From the Biological Laboratory of Bryn Mawr College.

A STUDY OF THE GERM CELLS OF APHIS ROSÆ AND APHIS ŒNOTHERÆ.[1]

BY

N. M. STEVENS.

Associate in Experimental Morphology, Bryn Mawr College, and Research Assistant, Carnegie Institution of Washington.

WITH 4 PLATES.

This work was undertaken in connection with a series of experiments on sex-determination in Aphids. On approaching the subject, it was found that very little is known concerning the ovogenesis and spermatogenesis of these insects. Blochmann ('87) showed that the parthenogenetic egg gives off one polar body, and the winter egg two; but I find no account of the number of chromosomes or the method of reduction in either egg, nor is there any published work on the spermatogenesis of Aphids. The present paper will be devoted mainly to the germ-cells, a few points in the early stages of parthenogenetic development being considered incidentally.

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I. MATERIAL AND METHODS.

Sections of Aphids from various host plants—the rose, English ivy, honeysuckle, alder, arrow-head, Œnothera, and hop, were examined; but Aphis rosæ proved the most satisfactory for study of the ovogenesis of both parthenogenetic and winter eggs, and Aphis œnotheræ for the spermatogenesis.

Both the Aphids and the winter eggs were killed and fixed with Gilson's aceto-chloroform-sublimate mixture, which gives far better results than any other method tried. Very small Aphids were embedded whole; larger ones after removing the head and thorax. Sections were cut from 5 μ to 7 μ thick, and stained with Delafield's hæmatoxylin and orange G, or with Heidenhain's iron-hæmatoxylin. The former method of staining gives remarkably good differentiation for ordinary purposes. The latter is more satisfactory for the study of ovogenesis and spermatogenesis. Many slides stained at first with Delafield and orange, were re-stained with iron-hæmatoxylin for more careful study.

II. PARTHENOGENETIC DEVELOPMENT.

1. *Female Line.*

Most of the stages in ovogenesis of parthenogenetic eggs and also early segmentation stages were obtained from sections of the unborn young of the viviparous female. For more advanced segmentation stages and early embryos very young Aphids were sectioned.

All the species examined have twelve ovaries in two groups as described by Balbiani ('69–'72). Oögonial mitoses were observed, but offered nothing of especial interest as the division figures are identical with other embryonic mitoses.

The resting oöcyte, before the growth stage of the ovum begins, has a very large nucleus in proportion to the size of the cell (Figs. 1 and 2). A large nucleolus occupies the center of the nucleus, and in addition to this the nucleus contains a linin reticulum and irregular masses of material, presumably chromatin, which does not take the hæmatoxylin stain at this stage.

One at a time the oöcytes at the posterior end of the ovary enlarge and push out into the oviduct, remaining, however, con-

nected by a stalk with the central core of the ovary (Fig. 1). As many as three eggs may be thus connected by stalks with the center of an ovary,—one egg just passing into the oviduct, another in maturation stage, and a third in 8 to 16-cell stage. As the oöcyte increases in size, the cytoplasm changes its staining reaction, coloring somewhat deeply with Delafield; and the nucleus shows various changes. The nucleolus disappears and the chromosomes become stainable at an early stage (Figs. 1, 3, 4, 5). As the egg rapidly enlarges the nucleus approaches the periphery at one side of the oval egg (Fig. 6). Just before maturation, clear vesicles appear in the cytoplasm and soon fuse forming large irregular spaces filled with a clear non-staining substance, presumably yolk material, separated from the general cytoplasm (Blochmann).

Fig. 7 shows the equatorial plate of a polar spindle in metaphase. There are 10 chromosomes of 5 different sizes. This is the somatic number, and there is therefore no synapsis or conjugation of chromosomes and no reduction in the maturation of the female parthenogenetic egg. The same number (10) and the same variation in size (5 pairs) is shown in the segmentation spindles and equatorial plate of Fig. 12. The maturation spindle in anaphase is well shown in Fig. 8; also the lacunar spaces in the interior of the egg.

The polar body is at first completely extruded from the egg and separated from it as seen in Figs. 8 and 9. It is distinctly a polar body not a "polar nucleus." Soon, however, it comes to lie within the boundary of the egg among the segmentation nuclei (Figs. 10 and 11). In such a stage as in Fig. 11, the polar body is easily recognizable by its clear cytoplasm and deeply-staining mass of chromatin. It lies in a sort of vacuole in the egg-cytoplasm. I have been able to follow the polar body as far as the stage shown in Fig. 13. The cytoplasm can no longer be distinguished from that of the egg; the chromatin mass is irregular in outline, usually stains less deeply and appears to be degenerating. I therefore feel sure that the polar body takes no part in the development of the embryo. Its inclusion within the egg is probably due merely to mechanical conditions, *i. e.*, to the pressure of the walls of the oviduct upon it, as it lies on the side of the oval egg.

There is only one point of especial interest in the later development, and that is the relation of the young embryo to the vitellaria.

This is shown in Figs. 14 and 15. Fig. 14 shows an embryo which has just begun to take in yolk (the "secundäre Dotter" of Will, '89). At the base of the embryo as figured are two conspicuous cells (*b*) which apparently guard a valvular opening in the wall of the oviduct, and recall the four guard-cells at the inner end of the embryonic pharynx of Planaria simplicissima. At the lower focus of the section the valve is slightly open and a small amount of yolk material has entered (Fig. 14, *a*). In Fig. 15 the valve is widely open and yolk cells are being taken into the embryo. Whether the embryo actively sucks in the yolk, or the yolk cells themselves are the active agents, it is impossible to tell, but the former seems more likely. Will ('89) describes the secondary yolk as forming in connection with the follicle epithelium and then being taken into the gastrula. In speaking of the work of Witlaczil ('84), he says, "Von seiner ganzen Darstellung ist nur das eine richtig, das der secundäre Dotter von Follikelepithel seinen Ursprung nimmt," and later he says, "Diese innerhalb der Epithelverdickung producirte Dottermasse ist es nun welche in das Ei eintritt und demselben den sogenannten secundären Dotter liefert. Dass es sich dabei nicht um eine Einwanderung von Zellen . . ."

The close resemblance in structure between the secondary yolk and certain large cells in the body cavity of the mother was easily observed in the sections, but the relation between the embryo and the vitellaria described above was first seen in connection with the egg of Aleurodes, a related form, where the relation is much more conspicuous than in the Aphid. It was later traced with certainty in the winter egg and in the parthenogenetic embryo of the Aphid.

No variation could be detected in the development of ova which produce winged parthenogenetic individuals. The winged young can often be distinguished before birth, and some of the same brood may be winged, others apterous. The winged parthenogenetic individuals are migrants and their appearance seems to be conditioned by the amount or the quality of the food supply.

2. *Male Line.*

The mothers of the males are apterous and cannot be distinguished externally from the apterous females which produce female offspring. The ovaries show no difference in structure, unless it may be in size and number of the oöcytes, and only one

polar body is given off. In studying the development of male[1] eggs, one is at a disadvantage so far as the amount of available material is concerned, because only eggs connected with the ovaries of females which contain embryos large enough to be recognized as male can be utilized, while in the female line the best eggs for study are found in abundance in the larger embryos. No polar spindles were found in the few male eggs obtained. Figs. 17 and 18 show the polar body as it appears in eggs containing 4 and 8 nuclei. There is no indication of fusion of two polar bodies. In Fig. 18 the chromosomes are not so fully fused as in Fig. 17, but this is the condition at a corresponding stage in the female eggs. Examination of the segmentation spindles in male embryos and of spermatogonial mitoses makes it certain that the full somatic number of chromosomes is present until we come to the spermatocytes when reduction occurs. This is what we should expect had it not been claimed by Castle ('03) that the female character which is usually dominant in parthenogenetic insects is removed from the egg with the second polar body, thus allowing the recessive male element to assert itself. There is no evidence in my material of any difference between the maturation of the female parthenogenetic egg and that of the male egg, and until I can procure more material and examine the point further, I shall assume that the method of maturation of all parthenogenetic Aphid eggs is the same, *i. e.*, only one polar body is produced and there is no reduction of chromosomes. There is no mixture of male and female young in the offspring of one individual: certain apterous females produce only females, either winged or apterous, and others produce only winged males.

The ovarian oöcytes, eggs and polar bodies in the male line (Figs. 16, 17, 18) are noticeably larger than those figured in the female line (Figs. 1–12), but this may be due to the fact that the drawings were made from different species; the former from Aphis œnotheræ, the latter from Aphis rosæ. The difference is one of size not of structure, and much the best and most abundant male material was obtained from Aphis œnotheræ, where parthenogenetic reproduction was wholly replaced by the sexual method early in October, and young males of all ages were abundant.

[1]Male is used here merely in the sense that these eggs produce males.

III. THE WINTER EGGS.

1. *Early Development and Growth.*

The material for study of the winter eggs was obtained by bringing into the laboratory rose twigs with broods of sexual females on the leaves. Adult males were usually found on the same leaves. These young females develop more rapidly in a warm room under glass, and soon begin to lay the fertilized eggs. The winged mothers of the sexual females were sectioned for the study of the ovaries of the sexual female embryos, and young sexual females for early stages in the development of the winter egg. Fig. 19 shows an ovary from such an embryo. It is considerably larger than the ovary of the ordinary parthenogenetic female embryo (Fig. 1), and in every case these ovaries show a large number of degenerating oöcytes at the posterior end (Fig. 19, *a*). These cells are more or less shriveled and stain deeply and irregularly. Whether this is simply degeneration of a large number—at least half—of the oöcytes of an ordinary parthenogenetic ovary in order that the remainder may have room for growth, or whether the parthenogenetic ovary may contain originally both eggs capable of parthenogenetic development and others capable of development only after fertilization, and the former degenerate in order that the latter may develop, I am unable to say. The number of oöcytes in the twelve parthenogenetic ovaries is certainly more than double the number of young ever produced by an individual, so that the latter supposition might be possible. Two cases observed in dissection, but never duplicated in fixed material, might support either view, and certainly tend to show that the ovary which produces parthenogenetic embryos and the ovary which produces winter eggs are originally identical. In two individuals egg-strings and winter ovaries with developing eggs were found associated in both groups of ovaries. The number in each varied—in one individual one group contained 5 egg-strings and one winter ovary, the other group 3 egg-strings and 3 winter ovaries; in the second individual each group contained 5 winter ovaries and one egg-string; on one side there was one parthenogenetic ovary with eggs and embryos (Fig. 20, *c* and *e*), on the other side was an egg-string consisting of two parthenogenetic embryos, a winter ovary and a young winter egg (Fig. 21, *a*, *b* and *e*). These individuals with mixed ovaries were found in

the greenhouse January 23, 1904, on a small rose bush which had lost its leaves and was nearly dead as a result of serving as a food plant for several generations of Aphids. They were the third generation from a winged female. Two or three others escaped dissection. Unfortunately only freehand sketches were made, and no other such cases were observed. One of these sketches is reproduced in Fig. 20 and a part of another in Fig. 21. Similar observations were recorded by Bonnet ('45) and by Leydig ('50), and Kyber ('15) observed sexual forms on the willow in June and on ripening grain in midsummer, but did not connect both parthenogenetic young and winter eggs with the same individual.

The oöcytes in the winter ovaries increase immensely in size before any eggs are given off, and the large nearly spherical ovaries are easily distinguished in dissections from the minute oval parthenogenetic ovaries (Fig. 20, *a* and *c*). The first egg is given off after the birth of the sexual female.

It is not my purpose to describe the growth period of the winter egg in detail. The nucleus, at first central, gradually moves to the periphery at one side, usually nearer the anterior end of the egg as it lies in the oviduct. When the egg has reached about one-half its ultimate size, yolk material from the vitellaria is taken in at the posterior end of the egg. There seems to be no such definite opening as in the case of the parthenogenetic embryo and of the egg of Aleurodes. Fig. 22 is an oblique section through an egg which plainly shows the relation between the yolk within and that without the egg. Entrance seems to be effected between any of the cells of the follicle epithelium, which is merely a part of the oviduct. Here again one wonders which is the active agent, the egg or the yolk cells, but it is impossible to tell. In the case of the embryo one is inclined to believe that it may actively suck in the yolk as the planarian embryo does, but there is nothing to indicate that the egg could have any such power. In the case of Hydatina senta, Lenssen ('98) describes the yolk cells as penetrating the egg by their own activity. The entrance of yolk seems to be associated with a definite size of the ovum and would therefore occur at a definite point in the oviduct. The relation between the oviduct and the yolk gland may therefore be a more definite one than the sections show. Entrance of yolk must be effected very quickly as cases where the process can be demonstrated are very rare; and one

finds no such intermediate stages as would necessarily appear if this secondary yolk were formed within the egg as described by Balbiani ('69–'72). The outlines of the yolk cells are lost and only fragments of nuclei are found (Fig. 22), while in Aleurodes several whole yolk cells with nuclei intact enter the oviduct below the egg and are later included in the posterior end of the egg.

The chromatin in the egg during its growth period offers no favorable conditions for study. In earlier stages it does not take chromatin stains, and in later stages the chromosomes are spherical and mingled with nucleoli of similar form and staining qualities.

2. *Maturation.*

The earliest stage found in the laid egg was that shown in Fig. 23—the equatorial plate of the first polar spindle, showing 5 chromosomes, the reduced number, of the same relative form and size as the chromosomes of the 5 pairs in Figs. 7 and 12. The manner in which the egg chromosomes are paired is not evident, but the two divisions appear to be longitudinal and identical with the maturation divisions of the spermatocyte where it is quite certain that they are paired longitudinally, and probable that the first division separates the paired chromosomes.

Fig. 24 shows the first maturation spindle in metaphase. One chromosome appears in both figures (stippled in *b*). In Fig. 25 the first polar body and the second polar spindle in metaphase are figured. A part of a chromosome appears in *a* and parts of two in the spindle of *b*. In Fig. 26, a later stage is seen; the chromosomes of the first polar body are massed together, those of the second (not yet separated from the egg) show the comparative size relation of Fig. 23, their position indicating a longitudinal division; the chromatin of the egg nucleus is becoming diffuse and less stainable.

The spermatozoön enters at any point, more often near the posterior end of the egg, and leaves a train of cytoplasm behind it, as it traverses the yolk preceded by an aster. Fig. 27 shows the male and female pronuclei, the former distinguished by the cytoplasmic path (indicated by arrows). I have never found the first segmentation spindle in my material nor indeed the divisions immediately following, though the resting nuclei of these stages have frequently been observed. In the later segmentation stages

it is impossible to distinguish the individual chromosomes in the spindle; they are crowded together and often are so united in metaphase as to resemble a spireme in one plane.

IV. SPERMATOGENESIS.

Nearly all of the material for the study of the spermatogenesis was obtained from the Œnothera. On the rose the young males are never met with in large numbers while on the Œnothera an abundance of all sizes may often be found on the still-blossoming tips of the flower spikes, while the sexual females are scattered over the leaves and stalks. Here also were found a few mothers of the males. The few young males from the rose showed the same number and relative size of chromosomes.

The testes, as seen in dissections, have 6 lobes corresponding to the 6 ovaries in each group. The lobes are much larger than the ovaries, and as the spermatogonia are somewhat smaller than the oögonia, the number of mitoses leading to the spermatocyte must be several more than in the case of the oögonia. Only spermatogonial divisions are found in the embryos, and the last such division often, if not always, occurs after birth. Fig. 28 shows a resting spermatogonium; Figs. 29 and 30 spermatogonia just before division. In Fig. 30, 9 chromosomes of characteristic form and size can be recognized, one of the smallest not being visible. The equatorial plate, as also in most somatic mitoses, is too crowded for distinguishing either number or form of chromosomes.

The resting spermatocyte of the first order (Fig. 32) does not differ materially in appearance from the resting spermatogonium (Fig. 28) and indeed can often be recognized only by its relation to later stages. There is no evidence of so long a growth stage as in most forms. Closely associated with the first maturation mitosis and probably immediately preceding it are found the stages shown in Figs. 33 and 34. In Fig. 33, *a*, 8 of the 10 chromosomes are to be seen scattered through the nucleus; in *b*, *c* and *d*, chromosomes of the same form and size are seen paired longitudinally. Fig. 34 suggests the synapsis stage described for many insects. This stage is of much more frequent occurrence than that shown in Fig. 33, and appears to follow that, and immediately precede the first spermatocyte division which is shown in Figs. 35–39. Fig. 35, *a*, *b* and *c* show the 5 chromosomes in the equa-

torial plate of the first maturation spindle. All are connected by linin threads, and every possible arrangement of the 5 chromosomes is found in different cells. In *c* the longest chromosome is seen to be double and as a side view of the spindle in metaphase always shows the chromosomes double, it is probable that they come into the mitotic figure in that condition from the preceding conjugation stage. A second longitudinal split to form a tetrad cannot be detected at this stage. In Fig. 36, a side view and an oblique view of the equatorial plate show the double chromosomes. Two stages of the anaphase appear in Figs. 37 and 38. There is always one "lagging" chromosome in this division, and it certainly is not usually the longest, as I at first thought likely. In fact it appears in most cases to be either the second or third in size. After the two spermatocytes of the second order are fully formed, as shown in Fig. 39, the two daughter elements of this "lagging" chromosome are still connected by a thread extending through the cytoplasm of each cell. This phenomenon seems to be a peculiar characteristic of one of the chromosomes in this particular division. I have never seen an exception, nor have I ever seen anything similar in the second spermatocyte division or in fact in any other mitosis.

If, as I have supposed, this first maturation division simply separates paired chromosomes, it is possible that the pair that shows this peculiarity has a different linin connection from the others. In Fig. 35, it will be seen that the 5 chromosomes are united by linin threads into a chain with free ends. A side view (Fig. 36) shows two pairs connected by two parallel linin threads. Now if one of the end pairs always has its two elements connected by a single thread, we might expect such figures as 37, 38 and 39.

Fig. 40 is the equatorial plate of the second maturation mitosis, showing again the 5 chromosomes of characteristic form and size. Fig. 41 shows one of a few fortunate sections showing the daughter chromosomes in anaphase, and removing all doubt as to the kind of division, longitudinal or transverse.

Judging from analogy in other insects, I fully expected to find one longitudinal and one transverse division, but was soon convinced that both are longitudinal, and from such figures as 23 and 26 that the same is true in the maturation division of the winter egg. This point will be more fully discussed later.

The chromosomes retain their individuality in the spermatids for some time, but finally become massed together to form the sperm-head (Fig. 42). The development of the spermatozoön from the spermatid appears to be very simple, the head being formed mainly from the chromatin and the long, rather thick tail from the cytoplasm. None of the accessory structures described for other insects are present. No centrosome has been detected in any mitosis, and asters have been seen only in connection with the sperm-nucleus in the egg, the pronuclei, and the segmentation spindles of the winter egg. There is no trace of anything that could be called an "accessory chromosome" (McClung, '02). The nucleolus appears to be of the same character throughout, appearing in resting cells and disappearing in mitosis. That it is not a chromatin nucleolus, or karyosome, is shown by the fact that the chromosomes in many cases are visible before it disappears (Fig. 29) and with the Delafield-orange combination the nucleolus invariably takes the orange stain while the nuclear reticulum stains with the hæmatoxylin.

V. GENERAL DISCUSSION.

1. *Mendel's Law, and the Individuality of the Chromosomes.*

It appears that in the Aphids studied there is a series of 5 chromosomes of different shape and size in the germ cells of the sexual generation: the maternal or egg-series is exactly equivalent to the paternal or sperm-series. The chromosomes show the same relative form and size throughout the maturation divisions. The two series of chromosomes meet in fertilization, and throughout the parthenogenetic generations, both female and male, we find the double series, 10 chromosomes of five different sizes. In the spermatocytes, and presumably in the oöcytes, the chromosomes of the double series are paired, and in one of the maturation divisions, apparently the first, the paired chromosomes are separated. Supposing that the different chromosomes have different physiological values, or represent different hereditary characters, as maintained by Boveri ('02), we find in the behavior of the chromosomes in the germ cells of the Aphid exactly the conditions required by Mendel's Law of Heredity. The characters represented by the 5 constantly different chromosomes would be segregated at

each recurrence of sexual reproduction, giving germ cells pure with respect to each of the 5 different characters or sets of correlated characters represented by the five chromosomes. During the whole series of parthenogenetic generations the same paternal and maternal series of chromosomes is maintained by longitudinal division, there is no amphimixis and no apparent chance for variation unless it be a change in dominance of certain characters, due to external conditions; for example, the winged-character and the sex-character.

The constant recurrence of this single or double series of chromosomes of the same relative form and size, is one point more in support of the hypothesis of the individuality of the chromosomes, strongly advocated by Rabl ('85) and Boveri ('87, '88, '91, '02). Recent papers by Sutton ('02) on Brachystola and Baumgartner ('04) on Gryllus show similar form and size relations of chromosomes; but in the Aphid one has the advantage of working with a smaller number, where each individual chromosome can be distinguished from all others of the series appearing in a winter egg or a spermatocyte.

2. *Maturation of Parthenogenetic Eggs.*

In comparing the results of various authors on this subject, one meets with great variations in different parthenogenetic forms. In the drone bee (Blochmann, '88–'89) two polar bodies are found, and Petrunkewitsch ('01) states that reduction occurs in the second maturation division, the normal number of chromosomes probably being restored by a subsequent longitudinal splitting of the chromosomes without mitosis. In Liparis dispar, Bombyx mori and Ocneria dispar (Platner, '88–'89, Henking, '92), two polar bodies are given off by the occasional parthenogenetic eggs and both sexes are produced. Weismann ('91), in attempting to bring these cases into line with his views of maturation and fertilization, says, "The nucleoplasm of certain eggs possesses a greater power of growth than that of a majority of the eggs of the same species, while in the case of the bee every ovum possesses a power of growth sufficient to double its nuclear substance." Petrunkewitsch's explanation of the presence of the normal number of chromosomes in somatic cells of the male bee sounds more like an echo of Weismann's argument than like the result of actual observation.

In Rhodites rosæ (Henking) there are two maturation divisions but no reduction of chromosomes in eggs that produce females. Males are rare and the maturation of the male egg is not known. In Artemia salina (Brauer, '94) either one polar body only is formed, or a second division occurs and the resulting nucleus conjugates with the egg nucleus. Here again the female and male generations seem not to have been distinguished.

Weismann and Ischikawa ('88) found only one polar body in the parthenogenetic eggs of several rotifers and crustaceans, no statement being made in regard to the male generations. Mrazek ('97), and Erlanger and Lauterborn ('97) found that in Asplanchna, a rotifer, the parthenogenetic female eggs gave off one polar body, while the parthenogentic male eggs formed two, and there was no indication of a union of the second polar body with the egg nucleus.

In Hydatina senta (Lenssen, '98) the first maturation division in parthenogenetic female eggs goes only as far as the metaphase; there is no reduction, and the chromosomes (10 or 12) fuse to form the egg nucleus. In the male egg reduction occurs, 5 or 6 chromosomes appearing in the polar plates of the spindle. In Hydatina it is supposed that the first maturation division is suppressed in both the parthenogenetic and the sexual eggs. In the Aphid only one polar body is given off in the parthenogenetic egg—male or female—and there is no evidence of reduction in either male or female parthenogenetic egg.

Thus we find a series, beginning with forms where parthenogenesis is either occasional or continues for only one generation, when maturation appears to follow the usual course for fertilized eggs; and ending with Hydatina senta where the whole process is practically suppressed in the parthenogenetic female eggs, and the Aphid where one maturation division without reduction remains in both male and female parthenogenetic eggs. We are thus led to question the importance of the second polar body in determining the male sex, also to question the view that parthenogenesis is due to the suppression of one or both maturation divisions, and to suggest that the various degrees of suppression of maturation phenomena in parthenogenetic eggs may be a more or less simple and perfect adaptation to a necessity of continued parthenogenetic reproduction, *i. e.*, the retention of the full double series of maternal and paternal chromosomes throughout the parthenogenetic

portion of the cycle. The whole subject needs further investigation from the standpoint of the determination of sex.

3. *Determination of Sex.*

As the male sex cells of the Aphid contain no "accessory chromosome," McClung's theory of sex-determination need not be discussed in this connection. The question naturally arises whether the "accessory chromosome" is to be found in any of the parthenogenetic insects or crustaceans.

Castle ('03) in his recent paper on "The Heredity of Sex" attempts to place the sex-character in the same category with other hereditary chracters and to apply to it the principles of Mendel's Law of Heredity—dominance and segregation. He says on page 198, "A study of sex-heredity in parthenogenetic animals shows (1) that in such animals the female character uniformly dominates over the male whenever the two are present together," and on page 199, "With a single exception, we know that in uninterrupted parthenogenetic reproduction, as it occurs in the Daphnidæ and Rotiferæ at certain seasons, the parthenogenetic egg forms only one polar cell, and the animal developing from such an egg is invariably female, or more correctly ♂ (♀), the male character being recessive," and further on, "At the return to sexual reproduction, the parthenogenetic mother produces eggs which form a second polar cell, and from such eggs only males develop. It is clear, then, that in the second maturation division the female character has been eliminated from the egg, for were it still there, it must from its nature dominate."

As an exception Castle cites Rhodites rosæ, in which according to Henking the parthenogenetic eggs produce two polar bodies, but no reduction occurs, and therefore no segregation of sex characters. Castle assumes that the occasional egg which produces a male, does, in some way, eliminate the female character. Hydatina senta is also cited: according to Lenssen ('98) no polar body is formed and there is no reduction in the female egg, while in the male egg one maturation division occurs with reduction. Castle supposes in the latter case that the first maturation division is regularly suppressed as Sobotta ('99) has found to be the case in the mouse.

In the Aphid only one polar body is formed in the female and

also in the male parthenogenetic egg, and there is no reduction of chromosomes in either case. If the sex character resides in one of the chromosomes, it is certainly not eliminated from the male egg. How shall we harmonize the conditions in the Aphid with Castle's theory? We cannot argue as he does for Bombyx mori, Ocneria dispar and all normally diœcious animals that there is no uniform dominance of one sex over the other. How then shall we account for the appearance of males, if all parthenogenetic Aphids are sex-hybrids with the female character usually dominant? The only possible argument seems to be that certain favorable conditions (warmth and abundant food) determine that the female sex-character shall continue dominant, the male sex-character recessive; while certain other conditions (not yet definitely determined) cause the male sex-character to become dominant and the female character recessive. A similar change in dominance may be imagined to account for the presence or absence of wings in both the parthenogenetic and the sexual generations. According to this theory we must suppose all of the oögonia to be alike in their hereditary characters—all sex-hybrids as well as hybrids in respect to other maternal and paternal characters. In addition, we must suppose that in parthenogenetic eggs, which undergo no reduction, dominance of certain characters can be reversed by external circumstances. This may of course occur at a very early stage in the history of the germ-cells, making the eggs at the time of maturation virtually male or female.

The case cited of two Aphids, each containing both parthenogenetic and winter ovaries (Fig. 20), and also showing that a parthenogenetic ovary may, after giving off parthenogenetic eggs, change to a winter ovary, the sexual form, is strong evidence that the ovarian oöcytes of the Aphid may be affected by external conditions. Whether the degeneration of certain oöcytes in all ovaries which are to produce winter eggs, can be considered evidence that there are two kinds of eggs, those that produce parthenogenetic young and those that require fertilization in order that they may develop, is not clear.

Beard ('02) says, in discussing parthenogenesis, "When in a parthenogenetic form, a long series of one sex appears, the eggs of the other sex must have been either delayed or suppressed." According to Beard's theory we must suppose that in the Aphid there are both male and female eggs, or at least such germ-cells,

produced, and that all the male eggs are suppressed in the female generations while all the female eggs are suppressed in the male generations. There is no histological evidence of any such degeneration of the germ-cells.

In trying to fit the Mendelian theory of dominance, as elaborated by Castle, to the sex-conditions in Aphids, we meet with a peculiar contradiction in the fact that the same external conditions lead to the production of males and of sexual females. On the Œnothera this condition is very conspicuous, for in the autumn parthenogenetic reproduction is completely changed over into the sexual method of reproduction. Certain apterous individuals are producing male offspring, and at the same time or slightly earlier other winged individuals are producing the winter egg-layers. Three generations at least are involved in the winter egg production—an apterous generation followed by a winged generation and that by the apterous sexual females. In the case of the males only two generations are necessarily involved, an apterous generation and the generation of winged males. The food conditions which probably lead to the change in method of reproduction, may therefore differ in degree, the earlier conditions starting the sexual female line, and later conditions the male line. In favor of this argument is the fact that on the rose a scattered generation of sexual females is often met with before there are any males and before the regular female sexual generation appears.

It is perfectly evident that histological study of the germ-cells combined with observation of the living insects has not settled the question of sex-determination in the Aphid, but it has, in a way, cleared the field for interpretation of the results of experiment. We know (1) that there is no "accessory chromosome," and (2) that the formation of a second polar body with reduction of chromosomes does not occur in the male generation.

In the discussion of sex-determination by Cuénot ('99), Beard ('02), O. Schultze ('02), and Lenhossek ('03) we find that the evidence is overwhelmingly on the side of the view that sex is determined in the egg; but to the question how sex is determined in the egg, no thoroughly convincing answer has yet been given.

V. SUMMARY OF RESULTS.

1. There is no reduction in the number of chromosomes in the female parthenogenetic egg.

2. The 10 chromosomes form 5 pairs of different form and size.

3. The polar body, at first extruded from the egg, later becomes sunken in the cytoplasm, but takes no part in the development of the embryo.

4. The early segmentations are nuclear only, cell walls coming in at a later stage.

5. The young embryo takes in yolk material (the secundäre Dotter) from the vitellaria, through a definite valvular opening in the oviduct.

6. In the maturation of male eggs only one polar body is given off, and there is no reduction in the number of chromosomes.

7. The offspring of one parthenogenetic female are either all females or all males.

8. The ovaries of the parthenogenetic and of the sexual females may be originally identical in structure, as shown (1) by the presence of both kinds of ovaries in the same individuals; (2) by the degeneration of about half of the oöcytes in the posterior half of the ovary of the sexual female embryo. The significance of the latter phenomenon is uncertain.

9. The reduced number of chromosomes appears in the polar spindles of the winter eggs, and both divisions are longitudinal.

10. The somatic number of chromosomes appears in the spermatogonia.

11. Reduction in the spermatocytes is effected by longitudinal pairing of the chromosomes immediately before the first maturation mitosis.

12. The first maturation division probably separates paired chromosomes and the second is a longitudinal division of the original univalent chromosomes.

13. The delay in separation of one pair of chromosomes in the first maturation division is probably due to a different linin connection from the others, rather than to any physiological peculiarity.

14. There is no "accessory chromosome" in the male germ-cells of aphids.

Bryn Mawr College,
Dec. 20, 1904.

LITERATURE.

BALBIANI, E. G., '69–'72.—Mémoire sur la génération des aphides. Ann. Sc. Nat. Ser. 5, Zool. T. 11, 1869; T. 14, 1870; T. 15, 1872.

BAUMGARTNER, W. J., '04.—Some new Evidence for the Individuality of the Chromosomes. Biol. Bull. vol. viii, No. 1.

BEARD, J., '02.—The Determination of Sex in Animal Development. Zool. Jahrb. vol. xvi.

BLOCHMANN, F., '87.—Ueber die Richtungskörper bei Insekteneiern. Morph. Jahrb. vol. xii.

'88.—Ueber die Richtungskörper bei unbefruchtet sich entwickelnden Insekteneiern. Verh. naturh. med. Ver. Heidelberg, N. F., vol. iv, No. 2.

BONNET, CH., '45.—Traité d'Insectologie ou observations sur quelques espèces de Vers d'eau douce et sur les Pucerons. Paris. Durand.

BOVERI, TH., '87.—Ueber Differenzierung der Zellkerne während der Furchung des Eies von Ascaris meg. Anat. Anz., vol. ii.

'88.—Zellenstudien II. Jena Zeitschr., vol. xxii.

'91.—Befruchtung. Merkel u. Bonnet's Ergebnisse, vol. i.

'02.—Ueber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh. d. phys.-med. Ges. Würzburg. N. F., Bd. xxxv.

BRAUER, A., '94.—Zur Kenntniss der Reifung des parthenogenetisch sich entwickelnden Eies von Artemia salina. Arch. mikr. Anat., vol. xi.

CASTLE, W. E., '03.—The Heredity of Sex. Bull. of the Mus. of Comp. Zoöl. Harvard College, vol. xl, No. 4.

CUÉNOT, L., '99.—Sur la détérmination du sexe chez les animaux. Bull. Sci. Fr. Belgique, vol. xxxii.

ERLANGER U. LAUTERBORN, '97.—Ueber die ersten Entwickelungsvorgänge im parthenogenetischen und befruchteten Räderthierei. Zool. Anz., vol. xx.

HENKING, H., '92.—Untersuchungen über die ersten Entwickelsvorgänge in den Eiern der Insekten. Zeit. wiss. Zool., vol. liv.

KYBER, J. F., '15.—Einige Erfahrungen und Bemerkungen über Blattläuse. Germar's Magazin der Entomologie, t. 1–2c.

LENHOSSEK, M. v., '03.—Das Problem der geschlechtsbestimmenden Ursachen. Jena. Fischer.

LENSSEN, J., '98.—Contribution à l'étude du dévelopment et de la maturation des oeufs chez Hydatina senta. La Cellule, vol. xiv.

LEYDIG, T., '50.—Einige Bemerkungen über die Entwickelung der Blattläuse Zeit. wiss. Zool., vol. ii.

McCLUNG, C. E., '02.—The Accessory Chromosome—Sex-Determinant? Biol. Bull., vol. vii, Nos. 1 and 2.

MRAZEK, AL., '97.—Zur Embryonalentwickelung des Gattung Asplanchna. Jahresb. böhna Ges., vol. ii.

PETRUNKEWITSCH, A., '01.—Die Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienenei. Zool. Jahrb., vol. xiv.

PLATNER, G., '88.—Die erste Entwickelung befruchteter und parthenogenetischer Eier von Liparis dispar. Biol. Centralbl., vol. viii, No. 17.

'89.—Ueber die Bedeutung der Richtungskörperchen. Biol. Centralbl., vol. 8.

RABL, C., '85.—Ueber Zellteilung. Morph. Jahrb., vol. x.

SCHULTZE, O., '02.—Was lehren uns Beobachtung und Experiment über die Ursache männliche und weibliche Geschlechtsbildung bei Tieren und Pflanzen? Sitz. ber. der phys.-med. Ges. Würzburg. 1902.

SOBOTTA, J., '99.—Ueber die Bedeutung der mitotischen Figuren in den Eierstockeeiern der Säugetiere. Festschr. phys.-med. Ges. Würzburg. 1889.

SUTTON, W. S., '02.—On the Morphology of the Chromosome Group in Brachystola magna. Biol. Bulletin, vol. iv, No. 1.

WEISMANN, A., '63.—Die Entwickelung der Diptera im Ei. Zeit. wiss. Zool., vol. xiii.

'92.—Essays on Heredity. Vol. ii. Oxford. 1892, p. 174.

WEISMANN AND ISCHIKAWA, '88.—Weitere Untersuchungen zum Zahlengesetz der Richtungskörper. Zool. Jahrb., vol. iii.

WILL, L., '83.—Zur Bildung des Eies und Blastoderms bei der viviparen Aphiden. Arb. Zool. Zoot. Inst. Würzburg. Vol. vi.

'88.—Zur Entwickelungsgeschichte der viviparen Aphiden. Biol. Centralbl., vol. viii, No. 5.

'89.—Entwickelungsgeschichte der viviparen Aphiden. Zool. Jahrb., vol. iii.

WITLACZIL, EM., '84.—Entwickelungsgeschichte der Aphiden. Zeit. wiss. Zool., vol. xl.

DESCRIPTION OF PLATES.

With the exception of Figs. 20 and 21, the figures are all camera drawings, made with Zeiss obj. 2 mm., oc. 8, except Figs. 1, 14, 15 and 19, which were drawn with Zeiss obj. 2 mm., oc. 4, and Fig. 22, for which Zeiss obj. D, oc. 4 was used. Plates II and III have been reduced one-tenth.

Plate I.

Fig. 1. Parthenogenetic ovary from an embryo of Aphis rosæ. *a* = a developing ovum. *b* = stalk of the next ovum in the egg-string.

Fig. 2. Oöcyte from above ovary.

Fig. 3. Oöcytes at the beginning of their growth period, showing chromosomes but no nucleolus.

Figs. 4–6. Later growth stages of the oöcyte.

Fig. 7. Equatorial plate of the polar spindle, showing 10 chromosomes of 5 different sizes and shapes *a–e*.

Fig. 8. Polar spindle in anaphase. Polar body already separated from the egg.

Fig. 9. A later stage, showing the polar body (*p*), and the egg-nucleus (*n*).

Fig. 10. An egg containing the first segmentation spindle, and the polar body (*p*) somewhat sunken in the cytoplasm of the egg.

Fig. 11. Segmentation stage with resting nuclei and polar body (*p*) lying in a vacuole in the cytoplasm of the unsegmented egg.

Fig. 12. Segmentation stage, showing polar body (*p*) and mitotic figures. The chromosomes of the equatorial plate are lettered *a–e* to correspond to those of the equatorial plate of the polar spindle in Fig. 7.

Plate II.

Fig. 13. Later segmentation stage, showing polar body (*p*) probably degenerating.

Fig. 14. Young parthenogenetic embryo just beginning to take in yolk (*a*) through a valvular opening in the oviduct (*b*).

Fig. 15. A slightly later stage of the same process.

Fig. 16. Resting oöcyte from an ovary of Aphis œnotheræ, all the embryos male.

Fig. 17. Section of a segmenting egg from an individual containing male embryos, showing one polar body (*p*).

Fig. 18. Part of a section similar to the above, showing chromosomes not so completely fused.

Fig. 19. Ovary from a female embryo of the sexual generation, showing degenerating oöcytes (*a*).

Fig. 20. Freehand sketch of a mixed group of ovaries, consisting of five winter ovaries (*a*) with winter eggs (*b*), and one parthenogenetic ovary (*c*) with developing eggs and embryos (*e*).

Fig. 21. Freehand sketch of egg-string from the other side of the same individual, showing two parthenogenetic embryos (*e*), a winter egg (*b*) and a winter ovary (*a*).

Plate III.

Fig. 22. Oblique section of a winter egg, showing entrance of secondary yolk from without. *a* = yolk cell (nucleus in next section). *b* and *c* = fragments of nuclei.

Fig. 23. Equatorial plate of first polar spindle in winter egg of Aphis rosæ. The five chromosomes are lettered *a–e* corresponding to the five pairs in Figs. 7 and 12.

Fig. 24. First polar spindle, metaphase. The stippled chromosome is a part of one which appears in *a*.

Fig. 25. First polar body (p^1) containing five chromosomes, and second polar spindle. Two chromosomes appear in two sections *b* and *c*, and a part of one belonging to the first polar body is seen in section *a*.

Fig. 26. First polar body (p^1); chromosomes of second polar body (p^2); and female pronucleus forming (*n*).

Fig. 27. Male and female pronuclei. The arrows indicate the path of the sperm nucleus.

PLATE IV.

Fig. 28. Resting spermatogonium of Aphis œnothoræ.

Fig. 29. Spermatogonia preparing for mitosis, showing both nucleolus and chromosomes.

Fig. 30. Spermatogonium showing 9 of the 10 chromosomes, and no nucleolus.

Fig. 31. Equatorial plate of spermatogonial mitosis.

Fig. 32. Spermatocyte of first order in resting stage.

Fig. 33. Spermatocytes of first order, showing (*a*) eight of the ten chromosomes not paired and (*b*, *c*, *d*) chromosomes of the same size in pairs.

Fig. 34. Spermatocytes before division, possibly a "synapsis" stage.

Fig. 35. Equatorial plate of first maturation mitosis, showing variations in arrangement of chromosomes (*a*, *b*, *c*), and double chromosomes, (*c*) chromosomes lettered as in Figs. 7, 12 and 23.

Fig. 36. Side view and oblique view of equatorial plate of first maturation mitosis showing double chromosomes.

Fig. 37. Anaphase of first maturation mitosis showing "lagging" chromosome.

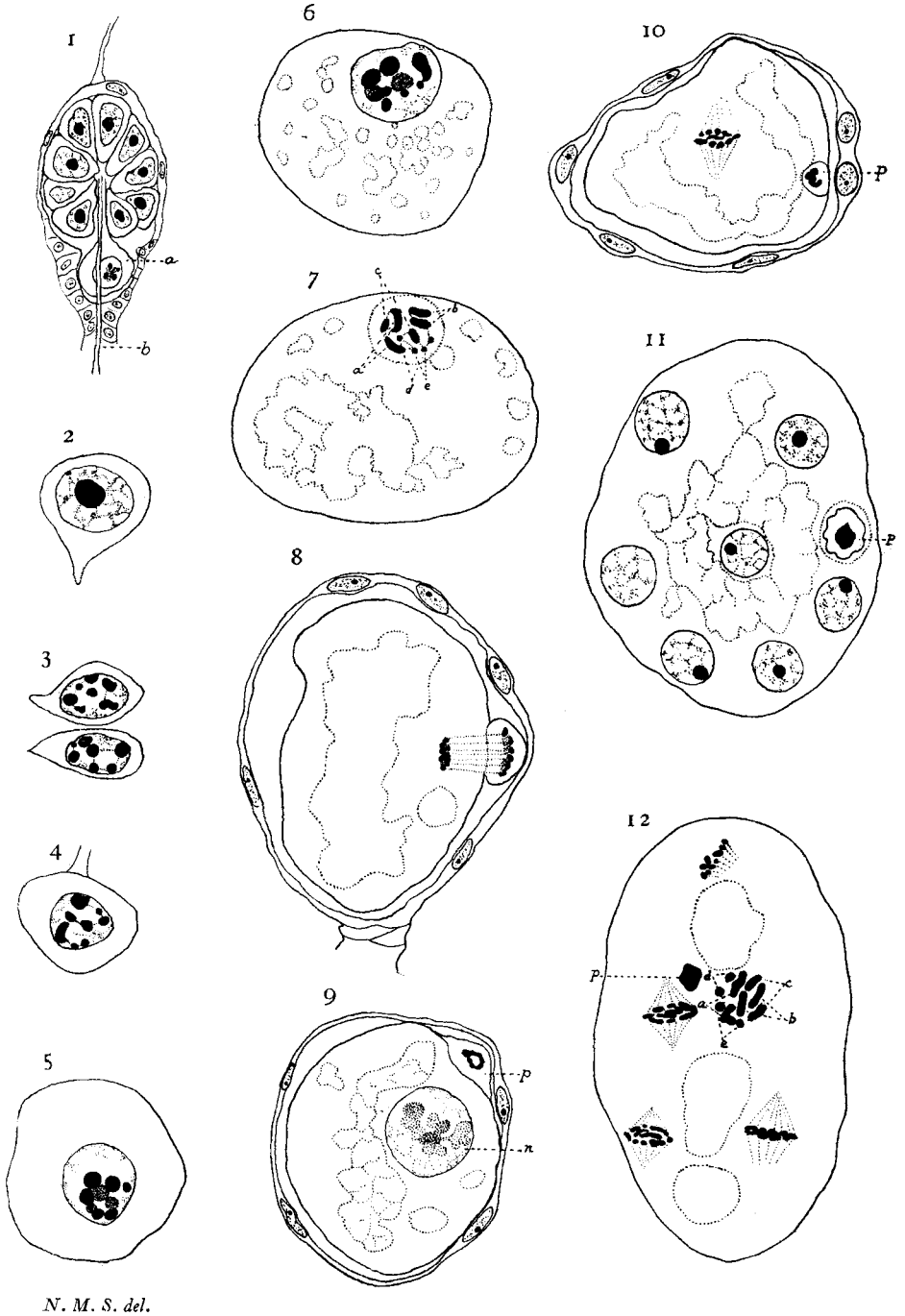
Fig. 38. Later stage of the same.

Fig. 39. A pair of spermatocytes of the second order in a partial resting stage, the lagging chromosomes still connected.

Fig. 40. Equatorial plate of second maturation mitosis, chromosomes lettered as in Figs. 7, 12, 23 and 35.

Fig. 41. Anaphase of second maturation mitosis.

Fig. 42. Spermatids in various stages.



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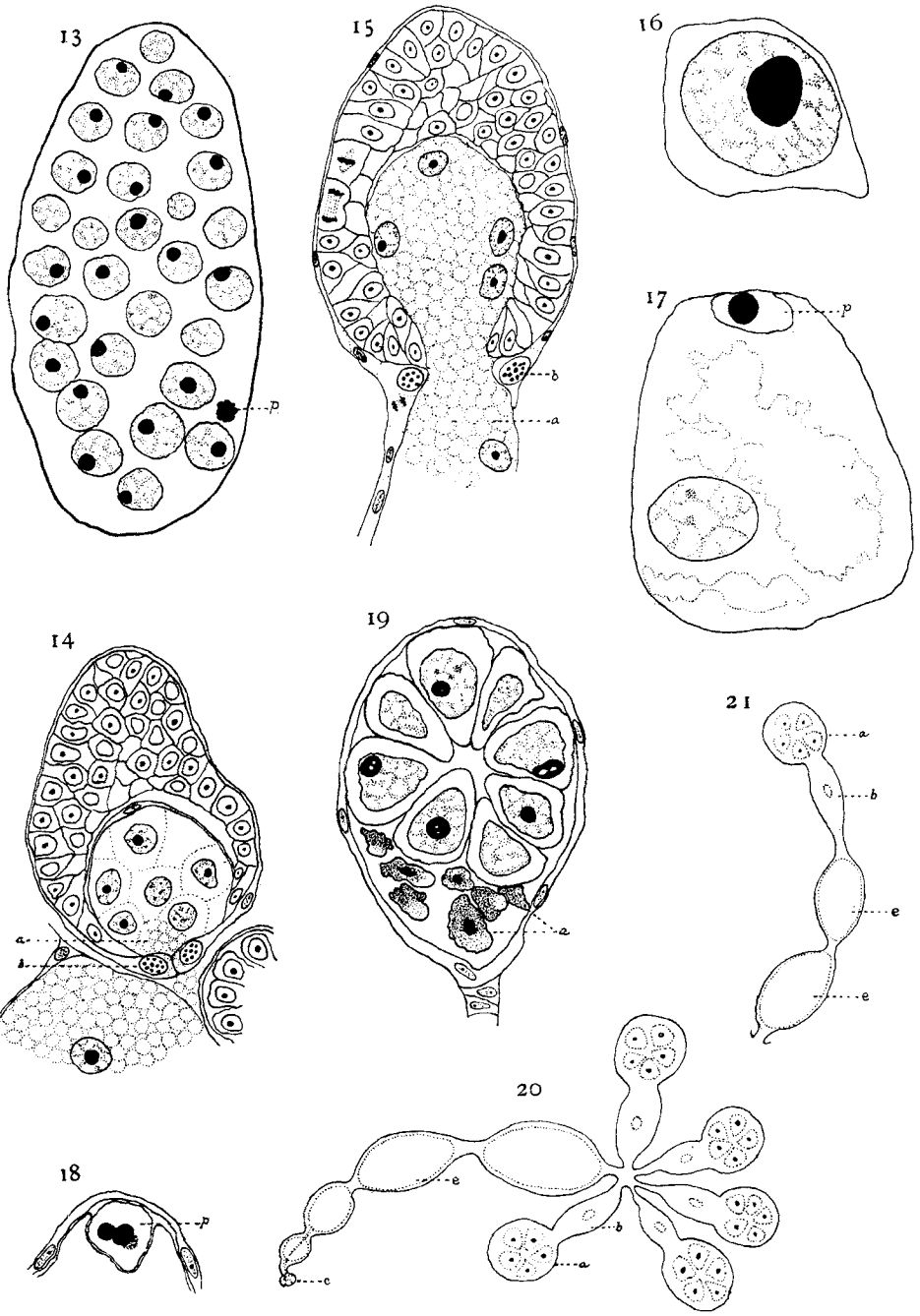

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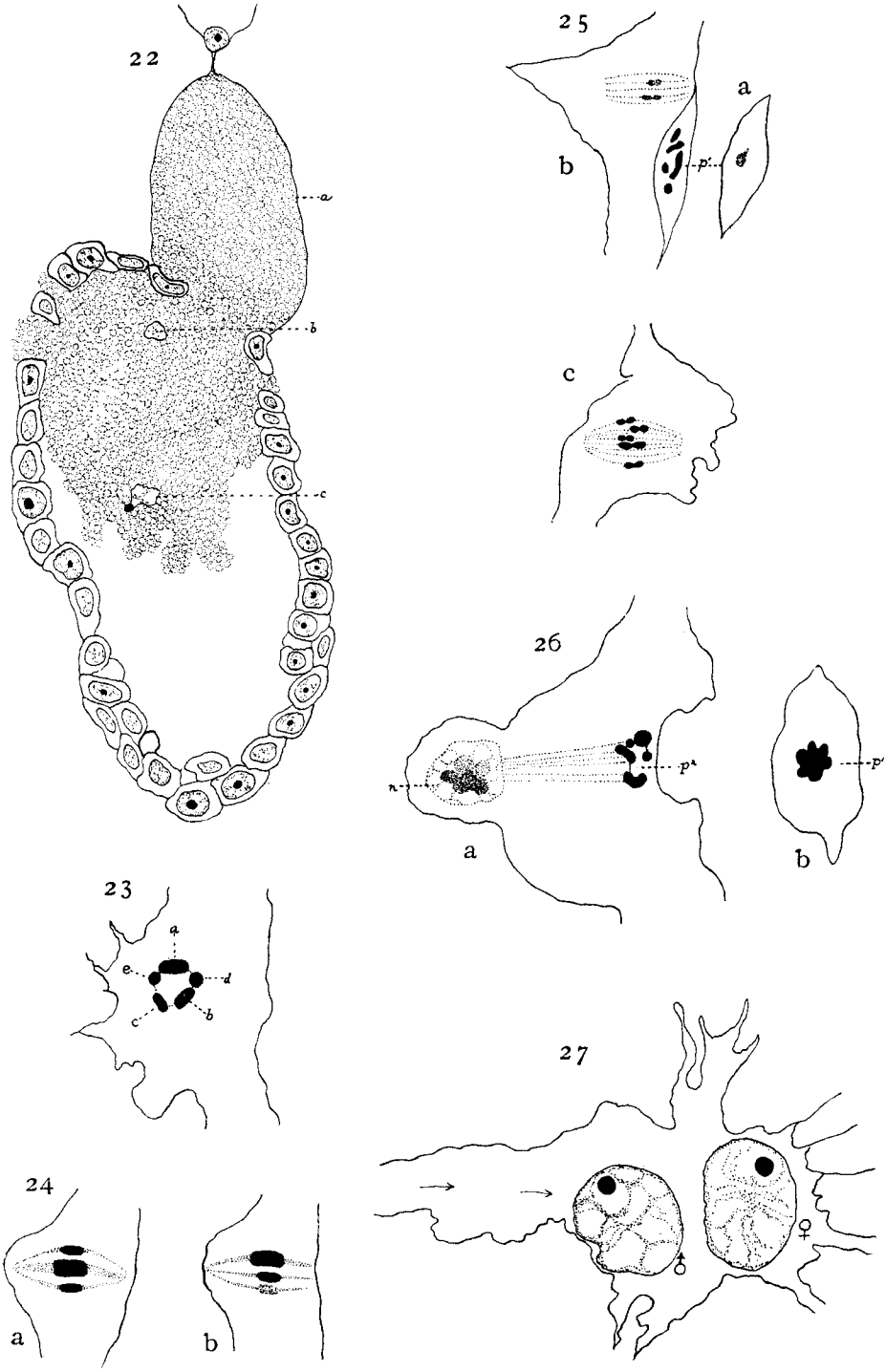

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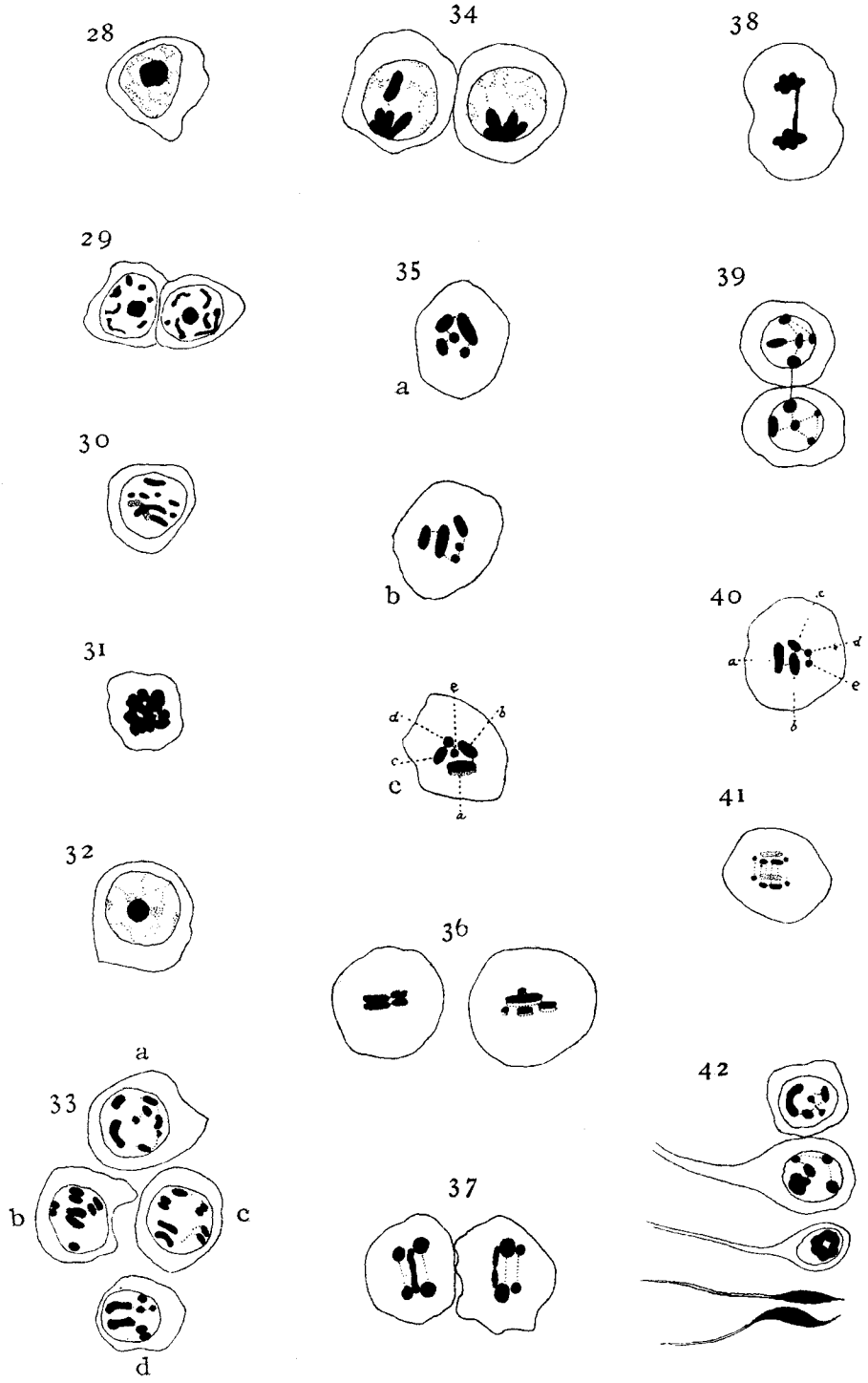

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