V. *On the Cytological Features of Fertilization and Related Phenomena in* Pinus silvestris L.

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[Plates 12–14.]

Introduction.

In spite of the attention which has of late years been devoted to the study of the histology of fertilization in plants, no one has hitherto investigated in detail the cytological features of this process in the Abietineæ.

The oospheres of this group, owing to their large size and the presence of a quantity of food material, present a strong resemblance to a well-known type of animal ova. The fact that in this type of ovum the phenomena of fertilization are usually both much more striking and much more easily followed than is the case among plants, suggested that the oospheres of the Abietineæ might be peculiarly favourable objects for study. It was with this idea that the work described in the present paper was begun in 1894.

But few observers have concerned themselves with the development of the oophyte and the process of fertilization in this group. In fact, we owe almost all our knowledge on these subjects to the researches of Hofmeister and Strasburger, especially the latter.

Hofmeister (2 and 3),* though he followed out most carefully the development of the embryo sac and corpuscula in *Pinus* and *Picea*, could make very little of the actual manner of fertilization. According to his observations the nucleus of the oosphere or "corpusculum" disappeared after a time, becoming dissolved, and in its place a number of free cells and nuclei made their appearance. It was one of these free cells which, as Hofmeister thought, was fertilized in some mysterious way by the pollen tube.

* The numbers in brackets refer to the List of Papers at the end.

It is clear that HOFMEISTER actually saw the generative cells in some cases (*cf.* 'Vergl. Unter.,' 1851, Plate 33, figs. 6 and 12; 'Engl. Transl.,' 1862, Plate 64, fig. 4; Plate 65, fig. 9), yet, confusing with the real cells other structures not of a cellular nature, he was led to the belief that the cells in the apex of the pollen tube were often very numerous (*cf. Ibid.*, Plate 33, figs. 11 and 12; Plate 32, fig. 5; *Ibid.*, Plate 64, fig. 3). At this time nothing was understood of the part played by these cells in fertilization. The tip of the pollen tube was supposed to remain always closed; thus, HOFMEISTER (2, p. 418), after stating that the tip of the pollen tube of *Pinus silvestris* often appears to be open and its contents discharged into the cavity of the corpusculum, adds: "This is plainly the result of mechanical rupture."

In an earlier paper (1) HOFMEISTER had observed a pit at the apex of the pollen tube in certain Abietineæ. This pit he figures in *Pinus Larix* (*Larix europæa* D.C.), but remarks that it seems always to be closed by the primary wall of the tube.

STRASBURGER in 1869 (4) pointed out that certain errors had crept into HOFMEISTER'S description of fertilization of the Abietineæ, errors which were largely due to the fact that HOFMEISTER examined his sections in water. STRASBURGER also used fresh material, but mounted his sections in white of egg. He came to the conclusion that HOFMEISTER'S cells and nuclei in the corpusculum were nothing more than granular masses produced in the cytoplasm by the disorganising action of water. All that he could see of the process of fertilization was a kind of cloudiness passing from the top to the bottom of the oosphere. At the base of the egg, a granular mass appeared, in which, later on, a clear spot, the fertilized nucleus, became visible, and then underwent division. STRASBURGER understood that the contents of the tube passed over into the oosphere, though he did not see the generative cells; he also observed the pit at the apex of the pollen tube.

Three years later, STRASBURGER (5) carried our knowledge of the phenomena of fertilization in the Abietineæ to a much higher level. He was enabled to do so by the discovery of the value of material fixed in alcohol. By the use of such material he was able to observe for the first time the nucleus of the oosphere before fertilization; he also observed the formation of one or more "primordial cells" in the apex of the pollen tube, and was the first to clearly distinguish them from other structures, but he was not clear as to their function, for he states that their shrivelled remains can be seen in the pollen tube after fertilization.

By this date the development of each of the corpuscula (archegonia) from a single prothallium cell, and the formation of the neck cells and of the ventral canal cell were well known.

In 1877 STRASBURGER (6) made a very distinct advance, for he figured the passage of the male nucleus into the egg, and also a stage which he thought represented the actual fusion of the male and female nuclei in *Picea vulgaris*. In the light of my own observations on *Pinus silvestris*, it is probable that this figure really represents an early stage of conjugation, before that of the actual fusion. In this paper

also, STRASBURGER figured the proteid vacuoles in the egg of *Picea*, and established a nuclear division in the pollen tube.

In 1879 STRASBURGER published his "Angiospermen und Gymnospermen" (7). This work did not add much to our knowledge of the cytology of reproduction in Abietineæ, but the existence of the ventral canal cell in *Juniperus*, which had formerly escaped observation, was established.

In 1883 GOROSCHANKIN (15), while working on the fertilization of *Pinus Pumilio*, made the striking observation that *both* generative nuclei passed from the pollen tube into the cytoplasm of one egg. He believed, however, that both these nuclei fused with the egg nucleus. GOROSCHANKIN also seems to have fallen into somewhat the same error as did HOFMEISTER, for he believed that the structures with highly refractive contents, which he found in the oosphere, were not real vacuoles, but of nuclear nature.

STRASBURGER (8), in 1884, by a study of *Picea vulgaris*, confirmed GOROSCHANKIN as to the passage of both generative nuclei into the egg, but made short work of his other contentions. He points out that it is quite out of analogy with other plants for both male nuclei to fuse with the egg nucleus, and that in *Picea vulgaris* he has actually observed that it is only one, the first entering, of the generative nuclei, which fuses with the egg nucleus, the other remains free in the cytoplasm of the egg and ultimately becomes disorganised.

STRASBURGER shows also that a study of the origin of the proteid vacuoles altogether disproves their supposed nuclear nature (*vide infra*, pp. 417).

In 1892 STRASBURGER published his well-known paper on the Pollen of Gymnosperms (9), in which he showed that BELAJEFF'S account of the formation and behaviour of the cells of the male gametophyte in *Taxus* held good in its main features for all the principal groups of Gymnosperms. In *Pinus* itself, the formation of the two generative cells from the "body cell of the antheridium," which takes place after pollination, was not actually seen, but from a study of the formation of the male gametophyte in the pollen grain, and a comparison of the whole series of events in other genera, no reasonable doubt remained that the formation of the two generative cells, which together with the "stalk cell" nucleus and the "pollen tube nucleus" were clearly figured lying at the apex of the pollen tube just above the egg, conformed to the general type.

The fact of this formation was established in 1894 by DIXON (14) in *Pinus silvestris*. DIXON, working in STRASBURGER'S laboratory, followed out the processes occurring in the pollen tube and pollen grain after pollination. He was the first to observe the passage of the two generative cells and of the two free nuclei into the egg. But the actual process of fertilization he failed to follow. DIXON also made some observations on the number of chromosomes in various cells of the oophyte, as well as in various sporophytic tissues. I cannot confirm DIXON'S work on this last point. The matter is fully dealt with in the body of the paper.

The present paper gives a fairly complete account of the act of fertilization and of the processes surrounding it, from the formation of the neck of the archegonium up to the stage of cell wall formation at the base of the egg. I have also counted the chromosomes in the first division of the pollen mother cells, in the division of endosperm cells, in the division of the central cell to form the ventral canal cell and oosphere, and in the first segmentation of the oospore nucleus, as well as in three types of sporophytic tissue.

Methods.

Material was collected at intervals of a week in the spring and summer of 1894 and the two following years.

Various fixing fluids were used:—Absolute alcohol, picric acid, mercuric chloride (a saturated solution, either in 1 per cent. acetic acid, or in a dilute solution of sodium chloride), chromic acid, Klein's fluid (chromic acid and spirit), Flemming's fluid (weak and strong formula), and Hermann's fluid. Of these, Flemming's strong formula gave by far the best results. Hermann's fluid, though fixing very well, caused much more blackening than the fluid of Flemming. Owing to the feeble power of penetration of Flemming's fluid, the best results were obtained only when the ovules were carefully cut open and the embryo sacs removed on a needle and placed directly in the fixing fluid; with a little practice this process can be carried on with fair rapidity, even in the case of young ovules.

Next to Flemming's fluid mercuric chloride gave the best results. Klein's fluid ($\frac{1}{6}$ per cent. chromic acid 2 parts, alcohol 1 part) also gave very good results.*

All material fixed with Flemming's fluid, after being washed in running water for 24 hours, and brought up carefully through the alcohols, was stained after sectioning with Flemming's triple stain (safranin, gentian-violet and orange). Material thus fixed and stained showed a sharpness of stain and clearness of detail which could be obtained by no other method.

Material fixed in mercuric chloride was mostly stained with Heidenhain's ammonium-iron-alum-hæmatoxylin, followed by fuchsin S. in watery solution. Material fixed in absolute alcohol was also usually stained in this manner. For washing material in running water the very convenient perforated porcelain cylinders described by Fairchild (29) were used. All material, however fixed, if not required for immediate use, was put up in Calberla's fluid (alcohol 1 part, glycerin 1 part, water 1 part). In this fluid the material will remain unchanged and retain its staining power for more than a year.

The thickness of the section was usually 5 to 10 μ. The slide was prepared to

* This fluid is well worthy of the attention of botanists, for it has a much higher penetrative power than that of chromic acid alone, and tissues, after its use, if thoroughly washed, stain well with both hæmatoxylin and aniline dyes. It preserves nuclear structures very well, though not possessing the power of fixing delicate cytological detail in the manner characteristic of Flemming's fluid.

receive the sections by first being smeared with a thin layer of egg albumen and glycerin; upon this a few drops of cold water were placed and the sections arranged on the layer of water; on gentle warming, the sections become perfectly flat, and after evaporation of the water remain adhering to the slide.

All material was fixed as quickly as possible after collection; warm days were preferably chosen for the latter purpose. The greater part of the material was obtained from the Botanic Gardens, Cambridge, and from the Royal Gardens, Kew. Apart from the use of other fluids, three parallel series of fixations were made in Flemming's fluid, mercuric chloride and absolute alcohol.

A few of the figures on Plate 14 are the work of Miss M. O. MITCHELL, who very kindly drew them from my preparations.

The photographs are due to the skill of Dr. E. C. BOUSFIELD, who has succeeded in obtaining prints showing the number of the chromosomes. Owing to the various chromosomes lying in different planes, the difficulty of obtaining such photographs is very great; that shown on Plate 14, fig. 38B, for example, required no less than five separate exposures.

FORMATION OF THE VENTRAL CANAL CELL.

The cell, which later gives origin to the neck cells, separates very early in development from the lower central cell. It is only after this separation that the central cell increases much in size, but its nucleus always remains at the place of origin, at the apex of the cell, close against the neck. In fig. 1 the undivided neck cell is visible above the central cell; the latter possesses a thin layer of protoplasm and a large central vacuole. The nucleus has been drawn out of its normal position by contraction, to which the large size of the vacuole renders the cell at this stage very liable.

The central cell soon begins to fill with very finely granular protoplasm, and the large central vacuole becomes replaced by a number of smaller ones (fig. 2). At the same time the neck cell divides. The neck of the archegonium consists nearly always of two tiers of cells (not one tier, as stated by STRASBURGER).* Fig. 2 shows this clearly. Although a double tier is the normal condition, yet a single tier was sometimes observed—but not in more than two cases out of all those examined. At the same time the cells of the layer immediately surrounding the central cell become densely filled with protoplasm, and the nuclei increase in size. The cells thus become sharply differentiated from the surrounding prothallial cells, and have the appearance of distinct sheaths to the archegonia.

Already at the stage shown in fig. 1, the wall of the central cell is thicker than that of the surrounding cells, and as the central cell increases in size the difference becomes still more marked (fig. 2). This thickened wall is very distinctly pitted;

* STRASBURGER (9) has later, however, figured an archegonium with two tiers of neck cells.

these pits can be made out even in section, but in surface view are very plain; when the wall is deeply stained, they appear as thin and lightly stained areas. GOROSCHANKIN (16) was able to trace protoplasmic continuity through the pits between the central cell and the surrounding sheathing cells.

With the increase in size of the cell, the nucleus also increases, both in size and in mass of staining material (figs. 3 and 4) and very soon begins to go through the stages preparatory to division.

Pari passu with this change in the nucleus, changes also take place in the cytoplasm of the central cell, it ceases to be finely granular, and becomes filled with large and deeply staining masses, culminating later in the formation of proteid vacuoles; but the number of these structures to be found in the cytoplasm of the central cell, before its final division, is usually very small.

The nucleus passes through the stages of a normal homotypic division. The chromosomes can be observed lying free in the cavity of the nucleus, before the nuclear wall has broken down: at this stage a number of cytoplasmic fibres can also be seen radiating from the lower portion of the nucleus and extending a short way into the main mass of the cytoplasm.

The chromosomes, which stain very strongly with safranin, hæmatoxylin, &c., have the form of straight rods when seen at the equator of the spindle (figs. 5 and 7). In fig. 5 they have already split longitudinally. The very distinct spindle is bluntly pointed at both ends (fig. 5); in some cases the upper pole seems to be actually pressed close against the wall of the cell. In fig. 5 a slight contraction has somewhat disturbed the natural relation of the parts, so that the spindle appears to project from the main mass of the protoplasm.

In later stages of division, the daughter chromosomes are distinctly V-shaped, as they move back towards the poles (figs. 6*a*, *b*, *c*, *d*, and *e*, and figs. 38*a* and *b*). Figs. 5 and 6 are dealt with more fully in the discussion on the number of the chromosomes (*vide infra*). When the chromosomes have reached the poles and the daughter nuclei begin to be formed, the spindle is seen to have become much wider by the addition of new fibres (fig. 8). In this spindle a very thin, but broad and sinuous, cell plate makes its appearance. This cell plate cuts out from the main mass of the central cell a small oval segment of protoplasm, the ventral canal cell (fig. 8). This cell plate, when very young, can be seen to be two-layered. The two layers have no doubt arisen by the splitting of an original single layer, and probably represent the two "Hautschichten" of the two new cells respectively. A similar condition has been described by STRASBURGER (13) in *Fucus*.

It is interesting to note the exact similarity between the two nuclei, the one the nucleus of the egg, the other that of the ventral canal cell. Such a preparation as fig. 8 strongly supports the ordinarily accepted view that the ventral canal cell is an arrested gamete, only differing from the egg in the possession of a much smaller quantity of protoplasm.

The Structure and Fate of the Ventral Canal Cell.

The ventral canal cell has a more or less oval form, both in the longitudinal section of the ovule (figs. 8 and 9) and as seen from above (fig. 10).

After the ventral canal cell has been cut off from the egg by the broad curved wall, the nucleus of the former surrounds itself with a membrane and goes through the earlier stages, at least, of the normal process of return to the resting condition (fig. 8); it never, however, reaches the completely resting state, but soon after its formation it loses its membrane and appears as a number of irregular rods and clumps, lying free in the protoplasm and giving the staining reaction of chromatin. Figs. 9*a* and 9*b* are two consecutive sections through the ventral canal cell of an oosphere which was just ready for fertilization. The nucleus is in the condition described, and in addition to the chromatin masses a nucleolar body is visible. In this preparation there is also present a curious wisp-like body. It consists of violet staining (kinoplasmic) fibres. This curious structure was observed in three or four cases, but no light was obtained as to its nature; the only thing that suggests itself is that it is the remains of a portion of the achromatic spindle present at the formation of the ventral canal cell nucleus, the latter having become disorganised without the disappearance of the spindle. If the two figures are compared, it will be noticed that the wisp-like body ends close to a curious spherical structure bearing some resemblance to a centrosphere.

The ventral canal cell usually presents the condition of a disorganised and broken up nucleus shortly after its formation. Later on it is often found separated by a short distance from the oosphere, and then consists merely of an oval mass of protoplasm containing the disorganised nucleus, which is now a uniformly staining mass, similar to the nucleolus. After the passage of the pollen tube through the neck of the archegonium all trace of the ventral canal cell is usually lost.

Dixon (14) states that the chromosomes which form the ventral canal cell nucleus remain completely separate. (It was by counting these that he deduced the number of chromosomes in the oosphere—see later on the question of the number of the chromosomes.) He figures a ventral canal cell containing twelve rods; he does not state, however, whether such a condition is to be explained by the chromosomes remaining separate at the pole of the spindle, at the time of the division which cuts off the ventral canal cell, or whether this condition is brought about later by a change in the nucleus, after the chromosomes have fused together in the normal way.

That the first explanation is impossible is shown by such a preparation as is seen in fig. 8, in which the chromosomes are fused together to form a definite nucleus; several such preparations were obtained.

The chromosomes which Dixon observed must then be considered to have been produced by changes in the nucleus subsequent to its passage into the partially

resting condition, and accordingly, to apply the term chromosomes to them at all seems of rather doubtful propriety.*

There seems then to be no reason to regard this formation of so-called chromosomes otherwise than as changes in a degenerating nucleus, and any evidence based on the counting of such "chromosomes" must be looked upon with the gravest suspicion.

A large number of ventral canal cells in various stages were examined, and in only one case (fig. 10, *a*, *b*) was anything to be seen which agreed at all with DIXON'S figure. In this preparation a number of threads, forming a tangled mass and lying free in the protoplasm of the ventral canal cell, are to be seen. These threads stain like chromatin, but there seems no reason to consider them as other than portions of a disorganised nucleus. Even if they be chromosomes, they do not confirm DIXON'S statement as to number, for although they cannot be counted with perfect accuracy, it is clear that there are more than *eight*, in fact the number seems to be even greater than *twelve*.

PENETRATION OF THE POLLEN TUBE.

The details of the passage of the pollen tube down the nucellus have been worked out by DIXON (14).

When the tube reaches the oosphere wall it seems to become fused with it (figs. 15 and 16); in no case was the pollen tube seen to pass beyond the wall, it never seemed to penetrate into the actual substance of the egg as has been so often described by older observers. In most cases after fertilization a cavity could be clearly seen in the upper part of the egg (figs. 16 and 17), a cavity which is usually considered to be caused by the actual entrance of the pollen tube into the cytoplasm. In the light of my observations, this cavity must be explained in some other way; it is very probably due to the sudden inrush of the contents of the pollen tube on the bursting of the pit-closing membrane (see below).

HOFMEISTER (1), STRASBURGER (4), and SCHACHT, have described and figured pits in the pollen tubes of various Abietineæ, but to these observers the pit always seemed to remain closed by the pit-closing membrane. DIXON (14) saw this pit very clearly in the apex of the pollen tube of *Pinus silvestris*.

I have been enabled to observe the perforation of this pit in a number of cases. Figs. 15 and 16 are median sections through the pit in the wall of the apex of the pollen tube soon after the passage out of the contents. In fig. 15 the end of the tube is slightly invaginated, and remnants of the pit-closing membrane are still

* The term chromosomes was invented by WALDEYER to apply to the segments into which the nuclear chromatin network became resolved *on division*. An extension of the term to clumps or rods to be found in the nucleus, in connection with any activity other than that of division, is unwarranted and liable to cause confusion.

visible. In fig. 16 a mass of staining substance is lying, partly in, partly out of the practically empty pollen tube.*

Maturation of the Oosphere Nucleus.

The oosphere nucleus, immediately after the formation of the ventral canal cell, is of very small size (fig. 8), very little larger than the nuclei of the cells sheathing the oosphere. At this stage it has the ordinary structure of a young daughter nucleus; it consists of a small number of chromatin bands and a few round nucleoli and is slightly oval in form (fig. 8), its long diameter being about 22 μ. It naturally resembles in all respects the other daughter nucleus, the ventral canal cell nucleus. The fate, however, of these two nuclei is very different, for while the latter nucleus hardly increases at all in size and very shortly becomes broken up, as described above, the oosphere nucleus passes through a series of changes, of which the most conspicuous is a great increase in size. I have applied the term *maturation* to the series of changes which the nucleus passes through from its first formation up to the stage in which it is ready for fertilization.

Even before the complete disappearance of the connecting fibres (Verbindungsfäden) maturation changes begin. In fig. 11 the nucleus is shown just beginning to move back towards the centre. It exhibits a very striking appearance; in its interior, within a distinct nuclear wall, a deeply stained chromatin mass is visible, which appears to be arranged in a somewhat spiral fashion (only *one* section of the nucleus is figured here); there is also a distinct nucleolus present, but over and above this, and filling up the rest of the nuclear cavity, is a peculiar granular substance, which, following Strasburger,† I have termed metaplasmic substance. It is not easy to distinguish this substance from the chromatin itself, for it stains in a very similar manner. If, for example, the preparation be stained with Heidenhain's iron-alum-hæmatoxylin, the whole nucleus and the protoplasm stain in the usual way a deep black, but after washing the stain completely out of the protoplasm, both the chromatin and the metaplasmic substance still remain black, giving one the impression that the nucleus is an ordinary nucleus containing no peculiar substance. On carrying, however, the washing still further, the real chromatin retains the black stain, while the metaplasmic substance loses its colour almost completely. By the

* It seems to have been generally overlooked that Goroschankin (15) as long ago as 1883 stated that he had observed the perforation of the pit in the pollen tube in *Pinus pumilio*; he gives, however, no figure.

† Strasburger (8) clearly recognised that the egg nucleus of the Abietineæ had a peculiar structure, in fact, he states that the nucleus, while moving back to the centre of the egg, fills itself with metaplasmic substance. He believed, however, that the metaplasm masked the real chromatin, and also that it was not generally distributed, but confined to the upper portion only of the nucleus: the metaplasm he considered to be of the same nature as the substance in the proteid vacuoles.

use of another stain (such as fuchsin S.), in addition, the metaplasmic substance takes a red tint, the chromatin substance remaining black.

The female nucleus moves very rapidly back towards the centre of the egg, so that the stage in which the nucleus is found in a position between the apex and the centre is rarely met with; accordingly the sharp distinction between chromatin and metaplasmic substance is not long visible, for as the nucleus moves towards the centre of the egg, it not only increases more and more in size, but becomes filled with a *uniformly* staining material. When the nucleus has reached the middle of the egg, instead of a sharply defined chromatin mass and a granular metaplasmic substance, there is found merely a uniform, rather wide meshed reticulum, filling the whole of the female nucleus, which is by this time of a huge size (fig. 18*a*). The reticulum stains uniformly in a chromatin-like manner.

An examination of a mature nucleus alone would lead one to believe that there had been an increase in the amount of chromatin *pari passu* with the increase in size of the nucleus. A consideration of the stage described above (fig. 11), shows clearly that this is not the case; the reticulum cannot be considered as consisting of chromatin material alone. That the chromatin only forms a very small portion of the mass of the female nucleus, a study of the stages of conjugation of male and female nuclei shows. From the huge female nucleus arise only a few small chromosomes, the ratio of whose total mass to that of the female nucleus is absurdly small (*vide infra* in the section on the Phenomena of Fertilization). An examination of female nuclei of oospheres which have failed to be fertilized, and which have begun to undergo disorganisation, also points to the same conclusion. In these nuclei (fig. 14) the main mass of the reticulum stains very slightly. The dark nuclear staining is confined to a few rod-shaped masses, which, no doubt represent the chromatin stock of the nucleus. Thus, although in the mature state the chromatin and the intruding substance (metaplasm) cannot be distinguished, yet that the two different substances really exist in the nucleus is shown very clearly, not only in the very young stages of the nucleus, but also during the changes which the nucleus goes through in fertilization, and during disorganisation.

It is true that, on washing the chromatin stain out of the reticulum of an adult female nucleus, a stage is finally reached in which a few granules alone remain stained. One cannot conclude, however, that these granules represent the real chromatin of the nucleus, for the same result can be obtained with ordinary vegetative nuclei (*e.g.*, the nuclei of the cells sheathing the oosphere).

The results obtained by FISCHER (22–24) in his experiments in fixing and staining, in which he shows how large a part physical condition plays in relation to staining reaction, must make one very careful in distinguishing substances by their various staining powers. The fact clearly remains, however, that the adult female nucleus consists mainly of a substance which is not chromatin—a substance which though, both on its first appearance and during the later stages of the nucleus, sharply dis-

tinguishable from the chromatin, yet in the mature condition of the nucleus can no longer be differentiated from it.

The most striking characteristic of this metaplasmic substance is, that at certain stages it has as strong, or almost as strong, an affinity for nuclear stains as has chromatin itself.

This metaplasmic substance first makes its appearance in the form of granules (fig. 11); later, however, as has been noted above, the granular appearance is lost, and the nucleus becomes filled with a uniformly staining meshwork. In later stages the metaplasmic substance may again partly take the form of granules (fig. 21).*

In the adult nucleus lying in the meshwork, a curious, hollow, vesicular structure, sickle-shaped in section, is to be seen (fig. 18*a*); this represents the nucleolus, which has enormously increased in size; sometimes, however, the nucleolus is represented merely by a number of large, deeply staining granules (figs. 12 and 13).

Although the female nucleus usually begins to grow in size and fill with metaplasmic substance immediately after its formation, yet in some cases the increase in size appears to be accompanied merely by vacuolation; such a young female nucleus is shown in fig. 12, where a number of large, deeply-staining granules, resembling nucleoli, together with a number of smaller staining granules, are to be seen lying in a large clear area. This stage, however, lasts only a very short time, for such a vacuolated nucleus soon begins to fill itself with metaplasmic material, and becomes an ordinary adult female nucleus. A vacuolated nucleus, which is in the process of filling itself with metaplasmic substance, is shown in fig. 13; there are present a number of large, deeply staining nucleoli.

As the nucleus moves back towards the centre of the egg, there is often to be seen a radiation of fibres from the nucleus towards the top of the oosphere. In well-preserved and well-stained preparations this radiation is sometimes very clear (fig. 12); some of the fibres forming it are very thick and seem to start from, or be attached to, the membrane of the nucleus. In one case (fig. 13) the fibres forming the radiation, though extending upwards to the top of the egg, were also arranged in a somewhat circular fashion round the nucleus.

Phenomena of Fertilization.

When the apex of the pollen tube reaches the oosphere, and the latter is in a fit state for fertilization, nearly the whole contents of the lower part of the tube pass

* Owing to the dense nature of the protoplasm and the resulting difficulty of direct observation of the fresh nucleus, it is impossible to be quite certain, in the light of our present knowledge of the action of fixing reagents, that the regular arrangement of the metaplasmic substance may not be, at least, in part, an artificial product of the method of preservation. The concordant results obtained by the use of alcohol, sublimate, and chromic acid, however, militate strongly against such a view. An examination, now in progress, of other members of the Abietineæ, will no doubt give additional evidence on this point.

into the protoplasm of the oosphere. The passage takes place through a well-marked pit in the wall of the tube, as has been described above. GOROSCHANKIN (15) in *Pinus Pumilio* observed that both the generative nuclei passed into the oosphere; STRASBURGER (8) established the same fact in *Picea vulgaris*. DIXON (14) was the first to observe that in *Pinus silvestris all the four nuclei* from the pollen tube (*i.e.*, the two generative nuclei, the pollen tube nucleus and the stalk cell nucleus) pass into the oosphere, though he was unable clearly to distinguish them one from the other as they lay embedded in the protoplasm of the oosphere.*

In a well-fixed and stained preparation all the four nuclei, together with a considerable number of starch grains from the pollen tube, can be clearly observed lying in the upper part of the oosphere. It cannot be doubted that cytoplasm also passes over into the oosphere, for each generative nucleus in the pollen tube is clearly surrounded by its own layer of cytoplasm, as can be observed in the stage when the tube is already in contact with the oosphere. It may here be noticed that small bodies staining deeply with fuchsin S. may be observed in the generative cell protoplasm. In fact they exactly resemble the bodies found in many meristematic cells, and freely set down as plastids. We cannot, of course, trace any connection with starch formation, but there is as much justification for calling these bodies leucoplasts as exists in many other cases. If leucoplasts are really present in the protoplasm of male generative cells, the general view that the male cell brings over no plastids to the egg appears to be directly contradicted. In addition to the cytoplasm belonging to the generative cells, it is probable that a certain amount of protoplasm from the apex of the pollen tube, in which the starch grains and nuclei are embedded, also passes over into the oosphere, since the whole of the lower part of the tube is practically emptied of its contents (figs. 15 and 16).

The behaviour of the four nuclei, after their passage into the oosphere, is quite easy to follow in good preparations. The two generative nuclei (round which the generative cell protoplasm can no longer be observed) are easily recognised by their large size. The pollen tube nucleus and stalk cell nucleus are, however, closely similar, and cannot usually be distinguished one from the other. One of the two generative nuclei moves forward alone towards the female nucleus, while the other three (invariably, as far as was observed) remain behind at the top of the oosphere. The movement of the male nucleus towards the female seems to be very rapid, for the stage in which it is to be observed on its course has been very rarely found. As it passes down the oosphere, the male nucleus increases in size, and this seems to be sometimes accompanied by an actual increase of stainable substance. Thus in fig. 18*a*, *b*, and fig. 39*a*, *b*, the generative nucleus which has reached the female possesses more stainable substance than its sister nucleus, which remains at the top

* In *Taxus baccata*, however, as observed by BELAJEFF (25), only a single nucleus, that of *one* of the generative cells, passes over into the cytoplasm of the egg. Evidence as to the behaviour of many other Gymnosperms is sadly wanting.

of the oosphere. In other cases, however, the increase in size seems merely to be due to vacuolation (fig. 19). No radiation or peculiar arrangement of protoplasm round the male nucleus, during its passage through the oosphere, could be observed, and no trace of centrospheres or of centrosomes was to be seen.

The ratio of the diameter of the male nucleus (which has a nearly spherical form) to the long axis of the (commonly ellipsoidal or egg-shaped) female nucleus at the time of fusion is usually about one-third, but cases have been observed in which it is as much as one-half.

The generative nucleus, which has taken no part in fertilization, shows a well-marked nucleolus (fig. 18*b* and fig. 39*b*). Two nucleoli, though much smaller, could also be observed in the other generative nucleus figured in 18*a* and 39*a*.

The actual process of conjugation is quite peculiar, the male nucleus actually pushing in the wall of the female, and coming to lie within the line of the original boundary of the latter, while the walls of both are still intact. In fig. 18*a* and fig. 39*a* the male nucleus has already begun to push back the wall of the female, but there is still a thin layer of protaplasm between them. (In this case the diameter of the male is less than one-third of the long axis of the female nucleus.) The two nuclei stain in an exactly similar manner, though the arrangement of the staining meshwork is not quite the same in both. The two nuclei, however, resemble each other in one respect. As was pointed out in treating of the maturation of the female nucleus, we cannot regard the whole mass of its stainable substance as chromatin, and the same may be said of the male nucleus. This is made clear at a slightly later stage of conjugation.

The male nucleus continues to penetrate the body of the female till it is almost completely enclosed by the latter, but actual fusion does not yet take place; in other words, the walls of both nuclei are still intact. This condition is to be observed in figs. 19 and 20. The male nucleus is here seen to be filled with a granular substance with slight staining power, while the chromatin is curiously arranged at the periphery in the form of short rods.

The female nucleus shows a similar state of things. Its interior also is filled with a granular substance quite like that of the male, while the whole of its chromatin is collected into a clump close beneath the male nucleus.

Thus the two nuclei after contact, but before fusion, undergo precisely similar changes. These changes give a clear proof of the existence of that metaplasmic substance whose gradual increase was traced during the maturation of the oosphere nucleus.

Scattered through this granular filling substance in both the male and female nuclei are to be found at this stage a number of deeply staining fine threads. With Heidenhain's iron-alum-hæmatoxylin and with gentian-violet, they stain like the threads of the achromatic spindle, and must be considered of kinoplasmic nature (using the word kinoplasm without any physiological connotation; in fact, applying

it merely to threadlike cytoplasmic structures with a special attraction for gentian-violet, etc.).

I believe these threads to be the first indication of the first segmentation spindle, and this view seems to be supported by evidence gained from the next stage of conjugation. Fig. 21 represents such a stage. The male nucleus is here seen to be completely embedded in the female, but though its outline is perfectly clear, and there seems to have been, so far, no fusion of substance, a definite wall can no longer be made out where it is in contact with the body of the female. Its posterior free surface, however, still possesses a definite wall, and the same may be said of the free surface of the female nucleus. We may therefore say that the actual fusion of the two nuclei has at length taken place. The male nucleus shows a well-marked nucleolus surrounded by a number of deeply staining rod-shaped chromatin bodies. A collection of similar chromatin masses is to be seen in the middle of the female nucleus. These bodies are clearly the same as those represented in a similar position in fig. 20. There can be little doubt that these bodies are really the chromosomes of the two nuclei. The stage represented in fig. 22, in which a longitudinally split chromosome is clearly visible, appears to follow closely upon that of fig. 21.

In fig. 21 a number of deeply staining straight (kinoplasmic) fibres are to be seen scattered about in the substance of both the male and female nuclei; these threads resemble in their staining reaction those of the earlier stage (fig. 20), and are evidently of the same nature. In this latter stage, however, the threads are much more numerous, and the granular substance in which they are embedded has correspondingly decreased. We must suppose, in fact, that new threads have been formed at the expense of the granular substance.

Both male and female nuclei are seen in the stage just described to be already preparing for the first segmentation division. In fact no resting fertilized nucleus is ever formed. The composite structure seen in fig. 21 is the nearest we ever get to it. But though the distinction is clearly quite arbitrary, we will, for the sake of convenience, describe the succeeding stages in which the first segmentation spindle is definitely beginning to be formed under the head of

Division of the Fertilized Nucleus.

Fig. 22 represents an early stage in the formation of the spindle. The wall of the composite nucleus has broken down, although its outline is perfectly clear; its whole cavity is filled with kinoplasmic fibres which at the periphery run in all directions, with here and there a definite bundle. In the centre, however, the fibres are thicker and have assumed the form of an irregular spindle. This spindle seems to belong to the type of tri- or multi-polar spindle described by Belajeff (26) and Farmer (27), and lately figured so clearly by Osterhout (18) and Mottier (19), who have shown that it is often the normal antecedent of the bipolar spindle.

The chromosomes lie scattered somewhat irregularly in the nuclear cavity, but the majority of them seem to have some relation to the irregular spindle. The oosphere is cut obliquely to its long axis, so that the exact arrangement is difficult to make out, but by carefully drawing all the chromosomes in the ten consecutive sections passing through the spindle (fig. 22 represents one of the sections), it was found that the whole of the chromosomes fell roughly into two groups which undoubtedly correspond respectively with the male and female groups. Many of the chromosomes have an open **V** shape; one such appears in fig. 22. There can be no doubt that the longitudinal splitting has here already taken place, the two daughter chromosomes remaining attached at one end only.

Fig. 23 shows about the same stage. The outline of the nucleus is still visible as a clear band in the protoplasm. The bipolar spindle is not fully developed and occupies merely the upper end of the nuclear area. The chromosomes are here very long and thin, and appear to be already longitudinally split as in the last figure.

Fig. 24 shows one pole of the first segmentation spindle with its group of daughter chromosomes. The spindle-fibres are very clear and there is a distinct radiation from the pole. Between the fibres lie a number of small staining granules. The chromosomes have the form of straight rods.

Plate 14, fig. 31, shows a later stage where the first segmentation nucleus is already formed. A delicate nuclear wall can be clearly seen, and also remains of the achromatic spindle. A number of larger and smaller granules, presumably of nucleolar origin, are to be found scattered around the nucleus, although the nuclear wall has been already formed. These have been cast out of the fertilized nucleus, but they do not return to the daughter nuclei (*cf.* ZIMMERMANN, 30) but disappear in the protoplasm.

The first two segmentation nuclei when newly formed are very small and have the usual character of young daughter nuclei (fig. 31); but almost immediately they begin to swell very considerably. For a time this swelling is due mainly to vacuolation, but later on they take up metaplasmic substance, just as the oosphere nucleus does during maturation. In fact they pass through exactly similar stages. The segmentation nuclei may reach to one-third the size of the unfertilized female nucleus.

The two first segmentation nuclei while lying free in the protoplasm of the egg, soon begin to divide. Fig. 25 shows this process just beginning. The chromosomes of both nuclei are already differentiated, but the spindles are not yet formed. The metaplasm in the form of a lightly-staining granular substance fills the nuclear cavities. Fig. 26 represents a later stage of one of these nuclei. The chromosomes are here seen on the spindle. The latter is surrounded by a clear space crossed by bands of protoplasm.

The division of the first two nuclei seems always to take place simultaneously (fig. 25). The four nuclei so produced lie quite free in the cytoplasm of the egg (fig. 27).

It is usually believed, from the observations of STRASBURGER* and others, that the fertilized nucleus in the Abietineæ moves down to the base of the egg and there divides, but in one of the later publications STRASBURGER (9) states that the fertilized nucleus (Keimkern) seemed in all cases to divide in its original position, its products moving down to the base of the egg. DIXON found also that the fertilized nucleus divided in the centre of the egg. It is clear from my observations that though a definite fertilized nucleus hardly exists as such, the first segmentation spindle appears in the position formerly occupied by the female nucleus. The two daughter nuclei themselves also undergo division before they begin to wander down to the base of the egg.

BEHAVIOUR OF THE SEGMENTATION NUCLEI IN THE PROTOPLASM OF THE OOSPHERE.

The four segmentation nuclei which lie free in the cytoplasm are at their first origin very small, but they soon increase in size, at first merely by vacuolation, but later becoming filled with metaplasmic substance (*vide supra*). After they have reached their full size they begin to move down towards the base of the oosphere. At this time a well-defined ring of cytoplasmic fibres (Filzschicht) appears round them. BELAJEFF (26) and STRASBURGER have described somewhat similar appearances in connection with the division of the nucleus.† In figs. 33 and 34 the nuclei show this layer of fibres very clearly, they also show the filling metaplasmic substance distinctly. Running through the metaplasmic substance deeply staining threads are to be seen. These threads have exactly the structure of the nuclear threads of an ordinary nucleus, namely, a number of chromatin granules embedded in a non-staining, or only slightly staining, matrix.

When the nuclei approach the bottom of the oosphere the sheath of fibres becomes lost, and in their place other fibres are found, which radiate out from the nuclei into the general protoplasm (fig. 36). At the stage shown in the figure the radiating fibres have no very definite arrangement, they all take their origin from the membrane of the nucleus, and most of them are directed away from the base of the oosphere.

At a later stage, when the four nuclei have arranged themselves in one plane at the base of the egg, the fibres also are confined to the lower part of the egg; the four nuclei then appear to lie in a mass of fibres, this mass rising to a little above the level of the nuclei.

* The stages relating to the changes in the oosphere of *Pinus* after fertilization, figured by STRASBURGER in 'Die Coniferen und Gnetaceen,' Taf. 8, figs. 14–18, and stigmatised by him as abnormal, in reality represent the normal procession of events.

† The structures described by BELAJEFF and STRASBURGER consist, however, of an actual network of interlacing fibres round the nucleus; the structures here described consist of a small number of fibres running circularly round the nucleus parallel to the nuclear wall (fig. 35).

After this, the two walls are formed at right angles to one another and to the base of the oosphere; each nucleus thus lies at the bottom of a kind of shaft which is open above (*i.e.*, towards the main mass of the oosphere). After the wall formation the fibres disappear. The exact part which the fibres play in the process of formation of the walls was not made out, but of their connection with it there can be little doubt. The whole process bears a striking analogy with the curious process described by HARPER (17) as occurring at the formation of ascospores in the ascus. In the case of the ascus the nuclei lie free in the protoplasm as in the egg of *Pinus*; their number is, however, 8. At the time of the formation of the ascospore wall round each of these nuclei fibres appear, which run in a circular manner round the nucleus at some distance from it; in these fibres the new wall makes its appearance. These fibres do not arise from all parts of the nucleus, but they take their origin from one point, the apex of the elongated nucleus, at which point there is apparently a centrosome.

In both cases we have the formation, apart from nuclear division, of cell walls round nuclei lying free in a large mass of protoplasm, and in both cases the formation is preceded by an arrangement of fibres which run from the nuclei concerned; in the one case the fibres run to a definite point of the nucleus, in connection, apparently, with a centrosome, in the other case the fibres arise generally from the surface of the nucleus.

Similar radiations were described by STRASBURGER at the formation of walls round the free lying segmentation nuclei in the oosphere of *Ephedra*, but in this case the fibres stand out equally all round at right angles to the surface of the nucleus. This difference may be connected with the fact that in *Ephedra* the segmentation nuclei lie perfectly free, and separated by a considerable distance from one another, while in *Pinus* the nuclei are closely aggregated together at the base.

ON THE STRUCTURE OF THE CYTOPLASM OF THE EGG AND ITS RELATION TO SPINDLE FORMATION.

Owing to the presence of numerous proteid vacuoles, and of both large and small staining granules and masses in the cytoplasm, the minute structure of the latter is not easy to make out.

In many of the preparations of younger oospheres signs of an underlying network in the dense protoplasm were observed, but these were not sufficiently clear to enable one to draw certain conclusions. In later stages of the oosphere, however, in which four segmentation nuclei have been derived from the fertilized nucleus, the structure of the cytoplasm could be clearly made out (figs. 28 and 29). When fixed with Flemming's fluid, and stained with his triple stain, the mass of large and small granules (which is the usual appearance of the protoplasm of the oosphere under a low power, or when badly stained), is seen to be permeated with a very delicate

reticulum. The reticulum appears as very fine lines stained blue with gentian-violet (fig. 28); in still more favourable preparations these lines resolve themselves into rows of very small stained granules (fig. 29). The meshes of the reticulum are not clear and transparent, as is usually the case with the cytoplasmic reticula described by BÜTSCHLI and others, but are filled with an amorphous substance stained light yellow, with here and there a darker staining granule embedded in its mass.*

The amorphous substance has the appearance of a coagulum, suggesting that the meshes of the cytoplasm were originally filled with proteid in solution, which has been coagulated by the action of the fixing reagents.

The reticulum to be found in the cytoplasm does not seem to be the optical expression of a foam structure (a Wabenbau such as described by BÜTSCHLI), for on the one hand the meshes are too large (the meshes of the reticulum described by BÜTSCHLI were always very small), and on the other hand they do not run in a sufficiently regular manner. One must conclude that the cytoplasm of the oosphere is really of a reticular nature, and that there is no evidence that it has honeycomb or foam structure.

I have been enabled to make out some points as to the relation of this reticulum to spindle formation in the division of the segmentation nuclei.

No trace of cell wall is to be found in connection with the spindles formed at the division of these nuclei; after division the spindle simply fades away into the general cytoplasm. Figs. 28 and 29 show stages in the disappearance of the spindle. In such a preparation as fig. 28 a definite spindle is still to be seen between the two segmentation nuclei; the spindle has no distinct periphery, but gradually passes over into the ordinary reticulum; the threads of the spindle can be distinctly traced into the threads of the reticulum. Fig. 29 shows a little later stage in this process of extinction of the spindle; the spindle in fact hardly exists as such, but is only to be distinguished as a reticulum with small meshes; this reticulum passes over directly into the wider meshed general reticulum.

These observations agree completely with those of WILSON (21) on the cytoplasm in the egg of the Sea Urchin. FARMER and WILLIAMS (20) made similar observations in the oogonia and oosphere of *Fucus*.

Such observations as these militate strongly against the view of a special spindle forming substance, either archoplasmic or kinoplasmic (*cf.* STRASBURGER (12)). They point to the fact that the spindle forming or kinoplasmic fibres result from a rearrangement of the ordinary cytoplasmic reticulum.

That the spindle fibres stain more deeply with gentian violet, &c., than the other

* It is this substance which makes it so difficult to render visible the reticular structure of the cytoplasm. If the washing out with orange G. is insufficient, the substance in the meshes remains blue, and obscures the reticulum; if the washing is carried too far, the blue of the reticulum becomes lost. When the differentiation is at its best the amorphous substance appears as transparent yellow masses, lying in the meshes of a blue reticulum.

reticular threads, &c., can be explained in the light of FISCHER'S researches, by their greater thickness, and perhaps greater density. It is quite unnecessary to suppose that the spindle fibres consist of a substance different in kind from that of the ordinary reticulum. It seems that, in the egg of *Pinus* at least, the so-called kinoplasm fibres are produced by a mere rearrangement of the fibres of the trophoplasmic recticulum.

ON THE NUMBER OF THE CHROMOSOMES.

On the Number of Chromosomes in the Oosphere.

The final division which differentiates the oosphere and ventral canal cell was met with in a number of cases, and in nearly all these the chromosomes could be counted. With properly stained microtome sections of well-fixed material, there is no very great difficulty in the process.

When the chromosomes are arranged at the equator of the spindle, which is very bluntly pointed and without polar radiations, they have the form of straight rods (figs. 5 and 7); later on, when the daughter segments move towards the poles, they have the form of rods sharply bent in the middle. It is at this stage that the chromosomes can be counted most easily.

* Figures 6*a*, *b*, *c*, *d*, and *e* are accurate representations of five consecutive sections through a spindle in which the chromosomes are moving towards the poles. The lower pole is represented in figs. *a* and *b*: the chromosomes there seen are those which will give origin directly to the oosphere nucleus. The bent condition of the chromosomes renders them extremely easy to count, for it is only necessary to pay attention to the angles; as long as these are left intact it is immaterial if the chromosomes are actually cut into portions by the knife, a condition of things which is almost sure to occur when the spindle is spread over a number of sections.

Six of these angles are to be observed in each section; one of them, however, seems to have been slightly broken by the knife. There are thus 12 chromosomes which go to make up the oosphere nucleus. The small separate straight portions visible in fig. 6*b* have been cut off the projecting legs of the chromosomes by the knife.

The chromosomes of the upper pole, those which go to form the ventral canal cell, are to be found in figs. 6*b*, *c*, *d*, and *e*; in *c*, *d*, and *e*, the upper pole alone is represented. In these four sections together, 11 chromosomes are to be clearly counted; the two portions marked with an asterisk represent, no doubt, the twelfth, which has been cut by the knife exactly through the angle.

Fig. 7 shows another division with the chromosomes arranged at the equator of the spindle; 11 can be distinctly counted, a twelfth is rather doubtful.

I have endeavoured to give here accurate drawings and microphotographs* of some

* Microphotographs of the preparations figured in 6*a* and *b* are to be seen on Plate 14, figs. 38*a* and *b*.

of the preparations on which the conclusions as to the number of chromosomes are based, instead of merely stating that such and such a number was observed.

In several other cases in which the chromosomes were observed and counted another method was followed. Owing to the thinness of the sections, the same chromosome was often found distributed in portions over a number of sections; from this cause and from their closely intertwined arrangement, it was impossible to count them in the ordinary way. The method used under these circumstances was as follows:—The various portions of chromosomes in the consecutive sections were all carefully drawn with a camera lucida, under a constant magnification, the lengths of these several portions were carefully measured, and the sum of these lengths gave the total length of all the chromosomes: it was always possible to obtain a view of one or two chromosomes which lay wholly in one section, the length of one of these gave the length of a single chromosome; the total length of all the chromosomes, when divided by the length of a single chromosome, will obviously give the number of the chromosomes. This number was always about 12 (11·7 and 11·5 in two of the cases in question).

Dixon concluded, from his observations, that the number of chromosomes in the oosphere nucleus was eight; he was, however, never able to count the chromosomes on the spindle directly, owing, as he says, to their feeble staining capacity. The evidence on which he relied was of a purely indirect nature, based on the number of bodies, which he calls chromosomes, to be found in the fully developed ventral canal cell. This evidence has been dealt with elsewhere, see section "On the Formation and Fate of the Ventral Canal Cell."

On the Number of Chromosomes in the Prothallial Tissue.

In spite of renewed attempts, I have not been fortunate enough to obtain the first division of the embryo sac mother cell, so that exactly where the reduction takes place cannot be stated; observations were made, however, on the number of chromosomes in the endosperm (Prothallial tissue).

Dixon (14), from his observations, concluded that the nuclei of young embryo sacs, and those at the base of older ones, had usually *eight* chromosomes, while, however, nuclei in the upper part of the endosperm, and those of the large and prominent cells sheathing the oospheres, possessed usually *twelve* chromosomes, though sometimes *eight* and *twenty-four*. I am unable to confirm Dixon's observations.

The cells surrounding the oospheres have very large nuclei with, in the resting state, a very distinct staining network, and many of them show with the utmost clearness the first stages of division: the coil stage, the transverse splitting of the single thread into chromosomes, the formation of the chromosomes from regularly arranged segments (Balbiani-Pfitzner granules), and the gradual disappearance of the nucleolus, can all be most sharply distinguished after fixing in Flemming's fluid

and staining with Flemming's triple stain. Very few of the nuclei, however, go further and reach the stage in which a distinct spindle is to be seen; most seem to become disorganised before they have succeeded in reaching such a stage. I have counted the chromosomes both when on the spindle and when, immediately after their formation, they lie free in the hollow of the nucleus, and in all cases where they could be counted with any hope of accuracy, *twelve* were to be found; the numbers *eight* and *twenty-four* were never found. DIXON'S figures suggest defective preservation, and I found as a matter of experience, that alcohol did not preserve their structure at all well; Flemming's fluid was the only fluid which gave satisfactory results. Plate 14, fig. 37, represents one section through a dividing nucleus of one of the sheathing cells of the oosphere; the chromosomes have been just formed, and they lie free in the cavity of the nucleus; nine whole ones are to be seen and portions of three others.

The chromosomes were also counted in the other cells of the embryo sac; this can only be done in the earlier stages before the archegonia and their sheaths are fully developed, for later on, the cells in the lower part of the embryo sac, cease altogether to divide and lose nearly all their contents. The counting is very difficult in these cells, owing to the small size and the close packing of the chromosomes, but from a number of countings, the usual number obtained was *eleven*, *twelve*, or *thirteen*, a result which may be taken to point to the number *twelve* as the usual number of chromosomes in the cells of the embryo sac.

On the Number of Chromosomes in the Pollen.

Material for this part of the work was found to be most easily obtained by the method which BELAJEFF used in his study of karyokinesis in *Larix pollen*. Living branches of *Pinus silvestris*, bearing male flowers with the pollen mother cells still undivided, were brought into the laboratory and placed in water; division then took place rapidly or slowly according to the temperature.

STRASBURGER (9) in 1892 stated that the number of chromosomes in the pollen of *Pinus silvestris* was twelve. In a later paper (10), owing to the results obtained by DIXON, he was led to renewed investigations on the pollen. As a result of this later work, he concludes that "both pollen mother cells and pollen grains of *Pinus silvestris* have only eight chromosomes;" he points out, however, that the counting is attended with great difficulty. I have examined chiefly the first division of the pollen mother cells. The irregular shape of the chromosomes and the fact that they overlap one another renders the counting very difficult; no attempt can be made to count them in a side view of the spindle; in good polar views, however, they can be counted, though with difficulty. In a considerable number of polar views of nuclei in the equatorial plate stage, I have been able to count undoubtedly *twelve* chromosomes, and in no case in which at all a good view could be obtained were less than *ten* to be

made out. Of a certain *five* countings made at one time, in *two* cases there were certainly *twelve*, in *two* others at least *eleven* could be seen, and in the fifth case at least *ten* could be made out. There is thus no reason to doubt that the number of chromosomes in the pollen mother cells is usually twelve, as STRASBURGER first stated.

Thus the male and female gametophyte agree with one another in possessing cells with the reduced number (12) of chromosomes.

On the Number of Chromosomes in the Fertilized Egg Cell.

The first division of the fertilized nucleus was met with three times in all, but in only one case (fig. 24) could the chromosomes be counted. In this figure one pole of the spindle is seen slightly obliquely from above, all the chromosomes are not represented in the drawing as they lie one above the other, but they can be made out by careful focussing ; there are then found to be exactly *twenty-four*.

The method of observation may be of interest,—the chromosomes were not counted directly in the preparation, but they were all rough drawn, their number being meanwhile neglected, then, only after it was certain that all had been included, was the number ascertained from the drawing.

My observations do not agree with those of DIXON, who found sixteen chromosomes in the first division of the fertilized nucleus.

On the Number of Chromosomes in the Cells of the Sporophyte.

Observations were made on the tissue of the nucellus, of the stem apex, and of the sporophytic tissue of the male flower.

The observations on the cells of the nucellus were few in number, the number that could be made out was always however *over twenty* ; here also the chromosomes are small and closely pressed together. In the stem apex and tissue of the male flower the number of chromosomes is seen at a glance to be much higher than in the embryo sac or pollen, and on counting they were always found to be *over twenty* in number, though absolute accuracy seemed here almost impossible. It seems allowable to consider here that the number is double that of the cells of the oophyte, namely, *twenty-four*. It is interesting to note that in the free floating tapetal cells, in process of absorption by the young embryo sac, huge sinuous equatorial plates, consisting of over *forty* chromosomes, were several times observed.

ON THE QUESTION OF CENTROSPHERES.

No centrospheres or centrosomes were visible at any stage of fertilization, though one would expect them, if present, to be clearly seen in the large mass of protoplasm

of the egg. It was partly with the object of studying centrospheres that this work was begun. The researches of FARMER, STRASBURGER, OSTERHOUT (15), MOTTIER (16), &c., have shown that the existence of centrospheres is very doubtful in plants higher than the Mosses, and my observations fully bear out their conclusions.

OVERTON states that he has found the endosperm of some Gymnosperms very favourable for the observation of centrospheres; I am, however, unable to confirm him in the case of *Pinus*. A curious structure, of unknown nature, having somewhat the shape of a centrosphere, was once seen in the ventral canal cell (fig. 9*b*), and a similar structure was also once observed between the male and female nuclei at the time of conjugation (fig. 20).

PROTEID VACUOLES.

These curious structures, so common in the oosphere in the Abietineæ, were very troublesome to the older observers, and led both HOFMEISTER and GOROSCHANKIN astray. The latter took them to be nuclear structures; this is hardly surprising, for they take up stain with such avidity that even in microtome sections of material, fixed and stained by the best methods, they often bear at the first glance a strong resemblance to nuclei.

The number of vacuoles in the oosphere varies very much, some oospheres being almost full of them, others possessing only a few scattered ones; as a rule they are not found in any number till after the formation of the ventral canal cell.

Under a high power, in fixed material, they appear as more or less spherical masses lying in the protoplasm, and causing interruption in the general cytoplasmic reticulum. The reticulum forms a delicate membrane or vacuole wall round them. Fig. 30 is an accurate representation of a proteid vacuole, as seen under a high power in a fixed and stained section; it appears to have a very complicated structure. It consists of a number of granules and masses, the granules shown deeply shaded have exactly the appearance of *nucleoli*, the irregular masses stain less deeply; some of the larger ones enclose smaller ones, and others show radiations from a central granule.

Similar nuclear-like granules and other irregular masses are to be found scattered about in the general protoplasm; the proteid vacuoles seem to be mere collections of these, separated off by a definite membrane. The scattered granules and masses may perhaps be considered partly as artefacts, due to precipitation of proteid by the fixing fluids.

CONCLUSION.

In all cases of fertilization hitherto described in plants, in either Cryptogams or Phanerogams, the male and female nuclei fuse while in the resting condition,* almost immediately after they come in contact, and form a definite resting fertilized

* MOTTIER ('Jahr. f. wiss. Bot.,' bd. 31, Heft 1, Tat. 3, fig. 24) has given a very beautiful figure of this fusion in *Lilium candidum*.

nucleus: no case has hitherto been observed in plants comparable to that of the fertilization of many animal ova (*e.g.*, *Ascaris*).* *Pinus*, however, is seen to depart from the usual plant type, and to agree very closely with such a type as that of *Ascaris*. The process of fusion of its nuclei is exceedingly slow; the male nucleus after coming into contact with the female gradually forces its way into the latter, till it becomes completely enclosed, but actual breaking down of the respective walls, *i.e.*, actual fusion, is for a long time delayed.

Before fusion has taken place the nuclei react upon one another, and traces of the first segmentation spindle appear as staining kinoplasmic threads (figs. 20 and 21).

The chromosomes of the male and female nuclei are found as two separate clumps, while the outlines of the two nuclei are still visible. At a later stage, the chromosomes from the male and female nuclei could be distinguished into two groups as they lay free in the protoplasm at the time that the first segmentation spindle was in the multipolar condition. At this stage the chromosomes have already split longitudinally (fig. 22).

As the process of fusion is very slow, and no definite fertilized nucleus formed, it is a little difficult to say when fertilization begins and ends. Fertilization may be considered as beginning before fusion, directly the male and female nuclei begin to react upon one another (a reaction made apparent by the rearrangement of the chromatin and the appearance in both nuclei of kinoplasmic threads): it can hardly be considered to be at an end till a stage is reached which is normal for the life cycle of an ordinary nucleus. This stage, in most cases of fertilization in plants, is that of the resting fertilized nucleus. In the case of *Pinus silvestris* no such resting nucleus exists, and such a stage is only reached when the half chromosomes, derived from the male and female nuclei respectively, fuse together at the poles of the first segmentation spindle; then, and not till then, can the process of fertilization be considered as complete.

The egg nucleus immediately after its formation goes through a curious process of maturation. In this process the nucleus increases very largely in bulk, and at the same time becomes filled with a metaplasmic substance (probably of proteid nature). This substance appears at first in a granular form, and can then be clearly distinguished from the chromatin thread. Very soon, however, the distinction between the chromatin and metaplasmic substance is lost, and the whole interior of the swollen nucleus is filled with a regular meshwork, which reacts towards staining reagents exactly like chromatin. In this condition the egg nucleus awaits fertilization. The chromatin of the egg nucleus can again be distinguished on fertilization, and can usually be clearly distinguished in unfertilized nuclei which have undergone degeneration.

* Since the above was written, BERLESE (43) has published a paper on the *Peronosporeæ*, in which he states, that in some members of that group, the male and female nuclei become resolved into chromosomes before fusion. He figures this condition in *Peronospora Alsinearum* and *P. effusa*.

The male nucleus during its passage towards the female nucleus undergoes similar changes, though to a less degree. The segmentation nuclei also undergo similar changes, but in them the chromatin can usually be sharply distinguished from the filling substance.

On fertilization and on the appearance of the chromosomes, the metaplasmic substance loses, in part, its strong attraction for stains; on division a portion of it seems to help in the formation of the achromatic spindle, while the remainder is lost in the surrounding protoplasm.

The *raison d'être* of this maturation process is not at all clear. It is true that part of the metaplasm seems to be used in spindle formation, but much more is taken up than can be used for this purpose, as is especially well seen in the case of the four segmentation nuclei, which, when moving down to the base of the egg, are usually packed with metaplasm (figs. 33 and 34); on division of these nuclei most of the metaplasm is thrown out, and seems to form a clear mass round the dividing nuclei, as STRASBURGER observed some time ago. When it is considered that all the nuclei which come to lie in the large mass of protoplasm of the oosphere, namely, the female nucleus, the male nucleus and the four segmentation nuclei, all go through these curious and, in part, seemingly fruitless changes, it is suggested that the changes may be due to the direct action of the environment, to some compelling action of the surrounding protoplasm. It is interesting to note in this connection, that the lower daughter nuclei (formed by division of the four segmentation nuclei), which are separated by cell walls from the main mass of the egg, undergo none of these changes. The increase in size of the nuclei is also, no doubt, in some way connected with the huge size of the egg, for STRASBURGER and others have shown that there is a distinct relation between the size of the nucleus and that of its cell. A comparative study of other Abietineæ in which, as far as observations go, a similar process seems to occur, will no doubt throw light on the physiological meaning of this process.

The observations of fertilization in *Pinus* support the ordinarily accepted view that the chromatin is the essential part of the nucleus, for though the actual masses of the male and female nuclei are very different, yet the masses of male and female chromatin and the male and female chromosomes are, as far as can be made out, exactly equal. The male nucleus naturally possesses a much larger percentage of chromatin than the female.

The nuclei of the male cells of Algæ, Mosses, Vascular Cryptogams and Angiosperms, usually contain no nucleoli, or, if these structures are present in the young condition, they disappear later (see ZACHARIAS, (31), p. 253, where numerous references are given, also STRASBURGER, 11). In the Gymnosperms, however, nucleoli in the male nuclei seem not uncommon. Thus, BELAJEFF (25), figures a nucleolus in the male nucleus of *Taxus*, just before its fusion with the female, and STRASBURGER (9) figures a number of male cells in the pollen tubes of various Gymnosperms, with distinct

nucleoli in their nuclei, at least in the young condition. He figures, however, without nucleoli the male nuclei of *Pinus silvestris*, when they lie just above the oosphere.

Fig. 18*b* shows a distinct nucleolus in the generative nucleus lying in the protoplasm at the top of the egg; a few small nucleoli can also be made out in the other generative nucleus of fig. 18*a*. A very distinct nucleolus is to be observed in the male nucleus at a later stage (fig. 21). There seems to be no doubt that the generative nuclei increase considerably in richness of chromatin contents after their first formation.

No trace of cilia was to be seen in the generative cells, and no trace of the structure called by WEBBER (42) a *blepharoplast*. Apart from the existence of cilia, however, the generative cells of *Pinus* and of the Abietineæ and Conifers in general do not differ widely from the antherozoids described in *Cycas* (36), *Gingko* (38, 39), and *Zamia* (41). They all consist of a cell, with a large nucleus of the ordinary structure, completely surrounded by a thin layer of cytoplasm; there is however a most striking difference in size of the respective cells. The generative cells of *Pinus* are nearly spherical and somewhat less than 40 μ in diameter; the antherozoids of *Gingko*, however, are 82 $\mu \times 49$ μ, those of *Cycas* a little larger, while those of *Zamia* reach a size of about 300 $\mu \times 280$ μ. The antherozoids hitherto described in Gymnosperms have a very slightly specialized form. They possess neither the condensed nucleus nor the elongated twisted form, nor the very reduced cytoplasmic body, all of which are so characteristic of the antherozoids of the Filicineæ, &c. They are little more than an ordinary cell of a somewhat oval form, with a large nucleus and a small amount of cytoplasm, provided with a spiral band of cilia: WEBBER'S figures (41) show this clearly. The generative cell of *Pinus silvestris*, if provided with a spiral band of cilia, would without further modification become a small antherozoid almost exactly of the type of *Zamia*. This slight degree of specialization of structure as compared with the antherozoids of Mosses and Ferns is correlated, no doubt, with the protected condition of the free antherozoids, and also with the much smaller distance through which they have to swim to reach the oosphere. It would seem that in *Cycads* and in *Gingko* the pollen tube has not yet reached perfection as the carrier of the generative cells, that is, the method of fertilization is not yet completely *siphonogamic*; therefore a certain amount of motility is necessary for the cells themselves. In *Cycas* and *Gingko* the cavity above the prothallium is filled with fluid, and the pollen tube never comes within a considerable distance of the archegonia, but the antherozoids escape into the fluid above the archegonia and thence make their way to the oospheres. In *Zamia*, however, the prothallial cavity contains only air: the pollen tube, however, seems actually to come in contact with the neck of the archegonium, then (as WEBBER believes) the tube bursts and itself supplies a drop of fluid in which the antherozoids can make their way, through the neck of the archegonia, to the oospheres.* It is a

* Although it is quite clear that the antherozoids of *Zamia* are capable, when set free from the pollen

very slight step from *Zamia* to the method of fertilization in all Conifers hitherto described (except *Gingko*), where fertilization is *completely siphonogamic*, the pollen tube making its way through the neck of the archegonium and carrying the generative cells directly to the oosphere. Taking this into consideration, and also the fact that the fertilization of Conifers has been worked out in detail in such a small number of cases, it is quite possible that antherozoids will be discovered in a number of other forms. Even if *Gingko* remains the only representative of the group, with actually motile antherozoids, it seems not improbable—unless we are to suppose that disappearance of cilia has taken place co-temporaneously with the attainment of perfect siphonogamic fertilization—that cilia in a functionless condition, or, at least, traces of them, will be observed in some of the generative cells of Conifers. It will be interesting to see if any trace of the structure called by WEBBER (42) a *blepharoplast*, which is so striking in the generative cells of *Zamia*, will be discovered later in the immotile cells of the Conifers. It is this important structure which gives origin, as BELAJEFF (32–35) has shown, to the ciliary band in the Filicineæ, Equisetineæ, and Characeæ; WEBBER (40, 41) also has described and figured most clearly the development of the ciliary band from it in *Zamia*, and HIRASE and IKENO (36) state that it performs the same function in the antherozoids of *Cycas* and *Gingko*.

The contents pass out from the pollen tube through a well-marked pit in its wall, by the rupture of the pit-closing membrane.

The cytoplasm of the egg shows a very fine reticular structure; no evidence could be obtained for the existence of a foam structure.

The first segmentation spindle is, in part, nuclear in origin. In later nuclear divisions in the egg the achromatic spindle seems to be formed by a mere rearrangement of the threads of the cytoplasmic reticulum.

A peculiar arrangement of cytoplasmic fibres round the four segmentation nuclei, when they move down to the base of the egg, seems to be connected with cell wall formation and to be analogous with the peculiar radiations from the ascospore nuclei, described by HARPER (17), in connection with the delimitation of the ascospores in the protoplasm of the ascus.

The number of chromosomes in the egg is shown to be 12, and the same number was found in the pollen mother cells; the cells of the female gametophyte have also 12 chromosomes. The number of chromosomes at the first division after fertilization was found to be 24. In the tissue of the sporophyte over 21 chromosomes could be counted, and presumably 24 are always present. Thus OVERTON'S generalisation as to the number of chromosomes in the two generations is completely confirmed.

tube, of prolonged swimming movements, it does not seem perfectly certain from WEBBER'S description that, in the normal course of fertilization, the pollen tubes were observed to stop short at the neck of the archegonium, (he does not figure the neck of a fertilized archegonium).

List of Papers.

1. Hofmeister. Embryobildung der Phanerogamen. Jahrb. f. wiss. Bot., Bd. I., p. 168, 1858.
2. ——— Vergleichende Untersuchungen (Engl. Transl.). 1862.
3. ——— Lehre der Pflanzenzelle, 1867, p. 120.
4. Strasburger. Die Befruchtung bei den Coniferen. 1869.
5. ——— Die Coniferen und die Gnetaceen. 1872.
6. ——— Die Befruchtung und Zelltheilung. Jenaische Zeitsch., vol. II., 1877.
7. ——— Die Angiospermen und die Gymnospermen. 1879.
8. ——— Neue Untersuchungen u.d. Befruchtungsvorgang bei den Phanerogamen. 1884.
9. ——— Ueber das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen, 1892, p. 34.
10. ——— The Periodic Reduction in the Number of Chromosomes, &c. Annals of Botany, vol. 8, 1894.
11. ——— Ueber Befruchtung. Jahrb. f. wiss. Bot., Bd. 30, Heft 3, 1897.
12. ——— Ueber Cytoplasmastrukturen, &c. Jahrb. f. wiss. Bot., Bd. 30, Heft 3, 1897.
13. ——— Kerntheilung und Befruchtung bei Fucus. Jahrb. f. wiss. Bot., Bd. 30, Heft 3, 1897.
14. Dixon, H. H. Fertilisation of Pinus Silvestris. Annals of Botany, 1894.
15. Goroschankin. Ueber den Bef.-Process bei Pinus Pumilio. Strassburg, 1883.
16. ——— Zur Kenntniss der Corpuscula bei den Gymnospermen. Botan. Zeit. Bd. 41, 1883.
17. Harper, R. A. Kerntheilung und freie Zellbildung im Ascus. Jahrb. f. wiss. Bot., Bd. 30, Heft 3, 1897.
18. Osterhout, W. J. V. Ueber Entstehung der Karyokinetischen Spindel bei Equisetum. Jahrb. f. wiss. Bot., Bd. 30, Heft 3, 1897.
19. Mottier, D. M. Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen u. Monocotylen. Jahrb. f. wiss. Bot., Bd. 30, Heft 3, 1897.
20. Farmer and Williams. Fertilisation and Segmentation of the Spore in Fucus. Proc. Roy. Soc., vol. 60.
21. Wilson, E. B. Archoplasm, Centrosome, and Chromatin in the Sea-Urchin Egg. Journal of Morphology, vol. 11, 1895.
22. Fischer, A. Untersuchungen über den Bau der Cyanophyceen und Bakterien. Alfred Fischer, Jena, 1897.
23. ——— Zur Kritik der Fixierungsmethoden und der Granula. Anat. Anzeiger, 1894.

24. FISCHER, A. Neue Beiträge z. Kritik der Fixierungsmethoden. Anat. Anzeiger, 1895.
25. BELAJEFF, W. Zur Lehre von dem Pollenschlauche der Gymnospermen. Berichte der Deutschen Botanischen Gesellschaft, Bd. 9, 1891.
26. ——— Zur Kenntniss der Karyokinese bei den Pflanzen. Flora, Ergbd, 1894.
27. FARMER, J. BRETLAND. Ueber Kerntheilung in Lilium Antheren, besonders in Bezug auf die Centrosomenfrage. Flora, Bd. 80, 1895, p. 56.
28. GUIGNARD, J. Nouvelles Études sur la Fécondation. Ann. des Sciences Nat. Bot., 7 Série, 1891.
29. FAIRCHILD, D. G. On a perforated porcelain cylinder as washing apparatus. Zeit. für wissen. Mikroskopie, 1895.
30. ZIMMERMAN. Beiträge zur Morphologie u. Physiologie der Pflanzenzelle. Bd. 2, Heft 1, 1893.
31. ZACHARIAS. Ueber das Verhalten des Zellkerns in wachsenden Zellen. Flora, Ergzbd., 1895.
32. BELAJEFF, W. Ueber den Nebenkern in spermatogenen Zellen und die Spermatogenese bei den Farnkräutern (Vor. Mitt.). Ber. d. Deut. Bot. Ges., vol. 15, 1897.
33. ——— Ueber die Spermatogenese bei den Schachtelhalmen. *Ibid.*
34. ——— Ueber die Aehnlichkeit einiger Erscheinungen in der Spermatogenese bei Thieren und Pflanzen (Vor. Mitt.). *Ibid.*
35. ——— Ueber die Cilienbildner in der spermatogenen Zellen. Ber. d. Deut. Bot. Ges., Bd. 16, Heft 5, 1898.
36. IKENO, S. Vorläufige Mittheilung über die Spermatozoiden bei Cycas revoluta. Bot. Centr., Bd. 69, Heft 1.
37. ——— Zur Kenntniss des sogenannten centrosomähnlichen Körpers im Pollenschlauche der Cycadeen. Flora, Bd. 85, 1898.
38. HIRASE, S. Untersuchungen über das Verhalten des Pollens von Gingko biloba. Bot. Centr., Bd. 69, Nos. 2 and 3, 1897.
39. HIRASE and IKENO. Spermatozoids in Gymnosperms. Annals of Botany, vol. 11, 1897.
40. WEBBER, H. J. Peculiar Structures occurring in the Pollen Tube of Zamia. Bot. Gaz., June, 1897.
41. ——— The Development of the Antherozoids of Zamia. Bot. Gaz., July, 1897.
42. ——— Notes on the Fecundation of Zamia and the Pollen Tube Apparatus of Gingko. Bot. Gaz., October, 1897.
43. BERLESE, A. N. Ueber die Befruchtung u. Entwickelung der Oosphäre bei den Peronosporeen. Jahrb. f. wiss. Bot., Bd. 31, Heft 2, 1897.

Description of Plates 12–14.

All figures were drawn by the aid of the Abbe Camera Lucida ; the magnification is given in each case.

All finer work was done with the help of an apochromatic immersion lens, Powell and Lealand, 1/12, N.A.1.4.

PLATE 12.

Fig. 1. Upper part of embryo sac, showing portions of two archegonia. The right-hand one shows the central cell with somewhat contracted contents, and above the yet undivided neck cell. × 150.

Fig. 2. Upper portion of older embryo sac. × 150. June 2nd.

Fig. 3. Upper portion of still older embryo sac. × 220. June 2nd.

Fig. 4. Upper portion of central cell, with nucleus. × 400. June 11th.

Fig. 5. Upper portion of central cell, with nucleus dividing. × 230. June 1st.

Figs. 6*a*, *b*, *c*, *d*, and *e*. Five consecutive sections through the dividing nucleus of the central cell. × 900. *a* shows only the lower (egg) pole of the spindle as the section is slightly oblique. *b* shows both the upper and the lower poles, while *c*, *d*, and *e* show the rest of the upper (ventral canal cell) pole. × 900. June 1st, 1896.

Fig. 7. A division similar to that of fig. 5, eleven distinct chromosomes and one doubtful one are visible. × 880.

Fig. 8. Central cell, with egg and ventral canal cell nuclei just formed. × 250. June 21st.

Figs. 9*a* and 9*b*. Two consecutive sections through ventral canal cell. × 600. June 19th.

Figs. 10*a* and 10*b*. Two consecutive sections, transverse to ovule, through free lying ventral canal cell. × 880. June 21st.

Fig. 11. Young oosphere with nucleus moving back from apex. Chromatin and metaplasm visible. × 560. June 21st.

Fig. 12. Apex of oosphere somewhat older than above. × 430. June 1st.

Fig. 13. Nucleus of nearly mature egg. × 430. June 6th.

Fig. 14. Unfertilized egg nucleus undergoing disorganization. × 250.

Fig. 15. Apex of pollen tube and surrounding nucellus tissue, showing pit, after the passage out of the contents of the tube. × 320. June 6th.

PLATE 13.

Fig. 16. A similar preparation more highly magnified. × 530. June 6th.

Fig. 17. Egg showing central female nucleus with one advancing male nucleus, and pollen tube and stalk cell nucleus behind. × 190. June 6th.

Fig. 18. Two sections through an oosphere in process of fertilization. June 19th.

18*a*. Section through oosphere showing, *b*, female nucleus, and *a*, entering male nucleus. × 170.

18*b*. Another section (a few sections removed from 8*a*) through same oosphere, showing one generative nucleus left behind at apex. × 170.

In both 18*a* and *b* there is visible the cavity made by the inrush of the contents of the pollen tube.

Fig. 19. Egg showing male and female nuclei in process of conjugation, and another generative nucleus in protoplasm at top of oosphere. × 80. June 19th.

Fig. 20. Conjugating nuclei, with surrounding protoplasm, of fig. 19, more highly magnified ; *a*, male nucleus, *b*, female nucleus. × 520.

Fig. 21. Male and female nuclei conjugating, a somewhat later stage than that of fig. 20 ; *a*, male nucleus, *b*, female nucleus. × 520. June 6th.

Fig. 22. Male and female nuclei fused, multipolar spindle visible. × 500. June 6th.

Fig. 23. A portion of the first segmentation spindle, the clear area marks the former limit of the female nucleus. × 470.

Fig. 24. One pole of the first segmentation spindle and the surrounding protoplasm ; the chromosomes have reached the poles. × 670. June 6th.

Fig. 25. The two first formed segmentation nuclei dividing again, chromosomes and metaplasmic substance visible. × 250.

Fig. 26. A spindle with chromosomes derived from one of the first formed segmentation nuclei. × 300. June 19th.

Fig. 27. Oosphere with four free segmentation nuclei. × 115. June 6th.

Fig. 28. A portion of protoplasm of the oosphere with two segmentation nuclei soon after division : remains of achromatic spindle between them. × 520. June 6th.

Fig. 29. Similar stage, but a little later, achromatic spindle just disappearing. × 450. June 6th.

Fig. 30. A proteid vacuole. × 600. June 19th.

PLATE 14.

Fig. 31. One of the daughter (segmentation) nuclei produced by the first division of the fertilized nucleus. A very young stage, with nuclear wall, remains of the achromatic spindle and nucleolar masses visible. × 850. June 21st.

Fig. 32. One of the small nuclei from the pollen tube, probably the stalk cell nucleus, lying in the protoplasm of the egg. × 600.

Fig. 33. Three segmentation nuclei on their way down to the base of the egg. The chromatin, the metaplasm, and the cytoplasmic fibres sheathing the nuclei are all clearly visible. × 230.

Fig. 34. A single nucleus in the same stage as that of fig. 33. The chromatin is arranged as threads in the metaplasm. The protoplasm outside is densely filled with granules. × 400.

Fig. 35. A portion of a similar nucleus more highly magnified. × 800.

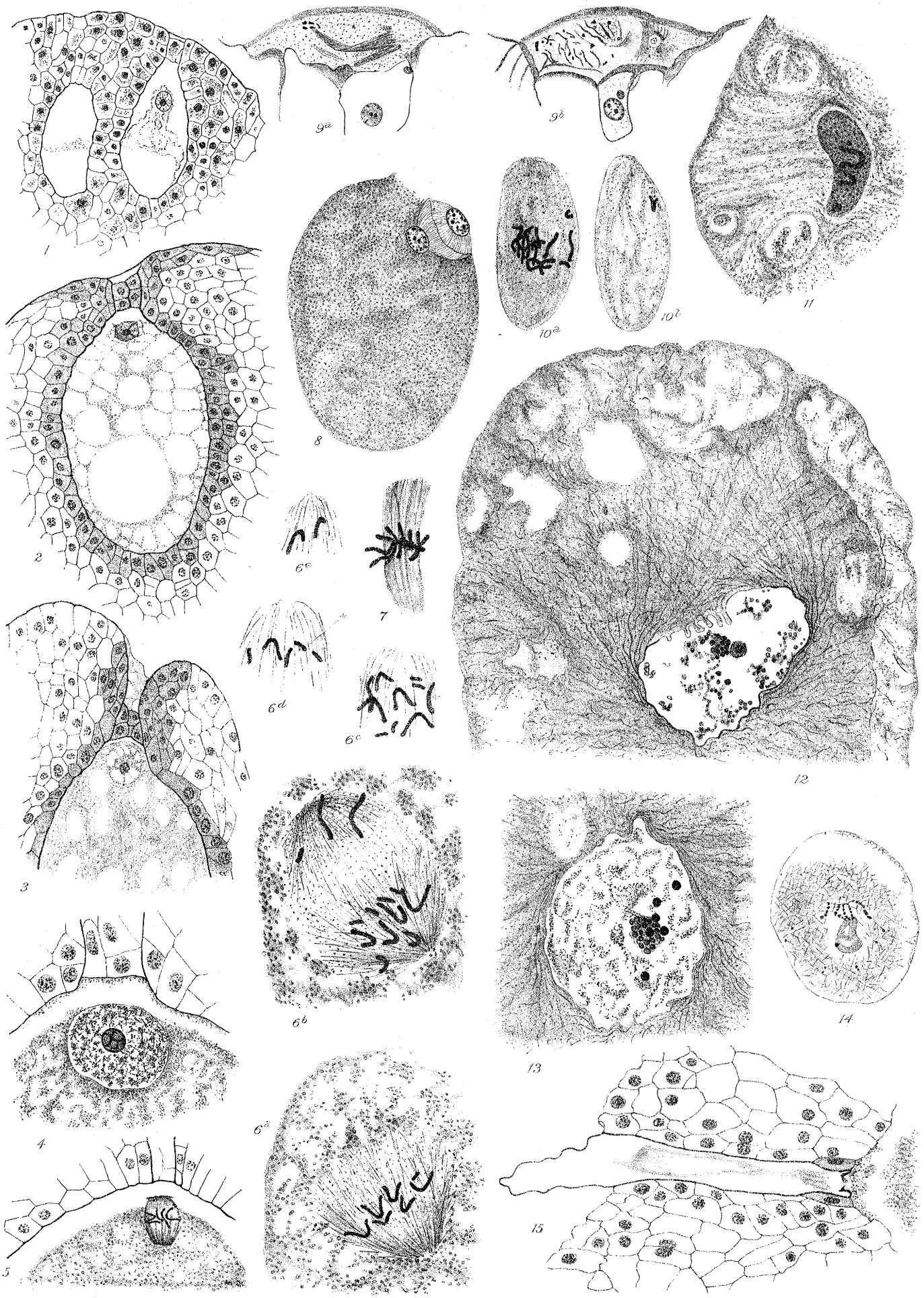
Fig. 36. The base of the egg, showing two of the segmentation nuclei and well-marked cytoplasmic fibres radiating from both of them. The nuclei are vacuolated, but contain little or no metaplasm. × 300.

Fig. 37. A nucleus in division, from one of the prothallial cells sheathing the oosphere. × 2450.

Figs. 38*a* and *b*. Microphotographs of two consecutive sections, the same as figured in Plate 12, figs. 6*a* and *b*. × 1000.

Figs. 39*a** and *b*. Microphotographs of portions of the sections figured in Plate 13, fig. 18*a* × 180, fig. 18*b* × 430.

* Before this microphotograph was made, the contents of the lower part of the female nucleus had become slightly contracted owing to accidental pressure on the cover glass.



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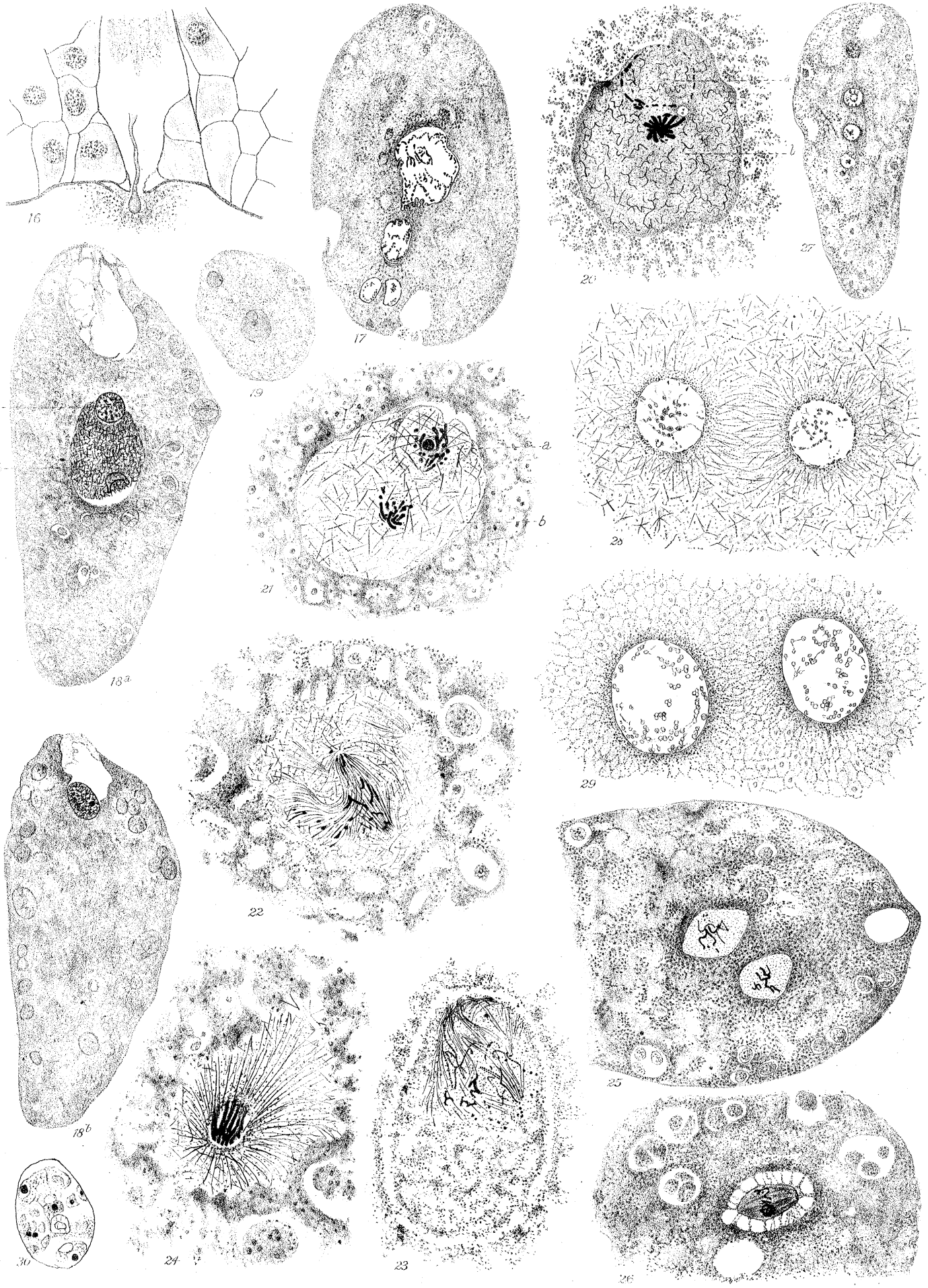
FERTILIZATION IN PINUS SILVESTRIS L.



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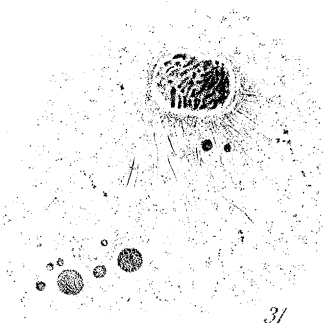

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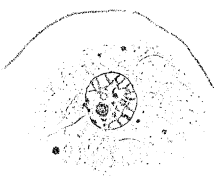

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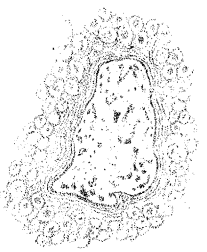

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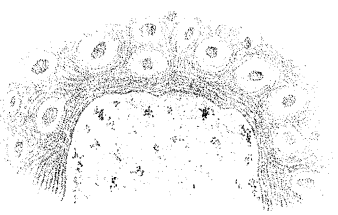

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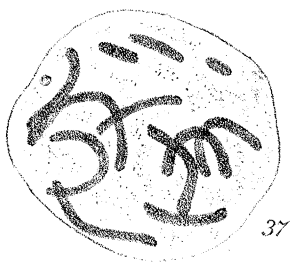

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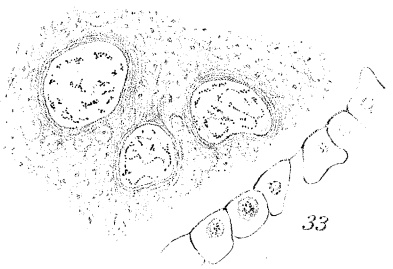

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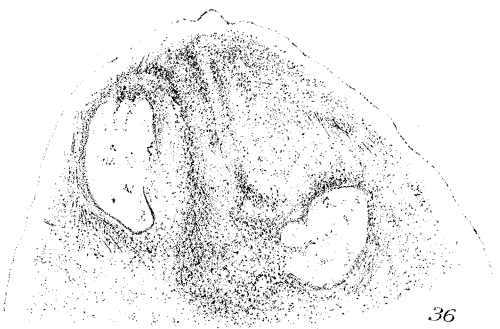

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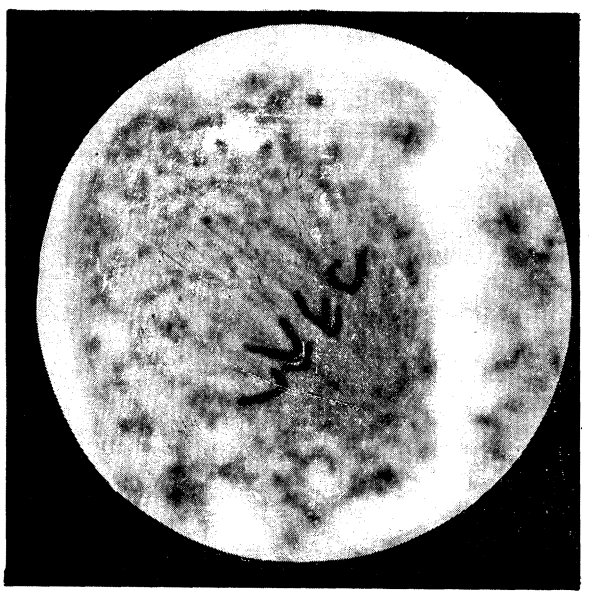

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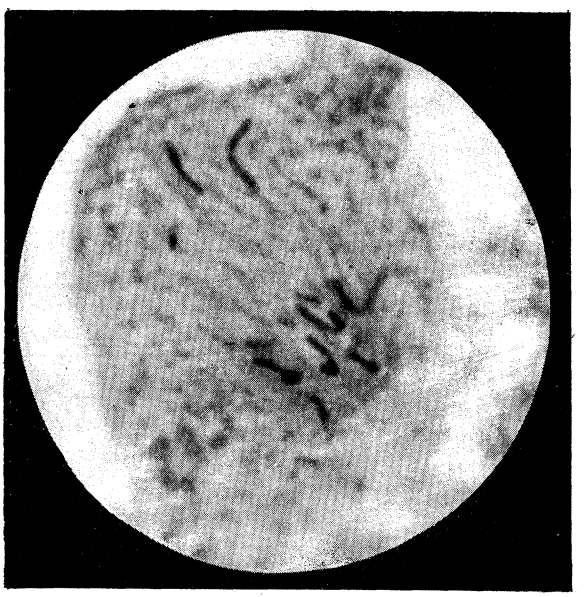

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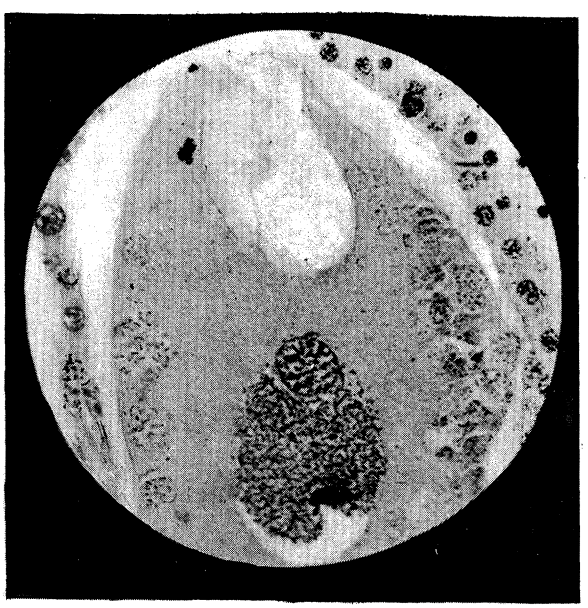

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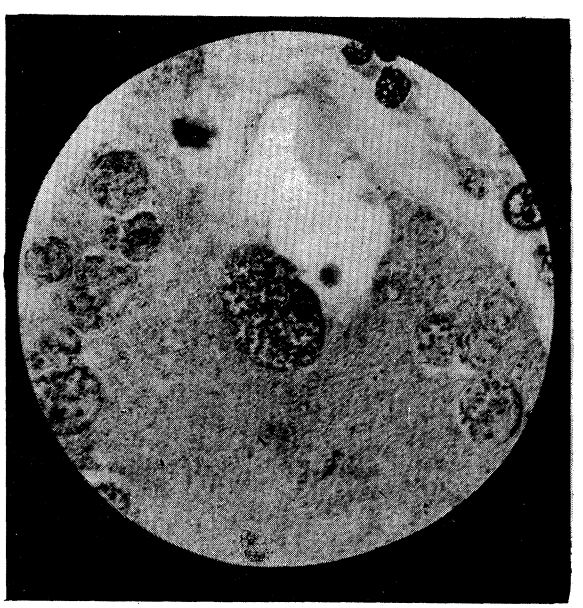

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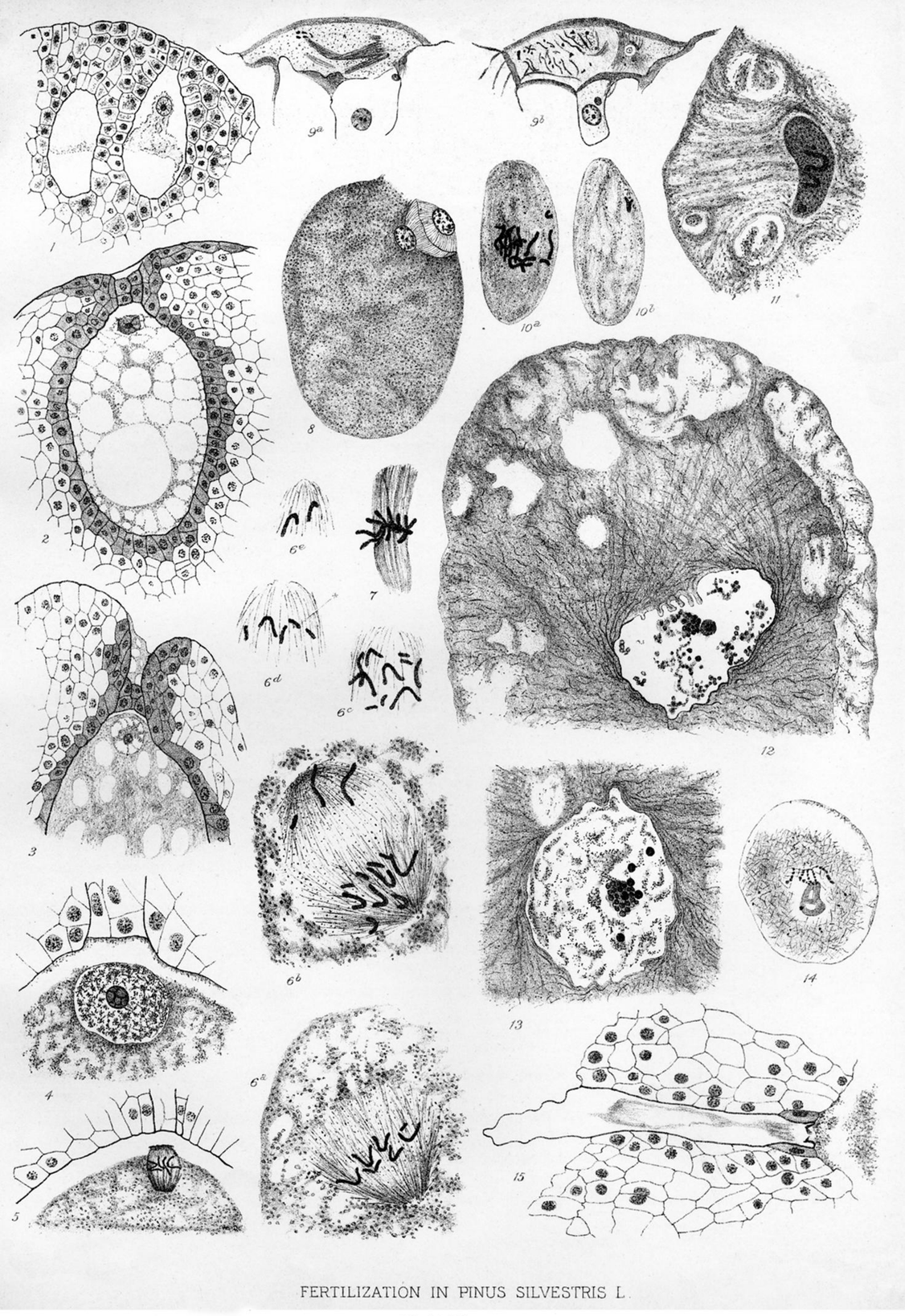
FERTILIZATION IN PINUS SILVESTRIS L.



FERTILIZATION IN PINUS SILVESTRIS L.

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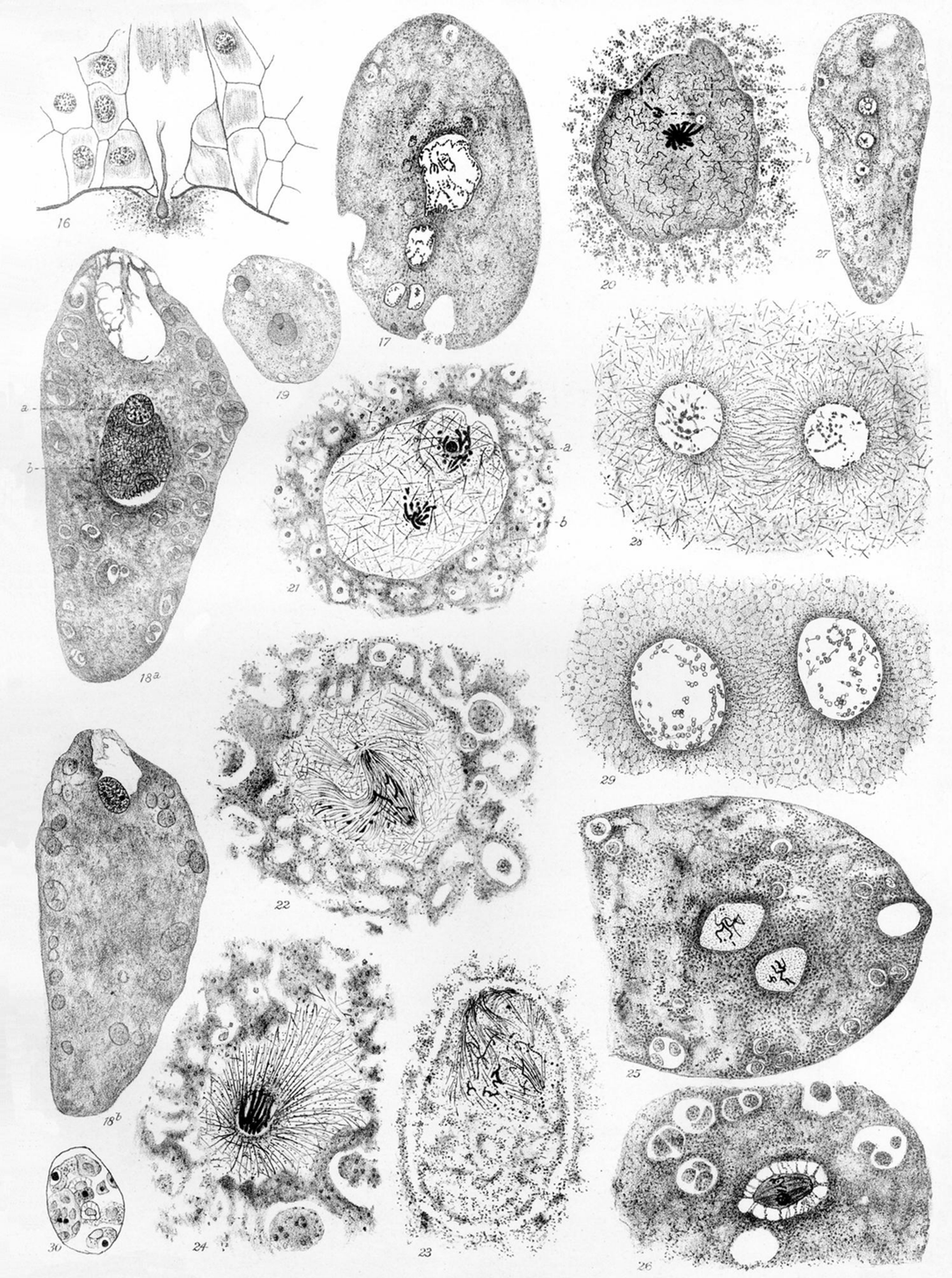

FERTILIZATION IN PINUS SILVESTRIS L.

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Fig. 18. Two sections through an oosphere in process of fertilization. June 19th.

18*a*. Section through oosphere showing, *b*, female nucleus, and *a*, entering male nucleus. × 170.

18*b*. Another section (a few sections removed from 8*a*) through same oosphere, showing one generative nucleus left behind at apex. × 170.

In both 18*a* and *b* there is visible the cavity made by the inrush of the contents of the pollen tube.

Fig. 19. Egg showing male and female nuclei in process of conjugation, and another generative nucleus in protoplasm at top of oosphere. × 80. June 19th.

Fig. 20. Conjugating nuclei, with surrounding protoplasm, of fig. 19, more highly magnified; *a*, male nucleus, *b*, female nucleus. × 520.

Fig. 21. Male and female nuclei conjugating, a somewhat later stage than that of fig. 20; *a*, male nucleus, *b*, female nucleus. × 520. June 6th.

Fig. 22. Male and female nuclei fused, multipolar spindle visible. × 500. June 6th.

Fig. 23. A portion of the first segmentation spindle, the clear area marks the former limit of the female nucleus. × 470.

Fig. 24. One pole of the first segmentation spindle and the surrounding protoplasm; the chromosomes have reached the poles. × 670. June 6th.

Fig. 25. The two first formed segmentation nuclei dividing again, chromosomes and metaplasmic substance visible. × 250.

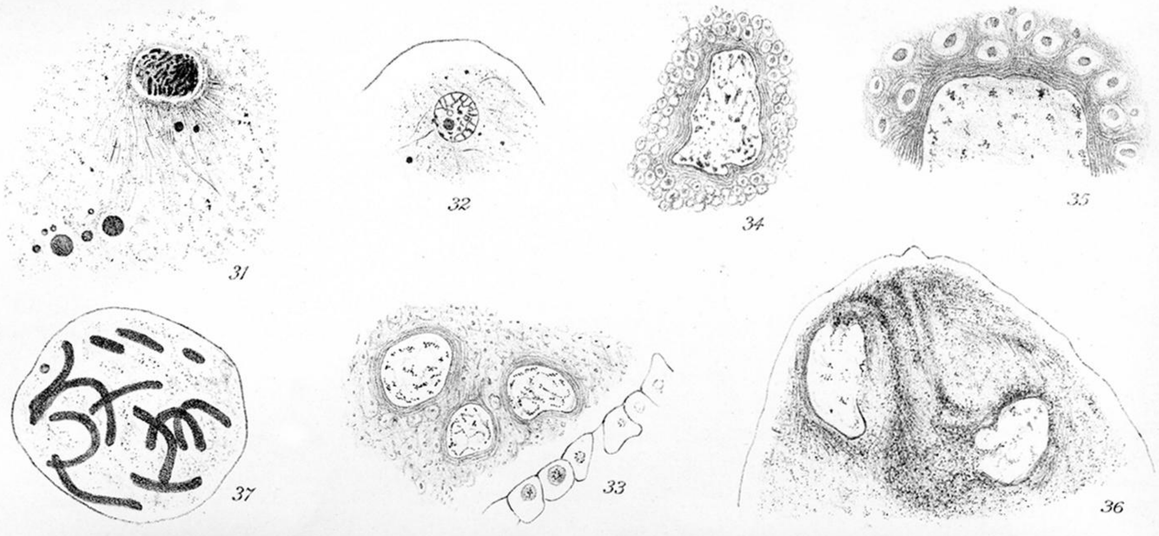
Fig. 26. A spindle with chromosomes derived from one of the first formed segmentation nuclei. × 300. June 19th.

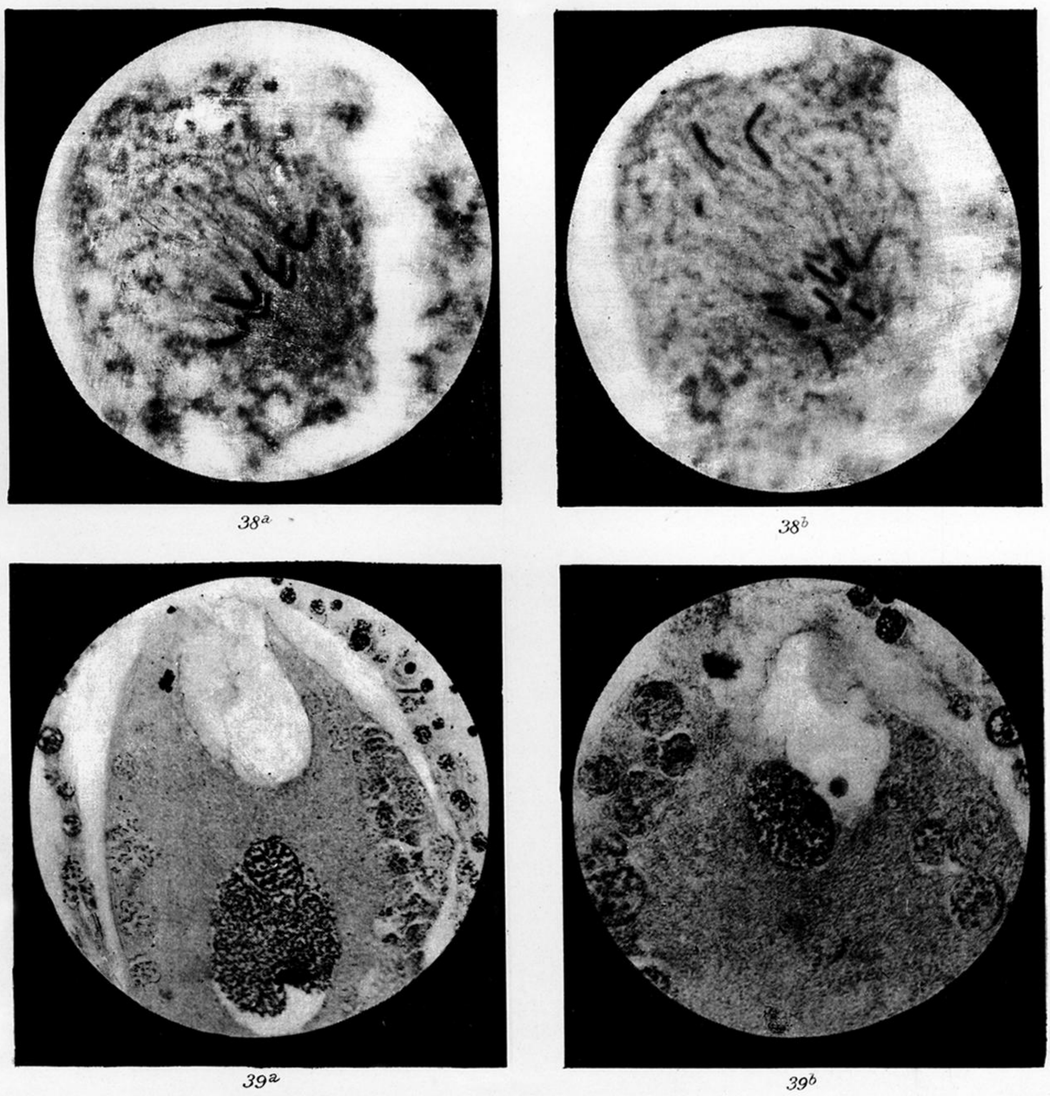
Fig. 27. Oosphere with four free segmentation nuclei. × 115. June 6th.

Fig. 28. A portion of protoplasm of the oosphere with two segmentation nuclei soon after division: remains of achromatic spindle between them. × 520. June 6th.

Fig. 29. Similar stage, but a little later, achromatic spindle just disappearing. × 450. June 6th.

Fig. 30. A proteid vacuole. × 600. June 19th.





FERTILIZATION IN PINUS SILVESTRIS L.

PLATE 14.

Fig. 31. One of the daughter (segmentation) nuclei produced by the first division of the fertilized nucleus. A very young stage, with nuclear wall, remains of the achromatic spindle and nucleolar masses visible. × 850. June 21st.

Fig. 32. One of the small nuclei from the pollen tube, probably the stalk cell nucleus, lying in the protoplasm of the egg. × 600.

Fig. 33. Three segmentation nuclei on their way down to the base of the egg. The chromatin, the metaplasm, and the cytoplasmic fibres sheathing the nuclei are all clearly visible. × 230.

Fig. 34. A single nucleus in the same stage as that of fig. 33. The chromatin is arranged as threads in the metaplasm. The protoplasm outside is densely filled with granules. × 400.

Fig. 35. A portion of a similar nucleus more highly magnified. × 800.

Fig. 36. The base of the egg, showing two of the segmentation nuclei and well-marked cytoplasmic fibres radiating from both of them. The nuclei are vacuolated, but contain little or no metaplasm. × 300.

Fig. 37. A nucleus in division, from one of the prothallial cells sheathing the oosphere. × 2450.

Figs. 38*a* and *b*. Microphotographs of two consecutive sections, the same as figured in Plate 12, figs. 6*a* and *b*. × 1000.

Figs. 39*a** and *b*. Microphotographs of portions of the sections figured in Plate 13, fig. 18*a* × 180, fig. 18*b* × 430.