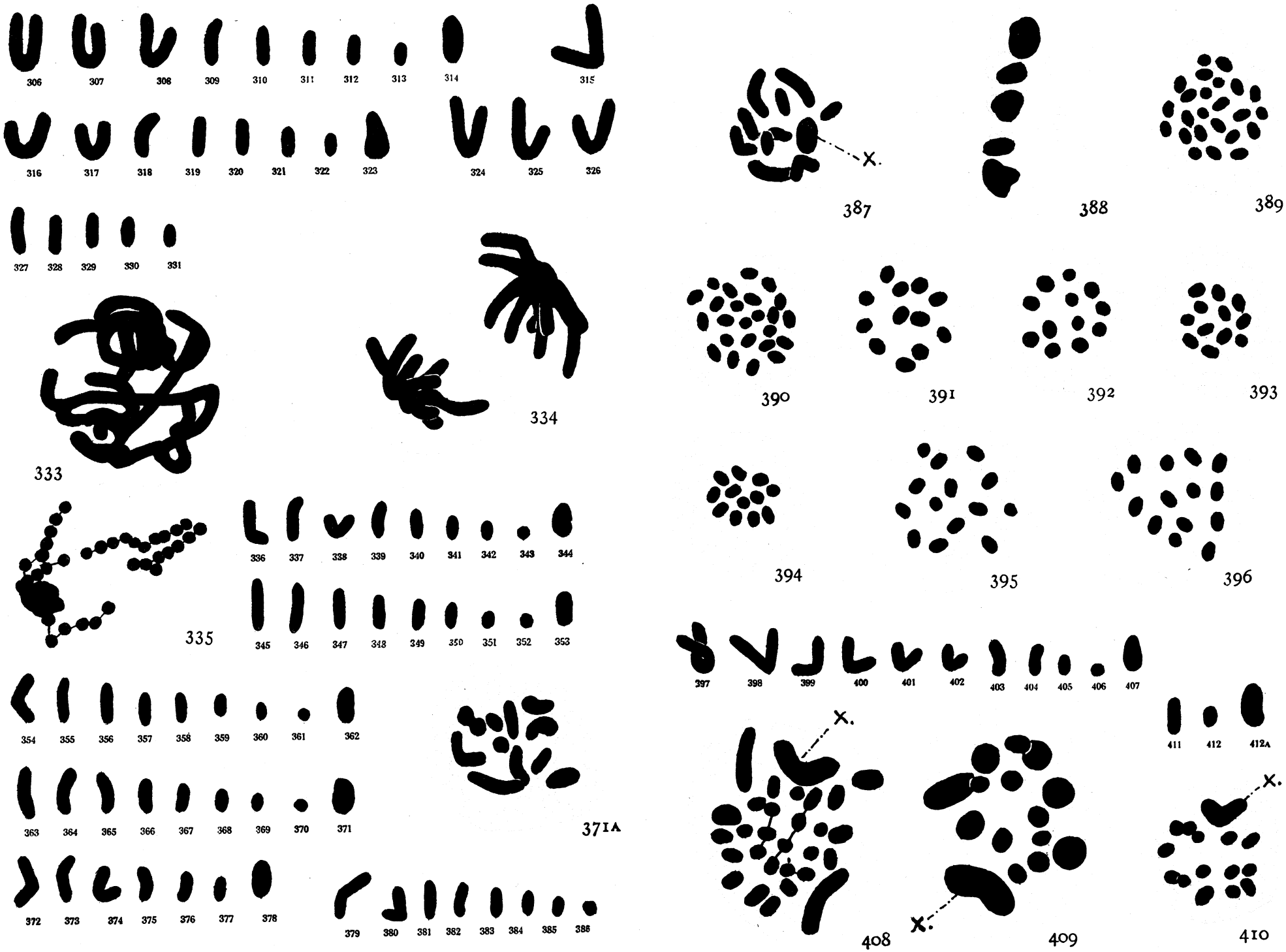
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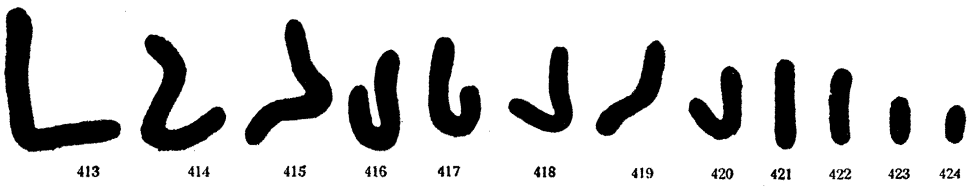
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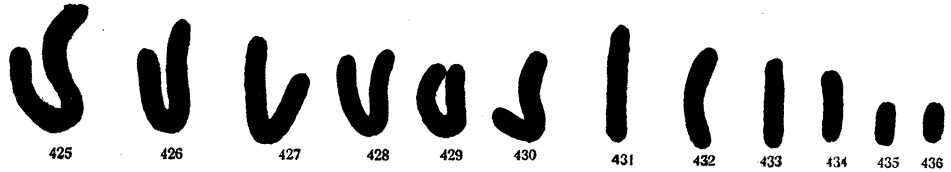
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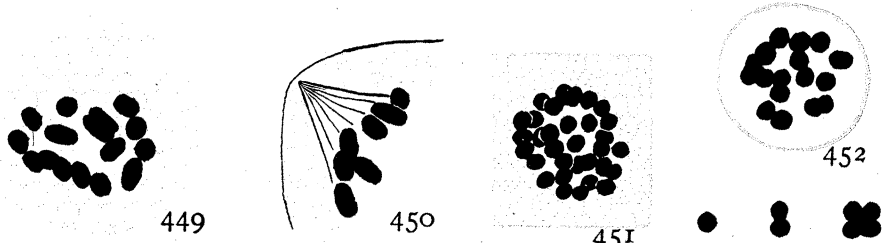
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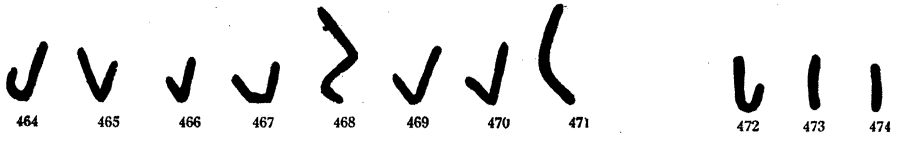
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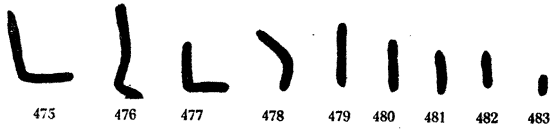
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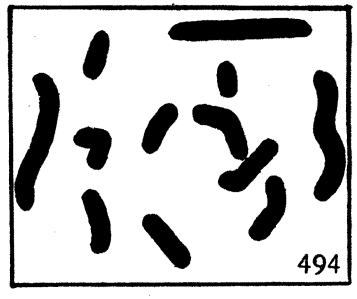
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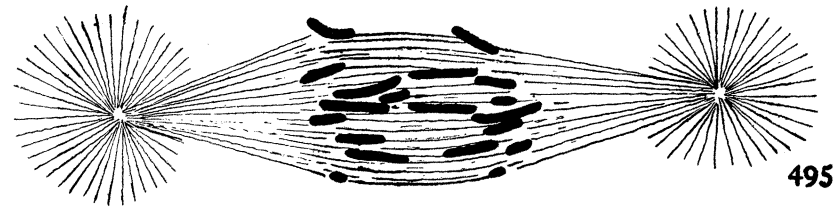
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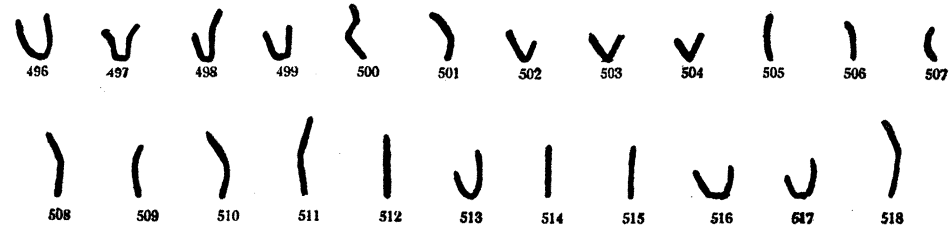
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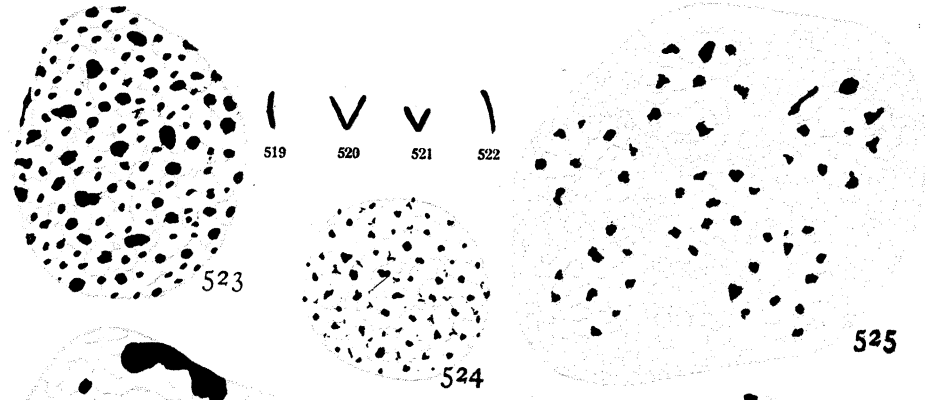
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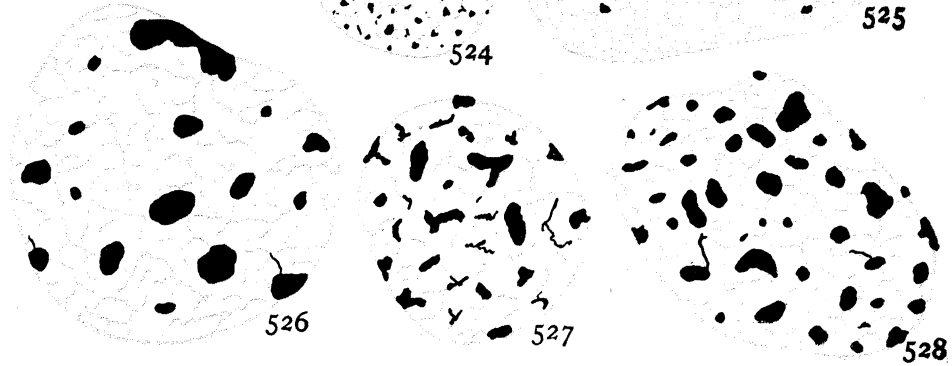
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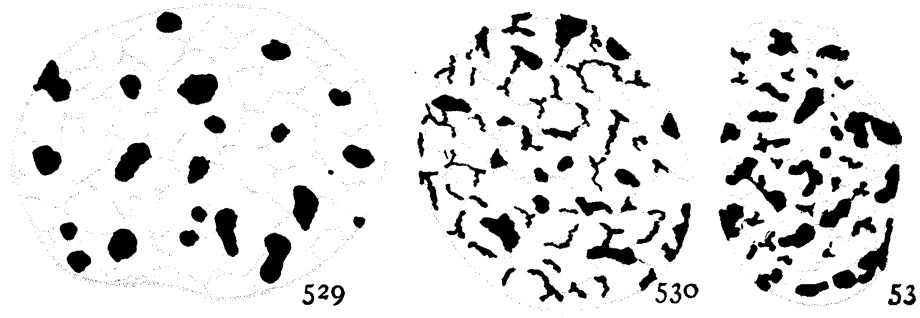
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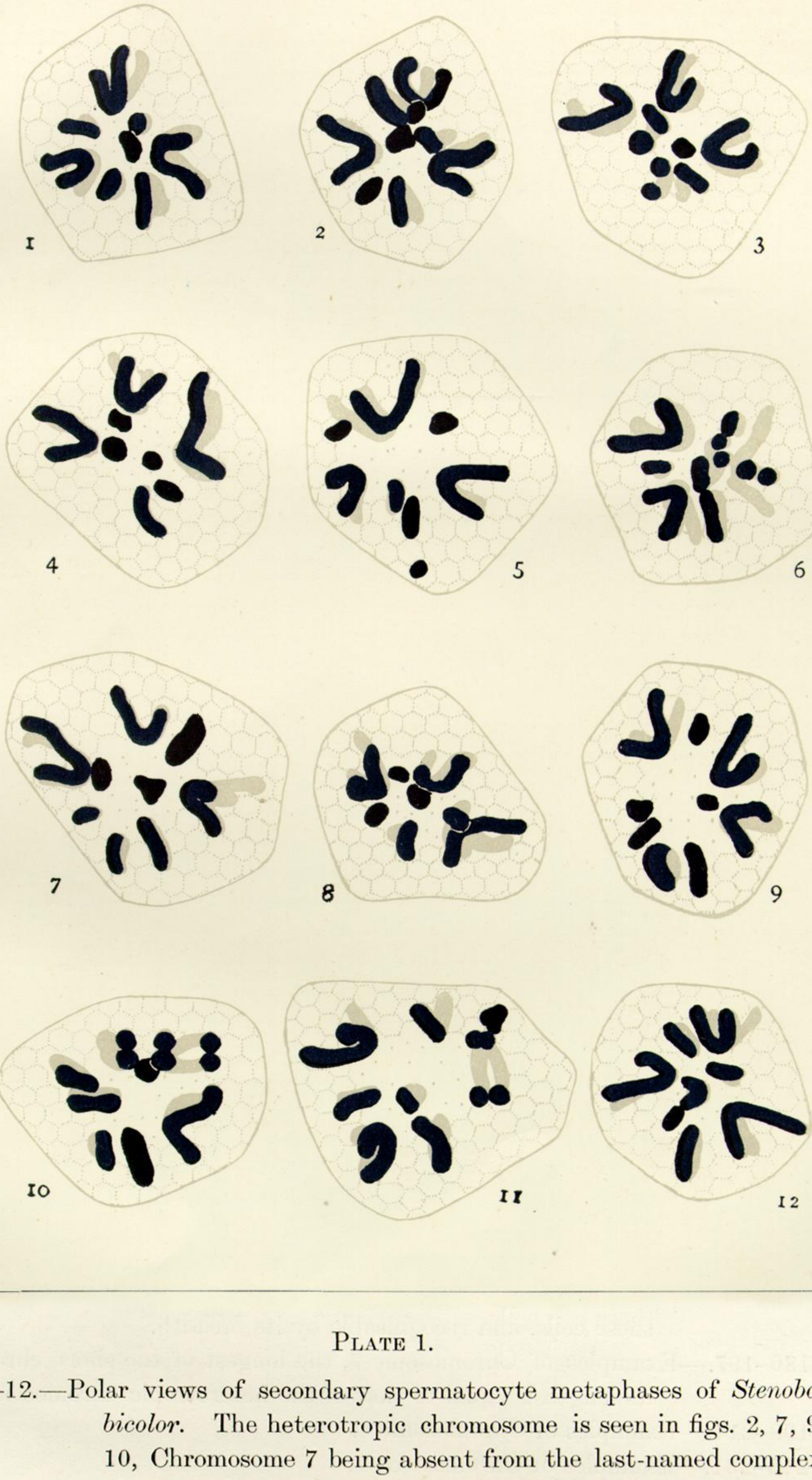

PLATE 1.

Figs. 1–12.—Polar views of secondary spermatocyte metaphases of *Stenobothrus bicolor*. The heterotropic chromosome is seen in figs. 2, 7, 9, and 10, Chromosome 7 being absent from the last-named complex.

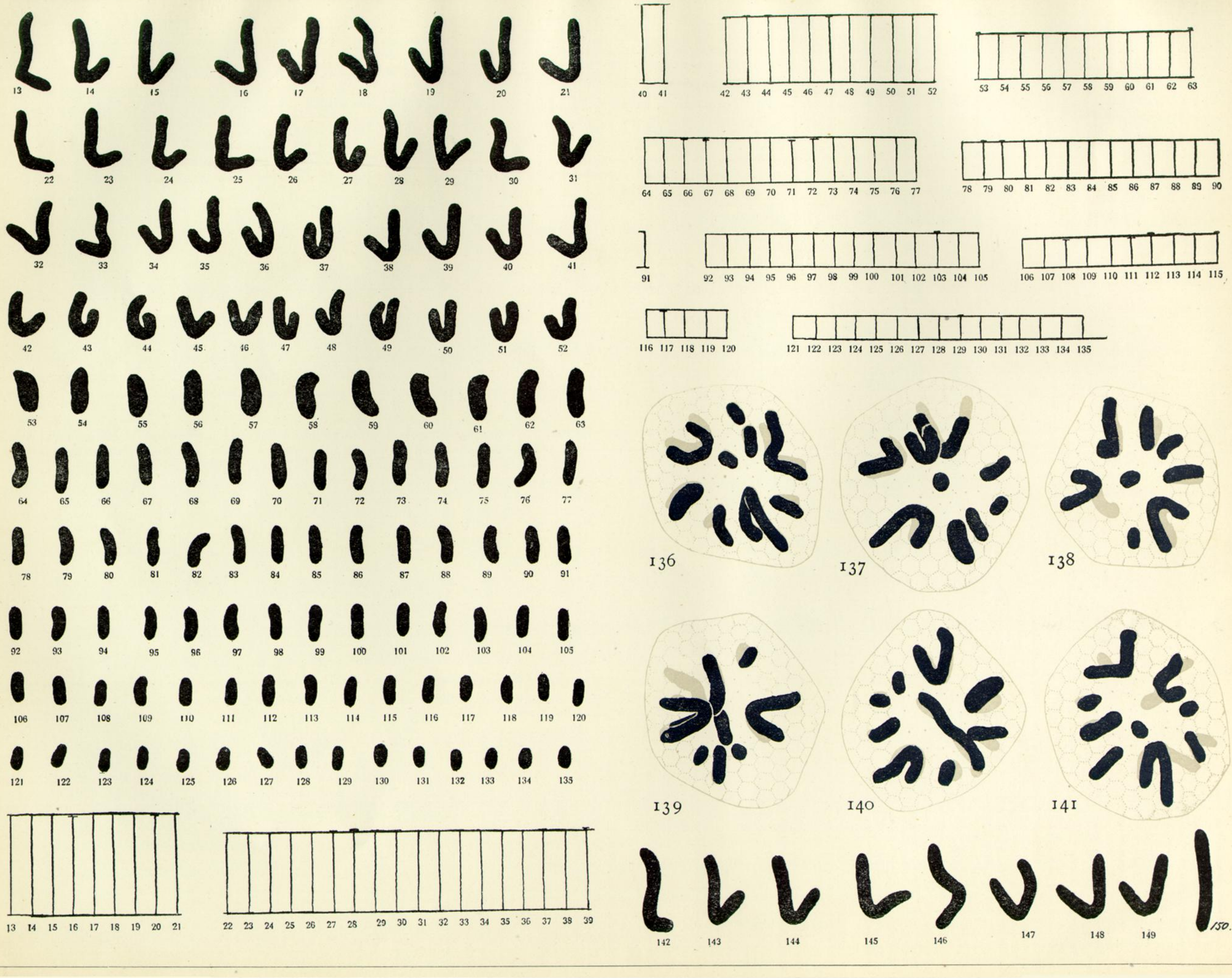

PLATE 2.

Figs. 13–21.—Examples of Chromosome 17, the longest of the complex.

Figs. 22–41.—Examples of Chromosome 13, the medium long chromosome.

Figs. 42–52.—Examples of Chromosome 11, the shortest of the three long chromosomes.

Figs. 53–63.—Examples of the heterotropic chromosome, found in 50 per cent. of these cells, and recognisable by its breadth.

Figs. 64–77.—Examples of Chromosome 7, the longest of the short chromosomes; its length is equal to that of the heterotropic chromosome.

Figs. 78–91.—Examples of Chromosome 6.

Figs. 92–105.—Examples of Chromosome 5.

Figs. 106–120.—Examples of Chromosome 4.

Figs. 121–135.—Examples of Chromosome 3, the shortest of the complex.

(N.B.—All the above drawings represent rods taken from secondary spermatocyte cells of *S. bicolor;* only those have been drawn that were lying at right angles to the microscopic line of vision. The lengths of the first 39 chromosomes are plotted out in vertical lines at the foot of this plate.)

Figs. 40–135.—Lengths of the corresponding chromosome rods; the lengths are represented by vertical lines, and prove that the complex can be divided into eight groups.

Figs. 136–141.—Polar views of secondary spermatocyte metaphases of *S. viridulus;* the heterotropic chromosome is seen in figs. 136, 137, 140, and 141.

Figs. 142–150.—Examples of Chromosome 17, the longest of the complex.

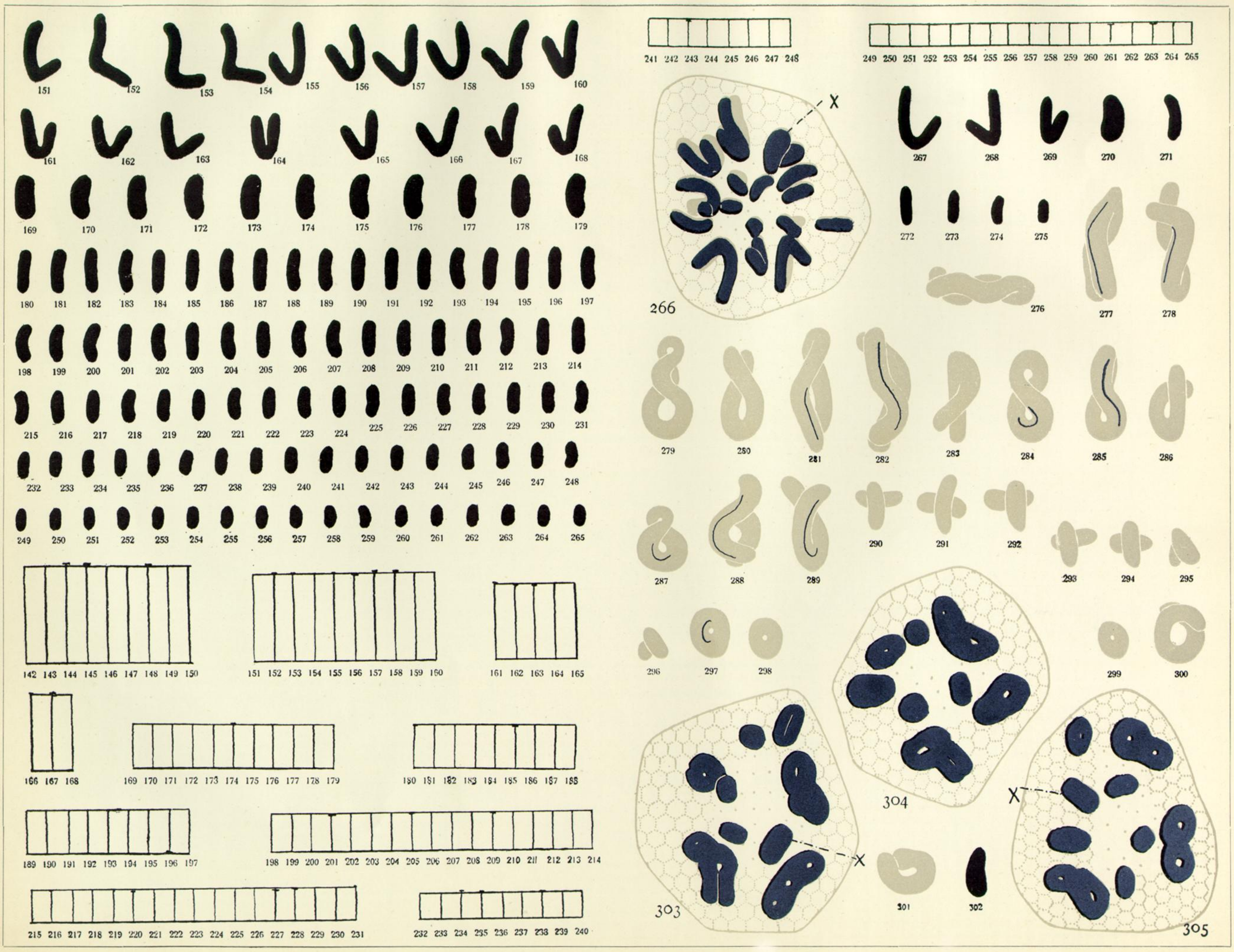

PLATE 3.

Figs. 151–160.—Examples of Chromosome 15, the medium long chromosome.

Figs. 161–168.—Examples of Chromosome 13, the shortest of the three long chromosomes.

Figs. 169–179.—Examples of the heterotropic chromosome, found in 50 per cent. of these cells, and recognisable by its breadth.

Figs. 180–197.—Examples of Chromosome 7, the longest of the short chromosomes; its length is equal to that of the heterotropic chromosome.

Figs. 198–214.—Examples of Chromosome 6.

Figs. 215–231.—Examples of Chromosome 5.

Figs. 232–248.—Examples of Chromosome 4.

Figs. 249–265.—Examples of Chromosome 3, the shortest of the complex.

Figs. 142–240.—Lengths of chromosome rods shown above; the lengths are represented by vertical lines, and prove that this complex also can be divided into eight groups.

(N.B.—All drawings have been made from secondary spermatocyte metaphases of *S. viridulus;* and, as in the case of *S. bicolor*, only those rods have been drawn that were lying at right angles to the microscopic line of vision.)

Figs. 241–265.—Lengths plotted out of the corresponding chromosome rods.

Fig. 266.– Polar view of spermatogonial metaphase of *S. bicolor.* Sixteen ordinary chromosomes are here seen, divisible into eight groups; the heterotropic chromosome can be recognised by its great breadth. A diagram showing the identity of the chromosomes is given on page 16.

Figs. 267–275.—Chromosome rods constituting the spermatogonial complex of *S. bicolor.* Fig. 270 is the heterotropic chromosome; the remainder show the eight length-groups, which are identical with those plotted out on Plate 2. Each spermatogonial complex possesses four rods of each length, *i.e.* two chromosomes.

Figs. 276–301.—Drawings of condensing chromosome filaments taken from the primary spermatocyte prophase of *S. bicolor.* The longitudinal split in component arms caused by the original cleavage of a single row of primary granules is shown by a black line in figs. 277, 278, 281, 282, 284, 285, 288, 289, and 297.

Fig. 302.—The heterotropic chromosome seen during this prophase; it has remained throughout the growth-period as a compact body, taking no part in the general dissociation.

Figs. 303–305.—Polar views of the primary spermatocyte metaphase of *S. viridulus.* Each shows three large and five small tetrads; the heterotropic chromosome is absent in fig. 304.

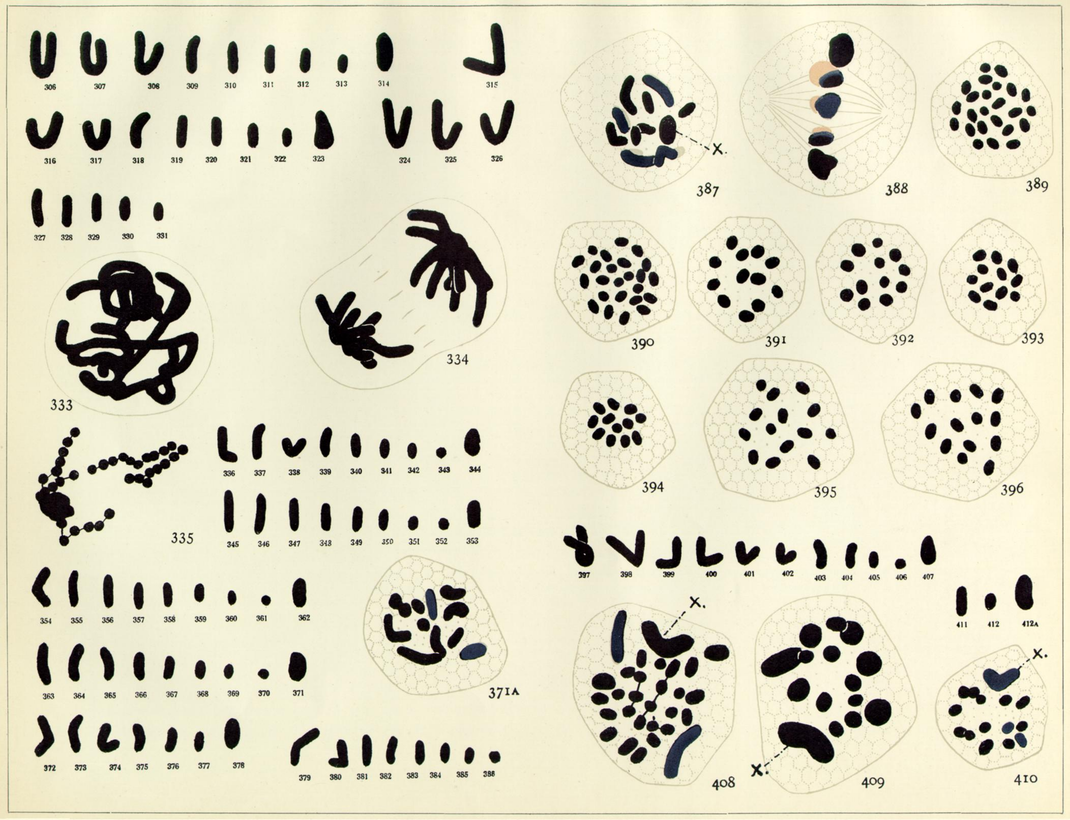

PLATE 4.

Figs. 306–314.—Chromosome rods constituting the secondary spermatocyte complex of *S. parallelus*. Fig. 314 is the heterotropic chromosome, which is found in 50 per cent. of these cells; the remainder show eight length-groups as in all previous cases.

Figs. 315–323.—Chromosome rods constituting the secondary spermatocyte complex of *S. bicolor* var. *nigrina*. Fig. 323 is the heterotropic chromosome, which is found in 50 per cent. of these cells; the remainder show the eight length-groups already found for members of the species itself.

Figs. 324–332.—Chromosome rods constituting the somatic complex of *S. parallelus*; they are seen to possess dimensions identical with those of the germ nuclei.

Fig. 333.—Condensing filaments of somatic cell taken from ovary of *S. parallelus*. This stage is immediately followed by segmentation into chromosomes, and shows the characteristic thread-width.

Fig. 334.—Lateral view of late telophase of somatic cell of *S. parallelus*. The thread-width is seen to be 0·83 μ.

Fig. 335.—Abnormal condition of chromosomes in ovum of *S. parallelus*, presumably due to flaking of yolk in the process of section cutting. The chromosomes are resolved into spheres of a diameter equal to that normally found in the genus.

Figs. 336–344.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus angustipennis*. The last is the heterotropic chromosome, recognisable on account of its great breadth; the remainder show eight lengths to which the 11 ordinary chromosomes belong.

Figs. 345–353.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus atlansis*. The last is the heterotropic chromosome, and the remainder show eight lengths.

Figs. 354–362.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus bivittatus*. The last is the heterotropic chromosome, and the remainder show eight lengths.

Figs. 363–371.—Chromosome rod-lengths in secondary spermatocyte complex of *Melanoplus packardii*. The last is the heterotropic chromosome, and the remainder again show eight lengths.

Fig. 371A.—Secondary spermatocyte complex of *Melanoplus packardii* seen from polar aspect. Ten ordinary chromosomes are present, and the heterotropic chromosome. It must be remembered that certain of the former are foreshortened.

Figs. 372–378.—Chromosome rod-lengths in secondary spermatocyte complex of *Arphia pseudonietana*; the last is the heterotropic chromosome, and the remainder show six lengths to which the 11 ordinary chromosomes belong.

Figs. 379–386.—Chromosome rod-lengths of secondary spermatocyte complex of *Melanoplus dawsoni*, showing eight lengths to which the 11 ordinary chromosomes belong.

Fig. 387.—Polar view of secondary spermatocyte metaphase of *Arphia pseudonietana*, showing one heterotropic and ten ordinary chromosomes; certain of the latter are foreshortened.

Fig. 388.—Lateral view of primary spermatocyte metaphase of *Arphia pseudonietana*. The tetrads are seen to be considerably larger than the chromosomes of the preceding figure.

Fig. 389.—Polar view of spermatogonial complex of *Forficula auricularia*, showing 24 chromosomes. This cell was fixed in Flemming's fluid.

Fig. 390.—Ditto.

Fig. 391.—Polar view of primary spermatocyte metaphase of *Forficula auricularia*, showing 12 tetrads. This cell was fixed in Flemming's fluid.

Fig. 392.—Ditto.

Fig. 393.—Polar view of secondary spermatocyte metaphase of *Forficula auricularia*, showing 12 chromosomes. This cell was fixed in Hermann's fluid.

Fig. 394.—Ditto. Fixed with Perenyi's solution.

Fig. 395.—Polar view of secondary spermatocyte complex of *Forficula auricularia*, showing 14 chromosomes. The widely separated chromosomes suggest that the polar axis is inclined slightly from the vertical, and that precocious division of certain chromosomes gives the appearance of a greater number than actually exists on the spindle. Fixed with Hermann's fluid.

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Fig. 409.—Primary spermatocyte metaphase of the same, showing the heterotropic and 14 ordinary chromosomes.

Fig. 410.—Secondary spermatocyte metaphase of the same.

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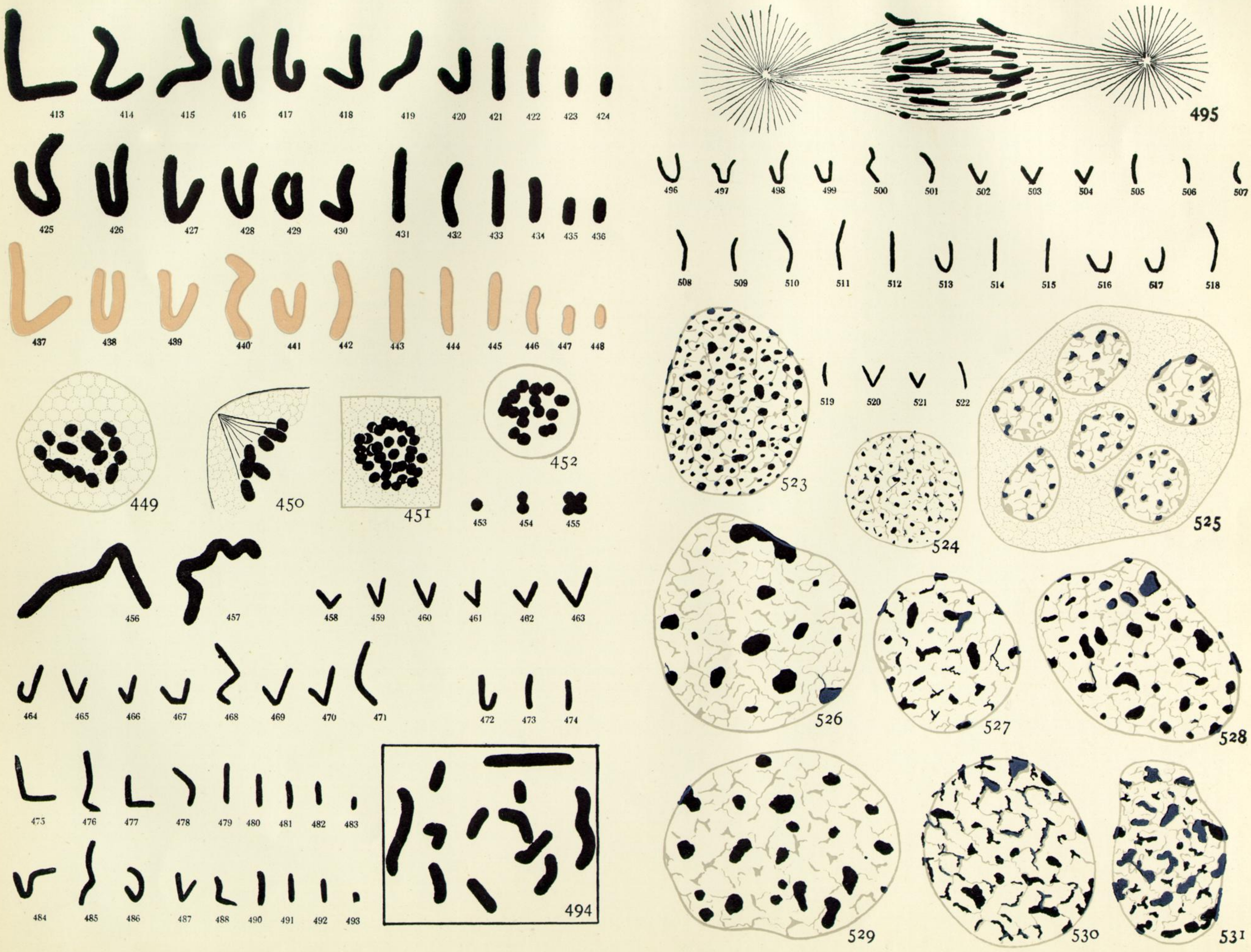

PLATE 5.

Figs. 413–424.—Chromosome rod-lengths constituting the secondary spermatocyte complex of *Triton cristatus.*

Figs. 425–436.—Ditto.

Figs. 437–448.—Ditto. Stained with iron brazilin.

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Fig. 450.—Telophase of secondary spermatocyte division of Helix, showing seven chromosomes.

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Fig. 452.—Polar view of secondary spermatocyte metaphase of Lumbricus, showing 16 chromosomes similar to those of the preceding figure.

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Fig. 454.—A dyad of Lumbricus.

Fig. 455.—A tetrad of Lumbricus.

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Figs. 472–474.—Chromosome rods of the maturing ovum in *Asterias glacialis.*

Figs. 475–483.—Chromosome rods constituting the complex of *Echinus esculentus* taken from maturing ova and 4-, 8-, and 16-cell stages of segmentation following amphimixis.

Figs. 484–493.—Ditto.

Fig. 494.—Chromosomes taken from several somatic cells of the human embryo (three weeks).

Fig. 495.—Telophase of segmenting blastomere in *Alcyonium digitatum*, showing chromosomes of same diameter as in Lineus, Echinus, and Asterias.

Figs. 496–507.—Chromosomes of *Euglena viridis.* The diameter is half that of the Nemertines, Echinoderms, and Cœlenterates.

Figs. 508–518.—Chromosomes of *Paramœcium caudatum.* The thread-width is equal to that of the preceding Protozoon.

Figs. 519–522.—Chromosomes of *Monocystis agilis.*

Fig. 523.—The resting stage in *Euglena viridis.*

Fig. 524.—Resting micronucleus in *Paramœcium caudatum.*

Fig. 525.—A cluster of resting spermatogonial nuclei of Lumbricus embedded in a common cytoplasmic mass.

Fig. 526.—Resting nucleus of a four-cell stage of segmentation in *Echinus esculentus.*

Fig. 527.—Resting nucleus of ovum in *Lineus lacteus.*

Fig. 528.—Ditto in four-cell stage of segmentation.

Fig. 529.—Resting spermatogonial nucleus in *Triton cristatus.*

Fig. 530.—Resting spermatogonial nucleus in *Stenobothrus viridulus.*

Fig. 531.—Resting nucleus of ovum in *Ascaris megalocephala.*

(N.B.—In all drawings in the above-mentioned plates the representation of the cytoplasm, where shown, is purely diagrammatic.)