CHROMOSOME STUDIES ON THE DIPTERA II. THE PAIRED ASSOCIATION OF CHROMOSOMES IN THE DIPTERA, AND ITS SIGNIFICANCE

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EIGHT PLATES

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INTRODUCTION

Attention was first called to the pairing of chromosomes in the Diptera by Miss N. M. Stevens during 1907 and 1908 in connection with studies upon the heterochromosomes of insects (Stevens '07, '08). Although primarily concerned with the heterochromosomes and maturation phenomena, Stevens nevertheless found the paired association of chromosomes, in the nine species she studied, so conspicuous as to warrant the statement that, "perhaps the most interesting point in the whole study is the pairing of chromosomes in cells somewhat removed from the sphere of the reduction process. This was found to occur in the ovarian follicle cells, the spermatogonia and some embryonic cells. This is not an occasional phenomenon, but one which belongs to every oogonial and spermatogonial mitosis"

(Stevens '08, p. 372). In a later paper on "The chromosomes in the germ-cells of Culex" (Stevens '10, p. 215), corresponding phenomena called forth a similar statement to the effect that "perhaps the most interesting point in the history of the germ-cells of Culex is the fact that, as in the Muscidae, pairing or synapsis, occurs in connection with each spermatogonial and oogonial mitosis as well as in anticipation for maturation." Although only able to study somatic mitoses to a very limited extent, Stevens surmised that, "it may therefore be true that pairing of homologous chromosomes occurs in connection with each mitosis throughout the life history of these insects" (p. 215). Now this would be a very important point to establish, as Stevens realized, and she doubtless would have followed it up had it not been for her untimely death in 1912. Most unfortunately, however, her work on the Diptera was stopped at its very beginning and many promising questions suggested by it have remained uninvestigated.

Nothing further appeared on chromosomes of the Diptera until 1914 when three papers were published, one by the author on Drosophila chromosomes; the others on the chromosomes on Culex pipiens, one by Miss Taylor, and one by Lomen. Both of the latter took exception to Stevens' conclusions that the chromosomes are paired in Culex and other Diptera, on the ground that the chromosome pairs which she described were really only precociously split univalent chromosomes. Their evidence on this point, however, is very inadequate, and their conclusions are surely erroneous (see pp. 244 and 245).

The purpose of the present paper is to describe in some detail the phenomena involved in 'chromosome pairing' in the Diptera, and to consider their bearing on current theories respecting the nature of the chromosomes and their rôle in heredity. Because of their remarkably definite paired association the chromosomes of the Diptera are especially suitable for studies on the relationships between individual chromosomes and on the qualitative characteristics of chromosomes as indicated by their behavior, but as I have mentioned in a previous paper (Metz '14) the technical difficulties involved in an extensive

cytological study of these insects have caused them to be generally avoided by cytologists. These difficulties, however, may very largely be overcome by care and persistence. Although certain principles must be observed in making preparations, the task is mainly one of securing and preparing enough specimens to get material in the proper stages and in sufficient quantity for study. No more difficulty is experienced in studying the nuclear phenomena, when the proper material is secured, than is the case in other insects; indeed the chromatic elements in the flies, when well prepared, appear with a brilliancy that is surpassed by very few objects.[1]

The observations included here are concerned chiefly with chromosomal behavior in somatic cells and in germ-cells outside the sphere of maturation. These cells I shall briefly term 'diploid' cells, in distinction to oocytes and spermatocytes. Since all of the 'diploid' cells agree in respect to the phenomena dealt with, no confusion should arise from such a terminology. Phenomena associated with the maturation processes are considered only in so far as they bear directly upon those in'diploid' cells. Likewise the relationships between the chromosomes in different species of flies are only briefly considered. I hope to return to both of these questions in subsequent papers.

In order to facilitate the treatment of the subject matter I will outline at once the main points considered in the paper, and will indicate in advance some of the conclusions attained. This may best be accomplished by taking account of certain genetic hypotheses which intimately involve the chromosomes and which have furnished the occasion for this investigation.

These hypotheses are all contained in one comprehensive theory which has recently been brought into prominence by the rapid development of Mendelism. According to this theory the chromosomes are complex, accurately differentiated bodies whose organization and behavior are directly correlated with the genetic factors located in them. In any biparental organism, the diploid chromosome group is composed of two equivalent,

[1] Except in maturation stages, which are often very unfavorable for study.

parental series (haploid groups), the individual members of which are respectively homologous and very similar to one another; and this involves the view that the chromosomes are present in bi-parental pairs (Montgomery, Sutton, Boveri). In addition it is supposed, in accordance with the conception of W. Roux that every chromosome contains a definite complement of serially arranged genetic factors, each responsible for one or more inherited characters—the complement of factors being the same or similar in homologous chromosomes (members of a pair) but different in non-homologous chromosomes. In order to explain the perpetuation of this duplex germinal constitution a process (reduction division) is assumed to occur during maturation whereby the members of each pair are separated from one another and segregated in different germ-cells.

From the cytological point of view the principal questions involved in this theory are as follows: 1) Can definite pairs of chromosomes really be distinguished? 2) If so, are the two members of a pair derived respectively from the male and female parents? 3) Are the two members of a pair actually similar to one another and qualitatively different from the others in respect to their physico-chemical constitution? 4) Do the two members of a pair actually separate from one another and go into different germ-cells during maturation?

Three of these questions, together with one other of a more strictly cytological nature—the question of synapsis—form the central points about which most of the facts considered in the present study may be grouped. The nature of the material prevents the detailed consideration of each question in the order given, but so far as possible the evidence is presented in accordance with this scheme. The evidence bears especially upon the first question, to which a definite affirmative answer is given. With respect to the second question judgment should, perhaps, be suspended until the genetic continuity of the chromosomes is established, but if this continuity be assumed, this question is likewise answered in the affirmative. Regarding the third question only indirect evidence is furnished, but this evidence lends support to an affirmative answer here also. The fourth

question is not directly involved in the present paper. In regard to the problem of synapsis the pairing phenomena in diploid cells, including final spermatogonia, clearly demonstrate that a side by side approximation of corresponding chromosomes (the essential feature of synapsis), actually does occur, although in this case it is not connected with maturation.

Throughout the course of this study I have profited greatly by the counsel of Prof. E. B. Wilson, under whose direction the work was begun, and to whom I have become increasingly indebted for many kindnesses.

MATERIAL AND METHODS

My observations are based upon a study of the chromosomes in about eighty species of Diptera, representing thirty-five genera and fifteen families, as given in the following synopsis.

ORTHORRAPHA

- Nemocera
  - Culicidae
    - *Culex pipiens* Linne.
- Brachycera
  - Stratiomyidae
    - *Ptecticus trivittatus* Say.
  - Asilidae
    - *Asilus sericeus* Say.
    - *Asilus lecythus* Walk.
    - *Asilus notatus* Wied.
    - *Asilus novae scotiae* Macq.
    - *Asilus sadytes* Walk.
    - *Ommatius marginellus* Fabr.
    - *Leptogaster badius* Loew.
    - *Erax aestuans* Linne.
    - *Erax rufibarbis* Macq.
    - *Dasyllis grossa* Fabr.
    - *Dasyllis thoracica* Fabr.
    - *Deromyia winthemi* Wied.
  - Bombyliidae
    - *Anthrax lateralis* Say
    - *Anthrax sinuosa* Wied.
    - *Spogostylum simson* Fabr.

CYCLORRHAPHA

Syrphidae

Eristalis tenax Linne.
Eristalis bastardi Macq.
Eristalis aeneus Fabr.
Eristalis meigeni Wied.
Volucella obesa Fabr.
Mesogramma marginata Say.
Toxmerus annulatus Loew.

Acalypterae

Micropezidae

Calobata lasciva Fabr.
Calobata nebulosa Loew.

Sepsidae

Piophila casei Linne

Ortalidae

Chaetopsis fulvifrons Macq.
Camptoneura picta Fabr.
Euxesta stigmatius Loew.
Euxesta anonae Fabr.

Trypetidae

Euaresta melanogaster Loew.

Sapromyzidae

Physegenua vittata Macq.

Drosophilidae

Drosophila.—27 species, many undescribed, see text.
Cladochaeta nebulosa Coq.
Scaptomyza adusta Loew.
Scaptomyza graminum Fall.

Sciomyzidae

Neuroctena analis Fullen.

Calypterae

Anthomyidae

Homalomya spp.
Fucellia marina Macq.
Ophyra leucostoma Wied.

Muscidae

Calliphora viridescens Desv.
Calliphora erythrocephala Meig.
Musca domestica Linne.
Muscina stabulans Fall.
Phormia regina Meig.
Lucilia sericata Meig.
Pseudopyrellia cornicina Fabr.

Sarcophagidae

Sarcophaga falculata Pand.
Sarcophaga tuberosa serraceniae Riley.
Sarcophaga dalmatina Schin.
Sarcophaga bullata Park.
Ravinia communis Park.
Ravinia peniculata Park.

Preparations have been made from gonads of both sexes, and somatic tissues of various kinds. Almost all of the latter represent embryonic stages, including eggs, larvae and pupae. The former have been taken from larvae, pupae or adults, or all three, depending upon the species. In some species all stages from early spermatogonia or oogonia to the formation of spermatozoa or eggs could be secured from adults, but in most cases it was necessary to use pupae or even larvae in order to obtain the desired stages. This is especially true of the family Drosophilidae. *In all cases the gonads or small bits of tissue were dissected out of the specimens and then fixed;* none of the specimens was fixed entire or partially intact. This fact is emphasized because it has been found that regardless of the fixative used, inferior results are obtained if tissues are fixed in situ.

Dissections were usually made in Ringer's solution except in the case of large specimens, when tissues were dissected out in the body fluid. Dissection in tap water was tried with fairly good results, but mitotic figures were less distinct after this treatment than after the use of Ringer's solution.[2]

For fixation Flemming's strong solution was found most satisfactory and was most frequently employed. Objects were fixed from ten minutes to three hours depending upon their size. Longer treatment was tried, but with less satisfactory results due to frequent osmication and distortion. In addition to Flemming's fluid various other fixatives were tried. Of these Hermann's platino-aceto-osmic, and Gilson's mercuric-nitric gave the best results (in many cases as favorable results as those obtained by the use of Flemming's fluid), especially when it was desirable to differentiate the chromosomes without reference to other nuclear structures. Sublimate acetic and Gilson-Carnoy's acetic alcohol with sublimate were found fairly satisfactory for somatic tissues, but were inferior for the gonads. Bouin's fluid (formol mixture) though frequently used, proved quite undesirable because of rts tendency to distort and produce

[2] Dissection in tap water has been recommended by Doncaster ('14) for Abraxis.

clumping of chromatic materials. Good fixation with this method was secured only in the case of eggs and occasional large pieces of somatic tissue where its penetrating power was advantageous.

To supplement the permanent preparations, temporary 'smears' were frequently made with the use of Schneider's Aceto-carmine (Stevens '08 pp. 359–360) which proved to be a valuable agent for rapidly determining whether or not materials contained stages suitable for study. Frequently, one gonad would be prepared in this way and if found to be in the proper stage of development, its mate would be fixed in Flemming. The aceto-carmine preparations often gave very good figures of metaphase chromosome groups, but were found to be unreliable for detailed study because of the frequent distortion incident to swelling or mechanical disturbance. Consequently, most of the observations included within this study are based upon fixed and sectioned material. Sections were made 5 μ thick, except in a very few cases where unusually large cells were found and a greater thickness was desirable. Nearly all slides were stained with Heidenhain's Iron Haematoxylin, either alone or with a counter-stain of eosin or light green. Safranin was used frequently, but gave less distinct images, and failed to differentiate the finer chromatic elements as distinctly as did the haematoxylin.

For the study of cleavage and early embryonic stages Drosophila eggs were used. These were fixed at different periods, from a few minutes to a few hours, after being laid. It was found necessary in most cases to puncture the eggs, in order to facilitate the penetration of the fixative. When the eggs were punctured, successful fixation was secured with Flemming, Gilson's mercuric-nitric, Bouin, sublimate acetic and Gilson-Carnoy, all of which were about equally favorable.

A large proportion of the species included in this study have been reared in the laboratory for one or more generations, and the cytological material which they have furnished has largely been derived from pedigree cultures. In a few instances material was taken from jars of food which had been set out-of-doors, but this was used only when the identification of larvae and

pupae could be determined by the flies which subsequently hatched from the food. In no case is there any question as to the genus of the flies concerned and only in a few cases is the species doubtful. Such cases are mentioned in the text. Of tne families Asilidae, Bombyliidae, Syrphidae, Sapromyzidae, Ortalidae and Trypetidae, only adult flies were used.

For the identification of the Sarcophagidae, the writer is indebted to Mr. R. R. Parker, for that of Culex pipiens to Mr. Fred. Knab, for that of the Drosophilidae to Dr. A. H. Sturtevant,[3] and for all other identifications to Mr. C. W. Johnson who has very kindly examined a large series of specimens.

REALITY OF CHROMOSOME PAIRING IN THE DIPTERA

Since Stevens' observations on chromosome pairing in the Diptera were more or less incidental to other features, and since her conclusions have been directly opposed by those of Taylor and of Lomen on Culex—material upon which part of Stevens' work was based—it seems desirable first of all to ascertain definitely whether or not the so-called pairing phenomena in flies do in reality represent the association of independent chromosomes. In the opinion of Taylor ('14) and of Lomen ('14) the duality of the chromatic elements in Culex (and hence by inference in the other Diptera), is due, not to a pairing of two chromosomes but to the precocious splitting of one. Hence they conclude that the haploid number is present in both germinal and somatic cells, and that the somatic divisions are essentially the same as the maturation divisions. According to their idea each chromosome divides in anaphase, giving rise to two daughter chromosomes which remain separated during the resting stage and prophase (thus simulating a pair), and go to opposite poles in the succeeding division.

Before considering the contentions of Taylor and of Lomen further, I will present some of the evidence that has led me to conclude that the double chromatic elements in flies are really

[3] Several species of Drosophila included here are undescribed, and are given Sturtevant's manuscript names.

pairs of chromosomes. This will make clearer the exact points at issue and facilitate subsequent discussion of the contrasting views. The evidence which I wish to present may be considered under three heads as follows:

In the first place the number of chromosome pairs in diploid groups is the same as the number of single chromosomes in mature germ-cells. Figures of the chromosomes in spermatocyte divisions, either first or second, or both, accompany those of diploid groups in most of the species included here, and speak for themselves in this regard. A comparison of figures 13 and 15, 27 and 33, 24 and 25, 44 and 48, 52 and 53, 74 and 77, 125 and 126, 137 and 139, etc., clearly shows the relation between haploid and diploid groups. In some species, the chromosomes are evident even in the spermatids leaving absolutely no doubt as to the number contained in the spermatozoa. It must be concluded, therefore, that fertilization results in a diploid group in which the members of two haploid groups have associated in pairs, unless we resort to the very improbable assumption that an eliminating process intervenes at some stage of fertilization to throw out half of the chromosomes or to fuse them together two by two. Even this assumption, however, is overthrown by the relations of the sex chromosomes described below.

Secondly, if the diploid metaphase group were not made up of pairs, but were composed of double, univalent chromosomes, the two elements of these double chromosomes ought to lie one above the other, not side by side, in polar view, and in early anaphase a haploid group should be seen going to either pole. As a matter of fact neither of these conditions is realized outside of the maturation divisions. The two members of a chromosome pair lie side by side in metaphase, as shown by the figures, except for an occasional displacement, and frequently all of the chromosomes (the double number), may be seen dividing (figs. 7, 8, 9, 16, 28, 32, 40, 77). The side by side association and the method of division are clearly shown in figures 1–5, 7–9, 17, 19–24, 37, 39–46, 77, 98 and 99, etc. Figure 1, for instance, is composed of five symmetrical pairs, the members of which lie side by side. Figure 2 from the same species, shows similar

features. Likewise in figure 3 the side by side arrangement is obvious. Figures 4 and 5, 17, 19 and 20, from species possessing another type of chromosome group, bring out the same relations. In each case the two members of a pair lie side by side, not one above the other—with the exception of one misplaced chromosome in figure 19. Similarly in figures 21–24, representing another type of group, the side by side pairing is very distinct. Other examples are given in figures 27, 28, 37, 39–46, etc. These figures are not selected from among many in which pairing is less evident, but are perfectly typical and represent the normal condition in their respective species.

The manner in which division takes place during late metaphase or early anaphase is shown by figures 7, 8, 9, 16, 28, 32, 40, etc. Figures 7, 8, and 9 represent the same type of chromosome group as do figures 4, 5, 17, 19, 20, namely, a group composed of two long U-shaped pairs, one straight pair, and one small spherical pair. In all of these figures each chromosome (save the smallest in 8 and 9) may be seen dividing equationally, in the ordinary manner. In figure 16 the mode of division in a similar group is seen at a somewhat later stage. The dark chromosomes are seen at a high focus, the light ones at a lower focus. It is evident that each member of the diploid group has divided and sent a daughter half toward either pole. The smallest pair cannot be seen in this figure. In figures 28, 32 and 40 the same process is indicated in the case of two other species. Earlier stages in the same species are represented in figures 27 and 39 respectively. The features indicated by figure 28 are brought out even more clearly by figure 32 (a side view at the same stage). In figure 32 each of the short chromosomes has divided, while the two long ones have split in preparation for division.

Passing now to the later anaphases it may be seen that during this period a diploid, not a haploid, group goes to each pole, and in many cases the two members of a pair of chromosomes are so clearly separated from one another that they cannot be considered the result of a precocious split as suggested by Taylor and by Lomen. This fact is demonstrated conclusively

in those cases in which the two members of a pair have become separated and do not lie side by side in metaphase. A few cases have been found in which the two members of a pair lie on opposite sides of the spindle. In anaphase, each of these is seen to have divided and sent a daughter half to either pole. Figure 29 (same species as 27 and 28), for instance, shows a metaphase in which the two large members lie on opposite sides of the groups. In figure 30 a similarly arranged group is seen in anaphase. It is perfectly clear from the position of the large chromosomes in figure 30 that the two large elements going to one pole are not sister halves of one chromosome, but are daughter halves of two separate chromosomes, else they could not lie on opposite sides of the spindle at this stage. A comparison with figures 27 and 31 shows how this differs from the normal condition in which the large as well as the small chromosomes are paired. The duality of the chromosomes in figure 31, if this figure were taken by itself, might be interpreted as indicating a precocious division of single chromosomes, rather than as indicating pairs of chromosomes, but other facts, as just described, preclude such an explanation. It is doubtless such appearances as those given by figure 31 that have led some authors to misinterpret entirely the nature of Diptera chromosomes.

Fully as convincing evidence is furnished by other cases in which the two members of a pair have become only slightly displaced, instead of lying on opposite sides of the spindle. Such cases are shown in figures 7, 9, 12, 16, 28 and others. Figures 7, 9, 12 and 16 are different stages in nuclei containing the same type of chromosome group. It is obvious that here one of the large pairs has been disturbed in such a manner that its two members resemble two horse-shoes placed side by side. According to the ideas of Taylor and of Lomen these two members should go to opposite poles, but it is clear that they do not. On the contrary each divides and sends a daughter half to either pole. Figure 12 represents a particularly interesting case, for here the chromosomes have all divided and the daughter halves have separated. The figure on the left represents the upper group, that on the right the lower group (displaced in order to

show the chromosomes clearly). Above them is a diagram showing the two groups in position as they appear in the section. Each chromosome in the one group is seen to be represented by a corresponding sister chromosome similarly oriented in the other. Such cases furnish unequivocal evidence that the two members of a pair are not daughter halves of a univalent prophase element, but are distinct chromosomes, and that they both divide equationally in metaphase.

In the third place, diploid groups in the males of species having an unequal X-Y pair, demonstrate by the morphological difference between X and Y that the pair is composed of two distinct chromosomes. A striking example of this is seen in the three species of Drosophila shown in figures 41, 42, 44 and 45 (compare with figs. 49 and 50) in which species the X-chromosome of the males is fully twice the size of its mate Y. It would be difficult indeed to imagine these being daughter halves of a univalent chromosome. The same features are also brought out by other species having unequal sex-chromosomes (figs. 85, 86, 88, 124, 135, 137, etc.), although the evidence is not always so striking as in the three species cited.

These lines of evidence, I believe, leave no escape from the conclusion that pairing of chromosomes is a reality in the species here considered. That the mosquitoes are no exception to this rule will be shown below when the different groups of flies are treated independently.

The essential difference between the above results and those of Taylor and of Lomen center around one particular feature—the behavior of the chromosomes in late metaphase and early anaphase. The other stages are not seriously disputed. The question, therefore, is whether the two metaphase elements separate from one another in anaphase, thus effecting a reduction division, as described by Taylor and by Lomen, or whether each divides and sends a daughter half to either pole as Stevens maintained. I believe that I have demonstrated the correctness of the latter conclusion in the above paragraphs, and need not dwell further on it. The difficulty in the work of Taylor and Lomen is due, I believe, to faulty fixation of their material.

In my experience good preparations have been obtained only when the gonads or small bits of tissue were dissected out and fixed separately—never when the whole insect, or a considerable part of it was fixed intact. The latter method, which is apparently the one used by Taylor and by Lomen, produces a clumping or running together of the chromosomes, which is exactly the kind of behavior that would cause pairs to give the appearance of single chromosomes. Any tendency toward fusion is especially apt to exhibit itself in the anaphases, and hence it is to be expected that such figures as those obtained by Taylor and by Lomen would result whenever the fixation was defective. I have frequently obtained such a result when the fixation was poor, especially after Bouin's, Gilson-Carnoy's or alcohol-acetic fixatives.

DETAILS OF CHROMOSOME BEHAVIOR DURING ONE CELL-GENERATION

The mutual relationship of homologous chromosomes during the various stages of cell division has been carefully studied in both somatic and early germinal tissues of several species, and it is believed that the main facts regarding this relationship are now evident. In brief they are these: In metaphase, either in somatic cells, oogonia or spermatogonia, the chromosomes lie in a flat equatorial plate, the two members of each pair, with occasional exceptions, being arranged side by side as described above (figs. 1, 2, 3, 17, 19, 20, etc.) Each of these chromosomes splits longitudinally, and during anaphase sends a daughter half to either pole, still associated with its mate from the other member of the pair. Figures have already been given (7, 8, 9, 16, 28, 40) showing the chromosomes in the act of splitting, or the daughter halves in the act of separating from one another, also figures (12, 30, 31, 95, etc.) showing later stages in which the halves have become well separated and are going toward their respective poles. Retention of the paired association during anaphase is evident in all, except those in which one or two pairs have been disarranged. In the telophase, the chromosomes become closely massed and rapidly lose their

staining capacity, so that very little can be determined about the behavior of individual chromosomes. It is significant, however, that these chromosomes normally enter the telophase in a closely paired condition (figs. 31, 95, 169, 171) and it seems highly probable that they retain this relationship during the transformations in the resting nucleus. Such a conclusion is rendered almost certain by their subsequent behavior in coming out of the resting stage. The earliest prophase or spireme stages in which the chromatic threads may be distinguished with any degree of clearness show these threads to be intimately associated in pairs (figs. 11, 14, 34, 58, 65, 70, 71, 78, 80, 91, 92, 100, 123, 130, 131, 155, 165); and from this time on they may be seen to retain this association during their condensation and contraction from early prophase up to the time at which definite chromosomes are formed ready to go on the spindle. Some of the earliest prophases in which the chromatic threads were well defined are shown in figures 58 to 63 (Calliphora). Each of the double threads in these figures represents a pair of chromosomes. In figure 62 all six pairs are shown (the smallest being very faint), but in the others only parts of the nucleus are represented. Figure 65 is a later stage showing the chromosomes more condensed and contracted, but still closely apposed in pairs. Figure 66 is a still later stage, in which the chromosomes are assuming their definite shape preparatory to disjoining and going on the spindle. It is followed by the late prophase and metaphase stages represented in figures 53, 54, 55, 56 and 57. These are succeeded in turn by the late metaphase and anaphase in which each of the twelve chromosomes divides equationally as described above. Other early prophases are shown in figures 100 to 102 (Homalomya). The chromosome group here is indistinguishable from that of Calliphora (five large and one small pairs). In figure 100 the long, delicate but double threads are clearly distinguishable. It is impossible to determine precisely how many double threads are present, for some are broken, but the number is clearly about five or six, certainly not ten or twelve. Part of a similar nucleus is shown in figure 101. One of the most interesting features about

these figures (100, 101) is the evident polarization of the chromatic threads. This appears to be characteristic of very early prophases, although such stages are seldom clear enough to draw. When this polarity is compared with that shown by telophases (figures 111 and 112), it is difficult to avoid the conclusion that the two are correlated,—that is, that the chromosomes reappear during prophase in the same relative position, and polarized in the same manner as in telophase. Prophases in other species similar to those cited above are represented by figures 70, 71, and 72 (Musca), 106–108 (Fucellia), 78–80 (Phormia), 91, 92 (Sarcophaga), 130, 131 (Anthrax lateralis) and 123 (Eristalis). These are all essentially alike and involve corresponding chromosome groups. Prophases, together with metaphases for comparison, in species having fewer chromosomes, are shown in figures 14 and 15; 11 and 4, 5; 34, 35, 36, and 4, 5; and 165 and 166.

As seen in the figures all stages subsequent to the condensation of the chromatic elements in early prophase are easily followed, although the behavior of the chromosomes differs slightly in different cases. Usually the association of the two members of a pair becomes loose long before contraction is completed. At this time the two threads are loosely and irregularly coiled about one another (figs. 34, 59, 71, 92), and as contraction proceeds they become more and more loosely associated (figs. 35, 72, 93, 94). Occasionally, however, a close association is retained up to a very late period of contraction (figs. 36, 66, 108, etc.), with the consequent production of figures which very closely simulate those of haploid groups. Such figures as these might readily create the impression of haploid groups in diploid nuclei. By the time spindle formation takes place the chromosomes are usually distinctly disjoined from their mates, although the paired association is still conspicuous and may be very close (figs. 15, 41, 68, 117, 132, etc.) Occasionally the process of separation has been carried on so far that pairing is very indefinite (figs. 153, 161), but such cases are decidedly exceptional. Soon after the chromosomes become arranged on the spindle they begin to show evidences of splitting in preparation for

division (figs. 8, 28, 40, 77, 99, etc.), and by the time the equatorial arrangement is completed they may all exhibit a longitudinal split. It is this stage that demonstrates unquestionably the presence of a diploid instead of a haploid group.

As shown by the figures, especially numbers 7, 28, 29, 68, it occasionally happens, as mentioned above, that the members of a pair appear in metaphase on opposite sides of the spindle, or separated from one another by other chromosomes. This disarrangement apparently takes place in late prophase while the chromosomes are becoming equatorially oriented. Several cases have been observed in which the members of a pair were partially separated by other chromosomes. and it seemed a question as to whether they would be forced completely apart, or would succeed in taking their places together. The frequent appearance of the condition in which the two members are on opposite sides of the plate appears to be due to their having approached the equator of the spindle vertically instead of horizontally, i.e., from one pole instead of from the side—and thus having been pulled diametrically apart, with their points of attachment near together but their extremities pointing in opposite directions. At first sight it would appear that chromosomes once separated in this manner would have difficulty in associating again, and that after many divisions all the pairs would be disarranged. An examination of chromosome arrangement in late anaphase indicates one reason at least why such a confusion does not occur. During this stage the chromosomes are drawn out in a slender cone with their apices brought close together at the pole. As a result all of the chromosomes are rather closely approximated throughout their lengths, and an ample opportunity is afforded for the reunion of separated members of a pair, even if they previously lay on opposite sides of the spindle.

In my paper on Drosophila chromosomes (Metz '14, p. 56), I mention the apparent occurrence of a 'second conjugation' of chromosomes in early metaphase, after the separation which normally occurs in prophase. The details of this phenomenon were obscure at the time, and were left for further study. It

appears now, after a careful study of these stages in a large number of flies, that the so-called 'second conjugation' is of only occasional occurrence, and is not a uniform stage in the chromosomal activities. In some, if not all cases, it is simply a retention of the close approximation that existed in prophase.

When considered step by step, as has just been done, it is remarkable what a resemblance the above processes bear to those of maturation. In the early prophase stages of either somatic or gonial nuclei an almost exact simulacrum of diplotene nuclei is often found. This extends in some cases, even to definite polarization of threads within the nucleus, such as is shown in figures 100 and 101.[4]

PAIRING IN DIFFERENT TISSUES AND DURING DIFFERENT STAGES IN ONTOGENY

No attempt has been made in this study to examine in detail all of the somatic tissues in any one species. Various tissues have been dissected out at different times, however, and fixed with the gonads. In this manner I have been able to study division figures in most of the tissues of the body and during most stages of ontogeny. Among the organs and tissues definitely identified in these studies the following may be mentioned; embryonic brain, eyes, malpighian tubules and wing buds, and somatic as well as germinal parts of the testes and ovaries. I have also examined various bits of tissue taken at random from dissected larvae and pupae of various ages.

In addition to studying isolated pieces, I have studied sections of entire embryos (larvae) in which all of the tissues could be examined. Of course division figures were never visible in all the tissues of these total preparations, but they were frequently found in several parts of one object.[5]

In regard to the ontogenetic development I may state that I have examined all stages from the newly hatched larvae up

[4] See concluding paragraph, page 257.

[5] As mentioned under 'Methods' the figures in total preparations are poor, but they are sufficient to show whether the chromosomes are paired or single.

to the sexually mature fly in several species of Drosophilidae, Muscidae and Anthomyidae.

The results of all these studies on somatic tissues may be summed up in one sentence, namely, that in all tissues of the body and during all stages in development from the newly hatched larva to the adult fly the paired association of corresponding chromosomes is a universal characteristic. So far as I have been able to determine, the pairing phenomena are identical in all diploid cells, whether somatic, spermatogonial or oogonial, from the egg to the adult.

DIFFERENT SPECIES AND FAMILIES COMPARED

In order to determine whether the paired association of chromosomes is characteristic of all Diptera or whether it is restricted to certain individuals or groups, an attempt has been made to study representatives of all the principal divisions in the order. As a result, sixteen families ranging from among the lowest to the highest have been included in the survey. Some of these families are represented by one or two species, others by several species. Since the principal aspects of the pairing phenomena are essentially the same in all of the flies studied no attempt will be made to treat each individual species. Instead, a few characteristic members will be chosen as representatives of the respective families. Likewise, no attempt will be made to give a complete account of the chromosome behavior in each species treated. In many cases only enough figures are reproduced to show the nature of the chromosomes and their paired association.

For convenience the order of treatment of the families is the reverse of that given in the synopsis (i.e., from the highest to lowest instead of vice versa), except that the Muscidae will be considered before the Sarcophagidae.

Muscidae

Calliphora erythrocephala (figs. 51–66). Figures 51 and 52 represent the haploid group of this species, taken from first spermatocyte divisions. The group consists of four similar,

long chromosomes, one shorter chromosome and one small, spherical chromosome. Figures 53 to 57 illustrate corresponding diploid groups of the same species taken from ovarian (53–56) and somatic (57) cells. From these figures it is evident that for each single chromosome of the haploid group there is a pair of chromosomes in the diploid group, and that the members of this pair are in close proximity to one another. Earlier stages, showing the origin of the pairs in prophase, are given in figures 58–66. Some of the figures represent only sections of the nucleus, but others (58, 62, 63, 65, 66) are taken from uncut nuclei and include all of the chromatic material. In early prophase stages the five pairs of long chromosomes are clearly represented by the five long, double threads as shown in figures 58, 62 and 65. Frequently the small pair is concealed and cannot be distinguished, but in many cases it is as clearly evident as are the others (figs. 62, 65). The duality of the threads in early prophase is perfectly distinct in almost all cases. The figures given here are entirely typical of scores studied, and are taken from various tissues of the body all of which show the same phenomena in dividing cells. Very rarely a figure is found in which no duality can be seen in the threads (fig. 63), but it seems certain that this appearance is due merely to overstaining which conceals the true dual nature. Figure 65 is a good example of such a case. When first studied the members of this group appeared to be perfectly homogeneous elements and were drawn as such, but after the material had cleared in balsam a few months, the duality of the threads became very evident, as shown in the figure. I have no hesitancy, therefore, in considering figure 63 to be of the same nature, especially since it is almost the only clear case of its kind found.

During later prophase stages such as shown in figures 53, 55 and 66, the chromosomes rapidly contract, and condense, and the members of a pair dissociate somewhat in preparation for division. When they go on the spindle they form a flat equatorial plate, with corresponding chromosomes arranged side by side in the same plane. Only in exceptional cases, such as are in-

evitable under the circumstances, are the two members of a pair in any other relation than this during metaphase.

Musca domestica (figs. 68–72). In Musca the chromosomes are very similar in form and behavior to those of Calliphora, except in respect to the sex-chromosome pair, which is almost as large as the autosomes. Haploid groups of Musca have already been published by Stevens ('08, fig. 3). The accompany ing figures are taking solely from diploid groups to illustrate the pairing phenomena. They are all from ovarian tissue far in advance of maturation stages and may be said to represent the characteristic features of prophase and metaphase in early ovarian and somatic cells. Figures 68 and 69 are metaphases showing the six pairs of chromosomes in the equatorial plate. It will be noticed that in each figure the members of one pair of chromosomes are displaced and are not closely associated. These are in all probability the sex-chromosomes (XX). Prophases showing the early appearance and the disjunction of the chromosomes are represented in figures 70, 71 and 72. The former is from an entire, or nearly entire nucleus, the latter two are from cut nuclei, but each includes almost all of the chromatin.

Phormia regina (figs. 73–80). Haploid groups of this species are shown in figures 73 to 75 (second spermatocytes) and figure 76 (first spermatocyte). As shown in figures 73 and 75 in contrast to 74, the sex-chromosomes (smallest in each case), are very unequal. In figure 76 they may be seen separating from one another in the reduction division. Figure 77 is taken from a spermatogonial cell in early anaphase (or late metaphase), and shows the six pairs of chromosomes, corresponding to the six single chromosomes of the haploid group; each of these is split lengthwise in the process of division. In the center may be seen the unequal X-Y pair splitting in the same manner as are the autosomes. A comparison of this figure (77) with that of a similar stage in the reduction division (76) clearly brings out the relation between the two groups (haploid and diploid). Prophases from early ovarian tissue showing the origin and behavior of the pairs in preparation for division in

diploid nuclei are given in figures 78 to 80. They differ in no essential respect from those in Calliphora and Musca.

Likewise the other Muscidae studied (Muscina stabulans, Calliphora viridescens, Lucilia sericata, and Pseudopyrellia sp.) agree with those already described.

Sarcophagidae

Sarcophaga (figs. 81–97). Several species of Sarcophaga have been used in this study and have been found to agree so completely in respect to chromosome behavior that they will be treated as a whole. For specific references see explanation of figures on p. 270. Haploid groups from second spermatocyte divisions are given in figures 81 and 82, and from first spermatocyte divisions in figures 83 and 84. The last named is a side view showing the inequality of the X Y chromosomes at the time when they separate during reduction. Corresponding diploid groups are represented by figures 85–88 (spermatogonial), figure 89 (ovarian follicle cell) and 90 (somatic, embryonic cell). In the male groups (figs. 85–88) the difference between X and Y (smallest chromosomes) is plainly evident. Prophases showing the early appearance of the pairs, and quite comparable with those in the Muscidae, are given in figures 91 (somatic, two sections of same nucleus), and 92 to 94 (somatic). An anaphase from a similar cell (embryonic glandular tissue) is given in figure 95. It clearly shows the persistence of the paired association and indicates the relative positions occupied by chromosomes when they enter the telophase and subsequent resting stage. In this figure the spindle fibers are schematized, but the chromosomes as in other figures are drawn in their exact position. Figures 96 and 97 are taken from multiple groups (somatic) showing respectively 24 and 48 chromosomes. The former is significant because it shows tetrad aggregates instead of pairs (compare with figs. 85–90 and see pp. 252 and 253). In the latter the chromosomes are so massed together as to obliterate the associations.

Ravinia peniculata (figs. 98, 99). This species is indistinguishable from those of Sarcophaga in respect to pairing phenomena. Figures 98 and 99 are ovarian (early pupal) metaphases showing the six pairs of chromosomes essentially like those of Sarcophaga. The latter shows the metaphase splitting of the chromosomes very clearly (compare with figure 77).

Anthomyidae

Homalomya sp. (figs. 100–105). Particularly clear prophase figures have been secured in this species, both with respect to somatic and to spermatocyte divisions. The chromosome group is practically indistinguishable from that of Calliphora (figs. 51–57). Figures 100 and 101 are very early prophases from somatic nuclei, illustrating the configuration of the chromatic threads at this time. The former is from an entire, or almost entire nucleus, in which the bivalent (double), long drawn out threads, each representing a pair of chromosomes, are discernible. Attention is particularly called to the polarization of these threads and the resulting similarity in appearance between this somatic prophase and the synaptic stages accompanying maturation in many other animals. Figure 101 represents a similar stage from the same tissue, but includes only a portion of the nucleus. A later stage in which these threads lose their polarity and contract before giving rise to the metaphase chromosome pairs is shown in figure 102. In comparison with such somatic and spermatogonial prophases it is of interest to examine corresponding stages in the maturation divisions. Figure 103 is a portion of a second spermatocyte prophase and shows sister chromosomes closely intertwined preparatory to going on the spindle. In metaphase (fig. 104) they come to lie one above the other in the equatorial plane. Figure 105 is a second spermatocyte anaphase. In figure 103 only three of the chromosomes are represented, but in 104 and 105 the full (haploid) complement is present. The double elements in these cases are split univalten chromosomes, the two members of which separate in anaphase as shown in figures 104 and 105. It is important

to note that at certain stages in prophase the figures of all three (somatic, first maturation and second maturation) divisions are superficially very similar, although the actual processes in the three cases are very different.

Fucellia marina (figs. 106–110). As in the previous case, so in the present, the paired relationship of the chromosomes is essentially like that described for the Muscidae and Sarcophagidae, and requires no detailed description. A few somatic prophases have been reproduced to show the origin of the chromosomes in the former in the form of closely paired threads, and the subsequent disjunction of these into the less closely associated condensed chromosomes found in metaphase. Figure 106 is an early prophase showing the six bivalent threads. Figures 107 and 108 are somewhat later stages illustrating the separation of the threads. All three are complete (diploid) figures. The most interesting features observed in Fucellia are those shown by prophases containing multiple (probably tetraploid) groups (figs. 109, 110). Each chromatic aggregate in these, contains four (or eight) chromosomes instead of the usual pair, (compare with figs. 96 and 97 and see pp. 252 and 253).

Ophyra leucostoma (figs. 111–114). In most of the Diptera studied so far great difficulty has been experienced in analysing telophase figures. Usually the chromatin is so massed at this point that no details whatever can be distinguished. In the present species, however, a few figures have been obtained, which although far from satisfactory, are nevertheless sufficient to show something of the chromosomal behavior during this stage. Two of these are shown in figures 111 and 112. They suffice to show the loop or U-shape of the chromosomes, and suggest the process of reticulation that is taking place as the chromatin becomes diffuse. The polarity of these U-shaped threads bears a significant relation to the similar polarity evident in early prophase when the chromosomes reappear (figs. 100 and 101). The chromosome group and the pairing phenomena of Ophyra are practically the same as those of Homalomya and Fucellia. Figure 113 shows a late diploid (spermatogonial) prophase with six pairs of chromosomes, some of which already

indicate the metaphase split; and figure 114 shows a corresponding but somewhat later stage in the first maturation (reduction) division.

Sciomyzidae

Neuroctena analis (figs. 115, 116). There is nothing peculiar about the chromosomal behavior in the Sciomyzidae, so far as I have been able to determine. Several specimens of N. analis have been studied, with results comparable in every way to those already described. The two accompanying figures are sufficient to show the paired association and the relation between haploid (fig. 115, second spermatocyte) and diploid (fig. 116 spermatogonial) groups.

Trypetidae

Flies of this family, so far as my experience goes, are not favorable for chromosome studies. Nevertheless they present sufficiently clear figures to show that the paired association is present here just as it is in other flies. Most of my studies were made upon Euaresta melanogaster, material of which I secured in Cuba. The chromosome group of this species appears to be composed of six pairs similar to those in the Muscidae, although no figures have been found that are complete and at the same time clear enough to settle this point.

Ortalidae

No embryonic stages (larvae or pupae) have been secured from any members of this family, and consequently no somatic divisions have been studied. Spermatogonial and spermatocyte divisions have necessarily formed the basis of my observations on both of the following species, yet there can scarcely be any question that there is a definite correspondence between the phenomena exhibited by spermatogonia and somatic cells.

Chaetopsis fulvifrons (figs. 117–119). Chromosomal behavior in spermatogonia of this species corresponds fully with that described for ovarian and somatic cells in species of Drosophili-

dae (figs. 4–20) having a similar chromosome group. In Chaetopsis no good figures of early spermatogonial prophases have been secured, owing to the small size of the nuclei, and to difficulties in fixation. Metaphases, however, are distinct (figs. 117–118) and plainly show the paired arrangement of the chromosomes. These, when compared with maturation divisions showing the haploid group (fig. 119, first division) leave no doubt of the relations in this species.

Camptoneura picta (figs. 120, 121). Since C. picta shows pairing relations similar to those in the last named species it attracts attention only because it differs so markedly from Chaetopsis in respect to the number and size relations of its chromosomes. As a matter of fact Chaetopsis excites the greater interest, for Camptoneura has the chromosome group (fig. 120, diploid, and 121, haploid) found in several families (all those above mentioned, as well as the Sapromyzidae, Micropezidae, Sepsidae, Syrphidae, and one species of Bombyliidae), while the group found in Chaetopsis is found in no other species I have studied outside the Drosophilidae.

Sapromyzidae

Physegenua vittata (fig. 122). I have had difficulty in obtaining suitable material from Sapromyzid flies, but as in the case of the Trypetidae enough has been secured to determine the essential point—that the chromosomes are associated in pairs. Figure 122 (spermatogonium) represents one of the few complete polar views found. It is seen somewhat diagonally, with the result that some of the pairs appear to lie beneath the others, but in reality they form an almost flat plate, entirely comparable with those seen in the Muscidae, etc. The two small chromosomes are doubtless the sex-chromosomes (X Y), just as are the small ones in the Muscidae.

Drosophilidae

(See pp. 222–224, "Reality of chromosome pairing." For specific references see explanation of plates; also Metz '14.)

Syrphidae

Eristalis tenax. My studies in this species have included pupae as well as adults, and in both I have found the chromosome behavior to agree with that in the cases described above, and with Stevens' ('08) description.

Eristalis bastardi (fig. 123); *Volucella obesa* (figs. 124–126); *Mesogramma marginata* (figs. 127, 128). These three species are very different from one another in appearance, but their chromosomes appear very similar (save for minor details of size relations) and hence will be considered together. Figure 123 (Eristalis bastardi) represents part of a prophase figure showing the bivalent chromatic threads which are comparable in every way with those seen in Homalomya, Sarcophage, etc. Figures 124 and 125 are metaphases (spermatogonial) of Volucella, and clearly show the paired relationship. In the former one chromosome is missing, leaving a single member (in left margin of group) without a mate, but otherwise all are paired. This species is particularly interesting because of the different sizes apparent in its chromosomes. One pair is easily recognized by its large, and one (sex-chromosome) by its small size, and even the others show slight differences from one another. Figure 126 is a first spermatocyte division for comparison with the diploid groups; note the unequal X and Y chromosomes, which are paired in the diploid groups. Figures 127 and 128 (spermatogonial) of M. marginata are of significance only in showing the paired arrangement of the chromosomes.

Sepsidae

Piophila casei. There is no marked distinction between P. casei and the various species of Muscidae and Sarcophagidae, either in chromosome numbers and size relations or in the general chromosome behavior.

Bombyliidae

Anthrax lateralis (figs. 129–133). No more conspicuous cases of chromosome pairing have come to my attention than those

exhibited by this and other species of Bombyliidae. Figures 129 to 133 are only a few from among scores of similar ones studied. In all cases the five large pairs and often the small pair stand out clearly and show a close approximation. The figures need little explanation beyond that given already for preceding species. Numbers 129 to 131 are spermatogonial prophases showing the five long and one short double threads, which later loosen up and contract to form the metaphase pairs shown in figures 132, 133.

Anthrax sinuosa (figs. 134–140). This species is very interesting from several standpoints. In the first place it possesses chromosome pairs of various sizes (figs. 134–137), which clearly illustrate the pairing of corresponding chromosomes. Secondly the evident dissimilarity between A. sinuosa and A. lateralis in number of chromosomes, the former having 18, the largest group in any fly within my knowledge, and the latter possessing but twelve, presents the greatest divergence of this nature that I have observed between two species in one genus. Thirdly, the sex-chromosome pair is apparently one of the largest in the group, instead of the smallest, as has been the case in all of the above species exhibiting a conspicuous inequality between X and Y. Unfortunately I have been unable to identify the sex-chromosome pair in A. lateralis. If the small pair in A. lateralis (figs. 132, 133) is the sex-chromosome pair, as it is in many flies, then a remarkable difference exists between the sex-chromosomes of the two species, such a difference as I have found in no other closely related flies. Similar differences have been observed between related species of Hemiptera and Coleoptera, but seem to occur very rarely among the Diptera. In maturation divisions of this species (figs. 138–140) the short chromosomes show a tendency to become rounded, but the relative sizes are readily seen to correspond with those of the diploid groups. Figures 139 and 140 (second spermatocytes) appear to be respectively X- and Y- containing groups. As the spermatogonial figures (134–137) show, X is the largest chromosome present, while Y is smaller than the two largest autosome sizes. Comparing figures 139 and 140 it may be seen

that the latter contains three large chromosomes (X and the two largest sized autosomes), while the former (139) has only two large chromosomes but has an extra small member which must be Y. No sufficiently clear first maturation divisions have been found to show the X-Y relations of that stage, unless the apparently single element projecting from the largest chromosome in figure 138 is the unmatched end of X. If so, one of the smaller pairs is concealed. The figure is drawn just as it appears, but I am not sure of its significance.

Spogostylum simson (figs. 141, 142). No males of this species were secured, but very clear figures were observed in ovarian follicle cells. Two of these are given to indicate the similarity between the pairing here and in the other species. Figure 141 is a metaphase plate showing the diploid group and the association in pairs. Figure 142 illustrates a similar cell in prophase with corresponding chromosomes forming closely united double threads in the characteristic manner. As the figures indicate, this group differs markedly from both species of Anthrax in the size and form relations of its members. Apparently there is no dominating type of chromosome group in the Bombyliidae such as is seen in the majority of other families.

Asilidae

Twelve species of this family have been studied as indicated in the synopsis (p. 217), but only a few of them need be considered. Those chosen are selected particularly to illustrate the various numbers and sizes of the autosomes, and the varying degrees of inequality of the sex-chromosomes. Pairing is constant in all of them.

Asilus sericeus (figs. 143–145). This species has perhaps the most simple group found in the family, containing as it does only five pairs of chromosomes, and lacking any conspicuous inequality between the sex-chromosomes. Yet it is one of the most interesting groups I have found, for each pair appears to differ from all the rest in respect to size. The two large pairs are admittedly

very nearly the same size, but even they may be distinguished in some figures (note especially figures 143 and 144).

Asilus lecythus (figs. 146–148). Scarcely less striking in the matter of size differences is the evidence presented by this species. Upon close examination its seven pairs (or its seven single chromosomes in haploid groups) are seen to be definitely graduated in size from the smallest to the largest. The gradations are somewhat confused in the diploid groups by the unevenness and the flexures of some of the chromosomes, but in haploid groups (fig. 148, second division) the gradation is much more conspicuous. The sex-chromosomes, apparently, are not unequal.

Asilus notatus (figs. 149, 150). What has been said of the last species (A. lecythus) applies equally to the present one, except that the size differences between the larger pairs are scarcely distinguishable. Figures 149, 150 show spermatogonial and second spermatocyte groups of this species.

Leptogaster badius (figs. 151, 152). The diploid group of this species is shown in figure 151. As may be seen it consists of five pairs, only two of which may be differentiated by size. The largest of these is the sex-chromosome pair, whose members, as in previous cases are frequently not associated during metaphase. The haploid group is indicated by figure 152 (second division).

Erax rufibarbis (figs. 153, 154). In this species, also, five rather similar pairs of chromosomes are found. As in the previous case only the smallest and largest (sex chromosome pair) may be differentiated. Figure 153 shows the chromosomes in a flat plate and indicates their size relations. In spermatocyte divisions the chromosomes of E. rufibarbis show a decided tendency to condense and become rounded, but the size relations are nevertheless conspicuous (fig. 154). This tendency toward condensation extends even into the spermatids, thus enabling one to count the chromosomes with ease, and to determine without doubt the number of chromosomes carried by the spermatozoan into the egg.

Dasyllis thoracica (figs. 155–158). D. thoracica furnishes evidence very similar to that presented by Asilus sericeus. No two of its five pairs of chromosomes (fig. 156) appear to be the same size. The smallest and next smallest pairs are very distinct, as is also the largest. Possible confusion arises then, only in connection with the two intermediate pairs, but since one of these appears to be the X-Y pair its dimorphism, if the apparent dimorphism is real, serves to differentiate it from the other intermediate pair. I have been unable to obtain sufficient spermatogonial figures to determine definitely the sex-chromosome relations, but evidence from the first spermatocyte divisions makes it probable that the relations shown in figure 156 are correct. In the first spermatocytes (fig. 157), one of the intermediate pairs (corresponding to XY in figure 156) appears to have a univalent attachment (X in the figures) at one end, which strongly suggests the unpaired end of an X-chromosome. Analysis of the first spermatocyte group (fig. 157) then, reveals one small spherical chromosome (1), one small, elongate chromosome (2), one larger, symmetrical chromosome (3), one similar, but asymmetrical chromosome (4), and one largest chromosome (5), each distinct from all of the others. In the diploid group each of these is represented by a pair of chromosomes. A diploid group showing the intimately paired association in prophase, similar to that in the Muscidae, etc. is given in figure 155. A second spermatocyte, haploid group is shown in figure 158. The seeming duality of the largest chromosome here is simply due to the metaphase split, and is not related to the apparent sex-chromosome dimorphism of the first division.

Deromyia winthemi (figs. 159–164). The six pairs of chromosomes in this species (figs. 159–161) are graduated into four sizes, of which the largest and smallest are represented by one pair each, and the two intermediates by two pairs each. In some figures (161, 164) even these intermediates appear to be individually differentiated, but the distinctions are not great. The sex-chromosomes (X and Y) are very dissimilar, and, as shown by the figures, are more often dissociated than are homol-

ogous autosomes. Figure 162 shows the X and Y -chromosomes separating from one another in the reduction division. Figures 163, 164 are second spermatocyte groups showing the X-containing and Y-containing classes.

Stratiomyidae

Ptecticus trivittatus. No differences in chromosome behavior (so far as the paired association is concerned) have been found to distinguish this species from those previously considered. P. trivittatus possesses eight pairs of chromosomes, of which the smallest is the unequal sex-chromosome pair.

Culicidae

Culex pipiens (figs. 165–171). Since exception has been taken to the observations of Stevens on the chromosomes of Culex pipiens (see p. 221), I have made a careful study of this species in order to determine whether any fundamental differences exist between it and the higher Diptera with regard to chromosome pairing, but I am not able to find such differences. My studies are based upon spermatogonia and ovarian cells from larvae and pupae. In these I find the six chromosomes closely associated in pairs during prophase (fig. 165), dissociating somewhat in late prophase, and arranging themselves side by side in a flat plate during metaphase (figs. 166–168) just as in the other Diptera. There is no evidence whatever, in my material, of a separation (reduction) of the two members of a pair during anaphase such as described by Taylor and by Lomen. On the contrary, anaphase figures clearly show each chromosome dividing and sending daughter halves to the poles. Figure 170 (a portion of an early spermatogonial anaphase in side view) shows the manner in which each individual chromosome divides. Figure 169 shows a later stage of a typical anaphase (spermatogonial) also in side view, in which six chromosomes (three pairs) are each undergoing a division. At this stage the chromosomes are in the form of double V's each of which is a daughter chromosome attached at its apex to a spindle

fiber. In the figure (169) the V-shaped chromosomes are all seen edgewise, so that one arm lies almost directly below the other (indicated by light shading.) The lower arms of the pair on the left are not visible (apparently being cut off by the knife), but the other two pairs are entire and clearly show the method of division. It is perfectly plain that the two chromosomes in the pair on the left have completely divided, that those in the center have almost divided, while those on the right have only partially divided and show the daughter halves attached for some distance at their ends. Figure 171, in which only two of the three pairs are drawn, shows the same features. It is obvious that such figures as these could not possibly result from a division in which the two members of each pair went to opposite poles, even supposing them to split in early anaphase as conceived by Taylor and by Lomen. The figures reproduced here are only a few from among many studied, all of which present the same features.

There can be no question, therefore, that in the ordinary (diploid) mitoses in Culex, the two members of a chromosome pair, lying side by side in the metaphase plate (figs. 166–168), both split longitudinally in the equatorial plane (transversely to the axis) of the spindle, and that each sends a V-shaped daughter half to either pole, or in other words, that an equation division is effected. This is in direct opposition to the ideas of Taylor and of Lomen who concluded that the two members of a pair lie one above the other in metaphase, that they go to opposite poles in anaphase (effecting a reduction division) and that as they go they split in a line parallel to the axis of the spindle. As I have heretofore stated (p. 226) I believe that Taylor's and Lomen's errors are due to poor preparations in which the anaphase chromosomes were so massed together as to entirely conceal their true nature and behavior.

DISCUSSION

One of the most interesting chapters in the history of modern biological progress is that marked by the rise into prominence of the 'chromosome theory' of heredity. And contributory

to the development of this theory probably no single conception has been of more value than that which postulates a qualitative differentiation among the chromosomes (Boveri '01), and an individual homology between respective members of the two gametic groups (Montgomery '01). The growth of this conception is of particular interest in the present connection.[6] It was based, of course, upon the foundation laid by Van Beneden's 'law' ('83) of the equivalence of maternal and paternal chromosome groups, and upon the principles of chromosomal individuality and continuity developed by Rabl ('85), Boveri ('87, '88, '91), Herla ('93), Zoja, Van Beneden and others, but not until 1901 did it assume its present features. From Montgomery first came the idea that each chromosome in the spermatozoon has an equivalent mate in the egg, that fertilization brings the two together in one cell, and that maturation segregates them again into different cells—the gametes.[7] These conclusions were based upon a study of several Hemiptera (Protenor, Peliopelta, Zaitha), in which certain pairs of spermatogonial chromosomes, distinguished by size and shape, apparently became associated in synapsis and underwent segregation in the reduction division. The almost simultaneous and even more far-reaching observations of Boveri ('01) were from his well known experiments on dispermic sea-urchin eggs, in which he demonstrated a qualitative difference between the respective chromosomes in their effect upon development.

Further attention may be confined to features relating to chromosome pairing. The first of these is the discovery by Montgomery in 1904 and 1905 of a paired association of corresponding chromosomes in cells other than those involved in the maturation process. These observations were made upon Plethodon, and upon the Orthopteran, Syrbula, in the latter of which he found twelve of the twenty chromosomes to possess size differences enabling him to assort them into six groups of

[6] For a comprehensive review see Wilson '05, '14, Conklin '14, East '15, and Morgan, Sturtevant, Muller, Bridges '15.

[7] This conclusion was forecasted perhaps by Henking in 1891, and by Montgomery in 1900, but was first given definite expression by Montgomery in 1901.

two each. The members of these groups, according to his observations are already actually associated in symmetrical pairs in the last spermatogonial divisions and later, in the spermatocytes, undergo synapsis and reduction.

After once discovering pairing in spermatogonia he returned to the subject again in 1908 with additional evidence based on studies of Ascaris, and again in 1910 with more evidence on the Hemiptera.

In 1902 Sutton described a significant case (Brachystola) in which he believed that all of the chromosomes could be assorted into pairs according to size characteristics. It should be noted that the chromosomes in Brachystola are not actually arranged in pairs, and that the size differences between them are scarcely sufficient to make possible an accurate analysis; yet in spite of this the probabilities afford strong support to Montgomery's deductions. Further support was given by Janssens and Willems ('08), whose description of paired chromosomes in spermatogonia of Alytes corroborated that of Montgomery on Plethodon. Similarly, the studies of Wilson, Payne and others on the Hemiptera, of McClung and his students on Orthoptera, of Stevens on Coleoptera, and of various others, plainly demonstrated that in animals possessing chromosomes of different sizes and shapes there are always two (or multiples of two), of each kind (excepting the sex-chromosomes of the male).

Contemporaneously with these researches in the field of zoölogy, there was taking place a strikingly similar development along botanical lines. Indeed it is an interesting coincidence that almost simultaneously with Montgomery's discovery of pairing in the spermatogonia of Syrbula, Strasburger ('05) observed a like association in certain plants. He even went one step further than Montgomery in finding the paired association in somatic cells, entirely distinct from the germinal tissues. In embryonic nuclei of Galtonia candicans he found four small and eight large chromosomes, which exhibited an association in pairs. Likewise in Funkia Sieboldiana he observed twelve large and thirty-six small chromosomes showing a similar paired relationship. "Ich habe zu oft in den Geweben von Galtonia,

und noch haüfiger von Funkia in vorgerüchten Prophasen gleich grosse Chromosomen in Paaren nebeneinander liegen sehen . . . . " ('05, p. 19). The pairing in these cases, as in those of Montgomery, is seldom intimate, if one may judge from the published figures, but there can be little question that it is real.[8] Somewhat later paired chromosomes were recorded by Strasburger ('07) in root-tips of Pisum, by Sykes ('08) in Hydrocharis, Lychnis and Bryonia,[9] and by Overton ('09) in root-tips of Calycanthus floridus, where the chromosomes are said to be arranged in pairs not only during metaphase, but also (as prochromosomes) in resting stages and prophases. In Calycanthus, as in Pisum and some of the other cases, the size difference between respective pairs is not noticeable, but the pairing is very intimate, and if Overton's counts are correct there can be no doubt as to the essential facts.[10] In the same year Müller ('09) described a pairing of chromosomes in somatic metaphases of Yucca. The statements of Müller were soon challenged by Bonnet ('11) who maintained that since only two sizes of chromosomes were present, and there were numerous representatives of each, such associations as those described by Müller were probably due merely to chance. In view of Müller's recent work ('12) however, in which he describes unmistakable cases of pairing in other plants, it seems unlikely that he was mislead by purely accidental phenomena in the previous case.

In 1910 Strasburger described further cases of chromosome pairing in root-tips of Melandryum rubrum, Mercurialis annua, and Cannabis sativa, in each of which different sized pairs were evident, although the spatial association was not very conspicuous. Stomps ('10, '11) during the same period found a comparable pairing in Spinacia, a plant possessing three large and three small pairs of chromosomes. Similarly Nemec ('10) work-

[8] Confusion has arisen in some cases by the application of the terms 'pairs,' 'paired chromosomes,' etc. to split, univalent chromosomes, and in other cases by a difference of opinion between different investigators on the same material, but those cited here are all based upon reasonably good evidence.

[9] Sykes at the same time confirmed the observations of Strasburger on Funkia and Pisum.

[10] His conclusions have been disputed by von Schustow '13.

ing on the root-tips of Ricinus, Kuwada ('10) on Oryza sativa, Tahara ('10) on Morus alba and M. indica, and Ishikawa ('11) on Dahlia coronata all observed evidences of pairing in somatic cells. The observations of the three Japanese authors are particularly convincing because of the variety of sizes among the chromosomes with which they deal, and the symmetry of the pairs. Shortly afterward Gates ('12) records slight evidences of pairing in Oenothera and expresses his belief that pairing in somatic metaphases "is widespread in the sporophyte tissue of plants" (p. 1004). During the same year Müller ('12) in a comprehensive study of metaphase pairing in plants figures and describes the paired condition in more than a dozen species, several of which had not been treated previously. Among the species described by Müller the following furnish convincing evidence: Najas marina, Galtonia candicans, Listera ovata, Albuca fastigiata, Aloe Hanburyana, Eucomis bicolor, Bischornerea superba, Bulbine annua, Nerine rosea, Muscari botryodes, Scilla bifolia, Chinodoxa Luciliae, and Hyacinthus orientalis.

It can hardly be said, however, that the conclusions of these various authors have been received by cytologists without criticism or opposition. True, most of the critics have been simply sceptical, rather than openly antagonistic, but others have been radically opposed to some or all of the conclusions. Chief among the critics are Meves, Fick and Della Valle on the one hand, and Dehorne with his adherents on the other. Meves, Fick and Della Valle object to practically the whole chromosome theory (Meves '07, '08, '11, etc.) and hence incidentally to the hypothesis of chromosome pairing. Since it is not in the province of this paper to consider the whole chromosome theory, only the criticisms relevant to pairing will be reviewed. The others have been repeatedly and completely answered by previous authors (Boveri, Strasburger, Gregoire, Wilson, Montgomery, etc.). Meves has presented the arguments of himself Fick and Della Valle relative to chromosome pairing, in connection with a study of Salamandra ('11). As a result of this study, he concluded that the chromosomes can neither be assorted into pairs according to size, nor can they be said to arrange themselves

in pairs through side by side approximation. Upon this basis he decided that the entire hypothesis of chromosome pairing is a delusion. His attitude toward this matter, however, is so obviously biased as to discount very materially his whole argument. Dealing as he does with a chromosome group composed of large, numerous and almost uniform members it is little wonder that he finds no conspicuous evidence of their being differentiated into pairs. The wonder is that he attempts to draw conclusions of any final nature regarding this problem from material so evidently unsuited for its solution. The only answer to be given to Meves argument is that it does not accord with the facts as presented by organisms in which the chromosomes are sufficiently differentiated to be susceptible of analysis. The conclusions of Meves on this question have been directly controverted by von Baehr, Montgomery, Müller ('12), Lundegardh ('13) and others.

The criticism of Dehorne and his adherents is in the nature of an alternate theory, based upon the conclusion that all chromosomes are constantly dual or quadruple in form. Upon this basis 'pairs' of chromosomes are very readily explained as simply being halves of single chromosomes derived from a precocious split. If the two members are themselves split, then the single chromosome is represented by a quadruple element or tetrad. According to this theory each univalent, metaphase chromosome is represented by four parallel elements or two dyads. During anaphase these dyads separate from one another (passing to opposite poles) and then immediately split again to re-form the tetrad. Thus a quadruple structure is maintained throughout the greater part of any cell generation.

Such a theory, if true, would afford a very simple explanation of 'pairing'; but unfortunately it cannot be reconciled with the facts. In the first place Dehorne's evidence is directly contradicted by the actual history of the chromosomes as re-examined by Gregoire and Muckermann; and in addition, as pointed out by these authors and by von Schustow ('13) and von Baehr ('11) it takes no account of the relation between haploid and diploid groups or of the evidence furnished by the

sex-chromosomes, which shows that members of pairs, whether associated together or not, are separate and distinct chromosomes instead of daughter halves of single chromosomes.

The actual behavior of chromosomes in the Diptera shows with the greatest clearness that neither the criticisms of Meves nor of Dehorne can be valid in this group. The evidence leaves no doubt that the chromosomes are arranged in pairs and are paired in accordance with their size and form. In Dasyllis thoracica (figs. 155–158) for instance the five pairs include four sizes, of which the smallest, next smallest and largest are individually distinct. Similar relations are seen to exist in various other species, such as Asilus lecythus (figs. 146–148), Asilus notatus (figs. 149, 150), Deromyia winthemi (figs. 159–164), Neuroctena analis (figs. 115, 116), Volucella obesa (figs. 124–126), Mesogramma marginata (figs. 127, 128), Chaetopsis fulvifrons (figs. 117–119), Anthrax sinuosa (figs. 134–137), Spogostylum simson (fig. 141), Asilus sericeus (see p. 241) and certain species of Drosophilidae (see especially figs. 21–26). When the haploid and diploid groups of any of these species are compared they are seen to contain the same series of sizes, the former having one and the latter two representatives of each size. There can be little doubt, therefore, that each pair in the diploid group is composed of one paternal and one maternal member; indeed it only remains to establish the continuity of the chromosomes to make this a demonstrated fact.

Another question upon which the Diptera present definite evidence is that of gonomery. In contrast to the more or less continued spatial separation of the two parental chromosome groups found (Haecker, Van Beneden, Rückert, Conklin ('02), Blackman, Ferguson, etc.) in some organisms, the parental groups in the flies intermingle, and the corresponding chromosomes become arranged in pairs at an early stage in the cleavage of the egg,—perhaps during fertilization and before the first cleavage, although this has not been observed. The earliest stages which I have been able to study with accuracy are those immediately following the migration of the cleavage nuclei to the surface of the egg; and these show the chromosomes

definitely paired. A late prophase group from one of these nuclei is shown in figure 47 (note the association of X and Y). Subsequently to this stage pairing remains constant throughout the development of the fly.[11]

As to the causes of chromosome pairing in the Diptera very little may positively be said, but there are certain facts about the phenomena which should be considered in this connection. The facts indicate for instance, that pairing is not due to purely mechanical causes, but is dependent in some way upon the qualitative nature of the chromosomes. This conclusion seems evident from the fact that paired chromosomes are corresponding or similar chromosomes. It is difficult to conceive how purely mechanical forces can cause anything more than random pairing, while as a matter of fact the actual pairing is selective to the highest degree. That this association is not merely an assortment according to size is shown by the pairing of unequal sex-chromosomes in the males (figs. 41, 42, 44, 45, 86, 88, etc.), where X is often several times as large as Y.

A suggestion as to the significance of pairing may be obtained from tetraploid groups such as are found occasionally in embryonic somatic tissues.[12] One such is shown in figure 96. In this case there is twice the normal number of chromosomes (24 instead of 12), which means that in place of two chromosomes of each kind, there are four. On the assumption that homologous chromosomes associate together, these 24 chromosomes ought to associate in groups of four; and this is actually their arrangement. In figure 97 is shown a multiple group containing four times the normal chromosome number, or 48. In this case the chromosomes are so crowded together that their grouping is confused, and it is impossible to tell how they are associated. In prophase nuclei of a similar kind, however, the association of homologous chromosomes is clearly evident. Figures 109

[11] In respect to the intermingling of the two parental groups the flies thus agree with other insects such as Hemiptera, Orthoptera, Coleoptera, in which an intermingling occurs at least before the adult stage is reached, and presumably much earlier.

[12] These are only of sporadic occurrence, and in my material have been found only in a few cells or in small bits of tissue, never throughout the body.

and 110 represent such prophases, taken from a small bit of somatic tissue, apparently ectodermal, in which practically all of the nuclei contain a multiple group. These nuclei are very easily identified by their size as well as by their chromosome number. No complete metaphase figures were found among these particular cells, so I could not determine whether the nuclei contained quadruple (48) or double (24) the normal number of chromosomes. But the essential point is clear, that in each prophase nucleus the chromosomes appear in only six different aggregates, just as they do in ordinary prophases.[13] This means that here each aggregate is composed of four or eight chromosomes instead of the usual two. In the tetraploid groups two of the four chromosomes are sister halves of the other two, and hence are respectively similar to them in make-up. But all four of these chromosomes associate in essentially the same manner, i.e., paired chromosomes are indistinguishable from sister chromosomes in their manner of association. It is a natural conclusion, therefore, that the paired chromosomes bear much the same qualtitative relation to one another as do sister chromosomes (that they are qualitatively similar) and that their association is dependent upon, although not necessarily caused by, this relation.[14] Such a conclusion is in harmony with the known facts of cytology and genetics which indicate that corresponding maternal and paternal chromosomes are similar in composition.

If the paired association in diploid cells is an expression of the same underlying forces which bring about the association (synapsis) during maturation, the views here set forth are sup-

[13] Only five aggregates are conspicuous because one of the six is composed of the very small chromosomes. Strasburger ('07) obtained tetraploid groups in chloralized root tips of Pisum, and Stomps ('11) found such groups occasionally in Spinacia, but both authors describe the chromosomes as arranged in pairs instead of tetrads. Evidence is lacking on the crucial (prophase) stages, however, and such metaphase figures as are given may readily be interpreted as indicating association in tetrads, even though the association is not close. The question should remain open until tetraploid prophases are studied in these plants.

[14] See Lundegardh '15, who has come to very similar conclusions respecting the bivalent chromosomes of the heterotypic maturation division in plants.

ported by much evidence other than that from the Diptera. Two cases bear such a resemblance to those described in the Diptera that they will be briefly noted here. The most striking is that described by Wilson ('10) in Metapodius, certain individuals of which possess a supernumerary Y-chromosome or a supernumerary 'm-chromosome'. The extra chromosome in these specimens always "behaves according to its own kind" (p. 69), exhibiting a definite relation to those of its own kind, but to no others, in the pre-reduction stage of maturation.[15] Similarly Miss Woolsey ('15) has found that in a certain specimen of Jamaicana subguttata two small chromosomes act as the synaptic mate of one large bipartite chromosome which has apparently arisen by the union of two chromosomes corresponding respectively to the two with which it associates. These cases, like those of pairing in the Diptera, are readily explained upon the assumption that the association depends upon a qualitative likeness between corresponding chromosomes, but are difficult to interpret otherwise.

At first sight the conclusion that only qualitatively similar chromosomes associate in pairs might seem to be contradicted by the pairing of the unequal XY chromosomes in the males; but the contradiction, I believe, is apparent only. The studies of Stevens on Coleoptera and Diptera, and of Wilson, Payne and others on Hemiptera indicate that the XY pair when present has arisen either from an XY, XY pair, one member of which has lost its X-chromatin, or from a Y, Y pair, to one member of which X-chromatin has become attached.[16] In either case the X-chromosome of the Diptera may be looked upon as a Y-chromosome with X-chromatin added to it; and upon the view that similarly constituted chromosomes associate together the Y-portion of X would be expected to associate with the true

[15] See footnote 20, page 264.

[16] ". . . . we may, accordingly, think of the XY-pair as being essentially a YY-pair with one member of which the X-chromatin is associated." (Wilson '11, p. 87.) Genetic work on Drosophila indicates that the Y-chromosome in this fly is inactive (i. e. no factors have been found in it), but this does not necessarily mean that it is physico-chemically different from the Y-part of X.

Y-chromosome, or in other words the two sex-chromosomes would be expected to associate in approximately the same manner as do corresponding autosomes. In reality the association of the sex-chromosomes differs slightly from that of the autosomes, in that the former appear to condense earlier in prophase and become separated more frequently in metaphase than do the latter, but in essential features pairing is the same in both.

As suggested above the phenomena of chromosome pairing in somatic and primordial germ-cells appear to be closely correlated with those of maturation in spermatocytes and oocytes. The latter phenomena are obviously much more complicated than the former, and the association of the chromatic elements during synapsis is perhaps much more intimate than during the resting stage or prophase of somatic cells; but the similarity between the figures in the somatic cells of flies and those in germ-cells of many animals (including flies) makes it seem very probable that essentially the same cause is operative in both cases. If this be true it would seem that in the development of a fly each cell division is preceded by an attempt at synapsis. Or, in other words, the tendency to undergo synapsis is so marked as to bring about a close approximation of homologous chromosomes during each cell generation.

No positive answer can be given to the question as to why pairing outside the sphere of maturation should be exhibited by some organisms and not by others. Among animals a definite pairing of all the chromosomes in somatic as well as germ-cells is known to occur only in the Diptera. Among plants it has been reported in several orders. But whether the phenomena are really the same in the two kingdoms is not clear, for the details of the process in plants are still obscure. In the Diptera one of the most characteristic features of pairing is the close apposition of the early prophase threads, upon which subsequent behavior seems to depend. Whether a similar apposition is found in the prophases of plant cells, or whether a pairing takes place just preceding metaphase, is not certain. The observations of Stomps on Spinacia, of Overton on Thalictrum

and of Ishikawa on Dahlia strongly indicate that the former is the case in some instances. The first author describes a definite and intimate paired association as a normal condition in vegetative prophases of Spinacia (Stomps '11, p. 258).[17] On the other hand Müller ('12), who gives perhaps the most complete description of prophase stages in any plant exhibiting paired chromosomes (Najas marina), apparently considers the chromosomes to be single (though split) in prophase, and believes the real pairing to occur in metaphase. I am inclined to be skeptical about this interpretation, however, for the dual elements in his prophase figures bear a striking resemblance to those in the Diptera. Unfortunately his prophase figures do not include all of the chromosomes in a nucleus, and it is impossible to tell whether the number of double threads is haploid or diploid.

With respect to the other cases (among plants) in which pairing has been described, the evidence regarding prophase processes is still less satisfactory, and no conclusion of any weight can be drawn from it. The meagre data available from botanical sources tell little about the details of pairing, but they do indicate that it varies in extent or degree among different groups of plants.

In respect to animals a similar generalization may be made, for, although a conspicuous and uniform pairing seems to occur only in the Diptera, yet a varying degree of pairing is discoverable in other organisms. For instance in Hemiptera the 'm-chromosomes' and other morphologically distinct types are frequently associated in pairs, and in the Orthoptera a tendency toward pairing has been noted by Montgomery, Sutton and others.

[17] "Spinacia oleracea hat in den vegetativen Kernen ihrer diploiden Generation 12 Chromosomen aufzuweisen. Diese sind in Paaren angeordnet, und zwar nicht nur innerhalb der Kernplatten (fig. A.) sondern auch, wenn die Chromosomen in den Prophasen an der Kernwand liegen und sehr wahrscheinlich auch im Ruhezustande der Kerne. Denn sobald die Chromosomen sich in der Prophase einer Teilung aus dem Netzwerk des ruhenden Kerns herausgesondert haben (Prochromosomen sieht man im Ruhekern nicht) zeigen sie die paarweise Anordnung und bisweilen kann man beobachten, wenn in irgendeinem Paare ein Teil der beiden Chromosomen noch mehr oder wenier netzförmig ist, dass diese beiden netzförmigen Partien einander deutlich parallel liegen."

Whatever may be the fundamental cause of this phenomenon it seems certain from the evidence that its manifestations differ markedly in different organisms. There seem to be various intermediate conditions between that of intimate pairing (Diptera) and that of very slight pairing. It may be true therefore, that the tendency to associate in pairs is inherent in the chromosomes of multicellular organisms, being manifest in all the cells of some, but only in the maturing germ-cells of others. At all events it seems certain that the Diptera are not sharply differentiated from other animals by reason of any primary distinction in organization responsible for the pairing of their chromosomes.

Many of the questions suggested by this study are intimately involved with those of maturation, and can only be satisfactorily treated when the phenomena of maturation in the Diptera are better known. For this reason a full discussion of them will be reserved for a subsequent paper in which I hope to consider the maturation processes in detail, but in conclusion a word may be said as to the bearing of the pairing phenomena upon the theoretical question of synapsis. It is a significant fact that in the diploid cells of the flies a process may actually be followed which agrees in all essential respects with parasynapsis. In metaphase corresponding chromosomes, although arranged in pairs, are usually not closely applied; but in anaphase the members of these pairs often become associated side by side as they pass toward the poles, until the approximation becomes very intimate.[18] In these cases there can be no doubt about the reality of a process which, whether or not it actually corresponds to that of synapsis, certainly involves the essential features of a synaptic (parasynaptic) union, and removes the a priori objections urged against the conception of synapsis.

[18] With regard to the synapsis during maturation it may be noted that during the final spermatogonial anaphase (and probably oogonial also) the chromosomes behave in this same manner, and hence are brought into a closely paired arrangement before maturation.

SUMMARY AND CONCLUSIONS

1. The chromosomes of about eighty species of Diptera have been examined with especial reference to the phenomena of chromosome pairing. The species studied range from among the lowest to among the highest families in the order. In a large proportion of cases the studies include somatic, spermatogonial and spermatocyte, or somatic and ovarian cells.

2. In all of these species the chromosomes were found to be uniformly associated in pairs in diploid cells. The only irregularities were occasionally displacements involving one or two pairs.

3. The paired association was found to be characteristic of all tissues, somatic as well as germinal.

4. It was found to continue throughout all stages of cell division from earliest prophase to latest anaphase, being most intimate in the earliest and latest stages, and least intimate in metaphase. Telophases and resting nuclei were not favorable for study.

5. Association of paternal with maternal chromosomes apparently is effected in early cleavage stages (perhaps before the first cleavage), since in late cleavage stages the chromosomes are definitely paired.

6. The paired association was found to continue during all stages in ontogeny, from the egg to the adult.

7. Certain cases of multiple chromosome numbers (tetraploid or higher multiples) were found in occasional cells. In these cases corresponding chromosomes were associated together in prophase in aggregates of four, eight, etc., instead of being arranged in pairs.

8. In many species several (in some cases nearly all) pairs of chromosomes could be individually distinguished by characteristics of size and form.[18a] These pairs, with the exception of the sex-chromosomes in males, were in all cases symmetrical, i.e., composed of similar members.

[18a] Since this paper was sent to press I have found a species of Drosophila in which each pair of chromosomes is very clearly differentiated from all others.

9. In certain respects the pairing phenomena were found to present a striking similarity to synaptic phenomena. They give an actual demonstration of a side by side approximation of corresponding chromosomes.

These facts lend strong support to the conclusions:

1. That the paired arrangement of chromosomes is not due to a random assorting process, but is selective to the highest degree.

2. That each maternal chromosome becomes associated with a definite, similar paternal chromosome and with no other.

3. That chromosome pairing is dependent upon the qualitative nature of the chromosomes,—and more specifically upon a qualitative (physico-chemical) similarity between associating members.

BIBLIOGRAPHY

VON BAEHR, W. B. 1911 Contribution à l'étude de la caryocinèse somatique, de la pseudoréduction et de la réduction (Aphis saliceti.). La Cellule, 27.

VAN BENEDEN, E. 1883 Recherches sur la maturation de l'oeuf et la fécondation (Ascaris megalocephala). Arch. de Biol. 4, 265–640.

BOVERI, TH. 1904 Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns. Jena.

1907 Die Entwicklung dispermer Seeigel-Eier. Ein Beitrag zur Befruchtungslehre und zur Theorie des Kerns. Zellen-Studien, 7.

BONNEVIE, K. 1908 Chromosomenstudien I., Arch. fur Zellfor., Bd. 1.

BONNET, J. 1911 Sur la groupement par paires des chromosomes dans les noyaux diploides. Arch. f. Zellf., 7.

BLACKMAN, V. H. 1898 On the cytological features of fertilization and related phenomena in Pinus sylvestris. Phil. Trans. Roy. Soc., 190.

1904 On the fertilization, alternation of generations and general cytology of the Uredineae. Ann. Bot., 18.

CONKLIN, E. G. 1902 Karyokinesis and cytokinesis in maturation, fertilization and cleavage of Crepidula and other Gasteropoda. Jour. Aca. Nat. Sc. Phil., 12.

1914 The cellular basis of heredity and development. Pop. Sci. Mo., 85.

DEHORNE, ARMAND. 1911 Recherches sur la division de la cellule I. Le duplicisme constant du chromosome somatique chez Salamandra maculosa Laur. et chez Allium cepa L. Arch. Zellf., 6.

DONCASTER, HARRISON and. 1914 On hybrids between moths of the geometrid sub-family Bistonidae. Part II. Jour. Genetics, 3.

EAST, E. M. 1915 The chromosome view of heredity and its meaning to plant breeders. Am. Nat., 49.

FERGUSON, M. C. 1904 Contributions to the knowledge of the life-history of Pinus, with special reference to sporogeneses, the development of the gametophytes, and fertilization. Proc. Wash. Acad. Sc., 6.

GATES, R. R. 1912 Somatic mitoses in Oenothera. Ann. Bot., 26.

GRÉGOIRE, VICTOR 1912 Les phénomènes de la métaphase et de l'anaphase dans la caryocinèse somatique a propos d'une interprétation nouvelle. Louvain.

HAECKER, V. 1892 Die Eibildung bei Cyclops und Canthocamptus. Zool. Jahrb., 5.

1895 Ueber die Selbstandigkeit der väterlichen und mütterlichen Kernbestandteile während der Embryonalentwicklung von Cyclops. Arch. Mikr. Anat., 46.

1902 Ueber die Autonomie der väterlichen und mütterlichen Kernsubstanz vom Ei bis zu den Fortpflanz-zellen. Anat. Anz., 20.

1907 Die Chromosomen als angenommene Vererbungsträger. Ergeb. u. Fortschritte d. Zool., 1.

HENKING, H. 1891 Ueber Spermatogenese und deren Beziehung zur Eientwicklung bei Pyrrhocoris apterus. Z. wiss. Zool., 51.

ISHIKAWA, M. 1911 Cytologische Studien von Dahlien. Bot. Mag. Tokyo, 25.

JANSSENS and WILLEMS, 1908 Spermatogénèse dans les Batraciens IV. La Cellule, 25.

KUWADA, Y. 1910 A cytological study of Oryza sativa L. The Bot. Mag. Tokyo, 24.

LOMEN, FRANZ 1914 Die Hoden von Culex pipiens L. Jenaische Zeits. f. Naturw., 52.

LUNDEGARDH, H. 1912 Das Karyotin im Ruhenkern und sein Verhalten bei der Bildung und Auflösung der Chromosomen. Arch. f. Zellf., 9.

METZ, C. W. 1914 Chromosome studies in the Diptera. I. A preliminary survey of five different types of chromosome groups in the genus Drosophila. Jour. Exp. Zool., 17.

MEVES, FR. 1907 Die Spermatocytenteilungen bei der Honigbiene. Arch. f. mikr. Anat., 72.

1908 Es gibt keine parallele Konjugation der Chromosomen. Arch. f. Zellf. 1.

1911 Chromosomenlängen bei Salamandra nebst Bemerkungen zur Individualitätstheorie der Chromosomen. Arch. f. mikr. Anat., 77.

MOENKHAUS, W. J. 1904 The development of the hybrids between Fundulus heteroclitus and Menidia notata with especial reference to the behavior of the maternal and paternal chromatin. Am. Jour. Anat., vol. 3.

MONTGOMERY, T. H. 1900 The spermatogenesis of Peripatus (Peripatopsis) balfouri up to the formation of the spermatid. Zool. Jahrb., 14.

1901 A study of the chromosomes of the germ-cells of metazoa. Trans. Amer. Phil. Sc., 20.

1901 Further studies on the chromosomes of the Hemiptera heteroptera. Proc. Acad. Nat. Sc. Phil., 53.

1903 The heterotypic maturation mitosis in Amphibia and its general significance. Biol. Bull., 4.

1904 Some observations and considerations upon the maturation phenomena of the germ-cells. Biol. Bull., 6.

1905 The spermatogenesis of Syrbula and Lycosa with general considerations upon chromosome reduction and the heterochromosomes. Proc. Acad. Nat. Sc. Phil., 57.

1908 On the morphological difference of the chromosomes of Ascaris megalocephala. Arch. f. Zellf., 2.

1910 On the dimegalous sperm and chromosomal variation in Euschistus, with reference to chromosomal continuity. Arch. f. Zellf., 5.

MORGAN, STURTEVANT, MULLER, BRIDGES. 1915 The mechanism of Mendelian heredity. H. Holt, N. Y.

MUCKERMANN, H. 1912 Zur Anordnung, Trennung und Polwanderung der Chromosomen in der Metaphase und Anaphase der somatischen Karyokinese bei Urodelen. La Cellule, 28.

MÜLLER, H. A. Cl. 1909 Ueber karyokinetische Bilder in den Wurzelspitzen von Yucca. Jarhb. f. wiss. Botanik, 47.

1912 Kernstudien an Pflanzen I und II. Arch. f. Zellf., 8.

NEMEC, B. 1910 Das Problem des Befruchtungs Vorgänge. Berlin.

OVERTON, J. B. 1909 On the organization of the nuclei in the pollen mother cells of certain plants with especial reference to the permanence of the chromosomes. Ann. Bot. 23.

ROSENBERG, O. 1904 Ueber die Individualität der Chromosomen in Pflanzreich. Flora, 93.

RÜCKERT, J. 1895 Ueber das Selbständigbleiben der väterlichen und mütterlichen Kernsubstanz während der ersten Entwicklung des befruchteten Cyclopseis. Arch. micr. Anat., 45.

VON SCHUSTOW, L. 1913 Ueber Kernteilung in der Wurzelspitze von Allium cepa. Arch. f. Zellf., 11.

STEVENS, N. M. 1906 Studies in spermatogenesis, II. Carnegie Inst. Publ. 36.

1907 The chromosomes of Drosophila ampelophila. Proc. VII Internat. Zool. Cong.

1908 A study of the germ cells of certain Diptera, with reference to the heterochromosomes and the phenomena of synapsis. Jour. Exp. Zool., vol. 5.

1910 The chromosomes in the germ cells of Culex. Ibid., 8.

1911 Further studies on the heterochromosomes of the mosquitoes. Biol. Bull., 20.

STOMPS, TH. J. 1911 Kernteilung und Synapsis bei Spinacia oleracea L. Biol. Centralb., 31.

STRASBURGER, ED. 1882 Ueber den Teilungsvorgang der Zellkerne und das Verhaltniss der Kernteilung zur Zellteilung. Arch. f. mikr. Anat., 21.

1905 Typische und allotypische Kernteilung. Histologische Beiträge zur Vererbungsfrage. Jahr. wiss. Bot., 42.

1907 Ueber die Individualität der Chromosomen und die Pfropfhybriden-Frage. Ibid, 44.

1910a, Ueber geschlectsbestimmende Ursachen. Ibid, 48.

1910b, Chromosomenzahl. Jena, Gustav Fischer.

SUTTON, W. S. 1902 On the morphology of the chromosome group in Brachystola magna. Biol. Bull., 4.

SYKES, M. G. 1908 Nuclear division in Funkia. Arch. f. Zellf., 1.

TAHARA, M. 1910 Ueber Kernteilung bei Morus. Bot. Mag. Tokyo, 24.

TAYLOR, MONICA 1914 The chromosome complex of Culex pipiens. Q. J. M. S. No. 239 New Series, vol. 60.

WILLISTON, S. W. 1908 Manual of North American Diptera. New Haven, J. T. Hathaway.

WILSON, E. B. 1905 Studies on chromosomes, II. Paired chromosomes, idiochromosomes and heterotropic chromosomes in Hemiptera. Jour. Exp. Zoöl. vol. 2.

1910 Studies on chromosomes VI. A new type of chromosome combination in Metapodius. Jour. Exp. Zoöl., vol. 9.

1911 Studies on chromosomes VII. Jour. Morph., vol. 22.

1914 Croonian Lecture. The bearing of cytological research on heredity. Proc. Roy. Soc. B., 88.

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

PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures were drawn with the aid of a camera lucida, using a Zeiss 1.5 mm. apochromatic objective and compensating ocular no. 12, with tube length of 160 mm. The drawings are reproduced natural size. They are taken from sections cut 5 μ thick unless otherwise noted.

1 and 2 Drosophila virilis Sturtevant mss.,[19] diploid metaphase, ovarian cell.

3 D. ramsdeni Stt. mss., diploid metaphase, ovarian cell.

4 Scaptomyza graminum Fall., diploid metaphase, spermatogonium.

5 Same, ovarian cell.

6 Same, haploid, second spermatocyte.

7 Drosophila robusta Stt. mss., diploid, early anaphase, spermatogonium; polar view showing separation of daughter halves of chromosomes.

8 Same, ovarian cell; slightly earlier stage showing division of chromosomes.

9 Same; slightly later stage.

10 D. nebulosa Stt. mss., haploid, second spermatocyte prophase.

11 Same, diploid, ovarian cell.

12 Same, diploid, ovarian cell, two poles of anaphase; lower figures displaced in order to compare the two groups (upper pole at left, lower at right); upper figure a diagram showing the two anaphase groups as they appear in the section. The small, spherical members are not evident.

13 D. amoena Loew, haploid, late metaphase, second spermatocyte.

14 Same, ovarian cell, prophase, diploid group.

15 Same, diploid, metaphase, spermatogonium.

16 Same, diploid, early anaphase, ovarian cell,showing separation of daughter halves of chromosomes.

17 D. busckii Coq., diploid metaphase, ovarian cell.

18 Same, haploid, first spermatocyte.

19 D. ampelophila Loew, diploid metaphase, ovarian cell.

20 D. dimidiata Loew, diploid, metaphase, ovarian cell.

21 D. ornatipennis Will., diploid, ovarian cell, metaphase; this individual apparently possesses three small, spherical chromosomes.[20]

22 Scaptomyza adusta Loew, diploid, metaphase, ovarian cell.

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[19] See footnote 3, page 221.

[20] Apparently this case is comparable with that of the supernumerary 'm-chromosome' described by Wilson ('10) in Metapodius, and results from non-disjunction of the small chromosomes in one of the parents. Unfortunately only two or three good figures were found in my specimen (as is usually the case in flies), and although these show the same features they are too few to be demonstrative. It may be noted that the three chromosomes are associated together in each of the figures.

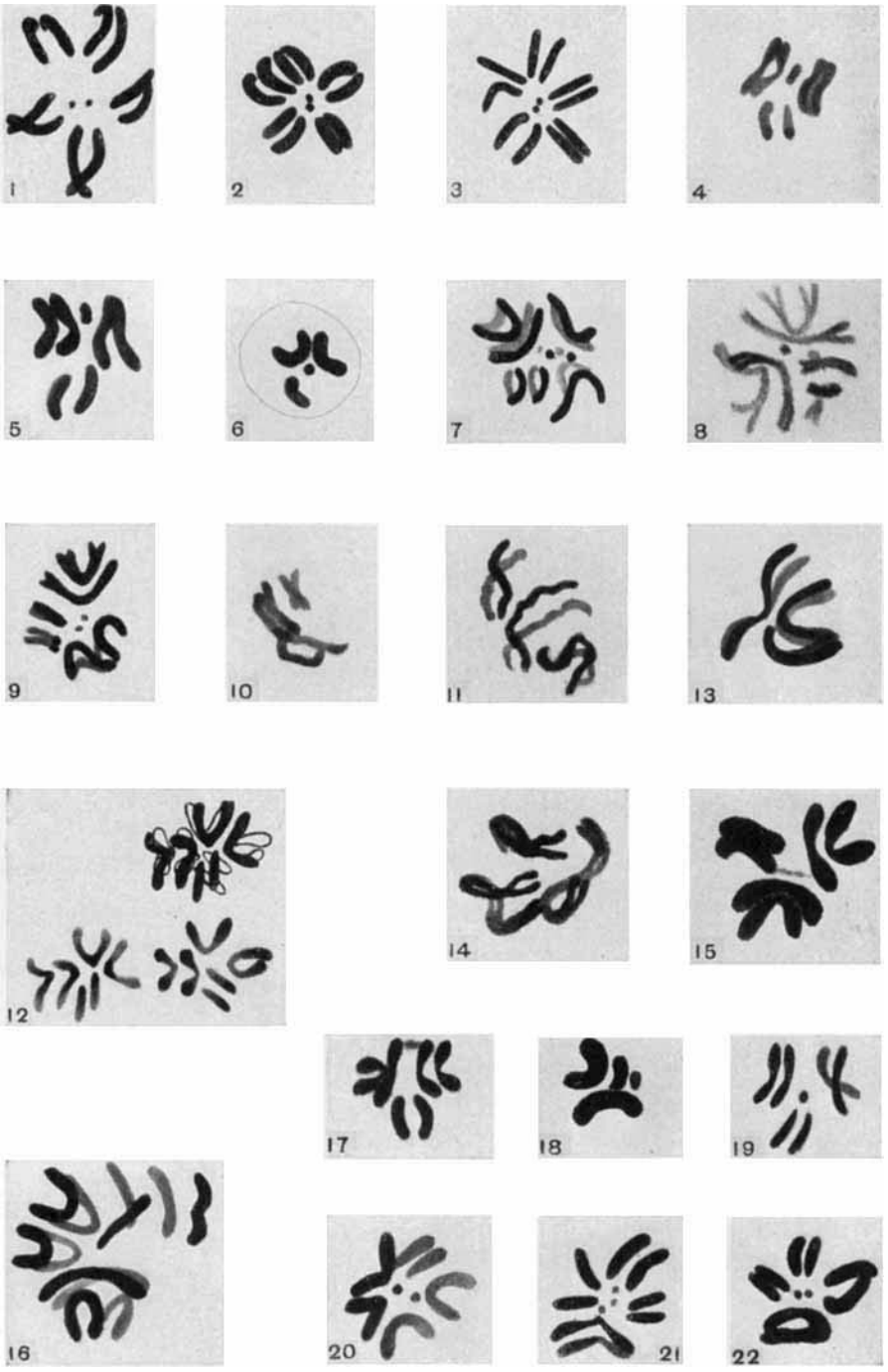
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PLATE 2

EXPLANATION OF FIGURES

23 Drosophila neglecta Stt. mss., diploid, metaphase, spermatogonium; (small chromosomes not evident, unless represented by the small chromatic body in upper part of figure.)

24 Same, all chromosomes present; made from an aceto-carmine smear.

25 and 26 Same, haploid, second spermatocytes; aceto-carmine.

27 D. funebris Fabr., diploid, metaphase, ovarian cell.

28 Same; aceto-carmine smear, slightly later stage showing division.

29 Same diploid, metaphase, spermatogonium; note the separation of the two large chromosomes.

30 Same, diploid, ovarian cell, anaphase in side view; note separation of large chromosomes.

31 Same, with large chromosomes in their normal position.

32 Same, early anaphase, side view, showing division of the chromosomes.

33 Same, haploid, first spermatocyte anaphase in side view for comparison with figure 31.

34 D. procnemis Will., diploid, ovarian cell, prophase.

35 and 36 Same, slightly later stages.

37 D. tripunctata Loew, diploid, metaphase, ovarian cell.

38 Same, diploid, ovarian cell, two poles of anaphase in polar view, displaced for comparison of the two groups.

39 D. repleta Woll., diploid, metaphase, ovarian cell; aceto-carmine smear.

40 Same, late metaphase showing division of chromosomes, from section. The two round bodies at left of figure are chromatic (?) inclusions, not chromosomes.

41 Same, spermatogonium.

42 D. affinis Stt. mss. diploid, metaphase, spermatogonium.

43 Same, late prophase, ovarian cell.

44 and 45 D. obscura Fall. diploid, metaphase, spermatogonia.

46 Same, ovarian cell, (small chromosomes not evident).

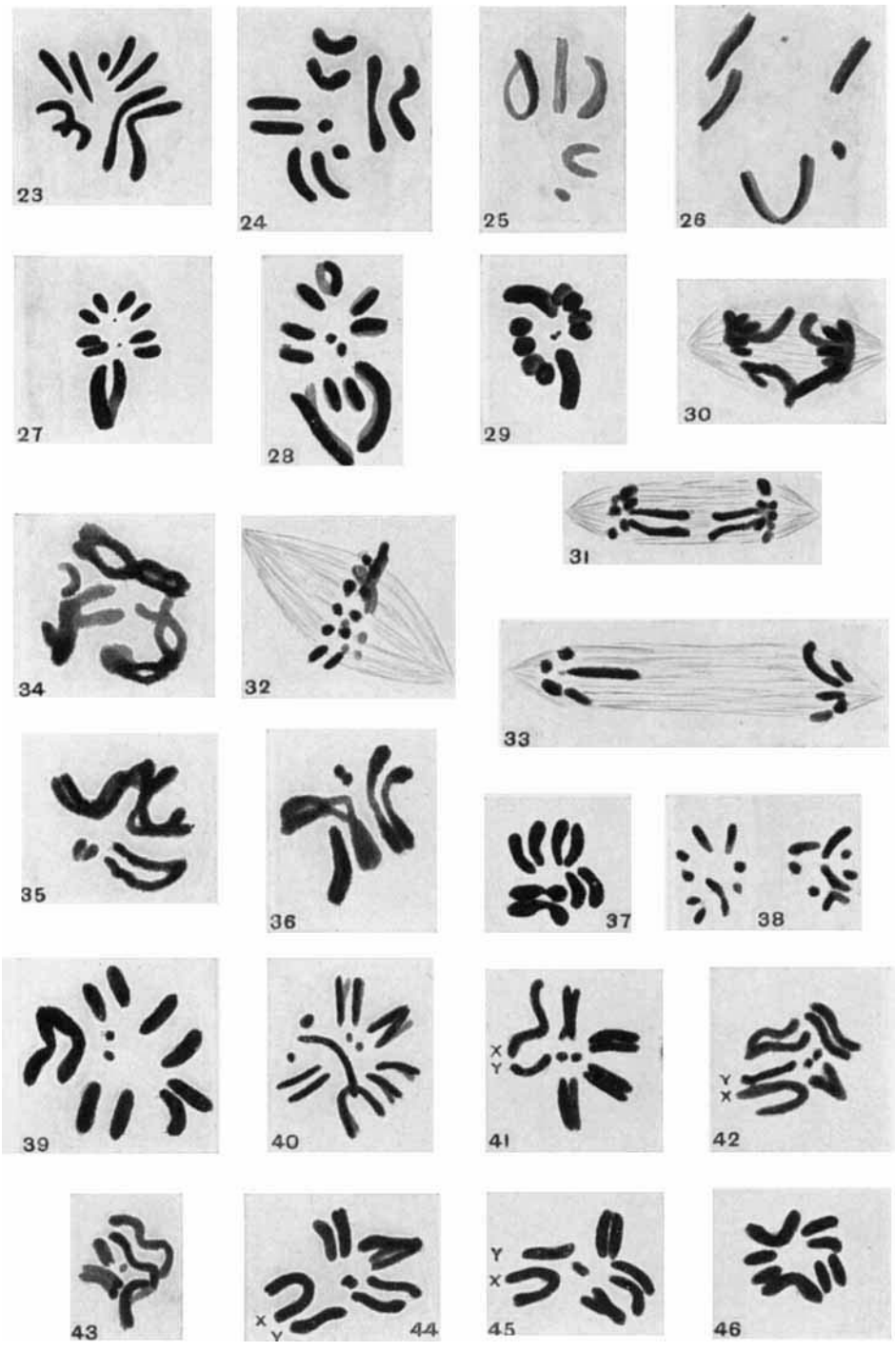
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PLATE 3

EXPLANATION OF FIGURES

47 Drosophila obscura, diploid, late prophase, from an embryonic cell during a late cleavage stage in the egg.

48 Same, haploid, metaphase, first spermatocyte.

49 Same, haploid metaphase, second spermatocyte, X-containing class.

50 Same, haploid metaphase, Y-containing class.

51 and 52 Calliphora erythrocephala, haploid, metaphase, first spermatocytes.

53 to 56 Same, diploid, metaphases, ovarian cells.

57 Same, somatic.

58 Same, diploid, somatic, early prophase, entire, or almost entire nucleus, one pair partly displaced in the figure to show all of the threads.

59 Same, somatic, only part of figure shown.

60 and 61 Same, somatic, two sections of one nucleus.

62 Same, somatic, entire or nearly entire nucleus.

63 Same, ovarian cell, entire nucleus.

64 Same, somatic, only three pairs represented.

65 Same, somatic, entire nucleus.

66 Same, later prophase, ovarian cell, showing separation of the two members of a pair in late prophase.

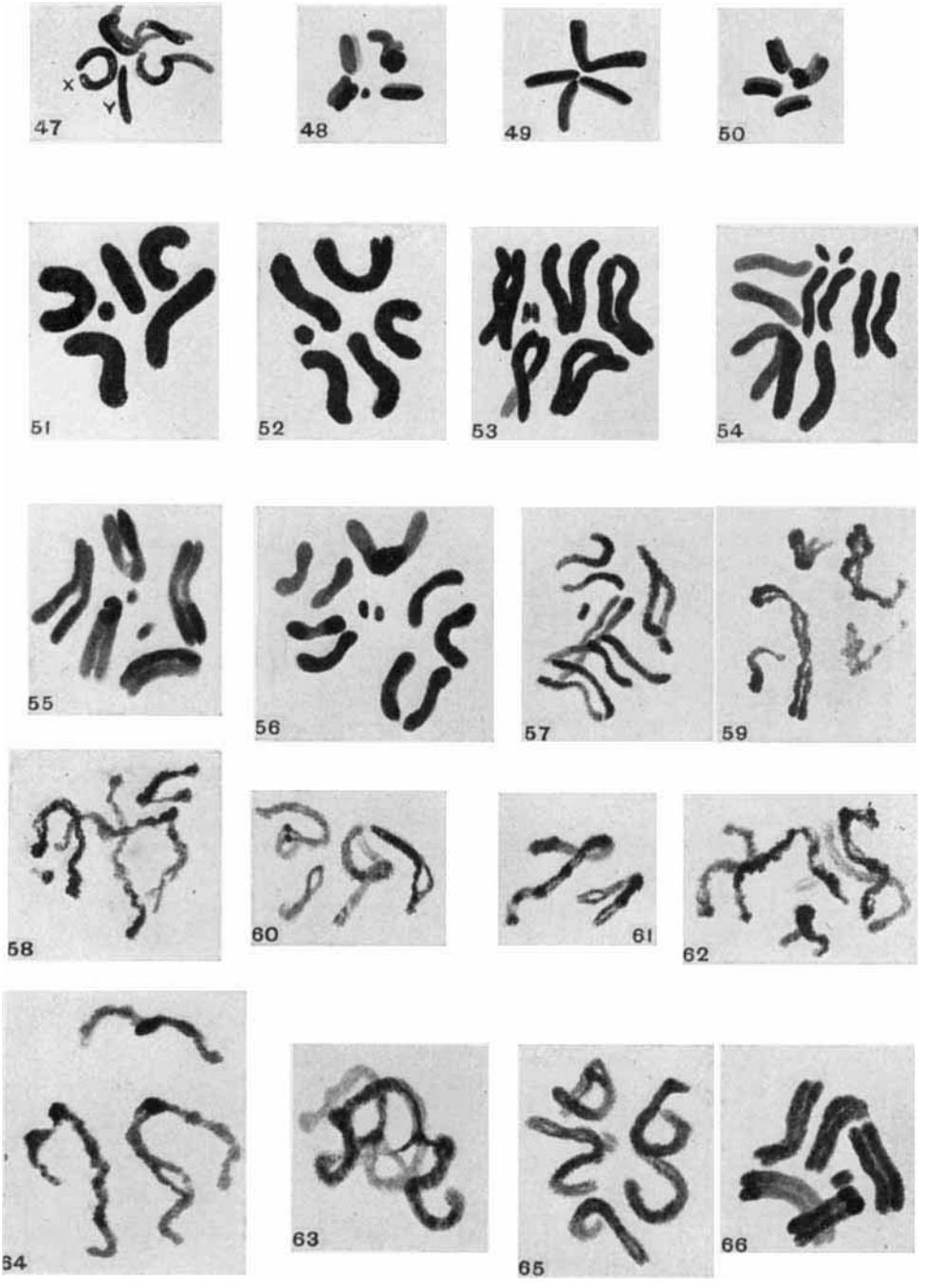
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PLATE 4

EXPLANATION OF FIGURES

68 Musca domestica, diploid, metaphase, ovarian cell.

69 Same, somewhat disarranged.

70 Same, ovarian cell, early prophase, entire or almost entire nucleus.

71 Same, nucleus not entire.

72 Same, late prophase, nucleus almost entire.

73 Phormia regina, haploid, metaphase, X-containing second spermatocyte.

74 Same, Y-containing group.

75 Same as 73, but slightly later, showing division of chromosomes.

76 Same, first spermatocyte, early anaphase, polar view, showing reduction division; note separation of X and Y (small pair).

77 Same, diploid, spermatogonium, late metaphase showing division of chromosomes.

78 Same, diploid, ovarian cell, early prophase.

79 and 80 Same, slightly later stage, nuclei entire, or nearly so.

81 and 82 Sarcophaga tuberosa serraceniae, haploid, second spermatocyte,

83 Same, first spermatocyte.

84 Same, side view, showing separation of X and Y at the reduction division.

85 to 88 Same, diploid, spermatogonia; two small members are X and Y.


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PLATE 5

EXPLANATION OF FIGURES

89 Sarcophaga sp., diploid, late prophase, ovarian follicle cell.

90 Same, somatic, small pair is XX pair in these figures.

91 Same, somatic, earlier prophase, two sections of one nucleus.

92 and 93 Same, slightly later prophase, entire nuclei.

94 Same, later stage.

95 Same, somatic, anaphase, side view showing both groups of daughter chromosomes; note the closely paired association.

96 Same, tetraploid metaphase; four small chromosomes are X chromosomes, from ovarian follicle cell.

97 Same, multiple group, somatic, apparently containing 48 chromosomes.

98 Ravinia peniculata, diploid, metaphase, ovarian cell.

99 Same, slightly later, ovarian follicle cell, showing splitting of chromosomes.

100 Homalomya sp., diploid, somatic, very early prophase showing bivalent threads, nucleus practically entire; note the polarization.


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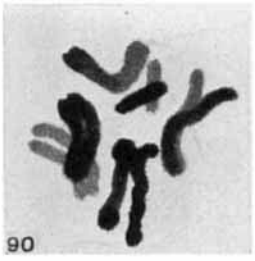
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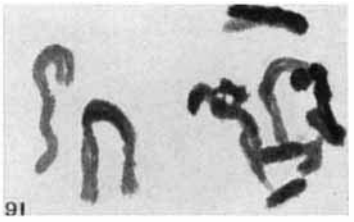
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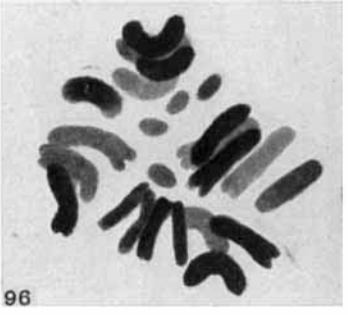
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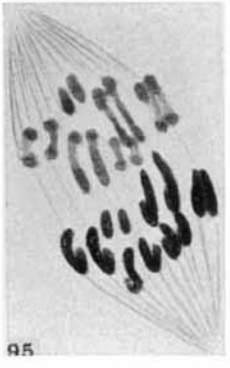
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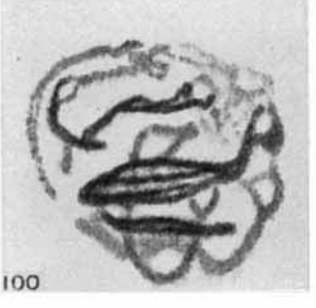
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PLATE 6

EXPLANATION OF FIGURES

101 Homalomya sp., diploid, somatic, early prophase, similar to figure 100; only portion of figure shown.

102 Same, slightly later stage, somatic, larval, nucleus not entire.

103 Same, haploid, prophase; only part of figure shown, second spermatocyte.

104 Same, haploid, second spermatocyte metaphase (late) showing division of the chromosomes.

105 Same, second spermatocyte, anaphase; entire nucleus.

106 Fucellia marina, diploid, somatic, early prophase, entire nucleus.

107 and 108 Same, somatic, later stages.

109 and 110 Same, somatic, prophases, multiple chromosome number, probably tetraploid.

111 and 112 Ophyra leucostoma, diploid, telophases, ovarian cells.

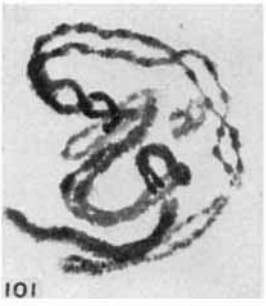
113 Same, spermatogonium, metaphase, showing division of chromosomes.

114 Same, haploid, first spermatocyte (reduction division), compare with figure 113.

115 Neuroctena analis, haploid, metaphase, second spermatocyte.

116 Same, diploid, spermatogonium.

117 and 118 Chaetopsis fulvifrons, diploid, metaphase, spermatogonia.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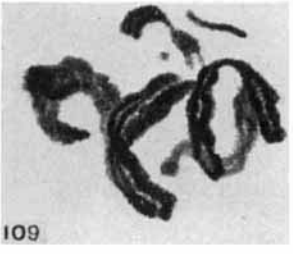
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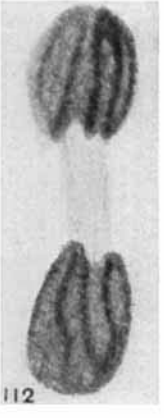
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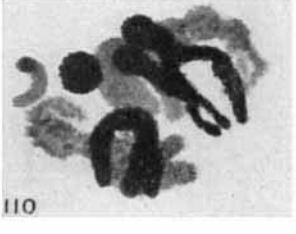
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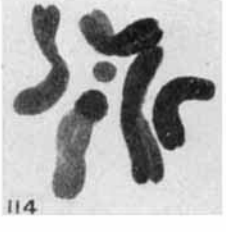
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PLATE 7

EXPLANATION OF FIGURES

119 Chaetopsis fulvifrons, haploid, metaphase,, first spermatocyte.
120 Camptoneura picta, diploid, metaphase, spermatogonium.
121 Same, haploid, metaphase, first spermatocyte.
122 Physegenua vittata, diploid, metaphase, spermatogonium.
123 Eristalis bastardi, diploid, early prophase, spermatogonium; nucleus not entire.
124 Volucella obesa, diploid, metaphase, spermatogonium, one chromosome missing.
125 Same, entire.
126 Same, haploid, first spermatocyte; small bivalent is XY pair.
127 and 128 Mesogramma marginata, diploid, metaphase, spermatogonia.
129 and 130 Anthrax lateralis, diploid, late prophase, spermatogonia; small pair not evident.
131 Same, earlier prophase, nucleus entire.
132 and 133 Same, metaphase.
134 to 137 A. sinuosa, diploid, metaphases, spermatogonia.
138 Same, haploid, first spermatocyte.
139 Same, haploid, second spermatocyte, Y-containing group.
140 Same, X-containing group.
141 Spogostylum simson, diploid, metaphase, ovarian follicle cell.
142 Same, early prophase.
143 Asilus sericeus, diploid, metaphase, spermatogonium.
144 and 145 Same, slightly later metaphases.
146 and 147 Asilus lecythus, diploid, metaphases, spermatogonia.

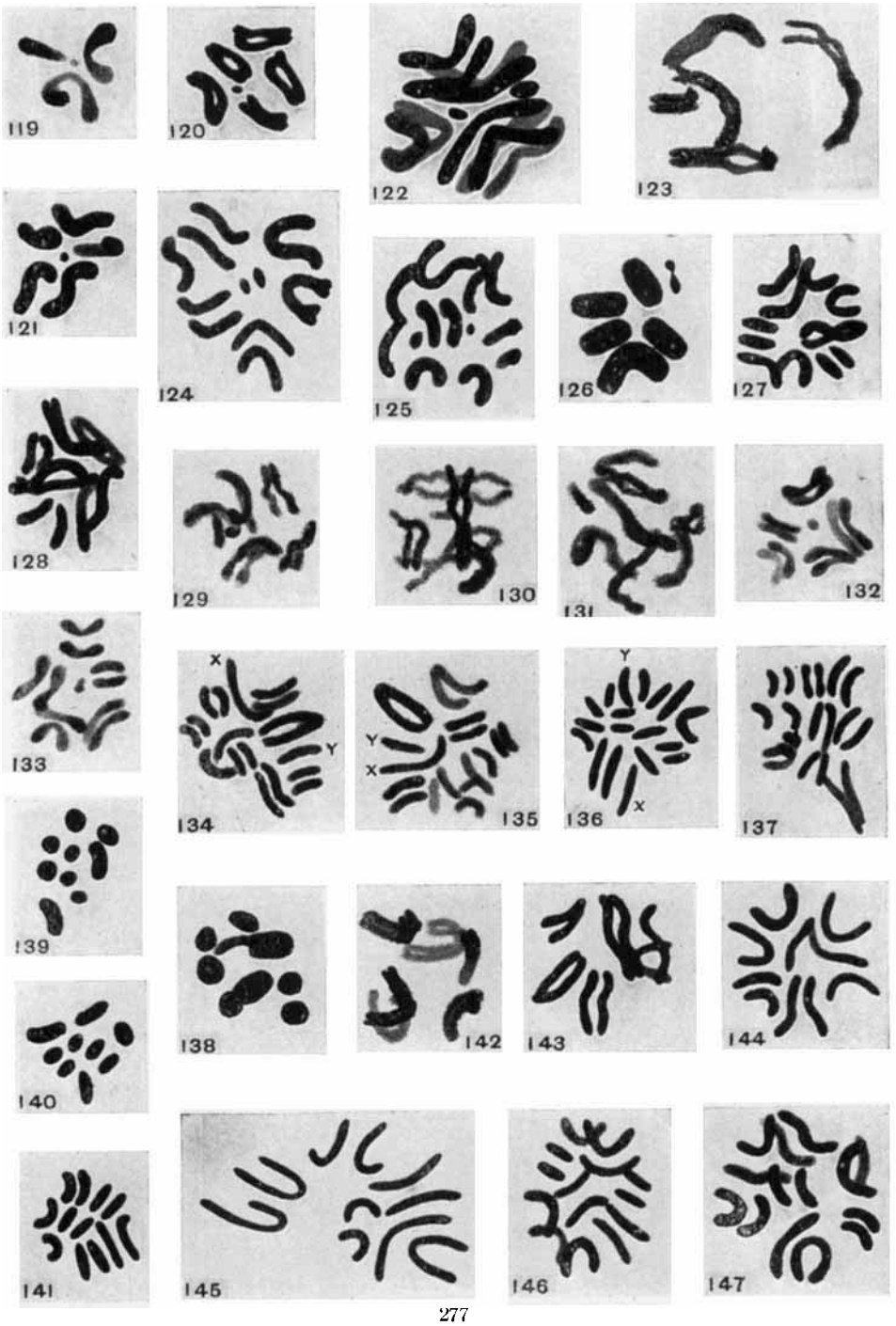
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PLATE 8

EXPLANATION OF FIGURES

148 Asilus lecythus, haploid, metaphase, second spermatocyte.

149 Asilus notatus, diploid, metaphase, spermatogonium.

150 Same haploid, metaphase, second spermatocyte.

151 Leptogaster badius, diploid, spermatogonium, metaphase.

152 Same, haploid, metaphase, second spermatocyte.

153 Erax rufibarbis, diploid, metaphase, spermatogonium.

154 Same, haploid, second spermatocyte.

155 Dasyllis thoracica, diploid, spermatogonium, prophase, entire nucleus.

156 Same, metaphase.

157 Same, haploid, metaphase, first spermatocyte.

158 Same, haploid, second spermatocyte.

159, 160 and 161 Deromyia winthemi, diploid, metaphase, spermatogonia.

162 Same, haploid, metaphase, first spermatocyte; note X and Y going to opposite poles.

163 Same, haploid, second spermatocyte, Y-containing group.

164 Same, X-containing group.

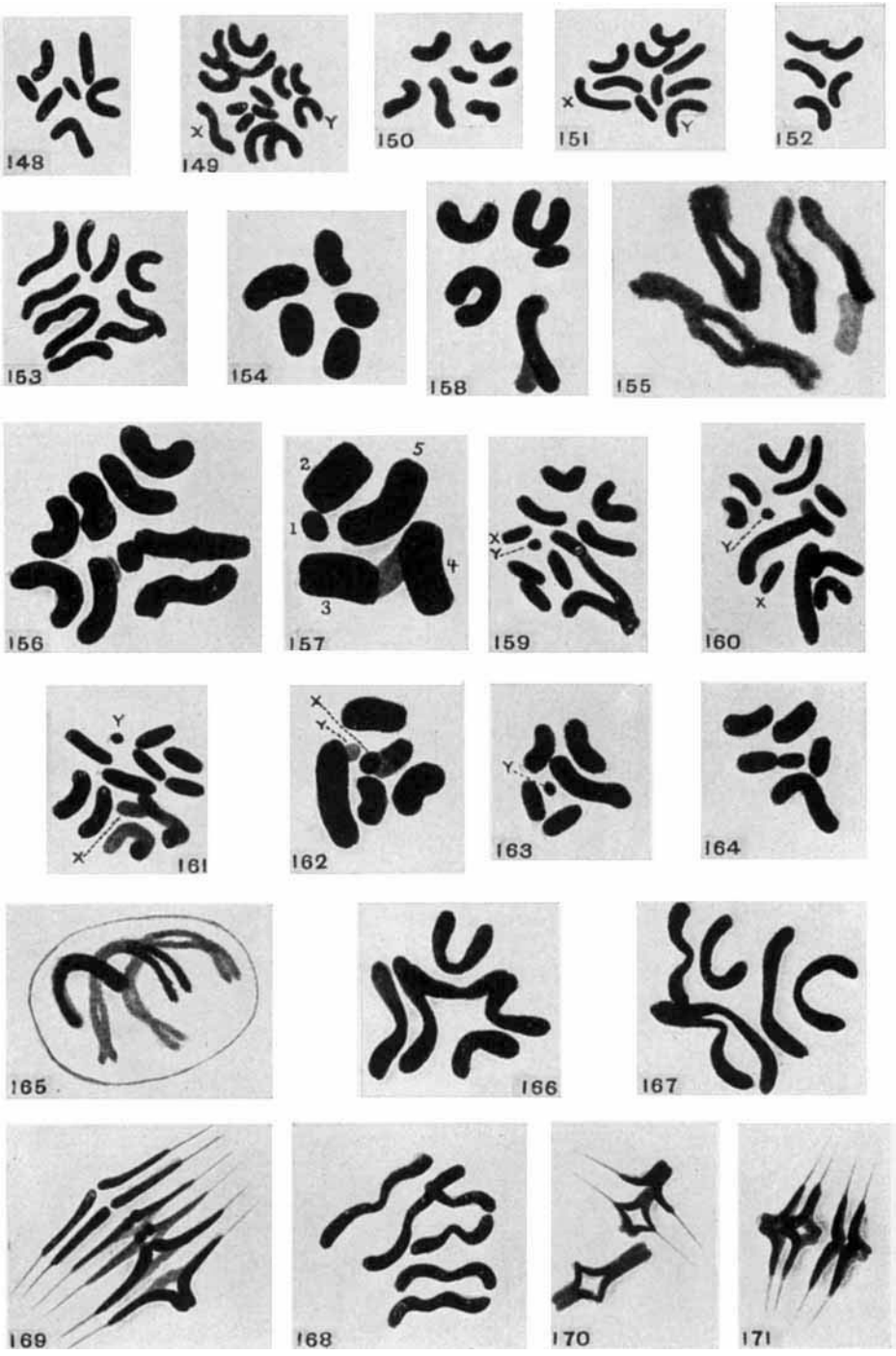
165 Culex pipiens, diploid, early prophase, ovarian cell, entire nucleus. Three pairs of chromosomes, note polarization.

166, 167 and 168 Same, diploid, spermatogonia, metaphase.

169 Same, anaphase, side view showing division of chromosomes and separation of daughter halves.

170 Same, earlier stage, side view showing manner of division; only three of the chromosomes are represented.

171 Same, same stage as 169; only two pairs of chromosomes represented.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