# Studies of Reproductive Elements.

## I. Spermatogenesis, Ovogenesis, and Fertilization in *Diaptomus* sp.

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*With Pl. I.*

## A. DESCRIPTIVE PART.

The species of *Diaptomus* on which the following observations were made is very abundantly found in an old pond in the University grounds. Its breeding occurs repeatedly at an interval of about two months during the colder half of the year.

### *1. Testicular Sac.*

By examining the testis under a microscope, we are struck with the variety of cellular elements found in it. Near to its two ends we find cells in active division, while its median part is filled with cells whose nuclei are in the resting stage. We can therefore divide the entire sac into different zones just as has been done by *Ed. van Beneden* and *Julin*,[1] and lately by *O. Hertwig*,[13] with the testis of *Ascaris megalocephala*. Following the example of these authors we shall speak of the blind end as the *formative zone*, the middle part as the *growing zone*, and the part near the vas-deferens as the *zone of ripening*.

In the formative zone, the nuclei are tolerably large, and with very *little* protoplasm. The chromatic elements are very much elongated and appear as so many minute rods. Many of the cells are in the state of division.

In the growing zone ("Wachsthumszone") the nuclei recede to the state of rest, and the germ-cells grow to nearly double their size after division in the first zone. This stage is, however, not so well marked in this animal as in *Ascaris*; we can nevertheless distinguish it pretty easily by the absence in it of karyokinetic figures.

And in the last zone we find again the active multiplication of the cells. "Jede Zelle zerfällt jetzt durch zwei Theilungen, die sich unmittelbar aneinander anschliessen, in 4 Elemente, welche sich direct zu den befruchtenden Samenkörpern umbilden."(13)

Fig. 1. represents a longitudinal section of a testicular sac not very highly magnified, the lower end of the figure showing the blind posterior end and the upper end the place where the vas deferens takes its origin. The three zones here enumerated can be clearly seen even by this figure. The entire lower third of the sac represents the formative zone in which are seen a few cells in division. The next zone is well characterized by the peculiar appearance of the chromatic elements and occupies the middle portion of the sac, while the last zone or the zone of ripening is seen at the anterior end, well characterized by the presence of many karyokinetic figures in it.

*a. The Formative Zone.*—This zone occupies nearly one-third of the entire testicular sac near its posterior end. The cells in this part are found partly in the state of division and partly in the state of the so-called "skein" stage (Knäuelstadium), showing thus very clearly that these are in active multiplication, as stated above (fig. 1 & fig. 2). The chromatic elements of the nuclei in the "skein" stage are found to be of very different thicknesses, owing to the different stages in which we observe them; and their number could not be exactly made out, except in the stages just before and during the division. At this stage each chromatic element assumes at first a rod-like shape, and then becomes constricted in its median portion, transversely to its long axis, thus resembling in shape a dumb-bell (fig. 2, left end). These dumb-bell shaped bodies are eight in number, and arrange themselves in form of a ring on whose periphery they lie. This is the stage of the spindle. It will be noted that the spindle appears different to the eye according to the methods of preservation we use. Thus preparations treated with hot-alcoholic sublimate (30 % alcohol to which a few drops of concentrated sublimate solution have been added) show no achromatic fibres connected with the chromatic elements, while those cells which are killed with aceto-picric-acid exhibit them very distinctly. The central bodies can also be clearly made out with Grenacher's haematoxylin. In the process of division each dumb-bell shaped chromosome elongates and becomes divided in its middle part, so that one half of the dumb-bell goes to one pole and the other half to the other. The two daughter cells arising by division of a mother cell contain each eight single chromosomes, each of which is half one of the original chromosomes. The only difference from the ordinary karyokinesis consists in the mode of division of the chromosomes, which generally divide longitudinally and not transversely.

Fig. 2 represents more highly magnified the extreme posterior end of the testis shown in fig. 1. We now see a number of primitive sperm-cells with very large nuclei, the chromatic elements of which appear as so many minute rods. At the left hand corner is seen one of the cells in division, in which five dumb-bell-shaped chromosomes are represented in a single row at the equator of the spindle; the other three, not here visible because lying at a lower level, could be made out by focussing the microscope. At the right hand I have drawn from the testis of another individual, a cell found in this region which represents a further stage of division, in which the chromatic elements are already separated from each other and lie near the poles of the spindle. I will call these primitive cells by the name of *primary spermatic cells* ("Ursamenzellen" of German authors). They generally divide two or three times, and thus give rise to the cells of the growing zone which will be spoken of as *sperm-mother-cells* ("Samenmutterzellen" of German authors).

*b. The Growing Zone.*—The cells at the beginning of this zone are relatively small and the eight chromosomes begin gradually to elongate and at the same time the nuclear membrane becomes distinct. Hand in hand with this change the cell-body enlarges, while the nucleus appears to get smaller and more compact, colouring very deeply with reagents, and at last becomes quite homogeneous, owing to the thick consolidation of the chromosomes. These stages are seen in figs. 3, 4, and 5. In fig. 3 the chromatic elements are just beginning to elongate, have continued to change in cells represented by fig. 4, and lastly in fig. 5 have become quite homogeneous.

The next change in the cells is the dissolution again of the elements (figs. 6 and 7, the former representing the polar view and the latter that from the side). Instead of a homogeneous mass we now see a number of elongated chromosomes irregularly lying in the centre of a cell, but generally more or less gathered to one side reminding one remarkably of the figures given by *Hertwig* in the same stages of development in the spermatic cells of *Ascaris megalocephala*. The only difference between them and our figures consists in the absence of a nuclear membrane in the latter. The chromosomes become shorter and thicker and assume at last a short rod-like shape just as we find them in primary spermatic cells preparing to divide. Fig. 8 represents these changes. At the left hand of the figure is seen a cell whose chromatic elements are somewhat shorter than those represented in fig. 6, and one at the right shows distinctly eight short chromatic rods.

The sperm mother-cells increase all the while in size up to the stage which we have called the zone of ripening ("Reifezone"), and which we will now proceed to describe.

*c. The Zone of Ripening.*—The eight chromatic rods now begin to be constricted at their middle point giving rise to eight dumb-bell-shaped bodies as before. These arrange themselves in the shape of a ring and each of them becomes divided into halves on the appearance of achromatic fibres as before (figs. 9, 10, and 11). In fig. 9 which is drawn from a specimen killed with hot picro-acetic solution, the eight chromatic elements are beginning to divide transversely, while in the cells represented by figs. 10 and 11 (treated with acetic acid solution of methylgreen) the division has proceeded a little farther. The large size of the cells represented by these figures is due perhaps to the pressure exerted upon them by the cover-glass. The cells resulting from this division contain therefore each eight single chromosomes, which now prepare to *divide directly without an intervening resting stage.* The eight single chromosomes in each of the cells after the division, arrange themselves in one plane, and mostly at the periphery of a ring as usual, but sometimes one or two chromosomes can be found in the interior of the ring. These cells now begin to be divided into two on the appearance of the attractive sphere, central bodies, etc., as usual in the karyokinetic cell division, but with this important difference concerning the chromosomes, that *each chromosome does not become divided into two as usual, but remains undivided during the division, so that four of the eight go bodily into one cell and the other four into the other.*

Figs. 12, 13, 14, 15, and 16 represent these changes. In fig. 12, which is drawn from a specimen treated with acetic acid solution of methylgreen, and shows the eight single chromosomes in the equatorial zone of a spindle, five of these are seen on one side while the other three lie on the other side. The cell body appears to be larger than those represented by figs 13, 14, and 15, which are taken from a specimen treated with picro-acetic acid solution. This difference in size is due partly to the pressure of the cover glass, but chiefly to the action of the acetic acid. Fig. 13 shows the polar view of a spindle at the stage of fig. 12. Of the eight chromosomes, seven lie at the periphery and one in the middle of a circle. In fig. 14 a similar spindle seen from the side is represented, while fig. 15 shows the cell-divsion nearly completed, four chromatic elements being drawn to one pole and four to the other pole. The cell-body is also nearly at the end-stage of its division. A similar stage is represented more clearly by fig. 16.

The nucleus of a cell after this division again acquires a membrane and the chromosomes become also indistinguishable, colouring very deeply and uniformly. At a place a little farther outward the nucleus again becomes uneven to the staining, and shows more or less distinctly indications of chromosomes coming to occupy positions at the periphery of the nucleus whose central part now shows a clear space. In this condition the sperm cells again begin to grow gradually, and the chromosomes come again to be seen more or less distinctly as very fine threads and lying rather irregularly in the nucleus. In this growth of the cell, the nucleus progresses more than the cell body, so that the latter becomes proportionally much reduced, and when the cell passes into the vas deferens no trace of the cell-body can be detected. The nucleus now assumes the characteristic elongated shape we find in the sperm cells contained in the spermatophore sac attached near the female opening. Illustrations of these changes are seen in figs. 16, 17, 18, 19, 20, 21, and 22, which are drawn from a testis and vas deferens of one and the same individual under the same magnifying power.

## 2. *The Ovarian Sac.*

The ovary of *Diaptomus* I examined, consisted of a median unpaired part—the ovary proper—and the oviducts arising on both sides of it. For the sake of convenience I have divided the contents of the ovary into three different zones as in the case of the testicular sac, although the distinctions are here not so complete as in the latter: *a*) *the formative zone* which occupies the extreme blind end of the sac; *b*) *the growing zone* filling the rest of the whole ovarian sac and part of the oviducts; and *c*) *the zone of ripening* occupying the oviducts alone. In fact the process of ripening does not end within the body of the animal: the second polar body, as was first observed by *Grobben*,(8) is formed after the eggs have been laid in the breeding-sac and attached to the base of the tail of a female. The ripening zone moreover overlaps the hinder part of the second zone.

*a. The Formative Zone.*—At the extreme blind end of the ovarian sac, which ends in a very narrow tube containing but a single row of germ-cells, we meet with cells quite similar to those we find in the posterior third of a testis. A comparison of figs. 23 and 24 with fig. 2 of the testis will at once show this similarity. In these cells of the ovarian sac, too, a relatively large nucleus contains chromatic nets which are certainly in the "skein" stage, but the karyokinetic figures are very scantily to be seen. Next follow the cells which to all appearance seem to correspond with the testicular cells as represented in fig. 3 and which are apparently in the beginning of a *dispirem stage*. The chromatic fibres become finer and finer till they appear as minute dots in the nucleus; at the same time a nucleolus appears within the nucleus and the egg gets into the next or the *growing zone.*

*b. The Growing Zone.*—Strictly speaking this zone begins from the point where the germ-cells get into the dispirem stage. The nuclear threads which now appear as minute dots become gradually fainter till at last they disappear completely from the view. The germinal vesicle together with the egg-cell grows larger and larger, and the germinal spot which always remains single also enlarges. As has been already stated by *Paul Mayer* of *Eupagurus*,[15] and after him by myself of *Atyephira*,[14] the growth of the germinal vesicle is relatively less than that of the cell-body.

Thus the ovary or germ-gland ("Keimdruse" of *Claus*) can also be divided into two parts in accordance with the description given by *Haecker* of *Cyclops*.[8] Just as in that case, the blind end is characterized, as above said, by the presence of a number of nuclei in division. This is the "erste Phase der Ovogenese" of *Haecker* and my formative zone. It is followed by the second part, the growing zone, which extends from here to the distal end of the oviducts. Here are passed two important stages of ovogenesis, the first of which is characterized by the appearance of a nucleolus within the nucleus and the disappearance of the chromosomes, and the second by the formation of the yolk-granules, and along with this the rapid enlargement of the germinal cells. It will perhaps be better to recognise in this zone two parts according to the stages of development of germ cells, as is done by *Haecker*, who takes as the first part that portion of a germ gland where the nuclear membrane appears and the contour of the nuclear thread becomes indistinct, and "zugleich findet sich ein Nucleolus vor, der Hand in Hand mit der Verwischung der Schleifenindividualität an Grösse zunimmt. Neben diesem grossen Nucleolus zeigen sich schon in sehr jungen Keimzellen regelmässig ein oder zwei runde Körper, die ich vorläufig als Micronucleolen bezeichnen möchte." This, as will be seen from my figures, corresponds exactly with what I have seen in the egg-cells of *Diaptomus*, the sole difference lying in the absence of the small bodies besides the nucleolus which he calls micronucleoli. These appear, in my case, first in eggs that are nearly ripe, and shortly before the formation of the first polar body, as will be seen later on.

In fig. 24, which represents a part of an ovarian sac, are seen on the right the cells of the formative zone, which towards the left gradually pass over into those of the growing zone. The chromatic elements, as will be seen, disappear as we pass from the blind end outward, while a distinct nucleolus comes into view.

The formation of yolk-granules and consequently the rapid development of germ-cells takes place first when the eggs get into the oviduct, just as in the case of *Cyclops*. As in that case also, all the egg-cells in the oviduct are at a given time in one and the same stage of development. *Haecker* says on this point: "die sämmtlichen Eizellen des nämlichen Individuums" are found "stets auf fast vollkommenen gleicher Entwicklungsstufe."

Of the origin of yolk granules I have not much to say. They originate, as I stated of the eggs of *Atyephira*, in the body of the egg-cell near the periphery rather than near the centre.

The next change in the development of the egg-cells is the appearance of a number of small bodies in the nucleus, besides the nucleolus, which correspond with the "Micronucleolen" of *Haecker;* there appear at the same time a number of dumb-bell shaped chromosomes exactly corresponding with those found in the spermatic cells. The nucleolus at this time is found to be much reduced in size and it shows very often a number of vacuoles of variable sizes. Like *Haecker* I am also at a loss to know whether the micronuleoli arise from the nucleolus or independently in the nuclear plasma. At all events the nucleolus here is in stages of regressive metamorphosis, and in many cells in these stages no trace of it can be seen within the nucleus. As with the similar developmental stages of spermatic cells, I will call the stage after this the *zone of ripening*.

Fig. 25 shows two neighbouring eggs of an animal in which the eight dumb-bell shaped chromatic elements have appeared. The germinal vesicle has now attained its largest size. In one of them, on the left hand side of the figure, we see besides the eight chromatic elements a germinal spot, (now greatly reduced in size), and nine small round vesicular bodies which certainly correspond to the micronucleoli of *Haecker*. In the germinal vesicle of the other egg, the germinal spot is no more to be seen. This figure is compounded from three successive sections, in such a manner that from the first and second sections the egg on the left side is drawn, while from the second and third sections that on the right side is drawn.

*c. The Zone of Ripening.*—As already stated, there is no distinction between the eggs in the last zone and those in the zone of ripening, and the eggs go through, in one and the same zone, the changes mentioned under the heading of the growing zone and the changes that are now to be mentioned; so that these two zones are not distinct in the ovarian sac, as they are in the testicular sac. The only reason for counting them separate consists in the advantage obtained when we come to compare these two kinds of germ cells together.

The egg cells, as mentioned above, now show distinct chromatic elements each in the shape of a dumb-bell. These are eight in number and are scattered about without any order in the nucleus. Shortly after the appearance of the chromatic elements the micronucleoli as well as the nucleolus disappear, and the nuclear membrane becomes also indistinct and finally disappears.

The eight chromosomes now arrange themselves on the periphery of a circle just as in the case of spermatic cells. The attractive sphere and the central bodies are not, as in the testicular cells, to be observed in preparations treated with sublimate-alcohol, but are very distinct in the cells killed with picro-acetic acid. Figs. 26-29 give the eggs in these stages. Fig. 26 represents only the small part of an egg, in which the spindle lies. The nuclear membrane has already disappeared, and no trace of the germinal spot and the micronucleoli is to be seen. Five of the dumb-bell-shaped chromosomes are seen in a curved row from this side, and the three others can be seen, by focussing the microscope, below these. In fig. 27 we have a polar view of such a spindle, while fig. 28 gives another spindle as seen from the side. These three figures are drawn from preparations treated with hot sublimate alcohol, and no trace of achromatic fibres is to be seen. Fig. 29 gives the spindle in the same stage as that of figs. 26, 27, and 28, but treated with picro-acetic acid. Here we see not only the achromatic fibres, but also a trace of the vesicular membrane quite distinctly.

This spindle is that of the first polar body, and travels gradually towards the surface of the egg, where each chromosome begins to divide transversely just as in the first division of testicular cells in the zone of ripening. This division gives rise to the first polar body, which is thrown out of the egg, while this is still in the oviduct. There are thus *eight chromosomes in the first polar body and the same number in the other half of the germinal vesicle which remains in the egg.* The latter begins to divide directly, but now in such a manner *that four of the eight chromosomes are separated off from the other four.* This is the formation of the second polar body, which thus exactly corresponds with the last division of the spermatic cells, as has been shown by *Platner*[16] in *Aulacostoma* and by *Hertwig*[12] in *Ascaris* eggs. These stages are represented by figs. 30-35. In fig. 30 the spindle of the second polar body is seen from the pole. The eight single chromosomes are clearly to be seen lying in the equatorial plane of the spindle. The first polar body is no more to be seen. Figs. 31, 32, and 33 show the spindle in more advanced stages. In the first two figures the chromatic elements are still very short, but in the last (fig. 33) they are rather elongated. Figs. 34 and 35 show the stage where the second polar body has entirely separated from the egg nucleus, which latter has now acquired a vesicular form. The four chromatic elements are more or less distinctly to be seen in the second polar body.

At the stage between the expulsion of the first and the second polar bodies the egg passes out of the oviduct into the breeding sac, so that the first polar body lies outside the egg-membrane, which is formed after its expulsion, and after the penetration of a spermatic cell into the egg, which takes place at the extreme distal end of the oviduct. That in *Copepoda* the first polar body lies outside the egg-membrane and therefore is not to be found in eggs that have come out of the oviduct was first shown by *Grobben*(8) in *Cetochilus* and afterwards by *M. Nussbaum*(16) in many *Cirripedæ*, in which it had been foreseen by *Weismann*.

### 3. *A Comparison of the Ripening Phenomena in Egg- and Sperm-cells.*

As is shown in preceding pages the correspondence in the development of the egg-cell and of the spermatic-cell is nearly as complete as in *Ascaris*, upon which *O. Hertwig*(13) has made a series of very fine observations. I say "nearly as complete," because here the corresponding zones are not so easy of comparison together as in the case of *Ascaris*. We can, however, follow them in each of the developmental phases, as we shall presently see.

*a. The Formative Zone.*—As above stated this zone is exactly alike in both sacs. The sizes of the germinal cells, their nuclei, and their chromatic fibres are so alike in the two sexes, that it would be difficult to say whether they belong to an ovary or to a testis. The only difference between them is that this zone is much shorter in ovary than in testis, and that more karyokinetic cell-divisions are present in the latter than in the former. *Haecker*(9) was also not able to see any "Mitose" in this region of the ovary of *Cyclops*, but he does not hesitate to affirm that such occurs here. A comparison of figs. 1 and 2 of the testicular sac with figs. 23 and 24 of the ovary will show the resemblance beyond all doubt.

*b. The Growing Zone.*—There appears in this zone a great difference between the egg- and spermatic-cells, just as in the case of *Ascaris*, of which *Hertwig*(13) says :—

"In diesem zweiten Abschnitt der Geschlectsröhren treten in der Anordnung der Eier und Samenzellen erhebliche Verschiedenheiten hervor. Erstere nehmen an Grösse ausserordentlich zu und ordnen sich dabei in einfacher Lage um eine central gelegene Rhachis an. Die Samenzellen dagegen, die sich zwar auch, aber in Vergleich zu den Eiern viel weniger vergrössern, sitzen zahlreichen Rhachislamellen mit Protoplasmafäden auf, ........"

This difference between the egg- and spermatic-cells in *Diaptomus* is still greater than in *Ascaris*, where the spermatic cells become also loaded with yolk-granules like the egg-cells, and grow to two to four times their original mass, whereas in *Diaptomus* the growth is very limited. But this difference in size is a point of no great importance in comparison with the much greater similarity existing between these two elements, in the complete disappearance of chromosomes and the resting condition of nuclei in both sexes. Here again occurs a slight difference between the two in regard to the absence or presence of nucleoli, as stated above, and this I think we shall be able to explain by the difference in growth between the two kinds of cells.

*c. The Zone of Ripening.*—This is the most important zone for us, because we find here in both egg- and spermatic-cells a kind of cell-division that we find nowhere else. This is the occurrence of *two successive nuclear divisions without an intervening resting stage and the reduction into half of the number of chromosomes in the second of these divisions.*

As observed first by *Platner*,(18) so far as my knowledge goes, and recently to a much greater extent by *Hertwig*(13) in *Ascaris megalocephala*, there occurs no case of such a division except in the genital cells, so that these processes can rightly be looked upon as corresponding stages of the egg- and spermatic-cells (*Platner*, *Hertwig*).

The nuclei that have been resting for some time in the last stage begin to be active in this stage. The chromosomes gradually come into view and begin to arrange themselves in a circle under the influence (?) of the attractive centres which also now appear. The number of chromosomes I counted to be eight. In the spermatic cells we can follow the steps from their dissolution and their gradual shortening till the formation of dumb-bell shaped figures. But in the case of eggs the first distinct appearance of the chromosomes shows them already to be in that form. The division of these produces two cells of equal size in the case of the male elements, while in that of the female one of the resulting cells is much larger than the other, and thus are produced the egg and the first polar body. In all cases, however, the resulting cells contain each eight single chromosomes. The nuclei of these cells do not, as said before, go through the resting stage usual with a karyokinetic cell-division, but *begin to divide immediately and produce in case of spermatic cells four cells each with four chromatic* *elements. In the case of eggs this second division occurs generally only in one of these cells—the egg-cell—, while in the other—the first polar body—this division is suspended, so that there are now formed three cells of unequal sizes—a large, single egg-cell and two small polar bodies, the first of which contains eight single chromosomes, while the second polar body and the egg-cell each contains only four.*

Thus there is a complete parallelism between the egg and spermatic cells. In both cases the primary germ cells contain eight single chromosomes, each of which divides transversely and produces many daughter cells—sperm mother-cells of males and ovum mother-cells of females. These contain each eight chromosomes like their mother-cells. They grow to a certain extent and then two successive cell-divisions take place in them, in both sexes, without an intervening resting stage, giving rise to four spermatozoa in one case and an egg-cell with two polar bodies in the other. There can therefore be no doubt that the egg-cell with two (or sometimes by division of the first polar body into two cells, three) polar bodies, corresponds exactly with four spermatozoa derived from a single sperm mother-cell, which again corresponds with the egg-cell before the formation of the polar bodies.

### 4. *Fertilization.*

During the formation of the second polar body the sperm-cell penetrates into the body of the egg-cell. The penetrating point seems to be quite irregular, but mostly near the place where the second polar body is placed. I have given three drawings of freshly laid eggs in the body of which a sperm cell is already present. In fig. 31 we see at the surface of an egg the second polar spindle, and near it to the right lies a spermatic nucleus, which has just penetrated into the egg ;

a small notch at the surface of the latter shows clearly the point where it entered. Both the polar spindle and the spermatic nucleus lie in a common protoplasmic mass. Figs. 32 and 33 are drawn from eggs in the same breeding sac as that from which fig. 31 is drawn. In both eggs the polar spindle is nearly in the same stage as in the egg represented by fig. 31, the only difference being that in fig. 33 it lies more in the centre of the egg, and that the chromosomes are more elongated than in the other. The position of the spermatic nucleus is, however, in both cases different. In fig. 32 it lies in the same pole of the egg as that in which the polar spindle is found, while in fig. 33 it has penetrated an opposite pole of the egg, although the path of its penetration is not to be seen in the figure.

The two nuclei resulting from the division of the second polar spindle, the nucleus of the second polar body, and the egg-nucleus, show great differences in appearance. That of the second polar body remains small and its four chromosomes coalesce into a single mass, stain very deeply by picrocarmine, haematoxylin, etc., while the nucleus of the egg soon becomes greatly enlarged, and its chromosomes show distinctly as four thread-like, V-shaped bodies (figs. 34 and 35). At first their number is quite distinct, but soon the chromosomes elongate, become twisted and send out many processes which anastomose with one another, and their number becomes indistinct (figs. 36-40). In figs. 34 and 35 the four chromosomes are still quite distinctly to be seen. In figs. 36, 37, and 40 they have begun already to send out processes, and in figs. 38 and 39 they appear as if broken up into a great number of elements. (The nucleus on the left of the figure is in both cases the egg-nucleus).

The changes which the spermatic nucleus goes through after it has entered into the body of an egg and before its copulation with the egg-nucleus, are almost the same as those of the latter, except during the first stages. At first the spermatic nucleus is relatively small and generally oblong, and stains very deeply and uniformly with colouring matters, but soon it shows within it a lighter space in which chromatic fibres become more or less distinct (figs. 31-33). At about the same time that the egg-nucleus begins to enlarge, the spermatic nucleus also swells up gradually, and assumes a spherical form, always remaining a little larger than that of the egg. The chromatic elements are very distinctly to be seen, but they are so much convoluted that it is very difficult to determine their number (figs. 36-40).

The nuclei of the egg and of the spermatozoa gradually approach each other till they come to lie close together (figs. 36–49), but the nuclear membranes continue distinctly visible up to the stage of the segmentation of the egg. The chromatic elements of the nuclei gradually pass, during these stages, from their so called resting stage to a state of activity again, and the individual fibres become more and more distinct. Their number is, however, no longer four but eight. Whether this is brought about by the transverse division of the elements or by a longitudinal division, I am not able to tell. Still I think I am quite justified in supposing that this doubling of the number of chromatic elements before the formation of a spindle is the same phenomenon as that observed by *Flemming*(7) and others in the division of many animal and vegetable cells, (*Flemming's* "heterotypische Form").

The entire process of the copulation of the two nuclei up to the beginning of the segmentation will be seen by looking at figs. 36-55. Fig. 36 represents only a small part of an egg in which both nuclei are to be seen lying at some distance from one another. The smaller upper one is the egg-nucleus and the larger one below is that of the spermatozoon; both are more or less in the "skein" stage. Fig. 37

shows the stage in which the nuclei are more closely placed than in the one represented in fig. 36, the right smaller one being the egg-nucleus and the left larger one that of the spermatozoon. Close to the smaller nucleus is seen a small spherical area with a minutė central body in the middle of it. This evidently corresponds with the central body with the attractive sphere around it. In fig. 38 the nucleus of the egg in proportion to that of the spermatozoon is relatively larger than those shown in figs. 36 and 37, and the four chromatic elements are more or less distinctly to be seen. The central body seems now to have become divided into two bodies lying still very close to each other. The section represented by fig. 40 did not pass exactly through the plane of the nuclei, but a little obliquely to it, so that they are seen overlapping. One of the central bodies, the lower one in the figure, lies below the spermatic nucleus and is seen through it. The central bodies are now quite separated from each other.

Figs. 41–45 are made from eggs killed with *Flemming's* solution of aceto-osmo-chromic acid, the chromatic elements of the copulating nuclei are very faintly stained by haematoxylin, but the contour of the nuclei, as well as the attractive spheres are very distinctly to be seen. These are placed in all these eggs, in contradistinction to the eggs shown in figs. 37–40, nearer to those nuclei which, from their relative position to the second polar body, appear to be those of the spermatozoon. In fig. 41, besides the second polar body, lying in the body of the egg, there is the first polar body which happened by chance to be carried with the egg into the breeding sac and came to lie outside the egg-membrane, just near the place where the second polar body lies. The number of chromatic elements within the first polar body is still distinctly to be counted as eight. Figs. 42–44 show three successive stages in the nuclear copulation ; and in fig. 45 we see two nuclei closely in contact with one another, while the spheres of attraction are found at the two poles of the plane of contact of the nuclei, which appear to be more or less elongated.

The sections represented by figs. 46–55 were all taken from eggs killed with hot 30 % alcohol to which a few drops of sublimate had been added. The chromatic elements and the attractive spheres are tolerably well to be seen, but the central body is not so clear as in the eggs killed with the picro-acetic acid solution. Fig. 46 shows the two nuclei close to one another, and the central bodies rather farther away from the nuclei than in the case represented by fig. 45. Numerous achromatic fibres are also well to be seen extending between the central bodies and the nuclei, in which are seen eight single chromosomes. In fig. 47 which is nearly in the same stage as the one represented by fig. 46, the eight chromatic elements are more elongated. The achromatic fibres from the attractive sphere on the right of the figure are attached to both the nuclei, but those from the other sphere of attraction are connected only with one of them, which therefore seems to lie nearer than the other to that sphere of attraction. This is more clearly seen in fig. 48, where we find the nucleus lying above in the figure to be pulled more by the central body on the right hand side, and that situated below more by the one on the left. The nuclear membrane, moreover, seems to have been dissolved at the pole where the achromatic fibres are attached. The eight more or less elongated chromatic bodies are seen in each of the nuclei. In fig. 49 both the nuclei are pulled equally by the central bodies and the chromatic elements are found lying at the centre of the spindle, whose achromatic fibres are now found indistinctly within the nuclei. The nuclear membrane is, however, still present except at the place of their contact. Fig. 50 shows a cross section of the nuclei in copulation as is represented by fig. 49. In fig. 51 the nuclear membrane has entirely disappeared, but the chromatic elements belonging to both the nuclei are still to be seen in two separate groups. In these three sections as well as in those represented by the next three figures the number of the chromatic elements is not at all clear. Fig. 52 shows a cross section of a spindle at the stage shown by fig. 51, more highly magnified. Figs. 53 and 54 are taken from the same lot of eggs as that represented by figs. 51 and 52, but the chromatic elements are fused together at the equator of the spindle, and are now no more to be distinguished as two separate groups.

Lastly in fig. 55 is found the first segmentation-spindle with the chromosomes just separating from the equatorial plane. Their number can be distinctly counted as eight in two rows, one of which at the right hand side happened to be pulled by the achromatic fibres of the left, and is seen with its one end at the right and the other at the left.

## B. SUMMARY.

1. The primary sperm cells correspond exactly with the primary egg-cells. Both contain eight chromatic elements.

2. In both cells the eight chromosomes become constricted transversely giving rise to eight dumb-bell shaped bodies. These arrange themselves in an equatorial zone and begin to divide in such a manner that half of each chromosome goes to one cell and the other half to the other. This kind of cell-division takes place two or three times and the resulting cells form the mother cells of eggs or spermatozoa, as the case may be.

3. These grow considerably ; after which

4. They begin to divide as before ; each chromosome dividing

transversely. This stage corresponds in the egg to the formation of the first polar body, which takes place exactly in the same manner as that of the first division of the sperm-mother cell. In both cases the original eight chromosomes become divided into two, giving rise to the daughter cells also containing eight chromosomes (*Weismann's* "Equationstheilung").

5. This is immediately followed by another division, without an intervening resting stage of the nuclei, and by reduction in the original number of chromosomes (*Weismann's* "Reduktionstheilung"). The eight chromosomes which at first are arranged in a single row become arranged in double rows of four each, and the cell begins to divide in such a manner that four chromosomes go to one cell and the remaining four to the other.

By divisions described under No. 4 and 5 a sperm-mother cell as well as an egg-mother cell increases to four cells, which in case of the former give rise to four spermatozoa, while in that of the latter a single egg cell with two polar bodies, results (or three when the first polar body divides).

6. During the formation of the second polar body the spermatic cell enters into the egg-cell. The nucleus of the sperm cell is, as a general thing, at first rather small and colours deeply and homogeneously, but soon the differentiation sets in and the four chromosomes become distinctly visible. The nucleus of the egg-cell remaining after the formation of the second polar body—the female *pronucleus* of *Ed. van Beneden*—shows at first distinctly four elements. These soon grow longer, show many convolutions, and pass into a "skein" stage.

7. The two nuclei gradually approach one another until they come in close contact, but they do not unite into a single piece before the equatorial plate is formed. The number of chromatic elements in each of the copulating nuclei is now found to be eight, exactly double

the number present in the maternal nucleus after the expulsion of the second polar body, and in the ripe spermatozoon.

This number goes unchanged to the formation of the first segmentation spindle, which gives rise to two first segmentation spheres, each of which contains eight single chromosomes.

## C. THEORETICAL CONSIDERATIONS.

Since the important publications of *Auerbach,** *Ed. van Beneden*, *Bütschli*, the *Hertwigs*, *Fol*, etc., investigations on the phenomena of fertilization have become more and more interesting, and now after the well known theoretical treatment of this and other kindred subjects by Prof. *Weismann*,[20] the formation of genital cells, their phases of ripening, and their copulation require exact and thorough study.

The most complete work in this direction is the beautiful investigation of *O. Hertwig* on the "Vergleich der Ei und Samenbildung bei Nematoden," published about a year ago. In this work is given for the first time a clear insight into the exact parallelism existing between the egg and the sperm-cells, the mode of their development, etc., every point in which corresponds so exactly with the descriptions given in this paper, that it seems almost superfluous for me to have published them. I have deemed it, however, worth while to record the results of my own investigations because the conclusions arrived at by *Hertwig* are regarded by some authors as demanding explanations other than those he has found for them.

There are, so far as my knowledge allows me to judge, three modes by which the "Reduktionstheilung" of sexual cells takes place. *Platner*[18,19.] in his various important publications on the sexual cells mentions an exceptional mode of cell-division in the last division of sperm cells and in the formation of the second polar body. He there says: "Das allgemeine Schema für die Zelltheilung verlangt, dass der Kern nach der Theilung wieder in das Ruhestadium zurückkehrt, das heisst sich aus dem Aster der Knäuel und aus diesem das Kerngerüst wieder rekonstruiert, und so findet man es auch überall. Nur in zwei Fällen findet hiervon eine Ausnahme statt. Der erste betrifft die Bildung des zweiten Richtungskörperchens und ist genugend bekannt. Es wird hier das Ruhestadium übersprungen. Aus der innern Tochterkernplatte der ersten Richtungsspindel bildet sich sofort die zweite Richtungsspindel, und auch die in das erste Richtungskörperchen übergegangene Kernhälfte zeigt häufig das gleiche Verhalten, das heisst sie bildet sich gleichfalls sofort wieder zu einer neuen Spindel in entsprechender Weise um. Die Theilung der zweiten Richtungsspindel wird dadurch zu einer Reduktionstheilung in Bezug auf die Quantität des Kernmaterials. Der zweite Fall dürfte weniger bekannt sein; er betrifft die letzte Theilung der samenbildenden Zellen. Auch hier wird das Ruhestadium übersprungen. Die letzte Theilung schliesst sich direkt an die vorhergehende an, indem sich aus der Tochterkernplatte sofort die neue Spindel bildet. Also auch hier findet eine Reduktionstheilung der Masse nach statt." He has thus, for the first time, clearly shown the truth of *Weismann's* theoretical conclusion as to the necessary occurrence of a reduction of nuclear elements at the beginning of each ontogenesis. In his well known essay on the "Zahl der Richtungskörper," etc. Prof. *Weismann*,[20] after discussing the subject of copulation, where the ancestral plasma ought to be doubled at each process, sees the necessity of its reduction to half of its original mass. The sharp insight of *Weismann* has brought out from *van Beneden's* and *Carnoy's* work the necessity of admitting the required reduction in the formation of the second polar body. He says: "In der Ausstossung der zweiten Richtungskörpers aber wird mit Recht eine Reduktionstheilung erblickt werden, durch welche die Hälfte der verschiedenen Ahnenkeimplasmen in Gestalt von zwei Kernschleifen ausgestossen würde." In another place he says: "Sollte ich mich aber selbst in dieser Deutung irren, so scheint mir doch die theoretische Forderung einer bei jeder Generation sich wiederholenden Reduktion der Ahnenplasmen so sicher begründet, dass die Vorgänge, durch welche dieselbe bewirkt wird, gefunden werden müssen, wenn sie auch in den bis jetzt bekannten Thatsachen noch nicht enthalten sein sollten."

This assumption of *Weismann* of a reduction of chromatic elements in the second polar body in the eggs of *Ascaris* and in general, is now in a wonderful way proved by *O. Hertwig*,[(12)] whose beautiful monograph has given me so much pleasure that I have read it over and over again, the more so, since his results are in exact parallelism with those obtained by myself in studying the sexual cells of *Diaptomus*, as stated above.

Thus the observations of *Platner*,[(17,18)] *Hertwig*[(12)] and myself show clearly that there is a kind of nuclear division in the formation of the second polar body as well as in the last cell division in spermatogenesis which is quite different from the ordinary karyokinesis, and which takes place in perfect accordance with *Weismann's* views.

*Boveri* also sees the necessity of admitting the "Reduktionstheilung" at each ontogenetic stage, but he lets this take place not in the formation of the polar bodies, but before it. On page 62 of his "Zellen Studien, Heft 3," he says:—

"Wann die reduzierte Chromosomenzahl zuerst auftritt und wie die Reduktion zustande kommt, dafür besitzen wir noch sehr wenige Anhaltspunkte. Betrachten wir zunächst die Eibildung, so können wir nur den *einen* Satz als sicher und allgemein gültig aufstellen, dass die Reduktion *spätestens im Keimbläschen* erfolgen muss. Denn bei der Bildung der ersten Richtungsspindel kommen die Chromosomen bereits in der reduzierten Zahl zum Vorschein. Falls also *Weismann* seine theoretische postulierte Reduktion der Zahl der Ahnenplasmen mit dieser thatsächlichen Reduktion der Zahl der Chromosomen identifizieren will—was nicht nothwendig ist—so muss er die Annahme, dass dieselbe durch die Bildung des zweiten Richtungskörpers vermittelt werde, aufgeben."

But as the observations of *Boveri* on this point in *Ascaris* were proved not to hold true by the above cited investigation of *Hertwig*, I will not treat of it again here, but will only refer the reader to his beautiful monograph.

The latest series of observations come from *Haecker*(8) and *Henking*,(9,10) both of whom also admit the introduction of a reduction of chromatic elements at the beginning of each embryogenesis. They admit, however, this to take place in the formation of the first polar body and not in that of the second. *Haecker* who studied the formation of the polar bodies in *Cyclops* gives a couple of very fine woodcuts, which lead one to think that the "Reduktionstheilung" takes place in the formation of the first polar body. As in the case of *Diaptomus*, the chromosomes in the sexual cells of *Cyclops* number eight. These divide longitudinally in stage A, and produce sixteen elements, every two of which lie close together and thus form eight groups of double elements. In stage B, they become separated into two groups each of four double elements, one of which serves for the formation of the first polar body. The remaining four double elements now arrange themselves to form the second polar spindle (stages C and D). *Haecker*, however, quite rightly does not regard his observations as to the stages in which the "Reduktionstheilung" takes place as conclusive. After discussing the possibility of *Boveri's* discoveries in eggs of *Tiana*, *Echinus*, *Carinaria*, *Pterotrachea*, and *Phyllirrhoi* being in accordance with his own view, *i.e.*, that the "Reduktionstheilung" takes place during the formation of the polar bodies, he concludes :—

"Die Reduktion findet also während der Ausstossung der Richtungskörper statt und es fragt sich nunmehr, welches Stadium im Speciellen die Reduktion darstellt.

"Die nächstliegende Deutung der Verhältnisse wird wohl folgende sein: die Längsspaltung der Chromosomen im Stadium A ist gewissermassen eine anachronistische, d. h. die normalerweise in der Aequatorialplatte der *zweiten* Richtungsspindel stattfindende Längsspaltung der Chromosomen, in gewöhnlichen Fällen also die secundäre, wurde in die Aequatorialplatte der *ersten* Spindel zurückverlegt, ein Vorgang, der nach Boveri's Befunden nichts Auffälliges bietet. Sieht man also ab von dieser (secundären) Längsspaltung, so übernimmt die erste Spindel aus dem Keimbläschen die ursprungliche, nicht reducierte *Achtzahl* der Elemente, um von diesen durch einen besonderen Vertheilungsprocess vier in den ersten Richtungskörper, vier in den Eikern abzuscheiden, ohne dass die primäre Verdoppelung der Schleifenzahl, wie sie sonst der ersten Spindel zukommt, auftritt. Nach dieser Deutung fände also die Reduktion bei der Ausstossung des *ersten* Richtungskörpers statt.

"Man kann aber den Thatsachen noch eine andere Deutung geben: die Spaltung der acht Chromosomen in *Doppelfäden* würde der primären Längsspaltung der Elemente entsprechen. Auch diese Deutung schliesst einen abweichenden Theilungsvorgang schon in der *ersten* Spindel in sich: anstatt dass jeder Pol von jedem Doppelfaden je *ein* Tochterelement an sich zieht, findet nach jeder Seite die Abscheidung *von vier Paaren* von Tochterelementen statt. Die vier im Ei zurückgebliebenen Paare liefern dann die acht einfachen Elemente, welche in der *zweiten* Spindel *zu je vieren* nach den Polen derselben attrahiert werden. Diese Deutung würde die eigentliche Reduktion *also erst in die zweiten Spindel verlegen.*"

Thus while *Hæcker* allows the probability of the occurrence of a "Reduktionstheilung" in the formation of either the first or the second polar body, *Henking* is quite decidedly of the opinion that this division takes place in the formation of the first polar body. In his latest work on *Pyrrhocoris apterus*, *L.* he gives a detailed description of the formation of spermatic elements and polar bodies, in which he says[10,11]:—

"1) Den Ursamenzellen entsprechen die Ureier. Beide Zellformen enthalten die für die Körperzellen charakteristische Zahl von 24 Chromosomen.

"2) Den unreifen Eiern entsprechen die Samenmutterzellen (Spermatocyten I. Ordn.). Beide wachsen erheblich heran, in beiden kommt es zur Ausbildung eines verhältnissmässig grossen bläschenförmigen Kernes, in beiden werden Dotterkügelchen erzeugt.

"3) Die Abschnürung des ersten Richtungskörperchens entspricht der ersten Theilung der Spermatocyten. In beiden Fällen kommt es zu einer (Weismann'sche) Reduktionstheilung, indem sich die Chromosomen „zwei reichig" aufstellen und zu je 12 Elementen in die neuen Zellen übergehen. Die typische Zahl 24 wird also hier durch einfache Trennung der chromatischen Elemente auf 12 reduciert.

"4) Die Ausbildung des zweiten Richtungskörpers entspricht der zweiten Theilung der Spermatocyten. Die 12 chromatischen Elemente werden unter Beibehaltung der Zahl durch Aequationstheilung direkt halbirt, ohne dass sich das Stadium eines ruhenden Kernes dazwischen einstellte. Die sofortige Theilung der Spermatocyten II. Ordn. wurde möglich, weil die vorhergehende erste Theilung nicht als normal anzusehen ist und weil die letztere wahrscheinlich bewirkte, dass sich gleich die auch für eine zweite Theilung nöthige (also doppelte) Zahl chromatischer Fäden an die Chromosomen anheftete."

*Henking* tries also to make his observations coincide with those of *Hertwig* upon *Ascaris* by giving different interpretations to the results obtained by the latter investigator. Whatever may be the interpretation in the case of *Ascaris*, my own case, given above, can not to my mind be made to coincide with his observation on *Pyrrhocoris*. I will not, however, assert the "Reduktionstheilung" in the formation of the second polar body and in the last cell division in the spermatogenesis to be the universal rule until a sufficient number of observations be obtained in other groups of animals and perhaps of plants too. Theoretical considerations concerning the phenomena of fertilization lead us in all probability to the "Reduktionstheilung" as occurring in the second polar body and in the last division of spermatic cells.

I will now consider very shortly the phenomena of fertilization. As stated above, the maternal and paternal nuclei do not unite into a single segmentation nucleus, as *Hertwig*(12) for the first time gives out, but remain separate from each other till after the complete formation of the segmentation spindle. Something of the kind had been already observed by various writers, but this fact was brought out in its full light only by the last work of *Ed. van Beneden* and *Julin* (1) on *Ascaris*. In the eggs of a copepod crustacea *(Cetochilus) Grobben*(8) observed for the first time, that the two nuclei do not become united with one another and form a single segmentation nucleus, but "stossen die beiden Kerne, Eikern und Spermakern, aneinander und sind nur noch durch eine zarte Wand voneinander geschieden. Auf dieses Stadium folgt sogleich die Bildung der ersten Kernspindel." But above all, the recent valuable investigations of *Boveri*(5) on this head in various groups of animals, as well as his enumeration of facts obtained by previous workers, make us accept this as the rule, and the perfect union of both the nuclei as exceptional cases only. To these we may add the observations of *Henking*(11) on the eggs of *Pieris*, as well as that of *Hæcker*(9) on *Cyclops*. My own observation on the conjugation of *Noctiluca*[14] can also be looked upon as a further contribution in this line.

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## Explanation of the Plate.

*Diaptomus sp.?*

*Fig. 1.*—Longitudinal section of a testis: the lower end represents the formative zone and the upper end is the place where the vas deferens takes its origin. $^{1}/$III × Seibert = 120.

*Fig. 2.*—The formative zone of the testis represented by fig. 1. more highly magnified. A karyokinetic figure at the right is drawn here from the corresponding part of another animal. $^{1}/$VII × Seibert = 850.

*Figs. 3–5.*—Represent successive stages after the last division of the cells in the formative zone. $^{1}/$VII × Seibert = 850.

*Figs. 6–8.*—Cells from the formative zone. In figs. 6 & 7 the eight chromosomes are still long and are difficult to count. In fig. 8 the chromosomes are much shortened in the cell on the right hand side. $^{1}/$VII × Seibert = 850.

*Figs. 9, 10, & 11.*—Show the first division of the sperm-mother cell. Fig. 9 is drawn from a specimen killed with picro-acetic acid, and coloured with hæmatoxylin. Three of the eight dumb-bell shaped chromosomes are seen on this side and five others on the other side. Figs. 10 and 11 are specimens killed with methyl-green-acetic acid. In both figures the eight chromosomes lie on the periphery of a circle and in both of them five elements lie on one side and the three others on the other. All drawn by Seibert $^{1}/$VII and magnified 850 times.

*Figs. 12–16.*—Show the second division of sperm-mother cells immediately following the last division without an intermediate resting stage. Figs. 12 & 14 show a stage just after the first division: eight single chromosomes are still in a single plane. Fig. 13 is the polar view of the same. Fig. 16 represents a stage a little more advanced than those of figs. 12 & 13. in which eight chromosomes lie in two circles of four each. Fig. 15 is more advanced and the cell itself has now begun to divide. Figs. 12–15 are drawn from specimens killed with picro-acetic acid, and fig. 16 with acetic acid methyl-green. All drawn by 1/VII Seibert = 850.

*Figs. 17–22.*—Last changes in the formation of spermatozoa. Fig. 17 is just after the second division of the sperm-mother cell. The nucleus is now found to stain homogeneously. In fig. 18 a cell on the left shows a vacuole in the nucleus; in the other two cells, as well as in that of fig. 19, traces of chromosomes are more or less distinctly to be seen. Fig. 20 shows two cells in which a big vacuole appears. In fig. 21 chromatic elements have become more distinct than in figs. 18 (two cells on the right) and 19: the nuclear membrane has become more or less indistinct. Fig. 22 represents four spermatozoa found in the vas deferens. All drawn by 1/VII × Seibert = 850.

*Figs. 23 & 24.*—Represent the posterior ends of two ovaries. In fig. 24 the extreme end is not represented. Both drawn by Seibert 1/V = 33.

*Fig. 25.*—Two eggs just before the formation of the first polar body. In both of them are seen eight dumb-bell shaped chromosomes scattered rather irregularly in the germinal vesicle. In the germinal vesicle of the egg on the left, a nucleolus and a number of small circular bodies—the micro-nucleoli—are seen, while in that of the egg on the right the nucleolus is no more to be seen. This figure is compiled from three successive sections. 1/V × Seibert = 330.

*Figs. 26–29.*—First polar spindles. Figs. 26, 28, and 29 represent the side view and fig. 27 the polar view. The nuclear membrane has already disappeared and the eight chromosomes lie in a ring. Figs. 26–28 were treated with hot-alcohol-sublimate and the achromatic fibres are not to be seen. In fig. 29 the achromatic fibres are well represented. All drawn with Seibert $^1/$VII=850.

*Fig. 30.*—A small superficial portion of an egg, just after the formation of the first polar body, and the second polar spindle is seen from a pole. Eight short chromatic elements are seen in a plane at the equator of the spindle which is not represented in the figure. Seibert $^1/$VII=850.

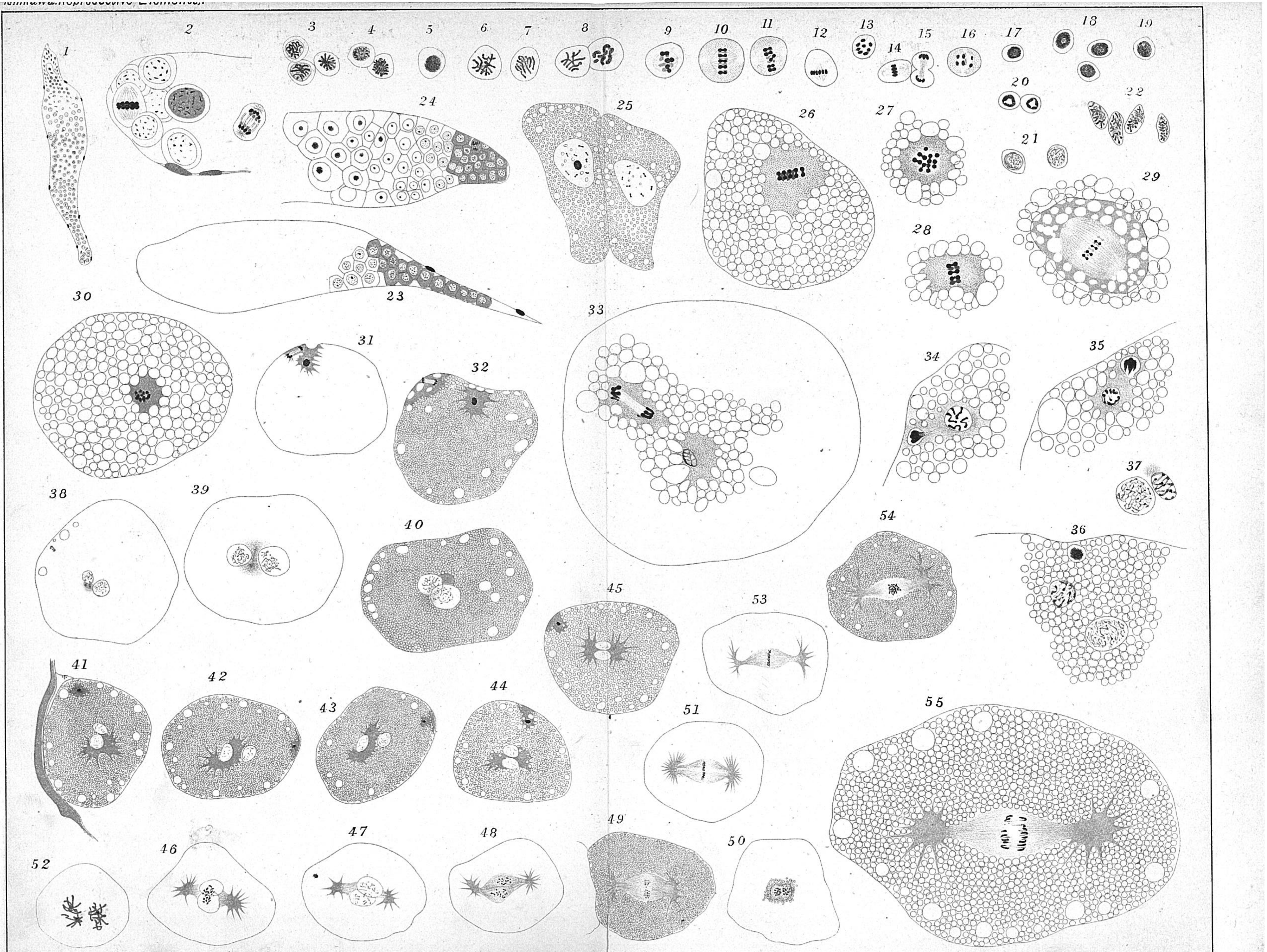
*Figs. 31, 32, & 33.*—Represent three sections in which the second polar spindle and a spermatic nucleus are present in the egg, and in relatively different positions. In all the spindles the four chromosomes are more or less distinctly to be seen. Figs. 31 and 32 are drawn by Seibert $^1/$V=330 times, and fig. 33 by Seibert $^1/$VII=850 times.

*Figs. 34 & 35.*—Division of the second polar spindle is nearly complete, and the egg-nucleus has swollen up a little. The four chromosomes are now more or less elongated. Both drawn with Seibert $^1/$VII = 850.

*Fig. 36.*—Piece of an egg in which the second polar body, the egg nucleus, and the spermatic nucleus are present. The four chromosomes in the egg-nucleus are much elongated and are joined together by "Lininfäden." The spermatic nucleus is now much larger than the egg-nucleus. Seibert $^1/$VII=850.

*Fig. 37.*—The copulating nuclei out of an egg, the larger of which represents the spermatic and the smaller the egg nucleus. At one pole of the egg-nucleus is seen a small central body with an "archoplasma" around it. Seibert $^1/$VII=850.

*Figs. 38–55.*—Represent various stages of copulation and the first segmentation spindle of an egg. In fig. 38 is seen the second polar body at the left hand upper corner. The two nuclei are approaching one another. Near the smaller one—the egg-nucleus—is seen two centrosomes not widely separated from each other. In fig. 39 the nuclei are much larger and the central bodies now lie between them. In fig. 40 the section did not pass exactly in the plane of both nuclei, which therefore are seen as overlapping one another. These three figures are drawn from specimens killed with picro-acetic acid solution. Figs. 41–45 are drawn from specimens killed with Flemming's solution. In fig. 41, the first polar body is by chance seen lying outside the egg-membrane. Figs. 46–55 are drawn from specimens killed with hot-sublimate-alcohol. In figs. 46 and 47 the two nuclei have still a distinct membrane. In both of them we see eight chromosomes. The sizes of the nuclei are still a little different from one another. In fig. 48 the nuclear membranes have become somewhat indistinct, and the nuclei themselves are more or less drawn by the attraction spheres. The relative positions of the latter and the nuclei are very interesting in regard to the manner in which the nuclei are drawn by the attraction spheres. In fig. 49 the two nuclei are drawn equally by the attraction spheres, and the chromatic elements lie at the equator of the spindle. Fig. 50 represents a transverse section of such a spindle. In fig. 51 the nuclear membranes have disappeared completely, but the chromatic elements of both the nuclei are still lying separated from one another. Fig. 52 represents a transverse section of such a spindle more highly magnified. In fig. 53 and 54 the two groups of chromosomes are no longer distinct from one another. Lastly in fig. 55 the chromosomes have just begun to separate from the equatorial plane into two new groups, each consisting of eight elements. All these figures are drawn with Seibert $^1/V = 330$, with the exception of Figs. 52 and 55 which are drawn with Seibert $^1/VII = 850$.

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