A study of the male germ cells in Notonecta, by Ethel Nicholson Browne

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ETHEL NICHOLSON BROWNE

Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University

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From the Zoölogical Laboratory, Columbia University

TEN PLATES

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I. INTRODUCTION

Gilson, in his study of the spermatogenesis of the arthropods in 1885, passed over Notonecta with the remark, "Les phénomènes de la spermatogénèse y sont fort simples et presentent peu de particularités dignes d'être mentionées" (op. cit., p. 123), adding that N. glauca possesses the longest and largest spermatozoa known. More recently, Pantel and Sinéty ('06) have published a copious memoir on "Les cellules de la lignée mâle chez le Notonecta glauca L.," and they, unlike Gilson, have found themselves "en presence d'un assez grand nombre d'images d'un aspect nouveau, parfois très inattendu ou même déconcertant" (op. cit., p. 90). Further work on this genus seemed to be warranted by the very peculiar appearances described by these authors, as well as by the acknowledged slight treatment of the maturation divisions in favor of the stages concerning the transformation into the spermatozoön. The problem was suggested to me by Prof. E. B. Wilson, to whom I wish to express my most sincere thanks for his valuable advice and criticism during the course of the investigation. This study is based on the three common American species, kindly identified by Mr. E. P. Van Duzee as Notonecta undulata (Say), N. insulata (Kirby) and N. irrorata (Uhler); the form used by the French authors was the European species, N. glauca. While the American species agree with N. glauca in presenting many very puzzling appearances, they differ from it in several important respects and also differ considerably among themselves. The two facts of main interest are, first, the presence of a karyosphere or body in which the chromatin is aggregated during the growth stages in all three species, as was noted also in N. glauca by Pantel and Sinéty; and secondly, the relation of the chromosome number to the species, a brief

summary of which has already been published (Browne '10). The present study deals only with the growth stages and maturation divisions, no attempt having been made to treat the later stages which have been elaborately worked out by Pantel and Sinéty.

II. MATERIAL AND TECHNIQUE

The material, consisting of the three species already mentioned, was collected during four summers at Woods Hole, Massachusetts. These species differ considerably from one another in size, in wing coloration and markings, and in other characters. N. insulata is the largest, with brown wings usually marked with two black bands. N. irrorata is slightly smaller, and its wings are black, mottled more or less with brown. N. undulata, the most common species, is considerably smaller than the other two and the wing color varies from a pure white to a white with one, two, or three black bands. In respect to germ cell production, there are two types. In N. undulata, all the stages of the spermatogenesis occur in the adult and even in the very young larva throughout the summer. In N. irrorata and N. insulata, during the greater part of the summer, the testis of the adult and late larva is filled with cells in the late growth stages, the younger cysts being empty except those at the very tip of the testis where a few spermatogonia occur. For only about a week during the summer are division stages found in these two species; after this the testis is filled with spermatids and spermatozoa. Pantel and Sinéty have noted the same slow evolution of the germ cells in N. glauca. Probably owing to this long period of growth, the cells of N. irrorata and N. insulata are larger than those of N. undulata. The great size of the cells coupled with the diagrammatic clearness of the spindle fibers and asters make the material exceptionally fine for the study of the maturation divisions. The French authors find it otherwise for N. glauca, stating that "La figure chromatique est d'un type malingre, dans les cinèses maturatives du Notonecta, et peu favorable à une analyse detaillée des phénomènes morphologiques" (op. cit., p. 136).

The only difficulty with my material has been the scarcity of spermatogonial divisions and early growth stages.

The testes are bifurcated coiled tubes lying on either side of the alimentary canal. They were dissected out in Ringer's solution and transferred at once to the fixing fluid. Flemming's strong fluid, Bouin, Carnoy, Gilson and corrosive sublimate were used with results that are favorable in the order named. Heidenhain's haematoxylin was used almost exclusively as a stain, though some saffranin preparations were made. In order to demonstrate mitochondria, some of the testes were fixed with Benda's modification of Flemming's fluid, and these were subsequently treated with his mitochondrial stain of sulphuralizarinate of sodium and crystal violet, used according to his original method. The results of the fixed preparations have been controlled by observations of the living cells both with and without intra-vitam stains. By dragging the testis over a slide and mounting in a drop of Ringer's solution, very good results were obtained. The mitochondria and karyosphere may at once very clearly be seen; and after half an hour or so, the chromosomes in division stages come out very clearly. This is probably due to the fact that some change has taken place in the chromosomes, and it may be that they are not visible in the living state. Such would seem to be the case from the fact that constant observation of anaphase spindles failed to reveal any progression of the chromosomes toward the poles. In some cases it was possible to count the chromosomes in these preparations.

III. CHROMOSOMES

A. Observations

As pointed out in my preliminary paper ('10), the study of the chromosomes in Notonecta has proved of much interest from the fact that the change in number from species to species can here be attributed to the relations of a particular chromosome. Briefly the results are as follows. In all three species there is present an unequal XY-pair of chromosomes which divide separately in the first spermatocyte division but are united in the

second, thus making the total number of separate chromatin elements one greater in the first than in the second division. In N. undulata, there are 14 chromosomes in the first division, 13 in the second, including two small chromosomes. In N. irrorata, there are 13 in the first and 12 in the second, including only one small one. In N. insulata there are either 14 or 13 in the first, and 12 in the second; when there are 14 in the first, there are two small ones, when 13 there is only one free small one, but the other small one can often be detected attached to the largest chromosome. This species thus appears to be intermediate in respect to the chromosomes between N. undulata with a larger number, and N. irrorata with a smaller number.

1. Notonecta undulata. In N. undulata, the typical, and I am inclined to believe, the invariable, arrangement in the first spermatocyte division is a ring of 12 surrounding two very small chromosomes. This is shown in polar view in figures 1 and 2, and in side view of a spindle from two adjoining sections in figure 3 *A, B.* Very frequently side views present the appearance shown in figure 4 *B*, the two pairs of small chromosomes lying in a straight line, as though on the same spindle fiber (*A*, *B*, *C* are serial sections of the same spindle). This is probably due to the fact that they lie very close together and the smaller of the two pairs usually precedes the other in division. In the peripheral ring can be distinguished one chromosome larger than the rest, one very small one slightly larger than the central ones, and ten of intermediate and intergrading sizes.

In the second spermatocyte division, side views clearly show the presence of an unequal *XY*-pair (fig. 5 *B*). Since these chromosomes have divided separately in the first division, as is the case in many other Heteroptera, there should be one chromosome less in the equatorial plate of the second division. That there are 13 chromosomes in this division is shown in side view in figure 5 *A*, *B*, *C* (from the same spindle), and in polar view in figures 6 and 7. (In the latter figure the *X*-chromosome is seen at a lower focus). In this division, *X* and *Y* always take up their position in the center of the spindle, as they do in other Hemiptera. A rather interesting phenomenon occurs in Notonecta

with regard to the *XY*-pair. The two components frequently fail to conjugate, and lie in the second metaphase side by side, on separate spindle fibers (figs. 8, 9). A little later stage is shown in figure 10 *A*, where the small component is evidently going to one pole, the large one to the other; these are frequently connected at this time by an oblique fiber (fig. 11). The size relations of the chromosomes are evident from an inspection of figures 5, 6, 7, 9, 10. The two smallest chromosomes which were in the center in the first division are now in the peripheral ring. The third small one of the first division was apparently the *Y*-chromosome. The largest chromosome is again evident in the peripheral ring. The *X*-chromosome is one of the larger chromosomes, probably the third largest. The size relations come out very clearly in figure 12 *A*, *B*, which are sister anaphase groups from the same spindle. It is apparent from these groups that *X* is present in one of the resulting cells, *Y* in the other, and since these cells develop directly into the spermatids and thence into spermatozoa, the latter must be of two types in respect to the chromosome content.

In the spermatogonial groups, there are 26 chromosomes (figs. 13, 14), among which a largest and a smallest pair can easily be recognized. There are two pairs of very small ones, evidently corresponding to the two small bivalents in the center of the spindle in the first spermatocyte division. There are two very large chromosomes corresponding to the one large one in the haploid groups. Then there is a pair slightly smaller than these and another odd large one. This is evidently the *X*-chromosome, and *Y* is distinguishable as the fifth small chromosome which clearly has no mate of its own size.

2. Notonecta irrorata. In N. irrorata, the typical arrangement is a ring of 12 chromosomes surrounding one small one (figs. 15, 16). The second small chromosome which occurred inside the ring in N. undulata is here lacking. Serial sections of a spindle in side view showing the total number and the typical arrangement, are represented in figure 17 *A*, *B*, *C*. A few cases have been observed where the components of the central pair apparently fail to conjugate and lie on separate fibers in the metaphase; these are distinctly univalent in contrast to the other bivalents

(fig. 18). Their behavior resembles that of the components of the XY-pair in the second division, which may or may not conjugate before going to the poles; and it is also analogous to that of the m-chromosomes of the Coreidae which conjugate very late and do not fuse. As in N. undulata, one chromosome in the peripheral ring is larger than the others, and it is here in some cases longitudinally split (fig. 16). There are in this species two small chromosomes in the peripheral ring.

In the second division, the presence of an unequal XY-pair in the center of the spindle is evident from side views (fig. 19 B). Serial sections of a spindle in side view (fig. 19 A, B, C), and polar views (fig. 20), show that there are 12 chromosomes, in contrast to the 13 of N. undulata. Here too, the components of the XY-pair may fail to conjugate before the second division, and lie on separate spindle fibers in the center of the spindle (figs. 21 B, 22). It is evident that X and Y are less unequal in size than in N. undulata, X being comparatively smaller and Y larger. The largest chromosome is distinguishable among the others, and also the three small ones of the first division (the one in the center and the two peripheral ones). The fact that the result of this division will be two kinds of cells (ultimately spermatozoa) differing in chromatin content in respect to one chromosome is apparent from sister anaphase groups (fig. 23 A, B).

Only one clear spermatogonial group has been found (fig. 24); the number here is 24, including three pairs of small chromosomes, corresponding to the three small ones of the spermatocyte divisions; the largest pair corresponding to the large one of the haploid groups; two other large pairs, and one odd large one. This is doubtless the X-chromosome; the Y-chromosome is indistinguishable, but must be one of the smaller intermediate ones.

3. Notonecta insulata. N. insulata has proved an extremely interesting species from the fact that two distinct types of chromosome groups occur in the first division, in approximately equal numbers and side by side in the same cyst. One type has 14 chromosomes including two small ones in the center, like N. undulata (figs. 25, 26); the other type has 13 chromosomes, including only one small one in the center, like N. irrorata (figs. 27, 28).

The discrepancy in number was very perplexing until consecutive sections of complete spindles were examined as they appeared in side view. It was then discovered that in many cases the discrepancy is accounted for by the fact that *the second small chromosome which appears in the center in the 14-type is frequently found attached to the largest chromosome in the 13-type.* In figures 29–31 *A*, *B*, *C* are shown serial sections of three spindles which have only one small chromosome in the center, the other small one being attached to the large chromosome forming the compound chromosome *Ma* (macrochromosome + small autosome). Polar views of the compound chromosome are rather difficult to obtain owing to the small size of the smaller component. Such a view, from a spindle cut somewhat obliquely, is given in figure 32 where both components show very clearly. In figures 33, 34 *A*, *B*, *C*, are shown spindles of the other type, where both small chromosome pairs are in the center and the large chromosome is not compound. In over thirty cases where the chromosomes have been counted in consecutive sections in side view, the apparent 13-type has been found to be due to the attachment of the second small chromosome to the large chromosome. *It is always this particular chromosome, the largest one, with which the little one is associated.* In many cases, however, when only one small chromosome appears in the center, the compound character of the large one cannot be detected, the two components having probably fused beyond recognition. When there are two small ones in the center, there are 14 chromosomes, and the large chromosome is never compound.

Besides the two small chromosomes at the center of the spindle (or one in the center and the other attached to *M*) it is clear from an inspection of the figures that there is always another small chromosome in the peripheral ring. Attention may also be called to the fact that the largest chromosome is usually longitudinally split, as it is occasionally in N. irrorata (figs. 25–28, 32).

In the second division, the number of chromosomes is always 12, so far as I have observed (figs. 35–37). As in the other two species, an unequal *XY*-pair is here present in the center of the spindle (fig. 37 *A*), and the components are frequently found side by side, having apparently failed to conjugate (figs. 38, 39).

They are more nearly equal in size than in N. undulata, in this respect resembling those of N. irrorata. The invariable number 12 is accounted for on the assumption that the second small chromosome which in the first division is sometimes separate and sometimes associated with the large one, has fused with it in all cases before the second division. Additional evidence is given by the fact that in most cases two of the chromosomes are considerably smaller than the others, one of these corresponding to the small central one, and the other to the small peripheral one of the first division (figs. 35–39). The large chromosome, however, presents an unexpected appearance. It gives no evidence whatever of its real composition of two very unequal parts, but it appears in the metaphase as a large quadripartite chromosome, as though each part into which it divides were composed of two *equal* parts (p. 89). The longitudinal split which was very noticeable in the first division in polar view marks the division plane of the second division. In figure 40 *A*, *B* are shown two sister plates of an anaphase; the groups are identical except for the middle chromosome, and it is evident that on this account two kinds of cells are produced which give two kinds of spermatozoa.

Unfortunately no spermatogonial groups have been found of which a satisfactory count could be made. The expectation would be either 26 single chromosomes, or 24 including two compound ones.

4. Notonecta glauca (*Pantel and Sinéty*). According to the account of Pantel and Sinéty ('06), there are in N. glauca sometimes 12, sometimes 13 chromosomes in the first division. They state that they are unable to account for this difference, but they also say, "elle (la couronne équatoriale) comprend un anneau périphérique, plus une ou deux unités situées au centre" (op. cit., p. 139). No figures of polar views are given but it seems probable from the statement that the discrepancy is here due to the presence or absence of a second small chromosome in the center, as in the case of N. insulata. They do not state in the text the number present in the second division, but figure 12 chromosomes. The writers mention no unequal XY-pair in the second division.

If this pair is absent, N. glauca differs radically from the other three species, and it may be that this discrepancy is analogous to that found in Metapodius (Wilson '09 a) where in different individuals of the same species a *Y*-chromosome may be present or absent; or in the mosquitoes, where there is a typical unequal *XY*-pair in Anopheles punctipennis, while in two other genera, Culex and Theobaldia, the differential chromosomes are absent (Stevens '11). It is possible that the *X* and *Y* chromosomes are present in N. glauca, but are of practically equal size as in Oncopeltus and Nezara hilaris (Wilson '11), and have been overlooked. Pantel and Sinéty call attention to the presence of an extra large chromosome, which they call the 'chromosome exceptionelle,' suggesting that it may be an accessory chromosome, but they are convinced that it participates in both divisions. This body seems quite similar in appearance and behavior to the large chromosome described in N. insulata. It is unfortunate that N. glauca cannot be brought into line with the three American species in regard to chromosome number, but the account of Pantel and Sinéty is inadequate to permit the attempt.

B. Discussion

1. The relation of chromosome number to species. It is doubtful whether every chromosome in the three species can be homologized individually, for the size relations are different in some respects. By comparing the spermatogonial groups of N. undulata and N. irrorata (figs. 13, 14, 24) it is evident that there are 5 large chromosomes in the former, and 7 large ones in the latter; and the *XY*-chromosomes are of different relative size in the three species. On the other hand, one largest chromosome can be traced throughout the history of all three species; likewise the smallest chromosome, not only by its size but especially by its position in the first spermatocyte division. We may also homologize the second small chromosome which is present in the first division of N. undulata, in the center of the group, with the one of similar size which is sometimes present in N. insulata in the same position. And since the steps in the process of fusion

can actually be observed in N. insulata, it seems reasonable to attribute its absence in N. irrorata to its permanent association with the largest chromosome. Representing the large chromosome, or macrochromosome by M, the two small autosomes by a, the unequal chromosomes by X, Y, and the larger autosomes by A, we may schematize the results as follows:[1]

	PRODUCTS OF THE FIRST SPERMATOCYTE DIVISION	PRODUCTS OF THE SECOND SPERMATOCYTE DIVISION
N. undulata	M + 9A + X + Y + a + a (14)	M + 9A + X + a + a (13) ♀ and M + 9A + Y + a + a (13) ♂
N. insulata	either M + 9A + X + Y + a + a (14) or Ma + 9A + X + Y + a (13)	Ma + 9A + X + a (12) ♀ and Ma + 9A + Y + a (12) ♂
N. irrorata	Ma + 9A + X + Y + a (13)	Ma + 9A + X + a (12) ♀ and Ma + 9A + Y + a (12) ♂

The scheme shows the intermediate condition of N. insulata between N. undulata with a larger number of chromosomes and N. irrorata with a smaller number. The large and small chromosomes in N. undulata are always separate, in N. insulata sometimes separate and sometimes associated, and in N. irrorata are presumably always associated. This may represent a progressive (or regressive) series, or the three forms may represent different modifications of a single original type.

The somatic characters do not afford decisive evidence concerning these three possibilities, although the wing color fits in with the view that N. insulata is an intermediate species. By substituting brown pigment for the white of N. undulata, the wing coloring and pattern of N. insulata is obtained; further, by substituting for this brown pigment, black, but leaving some of the brown as mottling, the wing pattern of N. irrorata is obtained. On the other hand, N. irrorata is intermediate in size between the other two, and N. undulata is intermediate in respect to the distance

[1] This scheme is identical with that published in my preliminary paper ('10) except that X, Y have been substituted for I, i.

between the eyes. It may be that the wing color is directly correlated with the fusion and separation of the two chromosomes, and that the other somatic differences are connected with other chromosomes, which, as I have stated, differ in size in the different species.

In the Acrididae, McClung ('05) has found that a particular genus, Hesperotettix, is distinguished from others of the family by a special arrangement of the chromosomes, by which the accessory is always associated with another chromosome forming a multiple element. He concludes that this arrangement "is genetically connected with the subsequently appearing characters" (op. cit. p. 326). This correlation of a multiple chromosome element and a generic difference in the Acrididae is directly comparable to the correlation of a multiple chromosome element and a specific difference in Notonecta.

The correlation of a definite number of chromosomes with a particular species is a well established fact throughout the animal and plant kingdoms, and is admitted by practically all cytologists, with only a few exceptions. In several cases, however, it has been shown that the number is constant for the individual, but differs for different individuals. This is sometimes due to the presence of 'supernumerary' chromosomes, as in Metapodius, Banasa calva, Diabrotica and Ceuthophilus (Wilson '09 a, Stevens '12 a, b), and sometimes to the fact that two types of chromosome groups occur within the species, one with twice the number of the other, as in the well known cases of Ascaris megalocephala, Echinus microtuberculatus and Helix pomatia, and as in Artemia salina, as recently pointed out by Artom ('11). Cyclops viridis is apparently a species in which different numbers occur in different varieties (Chambers '12). With these and possibly a few other exceptions, the number of chromosomes is a specific characteristic, although occasional fluctuations may occur.

Further, there are many cases where closely related species have the same number of chromosomes. For example, five species of Euschistus have the same number (Montgomery, Wilson); four species of Sagitta (Stevens '10), and three species of Ceresa (Boring '07). In other cases, related species differ only slightly

in number. For example, three species of Podisus have the diploid number of 16, two species 14 (Montgomery, Wilson). In this category belong the three species of Notonecta. But much wider differences in related species may occur, as for example, in two closely similar species of Banasa, of which B. dimidiata has 16 chromosomes, B. calva has 26 (Wilson '09 b). So in Thyanta, in which a distinction between two species has just recently been rediscovered by Barber, two types of chromosome groups occur, 27–28 in T. calceata, and 16 in T. custator. Among the phylloxerans, there is considerable variation; four species have 6 chromosomes in the diploid groups, one species has 8, one 12, and one 22 (Morgan '09). Similarly in the aphids, the haploid number ranges from 3 in the willow aphid to 16 in the maple aphid (Stevens '06). Likewise Braun ('09) found in fifteen species of Cyclops a wide range of number, from 6 to 22, although several species have the same number. In the Oenotheras, mutants have been found with 14, 15, 21 and 28 chromosomes (Lutz '12).

When we come to groups less closely related than species, marked differences in the chromosome number frequently occur. In the family Jassidae, the diploid number varies from 15 to 23 in different genera; in the Cercopidae, from 15 to 27; in the Membracidae, from 17 to 21 (Boring '07). In ten genera of the Chrysomelidae, there is a range from 16 to 36 (Stevens); in the Coreidae, from 13 to 27 (Montgomery, Wilson). These are a few of the many cases of divergence within a family. There are on the other hand, a few cases where a constancy obtains throughout as wide a group as a family, as for example, in ten genera of the orthopteran family, Acrididae (McClung '05, et al.), and in four genera of the opisthobranch mollusks. The constancy in number goes still further in some of the Amphibia, where all the urodeles, so far as examined, apparently have 24 chromosomes in the diploid groups.

It is therefore evident that while in some cases the chromosome number is the same for members of rather a large group, it is not necessarily the same for even very closely related forms. It is true in general, however, that closely related forms have the same

or very nearly the same number of chromosomes. This fact has led Montgomery and McClung to the view that the number and arrangement of chromosomes should be considered as an important character in taxonomy. More recently, McClung ('08) has expressed this view forcibly in his paper on "Cytology and Taxonomy." It is of interest in this connection to find that in the Oenotheras, according to Lutz ('12), all individuals having a given type of vegetative character have the same number of somatic chromosomes, irrespective of the origin of these individuals, whether hybrid or mutant.

That one method by which a change in the chromosome number has taken place is by the fusion or separation of particular chromosomes seems highly probable from the evidence given by Notonecta, where we have all the stages in the process in the three species. Such a process may also be indicated by the *d*-chromosome in Nezara (Wilson '11). A somewhat similar idea was put forth by Montgomery ('01) before the relation of the *X*-chromosome with sex had been established, to explain the occurrence of an odd number of chromosomes in the spermatogonial groups of some of the Hemiptera; the odd number representing, he believed, a transition stage between two even numbers. A change in number by a process of fusion has been advocated by McClung ('05) in regard to the multiple chromosomes in the Orthoptera. A change in number by a process of splitting has been advocated by Payne ('09) in the case of the multiple *X*-element in the reduvioids, and this may likewise apply to that of many other forms, such as Phylloxera, Syromastes or Ascaris lumbricoides, as has been indicated by Wilson ('11). A second probable method of change is by a process of progressive reduction and final disappearance of particular chromosomes, as was originally suggested by Paulmier ('99) in the case of the small *m*-chromosomes of the Coreidae, and later by Wilson in the case of the *Y*-chromosome.

These two methods will account for gradual and slight changes in the chromosome number. Such wide variations as occur in closely related species, e.g., in Banasa, Thyanta, and the phylloxerans, must be accounted for in some other way. Wilson ('11)

suggests that some sudden mutation has taken place, involving a new segregation of the nuclear material, and causing a change in number and size relations of the chromosomes, but not in their essential quality.

A fourth method by which either a slight or a radical change in the chromosome number might take place is by an abnormality occurring in mitosis, as has been suggested by several authors, either by an unequal distribution of the chromosomes to the daughter cells, or by an arrest of cell division after a division of the chromosomes. The former abnormality has actually been observed in the case of Metapodius (Wilson '09 a), and the Oenotheras (Gates '08, et al.). The possibility of the occurrence of the second abnormality is shown by the experiments of Gerassimow ('01) on Spirogyra, of Němec ('04) on Pisum and of Boveri ('05) on sea-urchin eggs, in which a monaster was produced instead of an amphiaster, the chromosomes dividing but not the nucleus, and the double number of chromosomes remaining in subsequent divisions. To this cause has been attributed the occurrence of triploid and tetraploid mutants in Oenothera(Gates '09, Lutz '12), and it seems probable that many of the cases of a double number of chromosomes occurring in closely related forms of some animals (e.g., Ascaris megalocephala), and many plants have been brought about in this way.

2. Temporary association and separation of chromosomes. The condition of temporary association and separation of particular chromosomes which occurs in N. insulata is of especial interest in comparison with other forms. In the first place, there are cases where the union and separation concerns the sex-chromosomes only. In these cases the *X*-element may consist of two or more components—in Acholla (Payne '09) and Ascaris lumbricoides (Edwards '10) as many as five—that appear as separate chromosomes in the diploid nuclei but become associated in the spermatocyte divisions and behave as a single accessory.

In a second category may be placed those forms where there is a temporary or permanent association of the sex chromosomes with other chromosomes. Sinéty ('01) was the first to describe a case of this sort in the phasmids Menexenus and Leptynia.

Here the accessory becomes attached to another chromosome in th first division, and goes over with it to one pole. McClung ('05) describes a similar relation for the acridian Hesperotettix and the locustid Anabrus. In Hesperotettix he found that it is always the largest chromosome with which the accessory is associated. In Mermeria, another acridian, a similar multiple element becomes further associated with another tetrad, and in division this complex acts as a single bivalent, with the anomalous result that entire tetrads pass to one pole. More recently, Boring ('09), Boveri ('09) and Edwards ('10) have found in the case of Ascaris megalocephala that the accessory may be free or may be indistinguishably united with another chromosome. Stevens ('11) similarly finds in one of the mosquitoes a close union of *X* and *Y* with a pair of autosomes in the spermatocyte divisions, while in the spermatogonia they may or may not be closely united with them.

In a third category we may place the form N. insulata where there is a temporary association and separation of two ordinary chromosomes (autosomes). That this association has some significance can scarcely be doubted when we consider that it is always two particular chromosomes that are united. If the union of the two chromosomes is the primitive condition, then the secondary separation might mean that certain characters are being segregated from other characters. If the separation is primary, the fusion of the chromosomes might mean that a certain series of characters which were entirely independent of another series have become linked with them. It is possible that just as the association of a sex-chromosome and an autosome may serve as a morphological basis for sex-linked inheritance as pointed out by Wilson ('11), the association of two autosomes may give the morphological basis for the cases of coupling that have recently been made known in experimental work. For example, Bateson and Punnett ('11) find that in the sweet pea, blue color and long pollen are usually combined, red color and round pollen, etc.; and in Primula, according to Gregory ('11), magenta color is coupled with short style. In Drosophila also, a linkage of a color and a wing factor has been found by Morgan and Lynch

('12). In order that the linkage may take place, however, in N. insulata, we must assume that a particular chromosome of one pair always associates with a particular one of the other pair and never with its mate. When these two chromosomes are permanently associated, as is probably the case in N. irrorata, one chromosome might serve as the basis of the linked characters.

3. The XY-pair. The observations on Notonecta add nothing new to the main facts in regard to the *XY*-chromosomes. The difference in size between the two components is much more marked in N. undulata than in the other two species; similar differences between related species have been found in Nezara, Euchistus and other Hemiptera. The only departure from the usual behavior of the *XY*-pair is in the failure of the two components to conjugate. Usually in the Hemiptera the components come together in the prophase of the second division, in contrast to all the other chromosomes which have paired before the first division. In Notonecta, however, in all three species this pairing frequently does not take place, and the two components of the *XY*-pair lie side by side in the metaphase of the second division and pass to opposite poles. A similar condition has been seen by Montgomery ('10) in Euchistus, but here it is apparently very exceptional for he found it only in one case out of 672. As to the time of conjugation of chromosome pairs, there is a graded series. The autosomes conjugate in the general synaptic period; the *m*-chromosomes undergo a late synapsis in the prophases of the first division; the *XY*-chromosomes in most Hemiptera do not finally conjugate till the end of the first division; the *XY*-chromosomes of Notonecta frequently do not ever form a definitive dyad. It is of interest, from the point of view of the mechanics of division, to find that a linear arrangement of the components of a chromosome pair is not necessary for their distribution to the opposite poles of the spindle.

IV. KARYOSPHERE[1]

A. Notonecta insulata

1. Formation of karyosphere. The growth stages of the primary spermatocyte are probably separated by a considerable interval from the last spermatogonial telophase. In the earliest spermatocytes observed (fig. 41), the chromatin is massed in a single large body, the karyosphere, and thin strands of linin are scattered through the nucleus. The first change to take place is the accumulation of chromatin on the linin threads (fig. 42). Although the source of this chromatin cannot be definitely ascertained, it seems most likely, from a study of many of these nuclei, that it comes from the karyosphere following the course of the linin strands and tending to aggregate at particular points. The threads from the karyosphere are more or less twisted, and show a distinct radial arrangement. The tendency of the chromatin to aggregate in clumps becomes more marked until the nucleus is filled with small chromatin masses connected with each other and with the karyosphere by thin deeply staining strands (fig. 43). During this process the chromatin masses are frequently approximated in pairs (figs. 42, 43). This fact suggests that the masses represent chromosomes which are conjugating. Although the evidence is not conclusive that synapsis takes place at this time, the whole process of the formation of these chromatin masses seems unintelligible otherwise. The number of the chromatin masses varies considerably in nuclei of the same cyst; the maximum number is however greater than the reduced number of chromosomes, although probably not as large as the somatic number. This is easily accounted for on the assumption that the pairing of different bodies takes place at different times, as seems

[1] The term 'karyosphere' is used in this paper in the sense in which Blackman ('03) first used it to denote a structure consisting of chromatin and other substances, such as linin and karyolymph. It is thus a broader term than karyosome or net-knot or chromosome-nucleolus which is usually applied to a mass of pure chromatin. 'Karyosphere' is practically identical with Carnoy's 'nucléole-noyau,' or miniature nucleus; it is however difficult to determine the presence of a membrane as is required by his definition. Although it is impossible to tell at all stages whether accessory material is present with the chromatin, the term karyosphere will be used throughout the discussion.

to be the case. This process in N. insulata is somewhat similar to that described by Arnold ('08) in Hydrophilus, and by Davis ('08) in some of the Orthoptera. The masses are perhaps comparable with those described in many plant cells as 'prochromosomes.[3]

After the stage of the scattered chromatin masses in N. insulata, a process of absorption sets in. The masses gradually decrease in size and the connecting strands become thicker, especially in the region of the karyosphere (fig. 44). By an absorption of all the chromatin masses, a spireme of approximately uniform thickness is formed which is irregularly coiled about in the nuclear cavity (fig. 45). The spireme is not formed here by an unraveling process as described by Janssens, Davis and others, but by a uniform distribution of the chromatin material along the connecting strands. A somewhat similar formation of the leptotene spireme has been described by Gérard ('09) in Stenobothrus.

The spireme now becomes arranged in loops which are more or less oriented toward the karyosphere and may connect with it at their apices (fig. 46). The karyosphere seems to act as a center of activity like the chromoplast of Eisen and Janssens. Later the loops take up a position on the nuclear wall, receding from the karyosphere which remains in the interior (fig. 47). The loops then become somewhat irregular, coiled and thicker; their staining capacity gradually diminishes until in faintly stained preparations only the karyosphere and a few scattered remnants of the loops take the chromatin stain (figs. 48–50). This change can be readily appreciated by comparing these three figures, which are from the same slide. The nuclear cavity is, however, filled with a flocculent reticulum, which is quite faint in lightly stained preparations, but is very noticeable and takes a deep chromatin stain in preparations that are less extracted.

The foregoing facts in Notonecta are extremely perplexing. Since the spireme is formed from the scattered masses, it must apparently at one time contain the essential elements of the chromosomes. A transfer of these elements into the karyosphere

[3] These are evidently similar to the 'massive bodies,' recently described in other insects by Wilson ('12), as occurring in Stage b.

may be afforded in two ways; by a flow along the whole length of the spireme and (for a brief period) by the connection of the curves of the loops with the karyosphere. After the loops become disconnected and oriented, they give somewhat the appearance of the 'bouquet,' though this is never so regular or clearly marked as in Batrachoseps, Tomopteris, etc. The polarized loops of the bouquet stage are in other forms the forerunners of the chromosomes. This may also be the case in Notonecta, but if this be so, the conclusion seems unavoidable that the fundamental material must subsequently return to the karyosphere, for, as will be shown, the chromosomes later arise directly from the latter. It is possible that this material flows back into the karyosphere along the faintly staining threads that can usually be traced from the loops; but it seems more probable that after the loops are disconnected, their substance does not enter the karyosphere. This conclusion is based on the fact that the loops withdraw from the karyosphere, and that some of the more remote threads keep the chromatin stain after the ones near the karyosphere have lost it. If this be so, considerable ground is given for the view that there are here two kinds of chromatin, corresponding with those designated by Lubosch ('02) as trophochromatin and idiochromatin. The chromatin, which is later to form the chromosomes is transferred to the karyosphere in one or both of the two ways suggested, while the rest of the chromatin becomes disconnected from the karyosphere and is represented by the flaky reticulum in the nuclear cavity.[4] In the case of N. glauca, Pantel and Sinéty have likewise concluded that the material in their 'moniliform cords' becomes achromatic and is spread through the nuclear cavity, taking no part in the chromosome formation. This material has no doubt a metabolic function during the enormous growth of the spermatocyte. From a comparison of figures 51 and 53 drawn to the same scale, it is evident that the surface of an equatorial plane of the full grown spermatocyte nucleus is approximately five times that of the youngest one, which means that its

[4] The paper of Vejdovsky ('12), containing very interesting observations, bearing on this and other subjects here treated, unfortunately came to my notice too late for his results to be incorporated in this article.

volume is nearly twelve times as great. About half of the increase in size takes place before the disappearance of the spireme, the other half after the karyosphere is fully formed (figs. 51, 52, 53). The growth of the spermatocyte in Notonecta is so great as to be comparable with that of an oocyte (as was noted also by Pantel and Sinéty), and it must involve a similar metabolic activity. It is well known that in the eggs of many forms, some of the chromatin is eliminated during the growth or maturation divisions; this is probably correlated with the metabolism of the cell. The diminution of the chromatin by a casting off of the ends of the chromosomes in Ascaris megalocephala and A. lumbricoides is probably of a similar nature. The ring in Dytiscus (Giardina '01) which during four divisions passes to only one of the resulting cells, i.e., the oocyte, has likewise been interpreted by Boveri ('04) and Goldschmidt ('04) as representing chromatin that is concerned in the nutrition of the cell. For a comprehensive account maintaining the existence of two kinds of chromatin, basichromatin and oxychromatin, see Stauffacher ('10).

2. Description of karyosphere. The appearance of the karyosphere varies considerably with the stage of growth, with different fixing fluids and stains and with the amount of extraction. In many preparations, especially those stained deeply with haematoxylin, no structure is evident; it is merely a round or approximately round mass of vesicular appearance (fig. 54 *A*). In other preparations an irregular contour and difference in staining capacity in different regions gives it a spongy appearance (figs. 54 *B*, 55). In haematoxylin preparations well extracted and in safranin preparations the structure is quite definite. The karyosphere consists apparently of dense, compact bodies of varying size embedded in a less dense matrix (figs. 53 *A*, 54 *C*, *D*), the former being probably the chromatin proper. When crowded these bodies give somewhat the appearance of a continuous spireme closely convoluted (fig. 53 *A*). In the younger stages, the karyosphere tends to have a vesicular appearance, and later the spongy or granular structure is more evident. In the living material, the differentiation of two sorts of material in the karyosphere is perfectly evident, the denser substance taking the form of com-

pact masses, either isolated or continuous, embedded in a less dense substance (fig. 56). In this condition the karyosphere persists during the whole growth period.

3. Dissolution of karyosphere. Just prior to the formation of the aster, the karyosphere tends to assume a more definite outline appearing in lightly stained preparations as a more or less spherical body in which darkly staining bodies are embedded. Figure 57 is from the same slide as figure 58, the former being an earlier stage. After the formation of the aster, the chromatin masses leave the karyosphere as compact bodies, either irregular in shape or threadlike (figs. 59–61). It is perfectly evident that the karyosphere is breaking up into its two constituents; the darkly staining chromatin bodies are passing out of the karyosphere, leaving the less dense, paler material which becomes rounded and now appears as a typical plasmasome. As the masses leave the plasmasome, they quickly proceed to the periphery of the nucleus where they take on the form of double threads, as will be described later. In figure 60 the plasmasome is entirely free of chromatin, staining a pale gray; some of the chromosomes are seen along the nuclear margin. The mass of chromatin that has just left the plasmasome, is the large chromosome, *M*, which has been mentioned previously as one element of the compound chromosome typical for this species. The plasmasome has usually one or several small vacuoles in the interior. The body gradually decreases in size and disappears in the late prophase.

B. Notonecta undulata

1. Formation of karyosphere. In the earliest spermatocyte, a small karyosphere is present, and the rest of the nuclear cavity is filled with a reticulum of linin (fig. 62). The reticulum increases slightly in staining capacity and takes on the appearance of a very thin spireme, often twisted in spirals; a leader is usually to be seen running from the karyosphere (fig. 63). This is undoubtedly the leptotene stage. The spireme becomes more heavily staining and tends to contract to one side of the nucleus; the threads are still very thin, and in some cases appear

to run parallel in pairs (fig. 64). This is probably a synizesis stage and it is likely that a conjugation of the parallel threads takes place, although this cannot be conclusively demonstrated. In the next stage, the spireme is somewhat thicker and becomes arranged in loops, oriented toward the karyosphere (fig. 65). The loops next become attached to the karyosphere at their apices; this gives the appearance of arms radiating out from the karyosphere (fig. 66), and affords an opportunity for the transfer of material from the loops to the karyosphere. Very frequently the threads give the appearance of being longitudinally split. The loops now recede from the karyosphere and become arranged on the nuclear wall while the karyosphere remains in the interior (fig. 67). The loops gradually disappear, and the nucleus is filled with an irregular reticulum which is appreciable when the staining is dark, but pale when the stain is more extracted, in contrast to the dark karyosphere.

The early history of N. undulata thus differs markedly from that of N. insulata. There is no formation of scattered chromatin masses, but instead a leptotene stage which is followed by a synizesis or contraction, after which a spireme is present which is quite similar to that of N. insulata. The fate of the material in the spireme offers the same difficulties as in N. insulata, but it seems probable that here too some chromatin passes into the karyosphere, and other chromatin is segregated out and furnishes the material in the nuclear cavity.

2. Description and dissolution of karyosphere. The karyosphere of N. undulata appears very much as it does in N. insulata; it tends to be vesicular in the early stages, especially in darkly stained preparations, but later appears granular or rope-like (figs. 68, 69). At the approach of the prophase the karyosphere becomes broken up into a variable number of small round bodies, giving the appearance of a mass of marbles (fig. 70). The mass loosens and from it project one or two longitudinally split threads (figs. 71, 72). That the thread may be formed directly from the balls which become arranged in pairs is evident from figure 72. As the double threads form, they go to the nuclear wall where they become the diffuse prophase chromosomes which will be

described later. In figure 73, some of the chromosomes already lie at the periphery while others are still being transformed from balls into threads in the interior. From this process it seems clear that the material of one particular chromosome may be broken up into more or less isolated bodies, which later arrange themselves and fuse together to give rise to a continuous structure. As the last chromosomes form, some of the substance of the original karyosphere is left behind as a plasmasome. This body must apparently be formed from material that was in the balls which segregates out in the process of thread formation. The plasmasome is not colorless as it is in N. insulata, but remains dark even in well extracted preparations, probably owing to the fact that some of the chromatin is left behind. The plasmasome has a vesicular appearance and is frequently vacuolated. It gradually decreases in size and disappears in the late prophase.

C. Notonecta irrorata

The earliest stage occurring in my material is represented in figure 74. This shows the presence of a looped spireme on the nuclear wall, more or less oriented toward the karyosphere. The spireme gradually disappears as in the other two species, probably giving rise to the flaky material in the nuclear cavity. The karyosphere at this stage is distinctly vesicular. Just before the aster forms, however, it breaks up into a mass of balls of an inconstant number which form threads, very much as described for N. undulata (figs. 75, 76, *A*, *B*). A plasmasome is formed during this process which sometimes takes a heavy chromatin stain (fig. 77), and sometimes appears grey and vacuolated; it gradually decreases in size and disappears in the late prophase.

D. Conclusions and comparisons

A karyosphere is apparently present in the three species of Notonecta which I have examined, throughout the entire history of the spermatocytes. In the very early stages and in the later stages, this is the only body in the nucleus that takes a deep chromatin stain, but there is an intervening stage when a chromatic spireme is present. It would appear that the chromatic

material comes from the karyosphere, and that later, at least that part of it which contains the essential elements, returns to the karyosphere. This flow of material back and forth from the karyosphere seems highly remarkable, and is probably concerned with a rearrangement of the chromatin particles, for which a particular structure (i.e., the spireme) is necessary. It is unfortunate that I have not been able to determine more definitely how the definitive karyosphere is formed. In N. glauca, according to Pantel and Sinéty, a pale nucleolus is present in the early stages and the chromatic material from the 'moniliform cords' condenses around it, a process similar to that described for some of the myriapods (Blackman '05 b, '07) and the dragon fly (McGill '06). In other myriapods, the accessory chromosome is the center around which the other chromosomes are deposited to form the karyosphere (Blackman '05 a, '07, Medes '05).

In Notonecta, the chromatin remains massed together in the karyosphere, in an apparently inactive state during a long growth period. It is usually possible at this time to distinguish the chromatin material as distinct bodies, not necessarily the individual chromosomes, embedded in a less dense (plasmasome) material. Such an intimate association of plasmasome and chromatin material, where the latter is recognizable as distinct bodies, has been described in some of the myriapods (Blackman, Medes), and in the case of the *XY*-chromosomes in some of the reduvioids (Payne '09) and in certain Coleoptera and Diptera (Stevens). In some cases, e.g., in Scolopendra heros (Blackman '05 a) and in Hydrophilus (Arnold '08), there is apparently no plasmasome material associated with the karyosphere. The distinction of two sorts of material is extremely apparent in N. insulata in the early prophase, when the chromatin leaves the karyosphere as compact masses, and the remaining material becomes a typical pale plasmasome. In N. irrorata and N. undulata, and apparently also in N. glauca, the dissolution of the karyosphere takes place a little differently, by breaking up at once into a number of separate elements. In either case, there can be no doubt that the material which forms the chromosomes comes from the karyosphere. The events described for Notonecta do not seem to me at variance with the hypothesis of the genetic continuity of the

chromosomes; it seems, on the contrary, altogether reasonable to suppose that the essential chromosome elements retain their identity throughout the entire process.

V. PROPHASES

A. General description

1. Notonecta insulata. It is of interest to trace the history of the chromatin from the dissolution of the karyosphere until the formation of the definitive chromosomes. After the irregular masses of chromatin have left the plasmasome, they pass from the interior of the cell to the nuclear membrane; and here the chromosomes pass through a diffuse stage before assuming their final form. At first they appear on the nuclear wall as longitudinally split rods, long, thin and somewhat curved (fig. 78); the rods are apparently made up of a linear series of granules (chromomeres) which give them an irregular contour. The usual prophase figures, rings, crosses, etc., are formed from the longitudinally split threads (fig. 79); they will be described in detail later. By a process of condensation are formed the definitive chromosomes which are typically dyad-like in appearance; their tetrad nature cannot be made out unless they lie in a favorable position and are very critically observed (fig. 80). During these stages, the chromosomes have remained close against the nuclear membrane, and it is from this position that they are drawn on to the spindle in the late prophase. In figure 81 they are seen irregularly arranged on the spindle, prior to their final grouping around the equator. Attention may be called to the fact that frequently in the late prophase, the small chromosome is found attached to the large one, forming the compound chromosome, to which reference has been made in an earlier part of the paper (figs. 80, 81).

2. Notonecta irrorata. The history for this species is practically the same. The thin longitudinally split rods (fig. 82) on the nuclear wall give rise to rings, crosses, etc. (fig. 83). While still on the nuclear wall, they condense into the definitive chromosomes, which later become irregularly arranged on the spindle (fig. 84).

3. Notonecta undulata. In this species also, the first indication of the final chromosomes is the presence on the nuclear wall of thin, more or less coiled threads (fig. 85). The prophase figures which these form are quite different, however, from those of the other two species. At first these have a very vague, spongy appearance and are coarsely granular (fig. 86). By a process of condensation, they become more compact and more definite in outline (fig. 87). The figures are quite irregular in shape, but in general consist of two bars, diverging or united at one or both ends. While in this stage, the nuclear membrane breaks down and the spindle fibers begin to form. The chromosomes are still quite irregular in shape after the spindle is fully formed (fig. 88), and do not assume their definitive form until the full metaphase.

B. Detailed description

1. Notonecta insulata. a. The ring. The *M*-chromosome is usually the last one out of the plasmasome, and is therefore in the interior of the nucleus at the time that all the other chromosomes are in a diffuse condition on the nuclear wall (fig. 60). Owing to this fact and also to its greater size, its history can be traced throughout the prophase and also during the first and second maturation divisions. Whereas the other chromosomes come out of the plasmasome in more or less irregular masses, the *M*-chromosome has the form of two rods, somewhat coiled about each other, but in general taking the same direction (fig. 89 *A–D*). The two rods untwist, and open out in the middle, usually becoming or remaining united at the ends (fig. 90 *A–D*). By opening out still more, a small ring is formed (fig. 91 *A–D*); frequently at this stage and occasionally earlier, a longitudinal split is present, in one or both half rings. If we term the original line of separation between the two rods a longitudinal split, this is the second longitudinal split. By this time, the *M*-chromosome has reached the nuclear wall, and at once a process of expansion sets in. The ring opens out until the enclosed space becomes relatively very large and the ring itself correspondingly thin (fig. 92 *A*, *B*). It

is usually broken at this stage into two half rings, each one corresponding to one of the original rods; and each half ring is clearly longitudinally split. The two bars of each half ring frequently intertwine, thus making a quite remarkable figure (fig. 92 *A*). There seems to be no fusion at the points of crossing and no connection of the elements of the two half rings inter se, so that the figure lends no support to Janssens' ('09) chiasmatype theory. After the stage of maximal expansion, a process of condensation sets in during which the enclosed space becomes smaller and the bars thicker; the first stage of the process is shown in figure 93 *A–F*. The second longitudinal split has become so pronounced that it entirely separates each half ring into two distinct elements. The quadripartite nature of the ring is especially noticeable at the juncture of the two half rings, for here the longitudinal bars diverge considerably. In the very late prophase, the *M*-chromosome appears as shown in figure 94 *A–D*; the space enclosed in the ring has become very much reduced, and the second longitudinal split is still in evidence. The chromosome becomes arranged on the spindle with its first longitudinal split in the plane of the equator and its second longitudinal split in the plane of the spindle axis. In a side view of the chromosome on a metaphase spindle, therefore, the second longitudinal split is not visible, since it lies in the plane of the paper (fig. 95 *A*, also figs. 29 *C*, 30 *C*, 31 *C*, 33 *C*, 34 *A*). If however one obtains an end view of the chromosome as it lies on the periphery of the spindle, i.e., so that the place of union of the four elements is in the line of vision, the second longitudinal split is clearly seen at right angles to the first (fig. 95 *B*). Also, in polar view of a metaphase plate, the second longitudinal split is so clearly marked, that the *M*-chromosome seems to consist of two distinct parts (fig. 95 *C*; also figs. 25–28, 32). The first division plane passes through the first longitudinal split. The second longitudinal split remains during the anaphase; figure 96 *A* is a view of the chromosome cut obliquely so that one of the components is at a higher level than the other; figure 96 *B* shows the compound chromosome *Ma* in end view. Figure 97 is a late anaphase showing the bipartite nature of the *M*-chromosome. This is also evident in

figure 98 where the chromosomes are being pulled on to the second spindle immediately after the completion of the first division. On the spindle, the *M*-chromosome lies so that the longtitudinal split is in the plane of the equator; the split therefore marks the line of division (fig. 99). The daughter groups in the late anaphase of the second division are shown in figure 100. The *M*-chromosome in the second division has rather a peculiar form for this stage. In the metaphase it looks like a tetrad, and after division like a dyad, but this bipartite appearance of the single element has probably no significance. The four chromatids have been distinct since long before the first division and each has retained much the same form throughout its history; this form happens to be a dyad-like structure.

To sum up: the *M*-chromosome starts as a double rod which opens out to form a ring; a second longitudinal split appears. In the first division, it divides along the first longitudinal split into what were two half rings. In the second division each part divides along the second longitudinal split which has remained since it was formed.

It is not only the *M*-chromosome that forms a ring, but the next largest chromosome goes through a similar history, as far as it can be traced. Starting with the open ring which is longitudinally split (fig. 101 *A*), it passes through stages in condensation, exactly parallel with those of *M* (fig. 101 *B–D*). In the metaphase of the first division, in side view, the longitudinal split is not visible since it lies in the plane of the paper (fig. 101 *E*), but in polar view it divides the chromosome in two halves (fig. 101 *F*). The line of division coincides with the plane between the two half rings. In figure 102 is shown a late anaphase group in which one may distinguish the largest and the next largest chromosomes, both longitudinally split. The split in both cases marks the line of separation for the second division.

In addition to the two large rings, there is a small ring in the prophase, which also has a longitudinal split (fig. 103 *A*, *B*). Its history has not been traced.

b. The cross. At the time that the *M*-chromosome is leaving the plasmasome, the other chromosomes are on the nuclear wall

in the form of thin double rods (fig. 60). The genesis of the cross from these bodies is as follows. The two segments open out into a *V*, each arm of which becomes longitudinally split (fig. 104 *A*). The arms of the *V* open out still further so as to form a double straight rod, the original space between the arms (i.e., the first longitudinal split) being represented only by the small opening in the middle of the two bars (fig. 104 *B*). There are evidently two methods by which a tetrad may be formed from this figure. The double bars may condense, while the connection around the central opening becomes very thin (fig. 104 *C*, *D*), or the connection around the central opening may become pulled out transversely, so as to form the cross-bars of a typical cross (fig. 104 *E*, *F*). In this case, half of each long arm and half of each short arm condense to form one element of the tetrad (fig. 104 *G*). The end result is the same in either case, a tetrad is formed in which the original longitudinal split is represented by the division line through the short axis and the second longitudinal split by the line through the long axis. In the metaphase, the tetrad lies with its long axis parallel with the spindle and its short axis in the plane of the equator. The first division therefore separates the two components of the original double rod. The vertical split is usually rather difficult to make out with certainty in the metaphase but in some cases is quite clear (fig. 104 *H*). This split becomes very distinct in the anaphase, and marks the line of separation of the second division (fig. 104 *I*).

c. The double rod. By a process of condensation, the original double filament forms a thick double rod, the two components of which lie parallel (fig. 105 *A*, *B*). These become united at one end, and straighten out to form a dyad (fig. 105 *C*, *D*). There is no clear evidence of the presence of a second longitudinal split. In the metaphase, the chromosome lies with its original longitudinal split in the plane of the equator, so that the first division separates the two components of the original double rod.

d. *XY*-pair. In figure 106 *A* is shown a diffuse cross which differs from the ordinary cross described above only in the fact that its longitudinal bars are unequal. It is possible, of course, that this is an ordinary cross of which part of one bar has been

cut off in the section. The same may be said of figure 106 *B* where the cross is more condensed. But the probability that these represent stages in the history of the *XY*-pair is suggested by the occurrence of an unequal tetrad in the late prophase (fig. 106 *C*, *D*). The two small components have, in this event, arisen from the longitudinally split short vertical bar of the cross, and the two large components from the split long vertical bar. The small components represent the *Y*-chromosome and the large ones the *X*-chromosome. Edward ('11) figures in Ascaris felis the *XY*-pair in the prophase quite similar to my figure 106 *B*. As stated previously, the *X*- and *Y*-chromosomes are separate in the first division and in figure 106 *D* from a late prophase they are already somewhat separated. A preliminary separation of the members of the *XY*-pair therefore takes place first in the prophase in advance of the other chromosome pairs. This may be correlated with the fact stated previously that the *X*- and *Y*-chromosomes are frequently found in the second metaphase side by side instead of joined together to form the usual unequal dyad.

2. Notonecta irrorata. The history of the ring in this species is the same as that described for the *M*-chromosome of N. insulata (fig. 107 *A–D*). The second longitudinal split remains here also during condensation, and although not seen in lateral view (fig. 107 *C*) is frequently visible in polar view of the metaphase (fig. 107 *D*; see also fig. 16). The crosses are likewise similar to those of the other species (fig. 108 *A–H*).

3. Notonecta undulata. A detailed study of the prophase figures in this species has not been attempted. They are evidently very different from those of N. irrorata and N. insulata and their irregular shape renders them difficult to trace. Some of these in the diffuse stage are shown in figure 109 *A–F*, and after they have condensed in figure 110 *A–F*.

C. Discussion

The prolonged discussion that has followed Flemming's original discovery of the open ring type of bivalent chromosome is even now not terminated, and the same is true of the cross described

by Paulmier and other early observers of the insects. In some respects Notonecta is not well adapted for the elucidation of this problem, owing to the difficulties attending the study of the chromosomes during most of the growth period. On the other hand, this form offers certain advantages in the fact that the formation of the rings and crosses may be clearly followed during the prophases. The facts here seen seem to leave no doubt that the rings are formed in essentially the same way as in the Amphibia and Tomopteris, though their relation to the original spireme can not be traced.

Those observers (Grégoire, the Schreiners, and many others) who accept a side-by-side conjugation, or parasynapsis, regard the ring as originating by the opening out of the longitudinally split spireme. Those observers (Paulmier, McClung, et al.) who accept an end-to-end conjugation, or telosynapsis, regard the ring as originating by the bending together of the split spireme at the two extremities. In either case, the final result is the same as far as the real significance of the ring is concerned. The plane between the two half rings passes through the synaptic point and therefore, according to most observers, a division in this plane means a reduction division, the division in the plane of the ring dividing it into two whole rings is longitudinal and equational. According to some observers, e.g., Paulmier, Montgomery, Farmer and Moore, and also most of the adherents of parasynapsis, the first division is reductional. McClung and his students, however, believe that in most Orthoptera it is the second division that is reductional. Bonnevie holds that the ring divides in its own plane in both divisions and that therefore there is no reduction; the rings of Enteroxenus, however, have been differently interpreted by the Schreiners ('07).

In Notonecta it is impossible to trace the chromosomes through the greater part of the growth period when they are aggregated in a karyosphere, but the evidence seems in favor of the heterohomeotypic scheme of Grégoire. The ring is formed from two parallel rods which probably represent univalent chromosomes. The first division separates the ring into two half rings, and is therefore probably a reduction division. The second division is in

the plane of the ring and is therefore probably an equation division.

In many cases the ring goes on to the metaphase spindle without further modification, e.g., in the vertebrates and higher plants, annelids, etc., but in some cases, e.g., the insects and copepods, it condenses into a tetrad, as first explained in detail by Paulmier ('98). In other forms where the ring condenses, the split is either entirely lost before the second division or is only faintly indicated by an indentation. In most forms in which no condensation takes place, the identity of the chromosomes is lost in the interkinesis, although in Tomopteris, the Schreiners ('06a) have traced the second longitudinal split with some degree of certainty to the second division. In Notonecta, the ring condenses to form a tetrad, but the longitudinal split remains most distinct. In N. insulata in the case of two chromosomes and in N. irrorata in the case of one chromosome, in the first metaphase, the chromosome is completely divided into two parts and it remains thus until drawn on the second spindle. The second division follows directly on the first, the telophase of the first being the prophase of the second. Since this split can be traced from the early prophase of the first division to the second metaphase when it lies in the equatorial plane, there can be absolutely no question as to its identity with the division line of the second division.

The cross is in principle the same as the ring, as first pointed out by Paulmier ('98) and as more recently discussed from the point of view of parasynapsis by the Schreiners ('06 a,b) and Montgomery ('11), the difference in form being due to the divergence of the two parallel rods from the ends (cross) instead of in the middle (ring). In the process of condensation the cross becomes a typical tetrad in contrast to the ring-tetrad, whose quadripartite nature is not detectable in lateral view since the second longitudinal split lies in the plane of the spindle. The similarity between the two is easily seen however, if we compare a lateral view of the cross-tetrad with an end view of the ring-tetrad (cf. fig. 104 *H* with 95 *B*). The first division plane passes across the short axis of the cross-tetrad, and if we consider each original parallel rod as a univalent chromosome this is a reduc-

tion division for this figure as well as for the ring-tetrad. The second division plane coincides with the second longitudinal split and probably means an equation division. In the case of the parallel rods, similarly, the first division separates the two original components. The evidence therefore, is in favor of the first division acting reductionally for all the autosomes. This is not true, however, for the XY-pair, the two components of which are finally separated in the second division. The fact that the reduction and equation divisions are reversed in the case of the XY-pair and of the X-chromosome has been noted in many other cases. With this exception and with the exception of the multiple element of Mermeria (McClung '05), and possibly a few others (Blackman '10), all the chromosomes are believed to undergo a qualitative division at the same time.

There is some evidence from N. insulata for Baumgartner's ('04) view that the form of individual chromosomes in the prophase is constant. The two largest chromosomes assume a ring shape, several of the large ones become crosses, one of the large ones a double rod, and a small one a ring; the smallest ones could not be traced. The Schreiners ('06) have also concluded that to a certain point the form of a particular chromosome is constant. Davis ('08) in the Orthoptera and Blackman ('10) in the myriapods, hold the same view. On the other hand, Bonnevie ('07) in Nereis and Foot and Strobell ('05) in Allolobophora, believe that the form of the chromosome is merely a matter of chance. Bonnevie states that rings are limited to chromosomes of a certain size, and Robertson ('08) has attempted to show in Syrbula that shape is dependent on size. From the fact that both very large and small rings occur in N. insulata, it seems that in this case, form is not dependent on size.

VI. MITOCHONDRIA

A. Observations

1. Late growth and division stages. An exhaustive study of this subject has not been undertaken, but a brief treatment is given because of a few observations that I have to offer. Owing to the

precarious nature of the mitochondrial stain of Benda, only a few clearly differentiated slides were obtained. The general tone of both cytoplasm and karyoplasm is a pale lavender or rusty red, the chromatin is a brick red and the mitochondria a deep purple. In the drawings, the lavender is represented as a pale grey, the brick red as a darker grey, and the purple mitochondria as black and dark grey. The earliest stage of N. insulata obtained is shown in figure 111; the chromatin is in compact masses in the karyosphere, and the mitochondria are scattered through the cytoplasm. A mass of mitochondria from which project fibers, is attached to the nuclear wall. A slightly later stage is shown in figure 112, where the karyosphere is breaking up, and the nuclear plate of mitochondria has disappeared. From these figures, it is evident that the mitochondria are of two distinct kinds, fibers and spheres. The spheres occur chiefly around the nuclear periphery, and frequently form a complete circle about it. The fibers usually occur further out in the cytoplasm and tend to aggregate in several dense clusters. The relation between the fibers and the spheres is shown in figure 113; the spheres have a curved rod at the periphery extending about half way around the circumference, the rest of the sphere is less deeply staining. By a gradual disappearance of this less dense substance, the sphere is converted into a fiber, or rather, the fiber which was already in the sphere becomes free. Whether the fibers always originate in this way, it is impossible to say. In figure 114 is represented a metaphase of the first division in side view. As the asters form, the mitochondria become pushed away from their vicinity although a few of the fibers take up a position along the astral rays. In the division stages, the mitochondria are quite evenly distributed through the cytoplasm between the mitotic figure and the cell wall, though there is usually a clear area at the periphery of the cell. The spheres and fibers are more intermingled than during the growth stages. When the cell divides, the mitochondria are divided en masse, so that each daughter cell receives approximately the same amount (fig. 115). There is no evidence that individual fibers or spheres divide, except possibly in the region of constriction. In the interkinesis the mitochondria

are distributed through the cytoplasm, so that in the second division they are arranged as in the first. They are divided again en masse when the cell divides.

2. Early growth stages: nuclear plate. In all three species of Notonecta, there is present during the greater part of the growth period, a characteristic deeply staining mass applied to the nuclear wall. This takes the chromatin stains of haematoxylin and saffranin, but is purple when stained according to Benda's method, and is evidently of mitochondrial nature. In the earliest growth stages, the body is more or less spherical, and may be closely applied to the nuclear wall, or may lie free in the cytoplasm (figs. 43, 44, 63–66). The mitochondrial body flattens down so as to form a plate on the outside of the nuclear membrane; it is in this form during the spireme stage (figs. 45–53, 67, 74). In N. undulata, at the time when the spireme is disappearing, there is a peculiar bulging of the nuclear membrane at the place where the nuclear plate is attached (fig. 68). On the nuclear side, chromatic substance is present in the swelling, and in the cytoplasm there is a mass of mitochondria in this region; this differentiation is clear with the Benda stain (fig. 116). Up to this time, there are practically no mitochondrial bodies present except the nuclear plate. The mitochondrial mass which appears outside the nuclear plate is composed of small spheres and fibers. The bulging very soon disappears, the plate flattens down again with the membrane (fig. 69), and the mitochondria become distributed through the cytoplasm (fig. 117). The nuclear plate gradually disappears; in N. insulata it becomes conical or spherical in the later stages and apparently may separate from the nuclear membrane (fig. 55).

In haematoxylin preparations, the plate when viewed from above appears as a spongy mass (fig. 53 *B*). When viewed from the side one or two small granules in many cases are seen projecting from the surface; these may be centrosomes. This is suggested further by the peculiar modification of the protoplasm in their vicinity, giving the appearance of an idiozome or attraction-sphere which lies as a cap over the nuclear plate. The origin of these granules cannot be conclusively shown, although in the early stages a small granule may be often detected in the modified

protoplasm near the mitochondrial body. It seems altogether probable that the centrosome becomes embedded in the mitochondrial mass at an early stage and remains in connection with it during the growth period. It is of interest to note that the orientation of the spireme is not toward the nuclear plate but toward the karyosphere; these two bodies may lie in any position relative to each other, the karyosphere being usually eccentrically placed.

B. Discussion

Mitochondria have been found in many invertebrates and vertebrates in both germ and tissue cells by many observers; Fauré-Frémiet ('10), Prenant ('10) and Montgomery ('11) have recently given comprehensive reviews of the subject so that only a few points will be touched on here. Most commonly, mitochondria appear as fine granules which have a tendency to arrange themselves in rods or chondromites (Meves '00). In some forms, the mitochondria form long fibers called by Meves ('08) 'chondriokonts.' In a few cases the mitochondria have been described as vesicles with a dense shell, e.g., by Meves in Pygaera ('00) and other forms, by Meves and Duesberg ('08) in the hornet, and by Gérard ('09) in Stenobothrus. In the latter case the mitochondria occur both in the form of vesicles and fibers and bear a striking resemblance during the growth period to those of Notonecta. In Notonecta it is perfectly clear that the fibers are formed not by chains of granules but directly from the vesicles by a disappearance of the surrounding substance; the dense shell described by the above named observers is probably the mitochondrial fiber in the sphere. Loyez ('09) has found in the egg of tunicates that the mitochondrial fiber develops into a yolk sphere; this is practically the reverse of what occurs in Notonecta.

In division the mitochondria usually appear to be divided en masse, as they are in Notonecta; but in some of the Protozoa, according to Fauré-Frémiet ('10), the individual mitochondria divide at the time of the division of the micronucleus. In some of the Metazoa they form a mantle of long fibers at the side of the spindle and are divided individually and equally. According

to the observations of Benda ('02) and others, no mitochondria occur in the spindle itself. In Notonecta this is true to a certain extent, but a few mitochondrial fibers lie along the inside spindle fibers.

In regard to the source of the mitochondria, the evidence from Notonecta leads to the conclusion that the first mitochondrial body is of cytoplasmic origin, that this becomes applied to the nuclear wall, and that by an interaction of this material and some of the chromatic material of the nucleus, the numerous mitochondrial bodies of the later growth period are formed. The mitochondria are not of nuclear origin in the sense of Hertwig for the chromatin in the nucleus and the mitochondria outside are of very different appearance. But it seems quite probable that their chief elaboration takes place under the influence of the chromatin since this accumulates in the region where they are formed and at the time of their formation. It seems to me probable that the mitochondria are merely early formed cytoplasmic structures which function in the mature sperm.

The observations on the nuclear plate in the American species confirm in the main the observations of Pantel and Sinéty on N. glauca. The 'archoplasmic vesicles' which they find scattered through the cytoplasm are no doubt the mitochondrial spheres described above; these form part of the acrosome of the sperm. The granular masses, 'matérial nebenkernien simple' which forms the principal foundation of the nebenkern, are probably identical with the masses of mitochondrial filaments which I have described; the fibrous nature of the masses is brought out by the Benda stain.

VII. SUMMARY

1. The most suggestive result of the foregoing observations is to show that in the case of Notonecta the change in the number of the chromosomes from species to species can be explained by the relations of two particular chromosomes. In N. undulata these two chromosomes are always separate, in N. irrorata always united to form a single body, while in N. insulata they may be separate in the first spermatocyte division, but are united in the second.

2. In all three species all the chromosomes are aggregated during the growth period to form a massive karyosphere, which consists of chromatic bodies embedded in plasmasome material. The precise origin of this body is somewhat difficult to ascertain, but the evidence indicates that it contains at least part of the early spireme.

3. In the prophases the chromosomes are formed from the karyosphere, which gives rise to dense chromatic bodies, which form diffuse double threads; these condense to form ring- and cross-tetrads, etc., whose entire history can in some cases be traced.

4. Mitochondria are present in the form of a flat plate in the early stages, and of spheres and fibers later; the fibers may arise directly from the spheres. The mitochondria are divided en masse with cell division.

September, 1912

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[1] In order to abbreviate the literature list, many papers have been referred to in general, and are not included in the list.

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PLATE 1[6]

EXPLANATION OF FIGURES

Notonecta undulata

× 2250

1–2 Metaphase of first division, polar view, showing 14 chromosomes, two small ones in center.

3 *A*, *B* Serial sections of spindle in side view, early anaphase, first division.

4 *A*, *B*, *C* Same, two central pairs arranged linearly.

5 *A*, *B*, *C* Serial sections of spindle in side view, initial anaphase, second division, showing 13 chromosomes, *XY* in center.

6–7 Metaphase of second division, polar view.

8–9 *X* and *Y* on separate fibers.

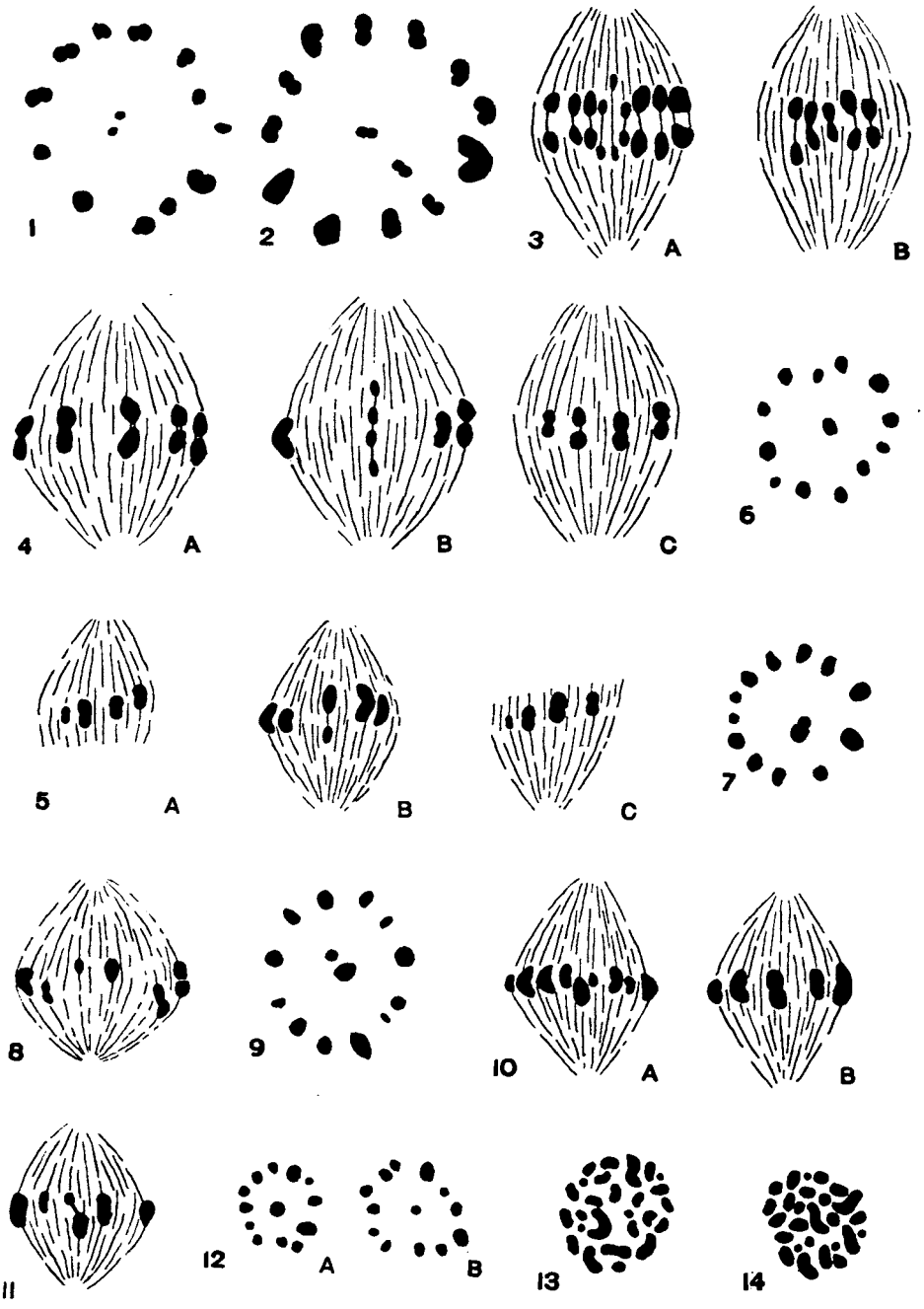
10 *A*, B Same in complete spindle.

11 *X* and *Y* connected by oblique fiber.

12 *A*, *B* Sister anaphase groups of second division, from same spindle.

13–14 Spermatogonial groups showing 26 chromosomes.

[6] All the figures (except fig. 113) were drawn with the camera lucida. In some cases, for the sake of clearness, overlying chromosomes have been displaced.



Browne, del.

PLATE 2

EXPLANATION OF FIGURES

Notonecta irrorata

× 2250

15–16 Metaphase of first division, polar view, showing 13 chromosomes, one small one in center.

17 *A*, *B*, *C* Serial sections of spindle in side view, early anaphase, first division.

18 Components of central pair on different fibers.

19 *A*, *B*, *C* Serial sections of spindle in side view, initial anaphase, second division, showing 12 chromosomes, *XY* in center.

20 Metaphase of second division, polar view.

21 *A*, *B*, *C* Complete spindle, *X* and *Y* on different fibers.

22 Same, polar view.

23 *A*, *B* Sister anaphase groups of second division, from same spindle.

24 Spermatogonial group, showing 24 chromosomes.

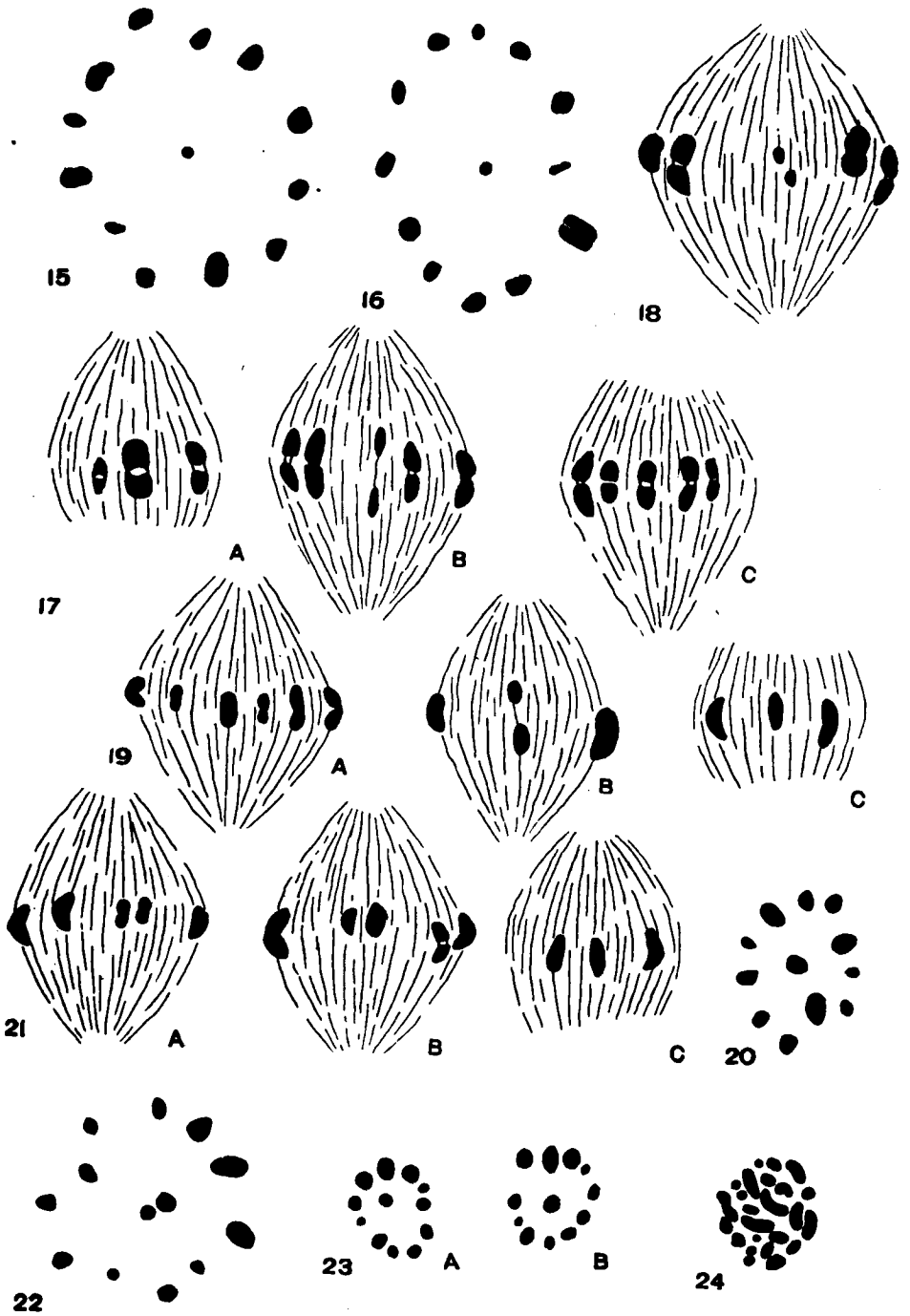


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PLATE 3

EXPLANATION OF FIGURES

Notonecta insulata

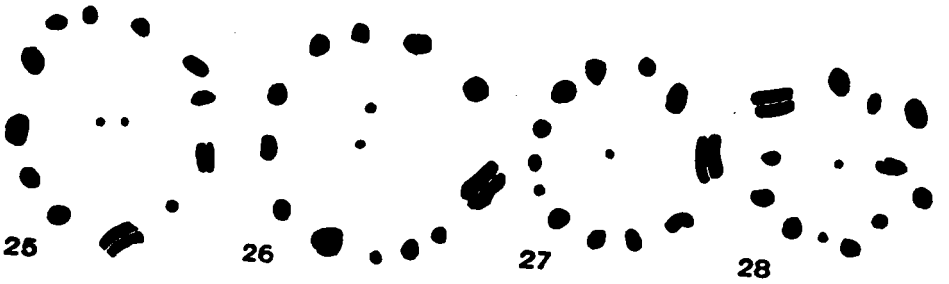
× 2250

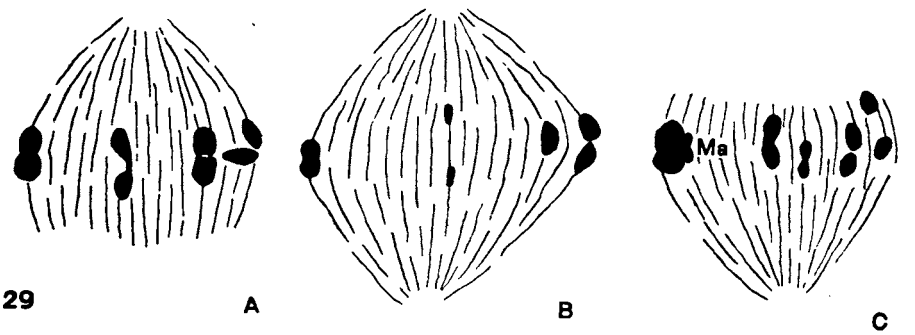
25–26 Metaphase of first division, polar view, showing 14 chromosomes, two small ones in center.

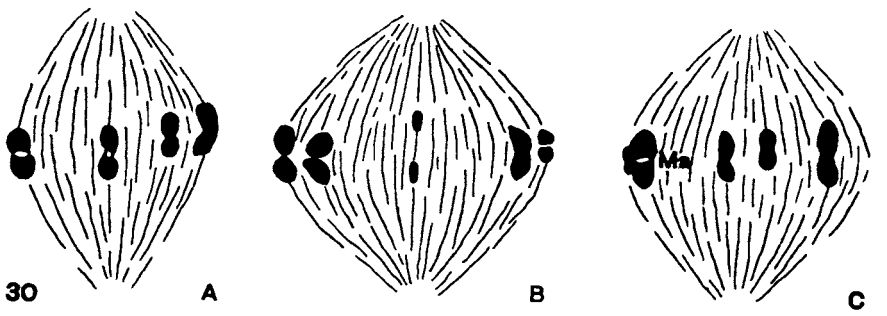
27–28 Same with 13 chromosomes, one small one in center.

29–31 *A*, *B*, *C* Serial sections of entire spindles in side view, showing one small pair in center, and compound chromosome Ma, consisting of largest chromosome and second small one.

32 Polar view, showing same.







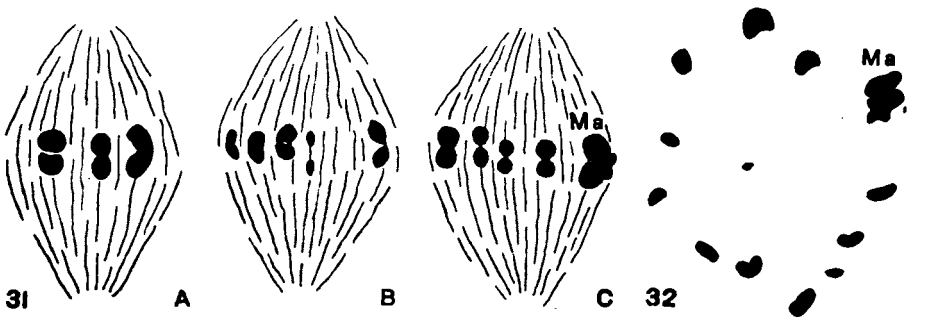


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PLATE 4

EXPLANATION OF FIGURES

Notonecta insulata

× 2250

33 *A*, *B*, *C* Serial sections of entire spindle in side view, showing two small pairs in center, components of Ma separate.

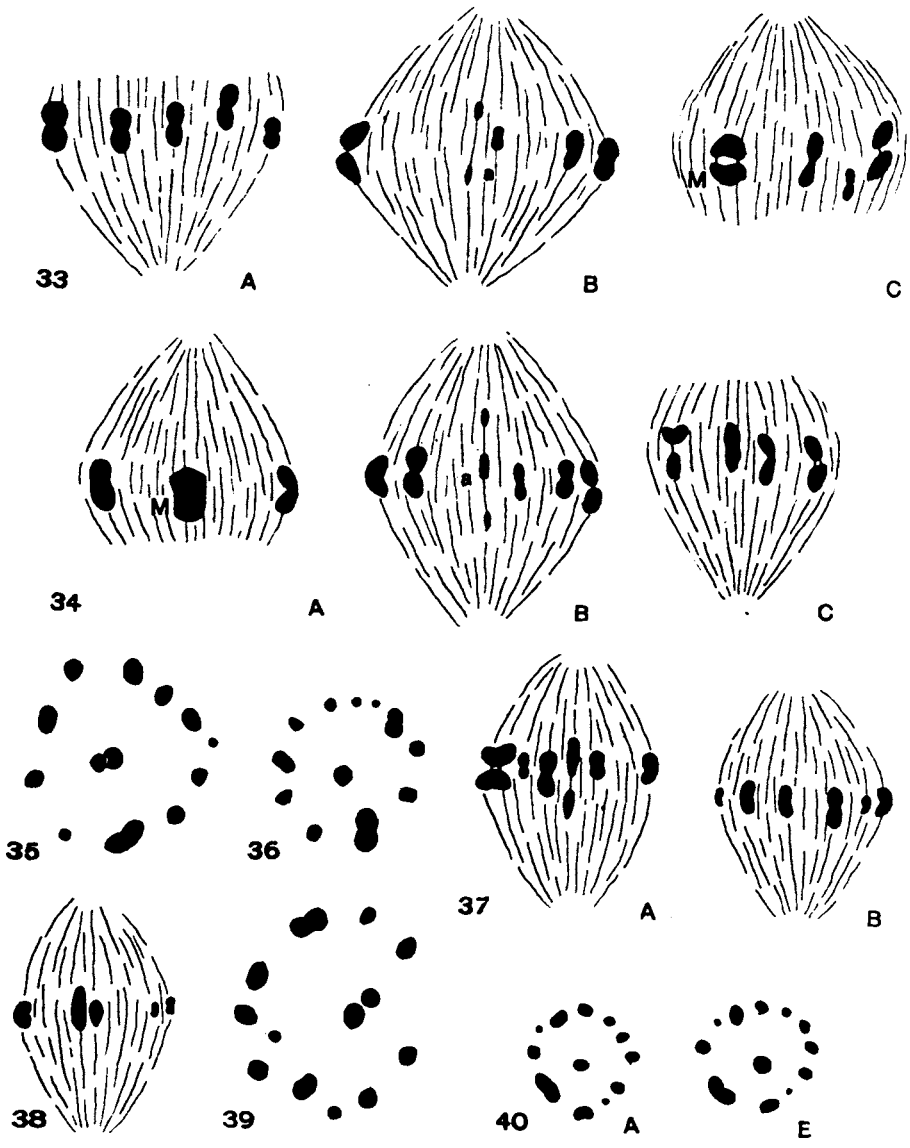
34 *A*, *B*, *C* Same, two small pairs arranged linearly.

35–36 Metaphase of second division, polar view, showing 12 chromosomes, *XY* in center.

37 *A*, *B* Complete spindle, initial anaphase, side view.

38–39 *X* and *Y* on separate fibers.

40 *A*, *B* Sister anaphase groups of second division, from same spindle.



Browne, del.

PLATE 5

EXPLANATION OF FIGURES

Growth stages. N. insulata

41 Very young spermatocyte. × 2250.

42–43 Formation of scattered masses of chromatin; mitochondrial body lies outside nuclear membrane in fig. 43. × 2250.

44–45 Formation of spireme; mitochondrial plate in fig. 45. × 2250.

46 Oriented spireme. × 2250.

47 Withdrawal of spireme loops from karyosphere. × 2250.

48–50 Disappearance of spireme loops. × 2250.

51 Same as fig. 41, drawn to scale of figs. 52–53, to show increase in size of nucleus during growth. × 1350.

52–56 Structure of karyosphere during late growth period. × 1350.

53 *B* Nuclear plate viewed from above. × 2250.

56 From living material. × 1350.

57 Irregular karyosphere before formation of aster; mitochondrial body has disappeared. × 1350.

58 Karyosphere rounded, after formation of aster. × 1350.

59 Chromatin leaving plasmasome. × 1350.

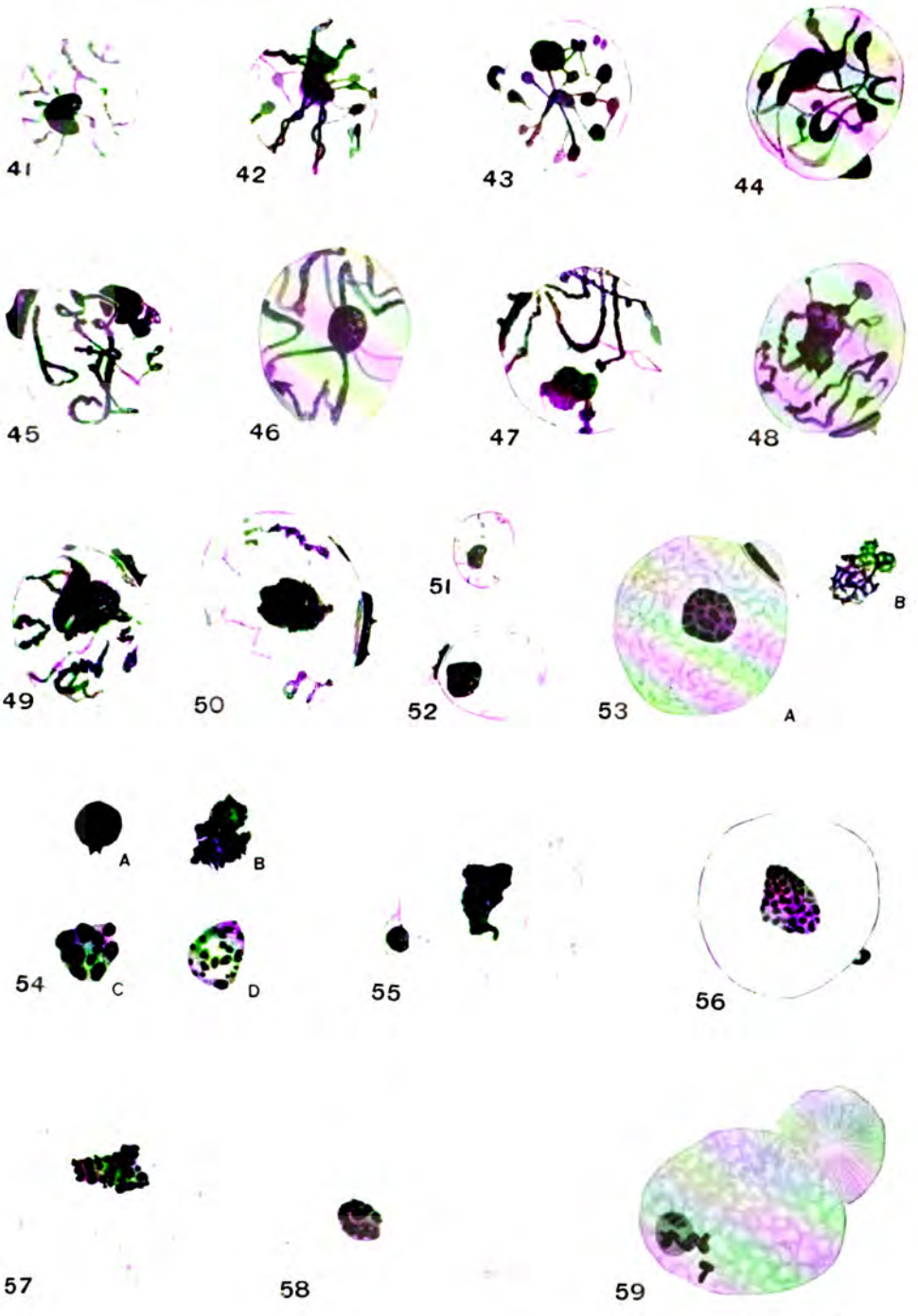


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PLATE 6

EXPLANATION OF FIGURES

Dissolution of karyosphere. N. insulata.

60 Last chromatin mass (*M*-chromosome) leaving plasmasome, diffuse chromosomes at periphery. × 1350.

61 *A–J* Chromatin leaving plasmasome. × 1350.

Growth stages. N. undulata

62 Very young spermatocyte. × 2250.

63 Leptotene spireme; mitochondrial body outside nucleus. × 2250.

64 Synizesis stage. × 2250.

65–66 Oriented spireme. × 2250.

67 Withdrawal of spireme loops from karyosphere; mitochondrial plate. × 2250.

68 Karyosphere fully formed; protrusion from nuclear wall in region of mitochondrial plate. × 1350.

69–70 Karyosphere breaking up into balls. × 1350.

71–73 Thread formation. × 1350.

Growth stages. N. irrorata

74 Looped spireme. × 2250.

75 Karyosphere broken up into balls. × 1350.

76 *A*, *B*. 77 Thread formation; deeply staining plasmasome in fig. 77. × 1350.

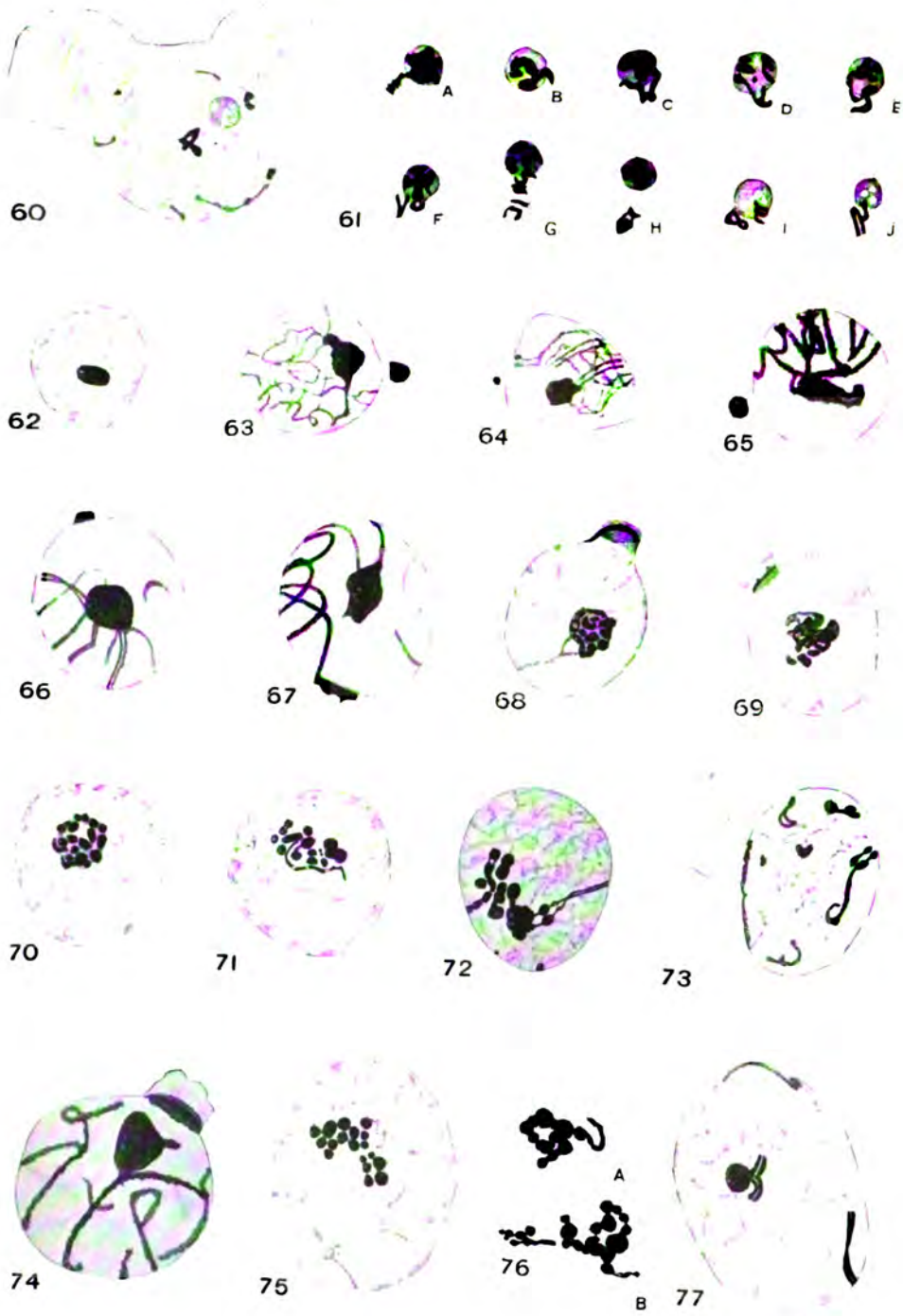

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PLATE 7

EXPLANATION OF FIGURES

× 2250

Prophases. N. insulata

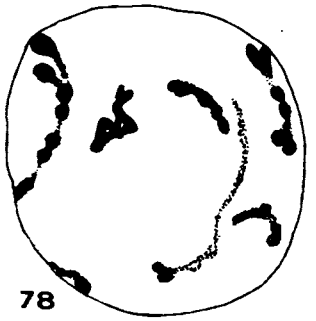
78 Early prophase, chromosomes diffuse on nuclear wall, *M* condensed.
79 Later prophase, rings and crosses.
80 Late prophase, chromosomes fully condensed.
81 Just before metaphase.

N. irrorata

82 Early prophase, diffuse stage.
83 Later prophase, rings and crosses.
84 Very late prophase, spindle forming.

N. undulata

85 Early prophase, diffuse stage.
86–87 Condensation, irregular figures.
88 Very late prophase.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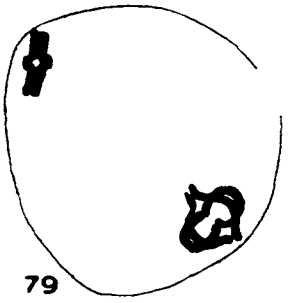
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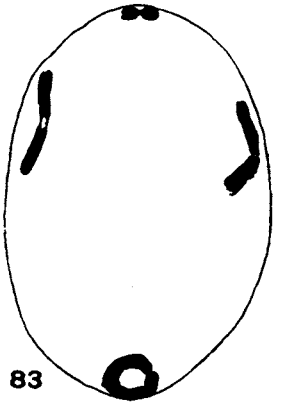
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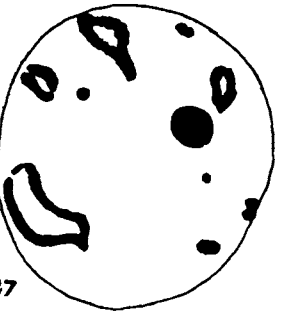
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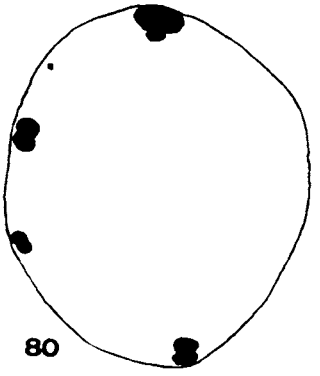
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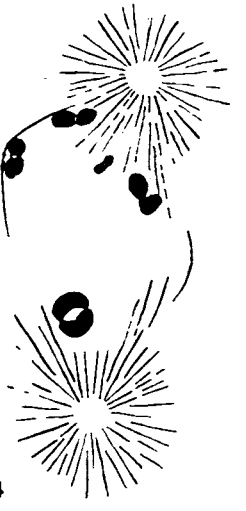
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PLATE 8

EXPLANATION OF FIGURES

× 2250

Ring tetrad. N. insulata

89 *A–D* *M*-chromosome as it leaves plasmasome.
90 *A–D* Opening out of two bars from middle.
91 *A–D* Small ring formed; in *B*, second split has come in.
92 *A*, *B* Open double ring.
93 *A–F* Ring condensing.
94 *A–D* Condensed ring of late prophase.
95 Ring tetrad in metaphase; *A*, side view; *B*, end view; *C*, polar view.
96 Ring tetrad in initial anaphase; *A*, slightly oblique view; *B*, end view, showing compound nature of chromosome.
97 Late anaphase, each part of *M* split.
98 *M* as it is drawn on second spindle.
99 Initial anaphase of second division, showing *M* dividing along split.
100 Late anaphase.
101 *A–F* Second largest chromosome forming ring tetrad; *D*, late prophase; *E*, metaphase, side view; *F*, polar view.
102 Telophase of first division, showing two largest chromosomes longitudinally split.

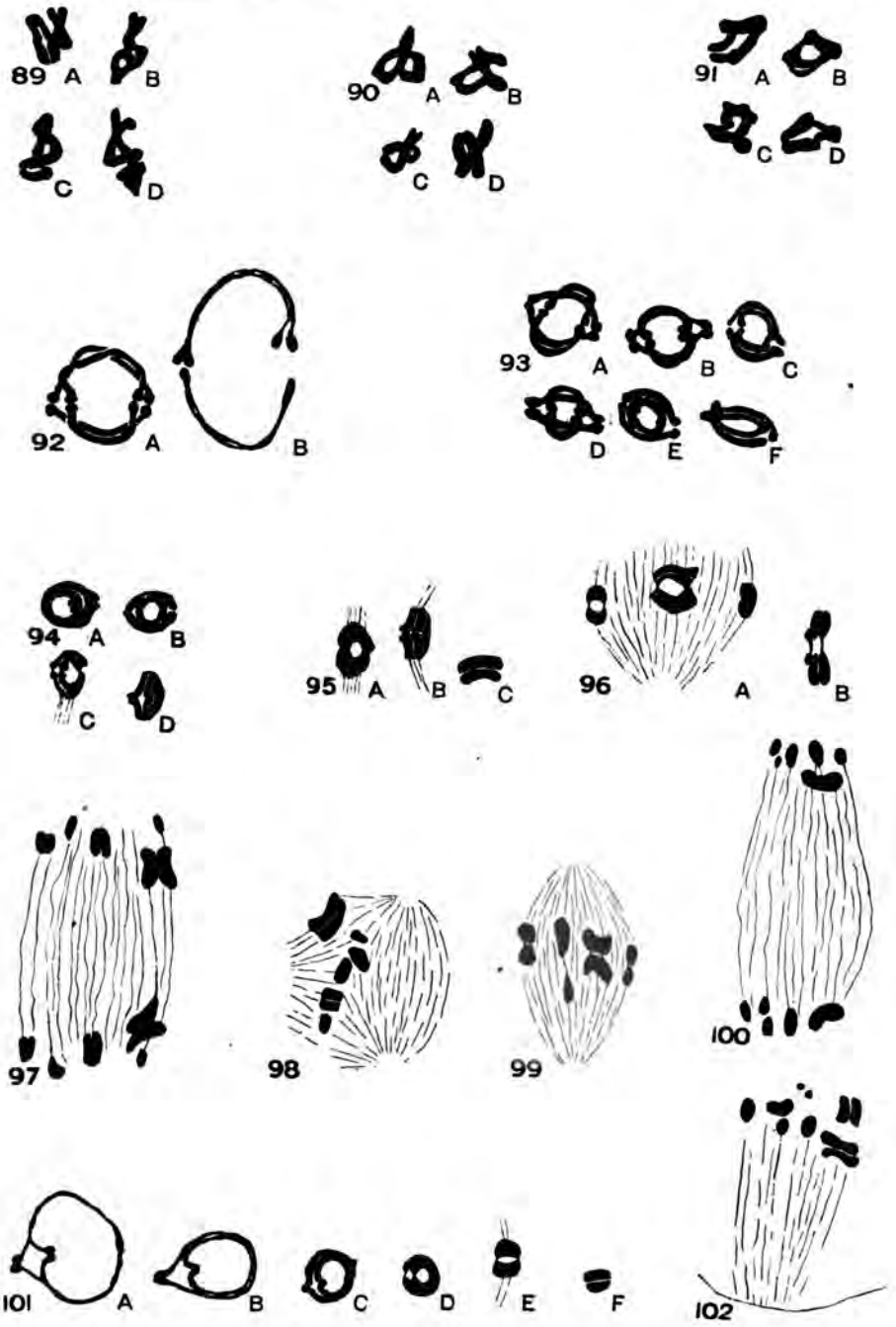

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PLATE 9

EXPLANATION OF FIGURES

× 2250

Tetrad formation. N. insulata (continued)

103 *A*, *B* Small ring.
104 *A–I* Formation of cross tetrad; *H*, in metaphase; *I*, in anaphase.
105 *A–D* Condensation of double rod.
106 *A–D* Condensation of *XY*; *D*, late prophase, elements separating.

Tetrad formation. N. irrorata

107 *A–D* Condensation of ring; *C*, in metaphase, side view; *D*, polar view.
108 *A–H* Formation of cross tetrad; *D*, in metaphase.

Prophase figures. N. undulata

109 *A–F* Diffuse stage.
110 *A–F* Condensed stage.

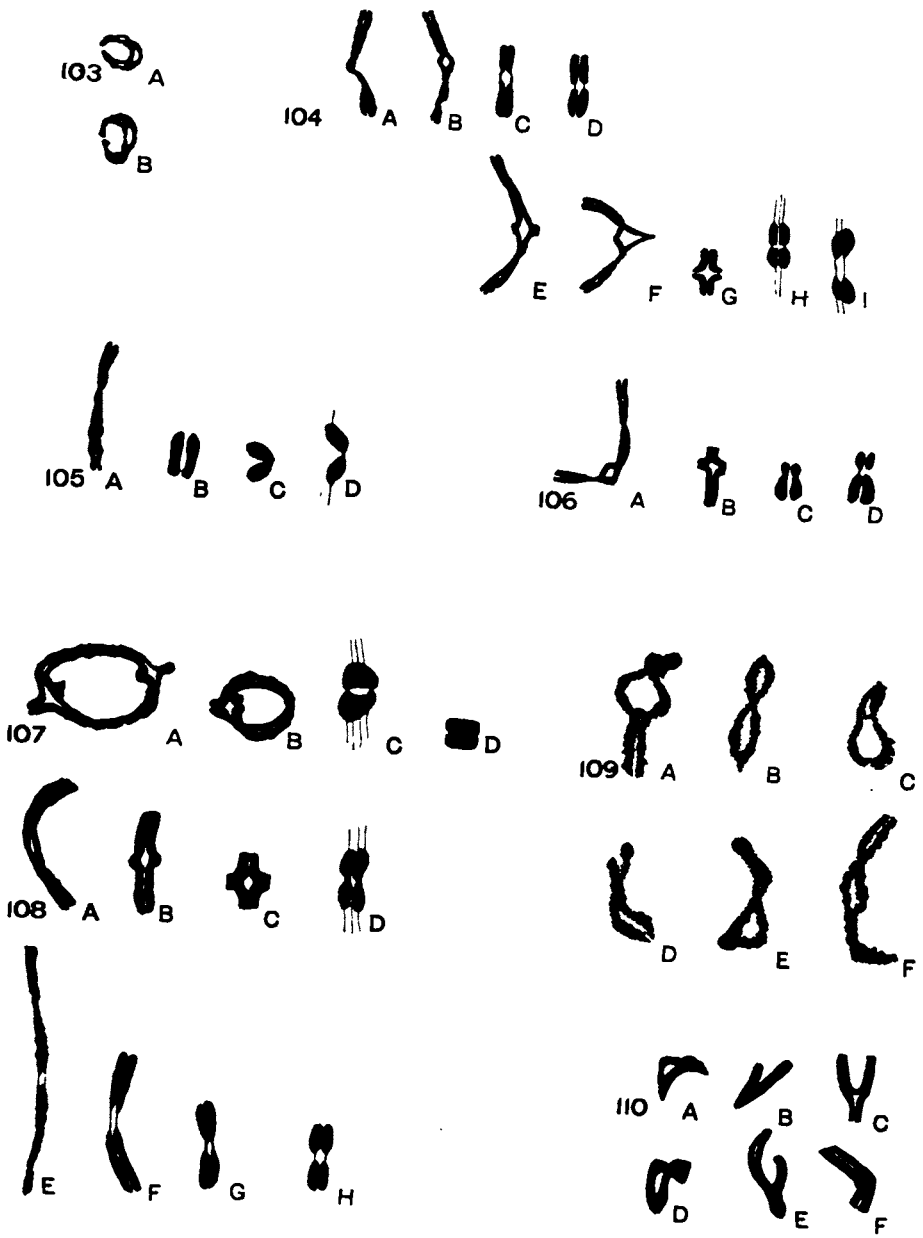

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PLATE 10

EXPLANATION OF FIGURES

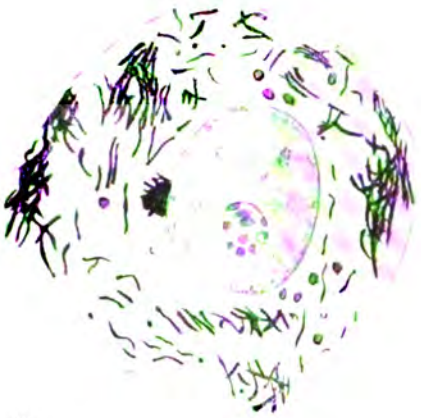
× 1350

Mitochondria. Chromatin represented by dark grey, mitochondria by black and grey. N. insulata

111 Late growth, mitochondrial mass attached to nuclear wall.
112 Later stage.
113 Transition stages between spheres and fibers. Free hand drawing.
114 Metaphase, first division.
115 Division of mitochondria en masse with cell division.

N. undulata

116 Early growth, formation of mitochondria near nuclear bulge.
117 Mitochondria becoming evenly distributed.



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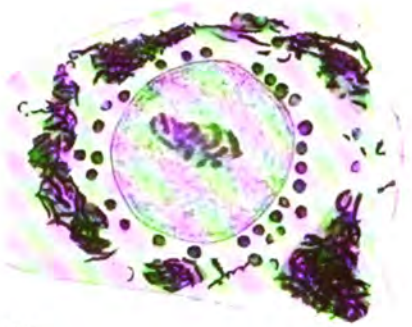

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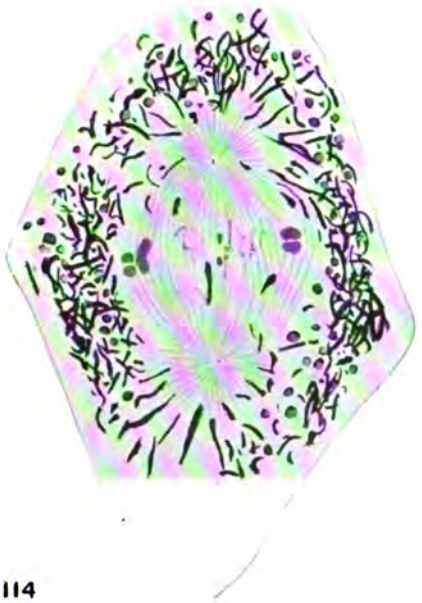

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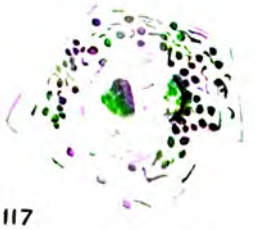

117

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VITA

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PUBLICATIONS

1909 The production of new hydranths in Hydra by the insertion of small grafts. Journal of Experimental Zoölogy, vol. 7.

1910 a Effects of pressure on Cumingea eggs. Archiv für Entwicklungsmechanik der Organismen, Bd. 29.

1910 b A method to test the hypothesis of selective fertilization (collaborator with T. H. Morgan and F. Payne). Biological Bulletin, vol. 18.

1910 c The effects of extirpation and transplantation of the reproductive organs of insects (a review). American Naturalist, vol. 44.

1910 d The relation between chromosome-number and species in Notonecta. Biological Bulletin, vol. 20.