Fertilization in *Sphaerocarpos*.

BY

H. W. RICKETT.

With Plates III and IV and three Figures in the Text.

INTRODUCTION.

THE details of the process of fertilization have been very thoroughly studied in examples of all the great groups of plants save the Bryophyta. In this group no complete cytological account of fertilization has been published for any form. A few scattered observations, ranging from the beginnings of cytology until recent times, make up the literature on the subject. This condition is partly due, no doubt, to technical obstacles in the study of the mature archegonium, owing to the formation of a large quantity of mucilaginous material through which the antherozoid passes to the egg and which renders fixation difficult.

The earliest account of fertilization in a Liverwort was given by Strasburger (1870) for *Marchantia polymorpha*. He described the egg as having an apical colourless receptive spot. He figured the swarming of antherozoids around the end of the neck of the open archegonium, in the extruded mucilage, and was able to distinguish single antherozoids in the neck. They were too small for him to follow farther. As to their fate when they reach the venter, he says:

‘Doubtless they are taken in by the receptive spot. Whether here, as in ferns, one spermatozoid is enough to complete fertilization could not be made out; from the size of the egg and the receptive spot in comparison with that of the spermatozoid, I might conclude that there are here a larger number of the latter taken into the egg.’

The first to concern himself with the cellular details of the sex organs of a Liverwort was Kruch (1891), who gave a careful and fairly complete account of the development of the sex organs and of fertilization in *Riella Clausonia*. His paper is of especial interest in that his account of fertilization can be very easily harmonized with that which is here presented for *Sphaerocarpos*, a form thought by many to be closely related to *Riella*.

He observed no receptive spot in the egg. He found that, of many antherozoids that enter the archegonium, only one penetrates the egg. This at once enlarges greatly, and becomes surrounded by a homogeneous hyaline area whose outer limit is not well defined. The body of the antherozoid then segments, first into four, then into eight threads; he thought that the eight threads result from the longitudinal division of the four seen in the earlier stage. The clear area now possesses definite boundaries, which thus outline the male nucleus. About the same time, the female nucleus also forms eight chromatin threads. The two nuclei become almost equal in size. He observed them in contact, but did not see their actual fusion. The entrance of an antherozoid causes the appearance of tangential divisions in the cells making up the wall of the venter.

Cavers (1904), studying *Fegatella conica* (*Conocephalum conicum*), by introducing a drop of water full of swarming antherozoids into a preparation containing mature archegonia was able to follow the opening of the archegonium, the extrusion of the mucilage, and the passage of the antherozoid down the neck. He could not distinguish the antherozoid after it had penetrated the egg. Large numbers of antherozoids reached the egg and caused the latter to exhibit a rocking movement.

Several stages in the fertilization of *Riccia natans* (*Ricciocarpus natans*) were observed and figured by Garber (1904). Numerous antherozoids were seen caught in the mucilaginous matter extruded by the archegonium. The egg is concave at the end nearest the neck, and, after the archegonium has opened, is somewhat shrunk away from the walls of the venter, though not nearly so much so as is usually described for *Riccia*. The fertilized egg swells and its end extends up into the neck canal. Garber saw the two sexual nuclei lying in the egg cytoplasm. The male is about half the size of the female nucleus, and stains more darkly because its chromatic contents are more closely crowded together. These contents include one or several large bodies and some smaller granules. The female nucleus contains a large body in the centre, and apparently a fine reticulum surrounding this. There are conspicuous plastids with starch grains all through the egg cytoplasm. According to his account, the male nucleus approaches the female nucleus and becomes embedded in it, but it is a question whether his figures really prove this point. In the absence of statements as to the thickness of his sections, or as to whether the two nuclei were at the same focal level, the nuclei may equally well be interpreted as being one above the other, no fusion occurring at this stage. As in *Riella*, the entrance of the antherozoid is followed by divisions in the cells of the venter, so that the latter consists of two layers of cells before the first division of the zygote.

Humphrey (1906) saw one stage in the fertilization of *Fossombronia longiseta*, which is noteworthy as being markedly different from anything

reported for any other Liverwort. He figures a cross-section of the archegonium and egg, with the antherozoid, a long, thin, curved body, lying in the egg cytoplasm, with one end in contact with the egg nucleus. This antherozoid resembles the free-swimming cell, and has no resemblance to a resting nucleus, or even to the antherozoid immediately after its entrance into the egg as described by Kruch and by the present writer. Humphrey reported that the egg of *Fossombronia* has a well-defined receptive spot, but did not describe the latter. He described the large egg nucleus as possessing but one nucleolus and a small amount of chromatin.

Meyer (1911) obtained several fertilization stages in *Corsinia marchantioides*, which showed the male and female nuclei lying side by side in the egg cytoplasm. In the earliest stages observed, the male nucleus has already rounded up and is surrounded by a clearly defined membrane. In its centre is a small mass of chromatin granules, from which linin threads extend towards the periphery. Later the chromatin granules become spread throughout the nucleus, which is now filled with a complicated fine network. The large egg nucleus is similar in structure. The cytoplasm of the egg is quite dense, and filled with vacuoles which are arranged somewhat radially about the nucleus as a centre. The egg was somewhat shrunken under the influence of the fixing fluid, and was surrounded by a clearly defined fertilization membrane after the entrance of the antherozoid.

Miss Black (1913) saw one stage in *Riccia Frostii* similar to that just described for *Corsinia*. The male nucleus lay in the egg cytoplasm beside the egg nucleus. Its structure was that of a resting nucleus. She also figures one egg which she interprets as having been fertilized and in which the nuclei have already fused. Its nucleus is large, and the chromatin is collected in a cord or in short segments, with a fine network occupying the remaining space. This, she says, is typical of a great number of eggs observed. She also reports the division, after the entrance of the antherozoid, of the cells that make up the wall of the venter—the basal cells usually dividing first.

Miss Graham (1918) figured male and female nuclei in the cytoplasm of the egg of *Preissia quadrata*. The male nucleus is smaller than the female. The chromatin in both is massed in the centre of the nucleus, and seems to be in the form of small masses or rods. She was chiefly interested in the discovery of centrosomes in the egg during fertilization, to which reference will be made in another connexion.

Woodburn (1920) shows one stage in fertilization in *Reboulia hemisphaerica*, which is general is similar to those just described. The nuclei are stated to be in the resting condition, though the figure is not very clear.

Sharpe (1921) figures one stage in the fertilization of *Anthoceros*. The male nucleus lies within the egg cytoplasm, in contact with the egg nucleus.

Both nuclei are in the resting condition. In the cytoplasm of the egg lies an elongated plastid.

In the Mosses the state of our knowledge of fertilization is even more unsatisfactory.

The entrance of the antherozoids into the archegonium was seen by Roze (1872) in *Sphagnum cymbifolium*, and by Arnell (1875) in *Disceium nudum*. The former figures many antherozoids clustered about the opening of the neck, several passing down the neck, and one in the venter just touching the egg. They enter the neck with the ciliated end ahead, and retain the cytoplasmic globule at the posterior end, carrying it with them into the venter. Arnell described the entrance into the venter of many antherozoids which impart to the egg a rocking movement. After this ceases, the surface of the egg is papillose, owing to the incompletely absorbed antherozoids. He was unable to distinguish the antherozoids after they had penetrated the egg.

Gayet (1897) also saw the entrance of many antherozoids into the archegonium of *Fissidens incurvus*, but discerned no rocking movement of the egg. Only one antherozoid penetrates the egg. This becomes first crescent-shaped, then spherical, and finally fuses with the egg nucleus, while the thin envelope of cytoplasm with which it has been surrounded fuses with the cytoplasm of the egg. The female nucleus possesses at this time four very distinct chromosomes, which seem drawn towards the male nucleus. Gayet followed the same history in *Bryum capillare* up to the crescent-shaped stage of the male nucleus. He also described abnormalities in the archegonia of various Liverworts, such as supernumerary ventral canal cells, and reported the fertilization of the ventral canal cell instead of the egg in *Marchantia*.

The Leeuwen-Reijnvaans (1908 *a*) described a remarkable series of events in the fertilization of *Polytrichum*. According to their account, reduction divisions occur in both antheridium and archegonium. The egg and the ventral canal cell then fuse, and the cell resulting from this fusion is fertilized by two antherozoids, this double fusion restoring the sporophytic number of chromosomes. The antherozoids maintain a slender form in the cytoplasm of the cell formed by the fusion of the egg and the ventral canal cell, but take on the appearance of resting nuclei when they come in contact with the female nucleus. The membranes of the three nuclei then disappear. In a subsequent paper the same authors (1908 *b*) described a similar 'double reduction' in *Mnium*. The spermatogenesis of *Polytrichum* has been investigated by Walker (1913), and that of *Mnium* by Wilson (1911). Both of these authors reported nuclear divisions of the usual type, without reduction. Walker also found that the egg and ventral canal cell of *Polytrichum* do not regularly fuse, and attributes the results of the Leeuwen-Reijnvaans to their technical methods.

In *Sphagnum subsecundum*, according to Bryan (1920), fertilization is replaced, at least in some cases, by a fusion of the ventral canal cell and egg. The possibility that a process of this type may be widespread among the Bryophyta makes it all the more desirable that the complete history of fertilization be followed in a number of forms.

MATERIAL AND METHODS.

The account that follows is based on a study of *Sphaerocarpos Donnellii* Aust. The plants used were grown on soil in three-inch pots, which were kept in Wardian cases in the greenhouses. Most of the cultures originated in single sporelings or in single plants isolated from larger cultures. Cultures of each sex were thus kept separate until it was desirable to bring about fertilization. Male and female plants were then transplanted to the same pot. After about a week they had become firmly attached and were flourishing in the new soil. By flooding these mixed cultures with sterilized distilled water, the plants having been kept submerged about fifteen minutes, numerous sporophytes were obtained. The sporophytes first became visible under a hand-lens as small, spherical, whitish bodies in the bases of the older involucres. The time necessary for them to attain this development varies from two to eight weeks from the time of flooding.

The fixing fluid that gave the best results was made according to Flemming's medium formula, modified by the addition of 3 per cent. urea crystals. Satisfactory preparations were obtained by using only the most healthy cultures, by fixing during the cooler seasons of the year, and by taking special precautions in dehydration and in paraffin infiltration.

To obtain a series of stages of fertilization in progress, plants from cultures that had been flooded were removed and fixed every few hours after flooding. Several trial sets of fixations sufficed to work out the best technique, and to disclose the approximate time involved in the process of fertilization. Four complete series were then made, covering periods from a few minutes to five days after flooding, at intervals of from two to four hours. These series were designated as *A*, *B*, *C*, and *D*. The account that follows is based on all four of these series, which after close examination proved to correspond very closely in all essentials.

The fixing plants were embedded in paraffin in the usual way, and sections were cut $7\ \mu$ in thickness. The stain most used was the Flemming triple combination of safranin, gentian violet, and orange G. The contents of the venter of the archegonium showed a marked affinity for the stains, especially for the safranin; so that it was possible only to dip the slide in the safranin and then necessary to destain in acid alcohol. An immersion of about a minute in gentian violet gave good results. Substitution of light green for orange G did not give as clear preparations. Heidenhain's iron-alum-

haematoxylin was also used. This stained the chromatin and nucleolus very heavily—almost too heavily—but left the cytoplasm practically untouched. When light green was used as a counterstain with the haematoxylin, the cytoplasm became visible, but could not be seen so clearly as with the triple stain.

Sections were made in a longitudinal vertical direction through the plants and seriated so as to include all of every plant. Only a few of these sections contained archegonia of the right age, and the others were discarded.

Free-swimming antherozoids were prepared for study by introducing them into a drop of water on a slide, inverting this slide over a phial containing 1 per cent. osmic acid, and allowing it to dry, afterwards staining in dilute gentian violet.

OBSERVATIONS AND DISCUSSION.

Since in *Sphaerocarpos* fertilization proved to be a slow process, and since the history was worked out from a large number of instances, it was found convenient to divide the process into a number of *phases*, each distinguished by some well-marked change in the male nucleus, in the female nucleus, or in both. The nature of these changes will be taken up as the phases are severally discussed. It must be borne in mind, of course, that these changes are in reality continuous, and that the limits of the phases are therefore purely arbitrary, constituting merely a method of handling the large amount of data. The number of cases in which fertilization was observed, with the distribution of these cases over the time covered by the process, and their classification into phases, is shown in Table I. Reference will be made later to the information deducible from such a statistical arrangement of the data.

Structure of the Mature Archegonium.

Mature archegonia appear, as has been pointed out elsewhere (Rickett, 1920), just behind the growing point of the thallus. Between them and the group of initial cells are other archegonia that have not yet reached maturity, and farther back on the thallus are others which, in the absence of fertilization, have disintegrated.

The structure of the archegonium is that typical for the Liverworts. The neck is regularly curved, often forward, owing to the more rapid division of the cells on one side. The venter is large and rounded, and consists, before fertilization, of a single layer of cells. The neck consists of six rows of cells, as described by Campbell (1896) and Cavers (1911). Hy (1884) and Gayet (1897), on the contrary, state that there are but five rows of cells in the archegonial neck.

TABLE I.

Cases of Fertilization.

<i>Phase.</i>	<i>Series.</i>	<i>Time after flooding.</i>		<i>No. of cases.</i>	<i>Phase.</i>	<i>Series.</i>	<i>Time after flooding.</i>		<i>No. of cases.</i>
		<i>Hrs.</i>	<i>Min.</i>				<i>Hrs.</i>	<i>Min.</i>	
1.	C	0	45	1	5.	C	28		1
	A	0	55	8		C	42		5
	C	1	30	9		C	44		1
	A	1	40	8		B	49		2
	C	2		4		B	52		10
				30		C	53	30	2
						B	56		8
						C	59	30	3
						B	60		7
						D	62	30	5
2.	C	4		6		C	64		1
	C	6		5		D	65	30	1
	A	7		4		C	66		29
	C	8		1		D	75	45	1
	C	20		2		D	84		1
	C	25		1					77
				19	6.	C	59	30	1
3.	C	14		2		B	60		4
	C	18		4		B	64		1
	A	18	30	2		D	71	30	1
	C	22		2		D	73	45	1
	C	28		1					8
	C	32		1	7.	B	60		3
				12		B	64		2
4.	C	28		2		D	65	30	8
	A	28		5		D	67	30	11
	B	30		5		A	68		9
	C	32		6		D	75	45	7
	B	36		1		D	84		1
	B	38		22		B	105	30	1
	B	40		9					42
	C	40		4	8.	B	64		2
	C	42		3		A	68		1
	C	44		4		D	65	30	1
	B	44		15					4
	C	46		3	9.	B	64		1
	C	48		5		A	100	30	4
	A	48		15					5
	C	50		1	10.	D	71	30	1
	C	52		2		D	84		2
	C	53	30	2		B	105	30	2
	B	64		1					5
	D	65	30	1					
	D	67	30	2					
				108					
									Total 310

The egg is large and ovoid, measuring about 40 by 20 μ . It does not quite fill the cavity of the venter. This is partly because of the disintegration of the ventral canal cell. There seems to be no considerable

shrinkage due to fixation, for in living archegonia freshly dissected out the egg may be observed through the wall of the venter as a darker body lying free in the cavity. The action of the fixing fluid is shown, however, by the slight irregularities in shape of several of the eggs figured. The cytoplasm of the egg is dense, of a fine structure, and stains very deeply. The nucleus lies in the centre of the egg, and is also of an ovoid form, measuring about 13 by 10 μ . In the telophases of the last division of the central cell, which gives rise to the egg and the ventral canal cell, the nucleus contains a small mass of very deeply staining chromosomes, so tightly packed together that nothing can be made out of their structure or number. In the resting nucleus of the egg the chromatin is still in a close mass in the centre, about the nucleolus, surrounded by a clear space which in turn is bounded by the nuclear membrane. Certain strands and granules are to be found in this clear area. Occasionally a nucleus is found having a more open structure, and in such cases the chromatin is seen to be still largely in the form of rods and threads. One nucleus of the latter type is shown in Pl. III, Fig. 2. In the centre is a large nucleolus.

There is no indication of any receptive spot in the cytoplasm of the egg. This corresponds with Kruch's (1891) account for *Riella*. A receptive spot has been described by Strasburger (1870) for the egg of *Marchantia*, by Humphrey (1906) for that of *Fossombronia*, and by Campbell (1888, 1918) for the eggs of *Targionia*, *Funaria*, *Pilularia*, and several ferns. In all these forms it is described as a more hyaline area of the cytoplasm near the apex of the egg. In *Sphaerocarpos* there are many cases (see especially Figs. 4 and 9) in which the distal end of the egg (the upper end in the figures) is flattened or even slightly concave. Garber (1904) described a similar concavity at the distal end of the egg of *Riccia natans*. It is possible that what is known as the 'receptive spot' appears only under the influence of certain technical methods. The flattened or concave portion of the egg in my preparations may be indicative of a local structural difference, which under a different fixation might appear as a hyaline area. There are conflicting statements as to the presence of a receptive spot in the eggs even of the same form, for Durand (1908) denies the existence of such a spot in *Marchantia*.

At maturity the four canal cells that fill the neck, together with the ventral canal cell, break down, and the mucilaginous material derived therefrom streams out from the mouth of the archegonium. This material may be seen in preparations as a densely stained, coagulated, somewhat stringy mass, filling the neck canal and part of the venter, and extending out from the neck. The egg is now ready for fertilization, and apparently remains so for only a short time. If not fertilized, the egg disintegrates, this change being evidenced first by a great increase in its (already marked) staining capacity, by a massing together of the nuclear materials, and

finally by a contraction of the whole egg into a small body which lies free at the base of the venter. During this process of disintegration the distal concavity is usually more pronounced than previously, while at the other end the egg is quite sharply pointed. The remainder of the archegonium persists unchanged in appearance for a long time after the egg has disintegrated.

The age of the archegonium may be judged approximately by the stage of development of the involucre. At the time of the maturity of the egg, the involucre is merely a cup around the base of the archegonium, extending sometimes to the base of the neck. Archegonia showing fertilization stages thirty-six hours after flooding are usually completely enclosed by the involucre, and after three days from the time of flooding the involucre extends a short distance beyond the end of the neck. The continued growth of the involucre is not dependent upon fertilization, although its form is considerably affected by the development of the sporophyte. A short distance behind the growing point archegonia containing eggs in the first stages of degeneration may be found, surrounded by involucres about thirty-six hours old (judging from the above account based upon cases of fertilization). From this time on the involucre enlarges rapidly, while the archegonium finally withers; so that the posterior part of the thallus is covered with the large cylindrical involucres, enclosing at their bases the remnants of unfertilized archegonia. The rate of growth of the involucre is so variable (compare Text-figs. 2 and 3) that estimates of its age based on its size, and consequently of the age of the archegonium based on that of the involucre, must be taken very cautiously. But it seems probable that the time during which an archegonium may be fertilized is limited to a period of from twenty-four to forty-eight hours after it reaches maturity, at which time the entrance of antherozoids is made more likely by the fact that the archegonium is not yet completely enclosed by the involucre.

An archegonium with its involucre is represented in Text-fig. 2. In this case the egg has been fertilized. Not all of the neck is shown, since its distal end curves, and portions of it appear in succeeding sections.

Each archegonium is usually surrounded by its own involucre; but on one plant I found two involucres each of which contained two archegonia. Two archegonia were found also which contained apparently two eggs each, side by side in the venter, and of about equal size. Several cases of similar abnormalities are to be found in the literature. Gayet (1897) reports that two superposed ventral canal cells are sometimes found in *Sphaerocarpos*. He found two ventral canal cells also in *Marchantia*, in this case side by side. Campbell (1896) reports the occurrence of two superposed ventral canal cells in *Geothallus tuberosus*.

Penetration of the Antherozoid into the Egg.

If one observes a male culture under a binocular microscope, many involucre may be seen to extrude from their tips small globules of whitish sticky material. These globules may be picked off with a needle or fine brush and deposited in a drop of water on a slide, or the whole plant may be transferred to the drop for a short time. The drop of water, upon examination with a high power of the microscope, proves to contain numerous antherozoids, which after a few minutes disentangle themselves from the surrounding viscous material and begin to swim around in the water. If killed and stained in the manner already described, their form is that shown in Text-fig. 1.

TEXT-FIG. 1. Antherozoids shortly after having emerged from the antheridium. $\times 1300$.

An antherozoid consists of a slender rod, variously curved or coiled, bearing two cilia attached near the anterior end, and a cytoplasmic vesicle at the posterior end. This vesicle is, apparently, soon lost. The cilia are not attached at the same point. At the anterior end of the body of the antherozoid is a less deeply staining portion. One cilium is attached at the anterior end of this body, the other just posterior to it. Measurements of the cilia of the antherozoids figured show a difference in length between the two cilia of each individual; the anterior cilium measuring quite constantly 44μ , the posterior one from 46.5 to 50.5μ ; an average difference of 3.6μ . The average length of the body of the antherozoids figured is 18μ , the individuals varying from 16 to 20μ . The body is 0.5μ thick.

Of all stages in fertilization the actual entrance of the antherozoid into the egg is the most difficult to observe. The neck at this time is filled with the mucilaginous material described above, and the space in the venter above the egg with the remains of the ventral canal cell; all of which forms

a dense, stringy, deeply staining material filled with various small bodies and granules. I have not succeeded in detecting the free-swimming antherozoid in its passage through these regions, though I have examined many slides made from material fixed at various short intervals after flooding. Neither have I seen a single case in which I can say with certainty that the antherozoid was penetrating the egg. The membrane of the latter is at this time very delicate, and, either naturally or as a result of fixation, the surface layer of the cytoplasm is somewhat 'frayed out' into the surrounding space; so that instead of a clear boundary of the egg there is a confusion of little strands and granules which seem to belong to the cytoplasmic reticulum. Among these bodies the entering antherozoid may in some cases lie concealed. The thinness of the egg membrane at this time has been noted also in *Riccia sorocarpa* by Kny (1866), who thought that the membrane is resorbed, at least at the distal end of the egg.

The first sign of the presence of the antherozoid in the egg is the occurrence, in fixations made fifteen, twenty, and forty-five minutes after flooding, of a slender curved body lying in the peripheral regions of the egg cytoplasm and hardly to be distinguished from the latter. Such a case is shown in Pl. III, Fig. 1. In several cases these bodies were parallel to the surface of the egg. Some were in the distal end of the egg (Fig. 1), some in the basal end, and still others in one side. This makes it doubtful whether the concavity sometimes seen in the distal end of the egg, described above, has any real relation to the entrance of the antherozoid. If it really represents the receptive spot described for other forms, then the receptive spot, in *Sphaerocarpos* at least, is not the only place at which antherozoids can penetrate the egg. It is noteworthy that these bodies which I have interpreted as antherozoids correspond in their variable position in the egg cytoplasm to the male nuclei clearly seen in later stages, which similarly may approach the female nucleus from above, from below, and from the side. In one case, a slender strand of the mucilaginous material that fills the venter extended across the ventral cavity from the archegonial wall to a point on the surface of the egg near where the antherozoid lay inside the egg.

First Phase.

The antherozoid first appears clearly and unmistakably discernible as a small, rod-shaped, often curved body lying in the egg cytoplasm, surrounded by a clear space of varying dimensions, whose boundaries are not clearly defined. This body is dense, staining most readily with safranin, and apparently homogeneous; I have found no evidence of a differentiation of its contents at this stage. It may be pointed at one or both ends, but usually both ends are rounded. It measures about 6 by $1.5\ \mu$. This stage is illustrated in Fig. 2.

Whether this is the whole antherozoid or only its nucleus could not be

determined. Neither at this nor at any later stage could I discern anything in the egg cytoplasm that might be interpreted as the remains of the cytoplasm of the antherozoid; yet it is certain that the rod-shaped body just described becomes transformed into a typical nucleus. Probably the cytoplasmic portions of the antherozoid are absorbed by the egg cytoplasm immediately after its penetration. Possibly the clear space mentioned above is occasioned by the occurrence of this change, though it seems more probable, in view of later changes, that it is due to an extrusion of material from the dense body itself. Taking what seems to be the most probable view of the origin of the latter body, I shall refer to it in future as the antherozoid nucleus, or as the male nucleus. A comparison of its dimensions with those of the free-swimming antherozoid shows that it has contracted greatly in length, while increasing in thickness.

In one case (one and a half hours after flooding), the male nucleus was not surrounded by a clear space, and was slightly more slender than usual. This is the only indication found of what must be an early stage in an extremely rapid process—the transformation of the antherozoid, or of its nucleus, into a large, dense, rod-shaped body. Indeed, the rapidity of all these first processes and changes is impressive. One case in which the male nucleus was already clearly distinguished in the usual shortened form was found forty-five minutes after flooding. The absence of clear stages showing the penetration of the antherozoid is no doubt partly due to the rapidity with which it takes place.

In another case (one and a half hours after flooding) the egg cytoplasm shows a slight depression in its surface, near one end of the male nucleus, which now lies within. This probably marks the place where the antherozoid entered the egg.

The egg at this time shows little change from the condition already described. The cytoplasm varies in appearance. In some preparations it has the appearance of a fine, complex reticulum embedded in a homogeneous mass (Fig. 2). This apparently reticular structure may of course be in reality alveolar. An alveolar structure does indeed appear in later stages; but in these cases the open spaces are large, regular in shape, and clear, whereas at the early stage now under discussion they are small, irregular, and take a deep stain. It seems probable, therefore, that the structure here is reticular in nature, with a dense homogeneous ground substance. Even at this early stage, the cytoplasm of different parts of the egg may vary in structure. In one case, the cytoplasm is noticeably denser about the nucleus, and shows a more open structure in the peripheral region. This is suggestive of what is to come later. A similar structure has been described by several authors. Gayet (1897) says that the egg of *Sphagnum* is formed of cytoplasm having a reticular structure, the meshes of which are denser about the nucleus. Campbell (1918) figures and describes the ripe egg of

Targionia as having a denser zone of cytoplasm surrounding the nucleus. Miss Graham (1918) reports that the outer zone of the egg of *Preissia* is more coarsely alveolar than the portion around the pronuclei. In *Nephrodium*, according to Yamanouchi (1908), the cytoplasm of the egg has a finely fibrillar structure, very dense about the nucleus and vacuolate towards the periphery.

It is of course impossible to draw from fixed material any final conclusion as to the actual structure of the protoplasm in the living state. But since the stages herein described were all seen in preparations made in exactly the same way, with the same reagents, and since, as will be seen, there is a progressive series of changes in the cytoplasm, it is evident that, if not the structure, at least the substance, of the cytoplasm must undergo changes during the process of fertilization. The whole series of changes lends support to the idea that there are two kinds of substance present in the cytoplasm, corresponding perhaps to Strasburger's (1892, 1893) 'trophoplasm' and 'kinoplasm', which at first are uniformly distributed throughout the cytoplasm, but of which one later becomes more or less localized and differentiated for its special functions.

Eggs observed at this stage measured about 50 by 20 μ . The average difference in length (10 μ) between these eggs and mature unfertilized eggs is probably significant, and indicates that the egg begins to enlarge at this time. This is in accordance with the account of Garber (1904) for *Riccia natans*, and of Campbell (1918) for *Riccia glauca* (?), both of whom described the egg as enlarging immediately after fertilization.

The female nucleus is also larger than that of the unfertilized egg, measuring about 17 by 12 μ . Its structure is essentially that already described. Fig. 2 shows an egg whose nucleus, although the antherozoid has already entered, contains traces of chromosomes which have persisted from the last preceding division. Usually, however, the egg nucleus has rather the appearance shown in Figs. 4 and 5, containing numerous small, irregular masses, granules, and rods, some scattered through the nuclear cavity, but most of them massed in the centre about the large nucleolus and almost hiding it. The nucleoli, of which there may be one or two, often appear irregular in shape, but this is probably due to the fact that masses of chromatin are grouped about and in contact with them.

There is no definite relation between the respective positions of the male and female nuclei. Just as, apparently, the antherozoid may penetrate the egg at any point, the male nucleus may lie in any part of the cytoplasm, and at any relative distance from the plasma membrane and from the nucleus of the egg. Most frequently the male nucleus is somewhat towards the distal end of the egg, and about midway between the egg nucleus and the outer boundary of the cytoplasm.

This phase extends through about two hours from the time of flooding.

Second Phase.

The next phase is marked by a change in shape of the male nucleus, which now becomes ovoid. This is shown in Fig. 3. Its structure and position do not change. It is of this form for about eight hours. It becomes somewhat shorter, being now about $5\ \mu$ in length; but its thickness almost equals its length, so that it is evident that a considerable enlargement has taken place.

The female nucleus is the same in appearance as in preceding and succeeding phases (Figs. 4 and 5). The egg cytoplasm is also unchanged in many cases, but in others it becomes coarser, showing a deeply staining, irregular reticulum. This is formed of threads of varying thickness, sometimes straight, sometimes very much twisted and curved, with granules of very various sizes at the points of junction of the threads and often at other points along them. This condition is more usual in succeeding phases, and is illustrated in Fig. 8, which represents the fourth phase. Such a structure may, of course, be considered as an alveolar one, greatly distorted by the fixing agent. My chief reasons for considering it a true reticulum are that a very clear alveolar structure is to be seen in later stages, and that eggs at this stage, though fixed in the same way, present quite a different appearance.

Third Phase.

During the next twelve hours, the male nucleus is approximately spherical (Fig. 4), and sometimes has the appearance of a dense mass of granules rather than that of an absolutely homogeneous body as heretofore. This structure may be especially evident at its surface, which is often rough, with tiny protrusions, instead of having a clear smooth outline. In most cases, however, the structure of the male nucleus is apparently the same as in the previous stages, except that the clear space surrounding it is now for the first time bounded by a definite membrane. This membrane is continuous with the cytoplasmic reticulum and is probably cytoplasmic in origin. The male gamete is now, therefore, represented in the egg by a typical nucleus, consisting of a nuclear membrane, a dense central mass containing chromatin, and a clear material filling the rest of the nuclear cavity; this clear material is analogous to the nuclear sap of the ordinary vegetative nucleus, and is probably formed by extrusion from the denser central mass of the nucleus.

The egg retains the same appearance as in the previous phases, as to both its nucleus and its cytoplasm (Figs. 4 and 5). Its surface has lost that 'frayed' appearance characteristic of the earliest stages, and a clearly defined membrane is present. Without microchemical tests, it is impossible to say whether or not a cell wall is present. In all later stages, and even in the young embryo consisting of several cells, there is no thicker boundary of the

egg or of the free surface of the cells of the embryo than appears at this time. Meyer (1911) describes and figures the egg of *Corsinia marchantioides* as forming about itself a distinct membrane after receiving the antherozoid. Comparing his figure with the fertilized egg of *Sphaerocarpos*, the egg of the latter takes up far less room in the venter, and what seems to correspond to the 'fertilization membrane' in *Corsinia* is the heavily staining mucilaginous material that filled the cavity of the venter and the neck during the first phase of fertilization and that remains as a dense layer appressed to the inner wall of the venter and filling the neck (Text-fig. 2). In a subsequent paper Meyer (1913) again refers to, and figures, the fertilization membrane, this time in *Plagiochasma rupestre*. Here the resemblance is even stronger to what I have seen in *Sphaerocarpos*, and the suspicion seems justified that the so-called 'membrane' may really be the mucilaginous material of the venter, which looks like a membrane belonging to the egg only when the latter completely fills the venter.

TEXT-FIG. 2. Archegonium surrounded by involucre and containing an egg in the fourth phase of fertilization. Thirty-six hours after flooding. $\times 320$.

A fertilization membrane has been described for several other Liverworts. Kny (1866) figures and describes a fertilized egg of *Riccia sorocarpa* as surrounded by a fairly thick layer or membrane. Garber (1904) says that the egg of *Riccia natans* surrounds itself with a membrane immediately after fertilization. The same is reported for *Fegatella conica* by Cavers (1904), and for *Targonia hypophylla* by Campbell (1918). Cavers says that the membrane is of cellulose. These authors do not, however, go into details on the subject.

The position of the male nucleus during this phase is in general the same as in previous phases, but in one instance the two nuclei were seen in contact.

Fourth Phase.

There now begins a marked change in the male nucleus. The condition mentioned above, in which the mass of this nucleus seems to be granular in structure, is more often seen, and often the structure is looser, so that distinct granules may sometimes be made out, with lighter areas between them (Figs. 10 and 11). At the same time, threads project from the edge of the dense part of the nucleus and extend across the clear space to the nuclear membrane (Fig. 8). Sometimes there are but one or two of these threads,

sometimes they are present throughout the nucleus, so that the latter has the appearance of a rough wheel with a large hub and irregular spokes (Fig. 7). Finally, the whole male nucleus may resolve itself into a compact reticulum which fills the whole of the space within the membrane, no part being noticeably denser than any other. This structure is illustrated in Fig. 12, a very early stage of the next phase. The reticulum is composed of granules linked together by threads which are almost as thick as the granules, so that the whole structure is still fairly dense and compact. The chromatic material of the nucleus now takes a violet colour in the triple stain, whereas in former stages it stained more readily with the safranin.

This series of changes is not found in succession throughout the period occupied by this phase. It seems probable that each nucleus must pass through these steps in order to attain the reticular structure; but some of the later stages are found in some nuclei at the beginning of the period, while others, perhaps in the same plant, still resemble those in the preceding phase. On the other hand, the earlier stages may be found throughout the period.

It is in this phase of the process, in fact, that the times at which the various changes take place begin to be variable. Series *A* and *B* furnish most of the cases in which the chromatic mass of the male nucleus is contracted and dense, while in Series *C* the more open reticular type of structure is more common. This variation between series suggests not only that the time necessary for the different phases may vary, but that the extent of the changes undergone by the nucleus may differ in different cases. It may be that in some cases the male nucleus never forms an open reticulum. The cause of this variation may lie in temperature or other external conditions, for each of the series was fixed at a different time of year from any of the others. And again, it is possible that the procedure of fixing varied sufficiently to account for these differences.

Another complication enters in the fact that in Phase 5, next to be described, the male nucleus seems to contract again, and presents an appearance similar to what is seen during Phase 3 (Pl. IV, Fig. 14). This makes it difficult to say, in the case of such a contracted nucleus occurring during the present phase, whether it is a delayed or an advanced stage. In one case, unfortunately torn in the preparation, the male nucleus seemed to contain a single much-coiled thread, along which were scattered granules in a single row.

Some variation begins to appear also in the female nucleus. In general, the chromatic substance is still massed in the centre, and little can be made out of its structure; but occasional nuclei are found in which more of the chromatin is distributed through the nuclear cavity in the form of threads and granules, with larger masses here and there. Typical nuclei of this phase are shown in Figs. 6 and 9. Sometimes also the changes characteristic

of Phase 5 are seen to be beginning here—a grouping of the chromatic material into the general shape of a continuous thread. In Series *A* most of the cases of fertilization 48 hours after flooding, a time which falls within Phase 5 in the other series, belong here as regards the structure of their nuclei.

In the cytoplasm the tendency towards an alveolar structure becomes more marked. There are also many cases showing the coarsely reticular structure described under the second phase (Fig. 8). Few eggs, however, show the fine, more homogeneous structure characteristic of the first phase. Some of the preparations belonging to this period show the beginning of a phenomenon which is characteristic of the next phase, and which will be more fully discussed later. This is a massing of a more dense and finely divided material in the cytoplasm near the poles of the female nucleus, leaving the rest of the cytoplasm often distinctly alveolar. The beginning of this process is shown in Figs. 9 and 12. Very rarely indications of this change are found in earlier stages. In Series *C* several eggs showing the characteristics of the second phase, and fixed only a few hours after flooding, show areas in the cytoplasm, near the poles of the nucleus, the substance in which seems more homogeneous than the rest of the cytoplasm and stains more lightly. Mention has already been made, under Phase 1, of a case in which the cytoplasm was denser and of a finer structure immediately around the nucleus, more open in the peripheral region. The most remarkable instances of the localization of a differentiated portion of the cytoplasm were found in Series *A*, 48 hours after flooding. In these cases there is an aggregation in the cytoplasm, in the regions normally occupied by the polar caps just described, of distinct granules of considerable size. They stain blue, and are conspicuous in the preparations. They are usually arranged in more or less regular rows radiating out from the poles of the nucleus. They are connected by a delicate system of slender threads. The whole structure seen under the low power of a microscope suggests at once two asters formed of radiating fibres; however, there is no distinctly recognizable body present at the centre of the radiations, either in this or in earlier or later stages. This condition is shown in Fig. 11. It is not universally found during this phase even in Series *A*, and is met with only rarely in the other series. As it sometimes occurs in later stages, further discussion of it will be postponed.

The condition of many of the female nuclei in eggs which show these cytoplasmic granules is peculiar. The contents are stained red instead of violet as usual, and are in the form of rounded bodies scattered throughout the nuclear cavity, with traces of a very fine reticulum. In Fig. 11 nine of these bodies are visible. The remainder of the same nucleus, seen in the adjacent section, shows several more. They have an appearance suggestive of small nucleoli, and recall the structures present in the nucleus in some of

the first stages of degeneration. This, together with the sporadic occurrence of the granules in the cytoplasm, suggests that the whole phenomenon may be artificial or pathological in nature.

It is the fourth phase which seems to correspond with most of the stages of fertilization in Liverworts observed by other workers.

Fifth Phase.

It was seen in the consideration of Phase 4 that there is a great variation in the time of occurrence of particular stages relative to the time after flooding. This variation now becomes even more marked, so that, although the next set of changes occupies roughly the next 20 hours (to 66 hours after flooding), this time may vary very greatly.

The male nucleus, as mentioned above, apparently undergoes a contraction, and appears once more as a small mass of granules surrounded by a clear area (Pl. IV, Fig. 14). It is probable that this condition is usually preceded by the open reticulate condition, since the majority of the cases of this phase was found in the same series (C) as afforded most of the instances of the reticulate condition.

The characteristic change in the female nucleus during this phase is the organization of chromosomes. The first indication of a change is that the chromatic substance, heretofore (in most cases) densely massed in one part of the nucleus, begins to spread out or unravel slightly, and becomes collected in several large masses and a larger number of smaller ones, which in their shape and position with respect to one another suggest that a continuous thread or spireme is being formed—or at least a series of chromosomes or short threads arranged roughly end to end. The substance of these groups of material is still a mass of fine granules and delicate coiled and twisted threads. The rest of the nuclear cavity is not entirely clear, for much of what seems to be the same stainable material is scattered through it, often in the form of a very delicate and rather regular reticulum bearing granules at the points of junction of the threads. Some slight indications of the beginnings of these changes can be seen in Fig. 13; the chromatic substance is still densely massed in the centre of the nucleus, but within it can be distinguished some more massive bodies arranged roughly in chains. In the nucleus represented in Fig. 14 an apparently continuous spireme has been formed. The outline of the latter is rough, and it is not homogeneous in structure. In many cases it seems to be continuous with the delicate reticulum which fills the rest of the nucleus. A nucleolus is not recognizable at this stage; if present, it does not differ from other structures of the nucleus either in form or in reaction to the stain. In Figs. 16 and 17 the spireme has apparently segmented into chromosomes; the outlines of the latter are much smoother than that of the spireme shown in Fig. 14. In Fig. 17 the X-chromo-

some described by Allen (1919) is suggested by a segment which is much longer and thicker than any of the others.

The two nuclei are usually in contact during this phase.

In many cases the staining method which best showed the nuclear details imparted to the cytoplasm only a very slight coloration, so that no details concerning its structure could be made out. In all cases, however, in which the cytoplasm was deeply enough stained to be carefully studied, certain well-marked changes were taking place in it. These changes are therefore assumed to be typical of this phase. The changes in question, as has already been indicated, involve the massing of a finer and more homogeneous portion of the cytoplasm at opposite ends of the egg nucleus. This differentiated material now takes the stain quite readily, but nothing can be made out of its structure save that it is practically uniform—apparently a mass of fine granules, possibly, in the living state, a colloidal liquid uniform in composition throughout its extent. In some cases there are faint indications of a reticular structure within these polar caps (Fig. 9). At their boundaries they merge into the cytoplasmic reticulum. The peripheral region of the cytoplasm is very vacuolate, and its substance is mostly concentrated into large and conspicuous ray-like structures, of a fibrillar nature, which radiate out from the boundaries of the polar caps. The whole appearance suggests very strongly a flowing of certain materials in the cytoplasm towards the female nucleus, and their aggregation there in a dense mass. A typical case of this process is shown in Fig. 9; another, in not quite so advanced a stage, in Fig. 12.

In the later stages the polar caps become more dense and apparently even more homogeneous, so that it is no longer possible to discern a reticular structure in them; they enlarge so as to surround completely the two nuclei and to occupy the larger part of the egg. The boundary of the material of the polar caps is now much sharper; outside it the cytoplasm is alveolar, the alveoli being fairly regular in shape and uniform in size. The condition is illustrated in Fig. 16. An indication of the ray-like structures formerly present is still seen in the presence of blunt, more or less conical projections from the dense into the alveolar cytoplasm, extending sometimes quite to the outer boundary of the cell.

In three cases the central mass of cytoplasm was replaced by a mass of granules, more or less radially arranged, as in those cases in Phase 4 described above.

It is while these changes are going on in the egg that the cells of the venter begin to divide, so that the wall becomes composed of two layers of cells. This division does not occur at the same time in all the cells, and the time at which it begins varies greatly. It might perhaps be expected that the beginning of these cell divisions would be correlated with some definite stage in the fertilization process. As a matter of fact, in the majority

of cases division of the wall cells does begin at the time that the polar caps begin to appear. Fifty such cases were counted; while only six showed a division in the wall of the venter at a time when the formation of polar caps had not begun. Twenty-seven eggs had formed their polar caps without any sign of division in the wall of the venter, but in many of these cases the polar caps were formed in advance of the usual time. It seems probable, therefore, that the division of the cells of the venter, if not stimulated by the changes just described in the egg cytoplasm, is at least usually coincident with them. The division is complete, and the venter consists of two layers or cells, in all cases observed in which the chromosomes are organized in the sexual nuclei.

Sixth Phase.

This phase includes the final steps in the organization of the chromosomes of both nuclei, the disappearance of the nuclear membranes, the splitting of the chromosomes, and the division of the latter to form the two daughter nuclei of the first embryonic division. Next to the penetration of the egg by the antherozoid, this is the most difficult of all the phases to observe. This fact is evident from a glance at Table I. Only eight cases are listed under this phase. Of these, four show the nuclear membranes intact, but enclosing fully organized chromosomes (Fig. 16).

The organization of chromosomes in the male nucleus was not seen. No transition stages were obtained between the granular condition last described and that of those nuclei which contained a clearly defined spireme or chromosomes lying within the nuclear membrane. The change is probably, therefore, very rapid; and the suggestion is inevitable that the spireme and chromosomes are organized during the contraction period of the nucleus—much as the *gemini* of the heterotypic prophase are organized in synizesis during the reduction division.

One case was observed in which the chromatic substance of the male nucleus was in the form of an apparently continuous spireme, coiled within the nuclear membrane. This is shown in Fig. 15. In another case, shown in Fig. 16, instead of a spireme there were eight distinct chromosomes, the regular gametophytic number for *Sphaerocarpos* (Allen, 1919). One of these is very small, and only by good fortune in the making of the section did it escape being masked by one of the larger ones. This is undoubtedly the Y-chromosome described by Allen. It is not a cross-sectional view of a long chromosome, since it disappears completely from view with a slight change in focus.

The female nuclei in these cases had not quite completed the organization of their chromosomes; the outlines of the latter were not quite so sharp, nor was their consistency apparently so uniform, as was the case with those of the male nuclei. Hence it would seem that, although the female

nucleus begins to organize its chromosomes earlier than the male, the completion of this process requires a longer time in the female nucleus, and that, before it is finished, chromosomes have been organized in the male nucleus with great rapidity. This difference may be connected with the large amount of material which is thrown off by the female nucleus and which takes no part in the formation of chromosomes. This material has been described under the preceding phase as visible in the form of small granules and in traces of a very fine reticulum scattered through the nuclear cavity; in the metaphase of the first embryonic division (Fig. 18) the cytoplasm contains many small granules which take the same stain, and which are in all probability the same bodies as were previously observed in the female nucleus.

In one of the cases above noted the central dense portion of the cytoplasm surrounding the female nucleus was replaced by a collection of the peculiar granules described under Phase 4, much as is shown in Fig. 11.

One preparation shows two groups of chromosomes lying in the cytoplasm, but is not sufficiently clear to enable me to determine whether or not the nuclear membranes are still present. Another preparation shows a dense mass of chromosomes surrounded by a slight clear area, but is also not sufficiently clear to be of great value. These two cases illustrate this phase without making very clear what is actually taking place.

Two preparations showed the chromosomes lying free in the centre of the dense central mass of cytoplasm. One of these belonged to Series *B*, and was fixed 60 hours after flooding. The chromosomes are in a small group, and only eleven could be counted with certainty (Fig. 18; one chromosome is in the adjacent section); the others (sixteen is the full sporophytic number) must either have been dragged out of place by the sectioning knife and lost, or were entirely concealed by some of the larger ones. The large X-chromosome is clearly visible. Two of the chromosomes seem to be split. Close examination of the cytoplasm showed it to be streaked in appearance, with alternate light and dark strips running in a fairly regular course. Definite fibres could not be seen. It is possible that this structure is an artifact resulting from the action of the fixing fluid on what was when living a typical spindle; but this suggestion is rendered less probable by the fact that the same preparations show spindles with clearly defined fibres in the vegetative cells of the thallus (Figs. 23 and 24), and in succeeding embryonic divisions (Fig. 22). It appears, therefore, as though the spindle of the first embryonic division were masked by a dense homogeneous material possibly nutritive in nature, or, more probably (to judge from the manner of its first appearance), a formative material in which the fibres originate. The streaked appearance of the whole mass of material may indicate the position of fibres which are themselves invisible as such.

The other preparation of a similar stage was found in Series *D*, fixed $73\frac{3}{4}$ hours after flooding. It is shown in Fig. 19. Fourteen chromosomes

are visible, of which two are thicker and less regular in outline than the others, and may each represent two chromosomes close together. Many of the chromosomes are split, the split being visible through only a part of the length of each chromosome. The large X-chromosome is plainly visible. One chromosome is very small, and, owing to the fact that it has divided, almost ring-shaped; this may be the Y-chromosome contributed by the male parent.

The most important question suggested by a consideration of these stages and of those immediately preceding them is: Do the sexual nuclei actually fuse, after the chromosomes have formed, but while their membranes are still intact; or do the membranes break down simultaneously, leaving the chromosomes free in the cytoplasm? On this point I have no positive evidence. No stages were found suggesting that fusion was taking place. When any nuclear membranes were present at all, the boundary separating the two nuclei (now always in contact) was always sharp and clear. The next stage found showed the chromosomes in one group, with nothing to indicate which were of paternal and which of maternal origin (except in the cases of the X- and Y-chromosomes), and surrounded by no membrane at all. If an actual fusion of the nuclei occurs, it must be very rapid, and the fusion nucleus must lose its membrane almost immediately. It seems more probable that there is no actual fusion, but that each nuclear membrane disappears independently of the other, though perhaps at the same time. In this connexion it is interesting to note that Miss Black (1913) reports that the nucleus of the fertilized egg of *Riccia* probably undergoes a period of rest, during which the chromatin is collected into a densely staining cord or series of segments, while there may be less deeply staining and more finely divided material scattered throughout the rest of the nucleus. This description at once suggests my cases, during Phase 5, in which the female nucleus is undergoing the first steps of spireme formation. Evidently *Riccia* differs from *Sphaerocarpos* in that its male nucleus has at this stage already fused with the female nucleus. The similarity is rather striking, and there is a possibility that, in *Riccia* as in *Sphaerocarpos*, the male nucleus is still separate from the female at this time, but was overlooked.

Seventh Phase.

This phase includes the anaphases and telophases of the first nuclear division of the zygote and the division of the latter into two cells. The wall formed in this division is always transverse, as in all other Liverworts (except *Anthoceros*) in which this division has been described.

The daughter nuclei during the telophases are much smaller than either of the sexual nuclei just before the division. Because of the degree to which their chromatin is massed together, I could not follow the details of reconstruction. The daughter nuclei shortly pass into a resting state, and then

except for their smaller size, resemble the egg nucleus in the first or second phase. The chromatin is arranged in the form of small granules and delicate twisted threads, without any definite reticular structure (Fig. 20).

As the daughter groups of chromosomes separate, and the daughter nuclei are formed, the central dense mass of the cytoplasm seems to pull out between them, leaving only some coarse strands extending from one daughter nucleus to the other. In some cases these strands are fine enough to resemble the central fibres of an ordinary spindle, though they are never very numerous. Each daughter nucleus is still surrounded by a mass of homogeneous cytoplasm, which radiates in all directions so as to form a sort of star-shaped figure. It is interesting to note that the figures of Campbell (1888) for *Pilularia globulifera* and of Meyer (1911) for *Corsinia marchantioides* illustrating two-celled embryos show a condition of the cytoplasm in the daughter cells very similar to that just described for *Sphaerocarpos*. The condition is illustrated in Fig. 20.

In seven cases, all belonging to Series *D*, the dense substance was replaced by masses of granules as previously described. These are not arranged regularly in rows, and seem larger and closer together than those, for instance, shown in Fig. 11; but they maintain in the cell the star-shaped arrangement just described. Often they are very closely massed upon the new wall separating the daughter cells.

No cases were observed in which the cell plate or dividing wall extended only partly across the cell. In all cases in which a division membrane was present at all, it cut the cell completely into two, and was fairly distinct and heavy.

The two-celled embryo measures about 75 by 25 μ , and is therefore considerably larger than the egg in the first phase of fertilization. The enlargement is gradual, the egg in each successive phase being a little larger than in the preceding one (compare Figs. 2, 8, 18, and 20. Many sections, of course, do not pass through the greatest length of the egg).

Eighth Phase.

In each of the four cases found showing a division in the two-celled embryo, one of the nuclei had begun to divide before the other. In three of these cases the upper nucleus was dividing, in the fourth the lower. Very distinct fibres were visible in three of these cases, arising in the cytoplasm of the dividing cell and spreading out from two fairly definite poles over the nuclear membrane. The chromatin was observed both in the form of a spireme, still enclosed by the nuclear membrane, and in the form of chromosomes arranged in an equatorial plate upon a typical spindle. The latter stage is illustrated in Figs. 21 and 22. Fig. 21 shows a polar view of the equatorial plate. The chromosomes are more slender than in the first embryonic division. Fifteen chromosomes can be counted; of these, either

of two apparent chromosomes may really be two whose ends are close together. The X-chromosome is plainly visible, also, close by it, the Y-chromosome. Fig. 22 shows the spindle very plainly, but the chromosomes are too close together to be counted. Even here, however, the X- and Y-chromosomes are evident, close together as in the case just described. There seems no doubt that dividing nuclei of the embryo contain the full sporophytic number of chromosomes (16). For comparison with these sporophytic groups I have included figures of division stages from cells of the wall of the venter. Fig. 23 shows an equatorial plate; the chromosomes (except the X-chromosome) seem to be cut in cross-section; eight of them are visible, seven being small rounded bodies, and the much longer and thicker X-chromosome extending up and down the spindle. Fig. 24 shows

an anaphase, in which eight daughter chromosomes are moving towards each pole of the spindle. In several cases the two daughter chromosomes of a pair are still attached to each other at one end. Here also the X-chromosome is easily distinguished.

TEXT-FIG. 3. Archegonium containing a four-celled embryo. Eighty-four hours after flooding. $\times 320$.

Ninth Phase.

This phase includes embryos which have undergone two cell divisions. The four cells are in all cases that I have observed arranged in a row, as illustrated in Text-fig. 3. This account agrees with that of Campbell (1918), but is contrary to the description of Cavers (1911), who says that the 'embryogeny is of the Marchantiales type'—that is, the young embryo passes through an octant stage. Some unpublished work of Miss E. M.

Ritchie indicates that there is some variation in the plane of the second division in the embryo of *Sphaerocarpos*. All the cells are of approximately the same size, and do not differ essentially in appearance from those formed by the first division, except that the cytoplasm is apparently alveolar in structure, and shows only slight traces of the dense material that surrounded the egg nucleus during Phases 5 and 6.

Tenth Phase.

This phase is merely a convenient grouping of all stages that were observed subsequent to those already described. The embryo has become a mass of cells, a longitudinal division having occurred in some cells. Since the present paper is concerned only with fertilization, no further discussion of embryological development will be included.

Time Relations.

Mention has already been made of the fact that sporophytes become large enough to be readily visible from two to eight weeks after flooding. This indicates that the speed of the process of fertilization and the rate of development of the embryo may vary under different conditions or in different cultures. The cultures that took the longest time (eight weeks) to produce visible sporophytes were those from which Series C was made. This fact coincides exactly with the cytological data, for, although the fixations in this series were carried on until 66 hours after flooding, no stages were found farther advanced than Phase 5, while in the other series fixations made 60, 64, and 65½ hours after flooding disclosed many examples of Phases 6 and 7 (Table I).

The earlier stages seem to proceed at a quite uniform rate, so that it is possible to assign definite time limits to the first three phases. Phase 4 shows considerable variation; and Phase 5 still more. Finally, Phase 6, which includes the actual division of the fertilized egg, seems to occur over a wide range of time. It is also noteworthy that the preceding phase (5) is quite prolonged, as though the changes that go on therein require a long time for their completion, while the division itself is rapid. It is evident that, after a long period of slow and gradual change, during which time the egg nucleus organizes its chromosomes, the egg cytoplasm takes on a definite structure, and the male nucleus is in a condensed state, the further processes leading to the first division—the formation of chromosomes by the male nucleus and the disappearance of the nuclear membranes—take place with almost explosive suddenness. Furthermore, the time necessary to complete the slow preliminary changes and to initiate the actual division varies very greatly with the individual. These time relations are illustrated (Table I) by the small number of cases of Phase 6 as compared with the number of cases referable to preceding and succeeding phases. This is not because fewer slides were made covering this time, since the instances of Phase 6 that were found occurred often at the same time, sometimes on the same plants as afforded numerous instances of Phases 5 and 7.

A few antherozoids are often seen in the cavity of the venter outside the egg in later stages, when fertilization has already taken place. These retain their normal form, and to all appearances were alive at the time of fixation. This fact suggests the possibility that some of the variation in time of the different stages in fertilization may be due to a variation in the starting-point—that is, that some antherozoids may not enter the egg until several hours after flooding. This, however, is rendered improbable by the uniformity in time of the first two or three phases. It is also possible, as is suggested by an exceptionally premature case of Phase 5 (Table I), that fertilization has sometimes taken place in the material studied before flood-

ing. This, however, must have been rare, if it occurs at all, again for the reason that only cases of Phase I are found within the two hours following flooding. We may therefore, I think, conclude that the variation in the times at which stages are found is due to variations in the rate of the process rather than in the time of its starting.

Doubtful Cases.

In a number of the cases listed in Table I, the male nucleus was not to be seen. The female nucleus and cytoplasm, however, showed all the changes characteristic of the fertilization process, at one phase or another. Since no eggs are found in this condition in material which has not been flooded or afforded other opportunity for fertilization, these are thought to be true cases of fertilization in which the male nucleus was accidentally lost in making the preparation. In one case the male nucleus was found apparently inside the female nucleus. Closer examination revealed the fact that it was at a higher focal level than anything else in the egg; and that in the next section there was a clear space surrounded by a distinct membrane, the latter in contact with the membrane of the female nucleus. Evidently the knife had caught the chromatin of the male nucleus without cutting it, dragged it out of its proper place, and left it in a section in which it did not belong. Something of this sort may have happened in some of the doubtful cases referred to.

Here arises the question whether parthenogenesis occurs. The number of doubtful cases referred to above amounts to about 6 per cent. of the total (19 out of 307 cases). If this proportion of all the sporophytes formed resulted from parthenogenesis, we should expect to find a fair number of sporophytes in purely female cultures. As a matter of fact, the reverse is true. In the great majority of female cultures no sporophytes are found; and in cases where they do occur, their origin is always traceable to a male plant that has somehow found its way into the pot, or to an accidental flooding of several cultures (including males) at the same time, or to some similar accident. It might be supposed that the act of flooding the plants may in some way furnish a stimulus by which a few eggs are enabled to develop parthenogenetically. This, however, seems rather far-fetched; especially as female cultures have often been flooded by accident, without the resultant appearance of any sporophytes.

There seems, therefore, little doubt that the development of an egg into an embryo is in all instances the result of a sexual process—that is, that parthenogenesis never occurs, and that cases that might seem to suggest it are due only to accidents of technique.

Polyspermy.

In something over 8 per cent. of the cases of fertilization observed, more than one male nucleus was present in the egg cytoplasm. These cases are

assembled in Table II. They were most numerous in Series *C* and *D*, and in Series *C* were found mostly in the earlier stages.

Most of these cases show two male nuclei instead of one. In the earlier stages both male nuclei frequently appear normal, and the egg is in the condition that is usual for the phase in question. In several cases there are differences between the two male nuclei. In one instance one was less homogeneous than the other, although the time elapsed since flooding was short (4 hours). In one very early case ($1\frac{1}{2}$ hours after flooding) no less than six male nuclei were observed in one egg, mostly of irregular shapes. In two other cases there were four; in one of these instances the shapes of the male nuclei were irregular; in the other, two of them were close together within one clear space.

TABLE II.
Cases of Polyspermy.

<i>Series.</i>	<i>Time after flooding.</i>		<i>No. of</i>	<i>No. of</i>
	<i>Hrs.</i>	<i>Min.</i>	<i>cases.</i>	<i>male nuclei.</i>
A	0	35	1	3
C	1	30	1	6
C	1	30	1	2
C	2		1	2
C	4		2	2
C	4		1	4
C	6		1	4
C	6		1	2
C	18		2	2
A	28		1	2
C	32		1	2
B	40		1	2
A	48		1	2
D	62	30	1	several
C	64		1	3
D	65	30	3	several
D	67	30	5	"
D	84		1	"
			—	
			26	

In slightly later stages there is more frequently a difference between the male nuclei. The difference suggests sometimes that one is developing in the normal way, while the development of the other is arrested in the earliest phases. In one such case, found 18 hours after flooding, one male nucleus has the appearance normal for this time; the other is long, narrow, and pointed at the ends, the form characteristic of Phase 1. In another case one male nucleus is distinctly smaller than the other. Sometimes, however, both of them, three in one case, appear quite normal. The presence of the additional male nuclei seems not thus far to have disturbed in any way the normal development of the female cell.

In the later cases of polyspermy, most of which come from Series *D*, the female nucleus shows signs of degeneration. This is evident in a lump-

ing together of its contents and a loss of finer structure. Similar changes have taken place also in the cytoplasm. The male nuclei in these instances have not reached an advanced stage of development, but appear as in the three earliest phases of the normal process (sometimes as in Phase 5), save that certain irregularities in shape are sometimes evident. The condition is illustrated in Fig. 25.

It seems clear that more than one antherozoid penetrates the egg in a fairly large proportion of cases. The occasional occurrence of polyspermy is perhaps connected with the rapidity with which the antherozoids reach the egg, and with the delicacy of the membrane of the latter. It seems clear also that the entrance of more than one antherozoid disturbs the normal process. The male nuclei themselves are arrested in development at an early stage, and the egg later undergoes degeneration, in much the same way as when it is not fertilized. There is a possibility, however, suggested by some of the above-described cases, that one antherozoid may continue its development in a normal way while the other remains in a condition characteristic of an earlier phase and finally breaks down and disappears. In any case, it is safe to conclude that never does more than one male nucleus take part in the formation of the zygote nucleus.

In many animals polyspermy is a normal occurrence. In other animals the entrance of more than one spermatozoon may be induced by various means; such artificial polyspermy usually causing a degeneration of the egg nucleus. In plants, however, polyspermy has been reported, so far as I know, only in *Fucus vesiculosus*, by Farmer and Williams (1898), and in *Onoclea struthiopteris*, by Mottier (1904). Farmer and Williams noted polyspermy in only three cases of a thousand studied. Their figure shows two antherozoid nuclei apparently fused with the egg nucleus. In *Onoclea* Mottier figures two antherozoids, one penetrating the egg nucleus in the normal way, the other lying coiled in the cytoplasm, surrounded by a clear area.

Cytoplasmic Radiations and Granules.

The cases that have been mentioned from time to time in the description, which show more or less regularly radiating rows of granules in the cytoplasm of the egg, are brought together in Table III. They appear only infrequently—in about 6 per cent. of the total number of cases observed—and in several different phases.

These granules are of spherical or ovoid shape, of considerable size, and seem to be, at least in many cases, united by fibres. The latter fact, as well as their general appearance, makes it improbable that they are of the nature of starch grains. They stain violet in the triple stain. In preparations stained with iron-alum-haematoxylin and light green, they appear as hyaline spheres, quite clear when in focus, black when out of focus.

TABLE III.

Cases showing Cytoplasmic Granules.

Phase.	Series.	Time after flooding.		No. of cases.
		Hrs.	Min.	
5	C	40		2
4	A	48		7
7	D	65	30	2
8	D	65	30	1
5	C	66		1
6	D	71	30	1
7	D	75	45	4
				<hr/> 18

It may be that these bodies represent an excess of food material in the cytoplasm. On the other hand, their appearance may be due to slight differences in the action of the fixing fluid, which in turn are perhaps due to slight differences in the condition of the cytoplasm. The substance that is usually present instead of the granules seems to be, as already mentioned, a dense, homogeneous, presumably colloidal material; and the appearance of a system of granules may be caused by a coalescence of one of the phases of the colloid, leading to the production of large droplets.

Both the rows of granules, and, in some cases, the radiating processes extending out from the polar caps, present under the low power of the microscope an appearance similar to that of two asters. It is interesting in this connexion to recall that centrosomes have been described in several Liverworts, notably in *Pellia* and *Marchantia*, in various tissues of both sporophyte and gametophyte. Centrosomes, with distinct radiating fibres, have been described in the fertilized egg of *Preissia quadrata* by Miss Graham (1918), and in that of *Riccardia pinguis* by Florin (1918). In the former case the radiations which form the aster are definite, easily visible fibres, and in the centre is a small definite body. In the latter they are apparently close rows of fine granules, which merge together in small dense masses ('centrosome-like bodies') near the poles of the nucleus.

In *Sphaerocarpos* such rays as are present, though fibrillar in nature in some stages, are much less regular than those described by these workers, and converge upon no definite bodies, but pass into the large dense masses of material to which I have referred as the polar caps. In later stages these rays are not at all fibre-like, and are continuous with the material which now surrounds the female nucleus. The whole appearance suggests, as I have said before, a flowing of material towards the female nucleus. But it is possible that an actual centrosome with its aster may lie concealed in the cytoplasm. At all events, it is evident that marked chemical and physical changes take place in the cytoplasm of the egg at both ends of the nucleus, which are later concerned with its division, and that these changes

may express themselves, in different plants, by influencing in different ways the structure of the cytoplasm; the difference may also be exaggerated by differences in technique.

The concentration of certain materials in the cytoplasm about the egg nucleus may be a phenomenon of general occurrence. Mention has already been made, under Phase I, of the variety of forms in which this has been partially described or figured. It is interesting also to note that Gayet (1897) describes the egg of *Sphagnum* as containing in its cytoplasm numerous rounded bodies, which he calls chromatophores. The cytoplasm of the fertilized egg of *Preissia quadrata*, as described by Miss Graham (1918), is in a similar condition, and the rounded bodies are more numerous around the nucleus. It is possible that the rounded bodies described by these authors bear the same relation to the denser portions of the cytoplasm noted by others as do the granules of the egg of *Sphaerocarpos* to the more usual homogeneous mass that surrounds the nucleus.

Fertilization in the Bryophyta.

The various cases, mentioned in the introduction to this paper, of fertilization in Liverworts observed and figured by previous writers harmonize very easily with the complete history as here presented for *Sphaerocarpos*. Although in most of the cases previously described only one stage, or several stages almost alike, were seen, and although various interpretations were placed upon these few stages, it seems possible that fertilization may follow the same course in many Liverworts as it does in *Sphaerocarpos*. At all events, in all cases (except two) so far described, the male nucleus enters into a resting condition before fusion with the female nucleus; and whether an actual fusion occurs, and in what condition the nuclei fuse, is not known with certainty in any Liverwort, not excluding *Sphaerocarpos*. Of the two exceptions mentioned, one is the case of *Riella* described by Kruch (1891), in which the male nucleus seems to proceed to the organization of chromosomes immediately after its entrance into the egg and while it is taking on a spherical form. More modern methods might perhaps throw more light on what actually takes place here. The other divergent case is found in *Fossombronia*, as reported by Humphrey (1906). Here the antherozoid, or at least its nucleus, retains its vermiform shape after entering the egg cytoplasm. Its subsequent behaviour was not observed. Some unpublished and as yet incomplete results of Mr. A. M. Showalter (to whom I am indebted for allowing me to see his figures) show what is apparently the same condition in *Riccardia pinguis*, and also seem to indicate that the still vermiform or coiled antherozoid penetrates the female nucleus, much as is the case in some ferns (Shaw, 1898; Yamanouchi, 1908). These two last-cited cases among the Jungermanniales suggest that the behaviour of the nuclei in fertilization may be quite different for the Jungermanniales and

the Marchantiales, which latter group includes all the other Liverworts in which fertilization has been thus far described except *Riella*. Should this suggestion be verified, it is important from a phylogenetic standpoint to note that the history of fertilization in *Sphaerocarpos* and *Riella* resembles that found in the Marchantiales.

If, as seems likely, there is no nuclear fusion, *Sphaerocarpos* constitutes in this respect a unique case among plants. Such a behaviour of the nuclei as seems to hold in *Sphaerocarpos* is characteristic of the gamete nuclei of many animals, reaching its extreme expression in such cases as that of *Cyclops*, wherein the male and female nuclei not only furnish independent groups of chromosomes, but these persist as two distinct groups, and can be followed through the division of the zygote, and in some cases through several succeeding divisions. Among plants, the closest approach yet noted to such a condition is found in *Pinus Strobus*, as described by Miss Ferguson (1904). Here the male nucleus is received in a concave depression in the surface of the (much larger) female nucleus. The chromatin is then organized into a spireme in each nucleus, while the boundary between the two is still very plain. The nuclear membranes then 'fade out', leaving the two spiremes on the spindle. The distinction between the two parental groups of chromosomes becomes lost in the equatorial plate of the first division.

Fusion of nuclei while the latter are in the spireme state has been described by several writers—as by Miss Weniger (1918) for *Lilium philadelphicum* and *L. longiflorum*, by Sax (1916) for *Fritillaria pudica* (in rare cases). The separate formation of chromosomes in two groups by the male and female nuclei after they have completely fused has been reported by Blackman (1898) for *Pinus silvestris*, by Murrill (1900) for *Tsuga canadensis*, by Hutchinson (1915) for *Abies balsamea*, and by Sax (1918) for *Triticum*. Chamberlain (1899) figures an egg of *Pinus Laricio* which contains two separate spiremes. In many of the higher plants that have been studied, however (Chamberlain, 1916; Sax, 1916, 1918), the two sexual nuclei fuse completely while in the resting state. This is apparently characteristic for the Thallophyta (a review of cases of fertilization in a number of the lower plants is given by Mottier, 1904). In *Polysiphonia violacea*, however, Yamanouchi (1906) reports that the male nucleus contains distinct chromosomes when it fuses with the female nucleus; the latter is in a resting condition. In the ferns, so far as known (Yamanouchi, 1908), the two nuclei fuse completely, and the fusion nucleus passes into a resting condition before the first division.

Sphaerocarpos furnishes yet another instance in support of the doctrine of the individuality of the chromosomes, since the eight chromosomes that are formed separately in each nucleus are undoubtedly the same as those that pass on to the spindle and finally form the daughter nuclei of the first

division. It is also worthy of note that, in spite of the immense size of the female nucleus and the much smaller size of the male, the chromatic material which actually enters into the sporophytic nuclei is contributed approximately equally by each parent (except for the difference between the X- and Y-chromosomes), since a large part of the substance within the female nucleus passes into the cytoplasm after the nuclear membrane disappears.

SUMMARY.

1. The antherozoid nucleus, after entering the egg, assumes the shape of a thick, curved rod surrounded by a clear space (Phase 1). After about two hours it takes first an oval shape (Phase 2), then, about eight hours later, a spherical shape (Phase 3). Its body is in these stages dense and homogeneous. These changes occupy in all about twenty-two hours.

2. The clear space surrounding the antherozoid nucleus becomes bounded by a membrane. The male nucleus now expands somewhat and its chromatic material assumes a more or less reticular structure, resembling that of the female nucleus at this time (Phase 4). In this condition the nuclei remain about twenty-four hours.

3. The female nucleus then proceeds to the slow organization of chromosomes, while the male nucleus again contracts and loses its visible structure (Phase 5). In the egg cytoplasm, meanwhile, dense caps of homogeneous material appear opposite each pole of the female nucleus. These processes occupy on the average about twenty hours, the time being very variable.

4. The polar caps in the egg cytoplasm enlarge until they completely surround the two nuclei, which now lie in contact. The male now organizes its chromosomes very rapidly, and the membranes of the two nuclei break down probably at the same time. Eight chromosomes can be counted in the male nucleus. A spindle cannot be distinguished in the cytoplasm on account of the dense mass of material there present. The first division proceeds very rapidly (Phase 6). About sixteen chromosomes can be counted. The first partition wall of the young embryo is transverse, as are the two succeeding ones in the majority of cases.

5. Polyspermy occurs in about eight per cent. of the cases of fertilization observed. This, at least usually, results in the degeneration of the egg.

In conclusion I wish to express my great indebtedness to Professor C. E. Allen, at whose suggestion and under whose supervision this work was done.

LITERATURE CITED.

- ALLEN, C. E. (1919): The Basis of Sex Inheritance in *Sphaerocarpos*. Proc. Amer. Phil. Soc., lviii. 289-316.
- ARNELL, H. W. (1875): An Observation of the Fecundation of Mosses. Rev. Bryol., ii. 114-15.
- BLACK, C. A. (1913): The Morphology of *Riccia Frostii*, Aust. Ann. Bot., xxvii. 511-52.
- BLACKMAN, V. H. (1898): On the Cytological Features of Fertilization and Related Phenomena in *Pinus silvestris*, L. Phil. Trans. Roy. Soc., London, B., cxc. 395-426.
- CAMPBELL, D. H. (1888): The Development of *Pilularia globulifera*, L. Ann. Bot., ii. 233-64.
- (1896): The Development of *Geothallus tuberosus*. Ibid., x. 489-510.
- (1918): The Structure and Development of the Mosses and Ferns, 3rd ed., pp. x and 708. New York.
- CAVERS, F. (1904): On the Structure and Biology of *Fegatella conica*. Ann. Bot., xviii. 87-120.
- (1911): The Interrelationships of the Bryophyta. New Phyt., Reprint No. 4, p. 203.
- CHAMBERLAIN, C. J. (1899): Oogenesis in *Pinus Laricio*. Bot. Gaz., xxvii. 268-80.
- (1916): *Stangeria paradoxa*. Ibid., lxi. 353-72.
- DURAND, E. J. (1908): The Development of the Sexual Organs and Sporogonium of *Marchantia polymorpha*. Bull. Torrey Bot. Club, xxxv. 321-35.
- FARMER, J. B., and WILLIAMS, J. L. (1898): Contributions to our Knowledge of the Fucaceae: their Life-history and Cytology. Phil. Trans. Roy. Soc., London, B., cxc. 623-45.
- FERGUSON, M. C. (1904): Contributions to the Knowledge of the Life-history of *Pinus* with Special Reference to Sporogenesis, the Development of the Gametophytes, and Fertilization. Proc. Wash. Acad. Sci., vi. 1-202.
- FLORIN, R. (1918): Das Archegonium der *Riccardia pinguis*, (L.) B. Gr. Svensk Bot. Tidskr., xii. 464-70.
- GARBER, J. F. (1904): The Life-history of *Ricciocarpus natans*. Bot. Gaz., xxxvii. 161-76.
- GAYET, L. A. (1897): Recherches sur le développement de l'archégone chez les Muscinées. Ann. Sci. Nat., sér. 8, v. 161-258.
- GRAHAM, M. (1918): Centrosomes in Fertilization Stages of *Preissia quadrata*, (Scop.) Nees. Ann. Bot., xxxii. 415-20.
- HUMPHREY, H. B. (1906): The Development of *Fossombronina longiseta*, Aust. Ibid., xx. 83-107.
- HUTCHINSON, A. H. (1915): Fertilization in *Abies balsamea*. Bot. Gaz., lx. 457-72.
- HY, M. (1884): Recherches sur l'archégone et le développement du fruit des Muscinées. Ann. Sci. Nat., sér. 6, xviii. 105-206.
- KNY, L. (1866): Über Bau und Entwicklung der Riccien. Jahrb. f. wiss. Bot., v. 354-84.
- KRUCH, O. (1891): Appunti sullo sviluppo degli organi sessuali e sulla fecundazione della *Riella Clausonia* Let. Malpighia, iv, fasc. ix, x. 1-23.
- VAN LEEUWEN-REIJNVAAN, W. and J. (1908 a): Über eine zweifache Reduktion bei der Bildung der Geschlechtszellen und darauf folgende Befruchtung mittels zwei Spermatozoiden und über die Individualität der Chromosomen bei einigen *Polytrichum*-arten. Rec. Trav. Bot. Néerl., iv. 177-220.
- (1908 b): Über die Spermatogenese der Moose, speziell mit Berücksichtigung der Zentrosomen- und Reduktionsteilungsfragen. Ber. d. Deutsch. Bot. Gesell., xxvi a. 301-9.
- MEYER, K. (1911): Untersuchungen über den Sporophyt der Lebermoose. I. Die Entwicklungsgeschichte des Sporogons der *Corsinia marchantioides*. Bull. Soc. Imp. Nat. Moscou, N.S., xxv. 267-83.
- (1913): Untersuchungen über den Sporophyt der Lebermoose. II. Die Entwicklungsgeschichte des Sporogons bei *Plagiochasma*. Ibid., xxvii. 597-615.
- MOTTIER, D. M. (1904): Fecundation in Plants. Carn. Inst. Wash. Publ. 15, pp. iv and 187.
- MURRILL, W. A. (1900): The Development of the Archegonium and Fertilization in the Hemlock Spruce (*Tsuga canadensis*, Carr.). Ann. Bot., xiv. 583-607.
- RICKETT, H. W. (1920): The Development of the Thallus of *Sphaerocarpos Donnellii*, Aust. Amer. Journ. Bot., vii. 182-95.

- ROZE, E. (1872): De la fécondation chez les cryptogames supérieures, et en particulier chez les Sphaignes. Bull. Soc. Bot. France, xix. 91-103.
- SAX, K. (1916): Fertilization in *Fritillaria pudica*. Bull. Torrey Bot. Club, xliii. 505-22.
- (1918): The Behavior of the Chromosomes in Fertilization. Genetics, iii. 309-27.
- SHARP, L. W. (1921): An Introduction to Cytology, pp. ix and 452. New York.
- SHAW, W. R. (1898): The Fertilization of *Onoclea*. Ann. Bot., xii. 261-85.
- STRASBURGER, E. (1870): Die Geschlechtsorgane und die Befruchtung bei *Marchantia polymorpha*. Jahrb. f. wiss. Bot., vii. 409-22.
- (1892): Schwärmsporen, Gameten, pflanzliche Spermatozoiden und das Wesen der Befruchtung. Hist. Beitr., iv. 48-156.
- (1893): Über die Wirkungssphäre der Kerne und die Zellgrösse. Ibid., v. 97-124.
- WALKER, N. (1913): On Abnormal Cell-fusion in the Archegonium, and on Spermatogenesis in *Polytrichum*. Ann. Bot., xxvii. 115-32.
- WENIGER, W. (1918): Fertilization in *Lilium*. Bot. Gaz., lxvi. 259-68.
- WILSON, M. (1911): Spermatogenesis in the Bryophyta. Ann. Bot., xxv. 415-57.
- WOODBURN, W. L. (1920): Preliminary Notes on the Embryology of *Reboulia hemisphaerica*. Bull. Torrey Bot. Club, xlv. 461-4.
- YAMANOUCHI, S. (1906): The Life-history of *Polysiphonia violacea*. Bot. Gaz., xlii. 401-48.
- (1908): Spermatogenesis, Oogenesis, and Fertilization in *Nephrodium*. Ibid., xlv. 145-75.

EXPLANATION OF FIGURES IN PLATES III AND IV.

Illustrating Dr. Rickett's paper on Fertilization of *Sphaerocarpos*.

PLATE III.

All figures were outlined with the aid of a camera lucida, at a magnification of 2,000 diameters. Lenses used were Zeiss comp. oc. 12 and Zeiss apochr. obj. 2 mm., N.A. 1.40. The sections were 7μ in thickness. The upper end of the figure in every case corresponds to the distal end of the egg.

Fig. 1. Antherozoid shortly after its entrance into the cytoplasm of the egg. Ten minutes after flooding.

Fig. 2. Egg showing the first phase of fertilization. Two hours after flooding.

Fig. 3. Male nucleus in the cytoplasm of the egg during the second phase. Seven hours after flooding.

Fig. 4. Third phase. Eighteen hours after flooding.

Fig. 5. Remainder of female nucleus of preceding figure.

Fig. 6. Female nucleus of egg during the fourth phase. Thirty-eight hours after flooding.

Fig. 7. Male nucleus during the fourth phase, showing open type of structure. From the egg containing the nucleus shown in Fig. 6.

Fig. 8. Fourth phase. Thirty hours after flooding.

Fig. 9. Fourth phase, showing the formation of polar caps in the cytoplasm. Forty-four hours after flooding.

Fig. 10. Male nucleus from the egg of Fig. 9 (in adjacent section).

Fig. 11. Egg during the fourth phase, showing radiating masses of granules replacing the polar caps in the cytoplasm. Forty-eight hours after flooding.

Fig. 12. Very early example of the fifth phase. Fifty-six hours after flooding.

Fig. 13. Remainder of female nucleus of preceding figure, showing the first indications of spireme formation.

PLATE IV.

Fig. 14. Male and female nuclei in the fifth phase. Sixty-two and a half hours after flooding.

Fig. 15. Male nucleus which has its chromatin in the form of a spireme. Seventy-one and a half hours after flooding.

Fig. 16. Early example of the sixth phase. Each nucleus a group of chromosomes surrounded by a membrane. Cytoplasm definitely organized for the first division. Sixty hours after flooding.

Fig. 17. Remainder of female nucleus of preceding figure.

Fig. 18. Sixth phase. Nuclear membranes have disappeared. Sixty hours after flooding.

Fig. 19. Sixth phase. Equatorial plate of first division. Seventy-three and three-quarter hours after flooding.

Fig. 20. Seventh phase. Two-celled embryo. Sixty-eight hours after flooding.

Fig. 21. Chromosomes in the second division of the zygote. Polar view of equatorial plate. Sixty-eight hours after flooding.

Fig. 22. Same as Fig. 21. Side view showing spindle. Sixty-four hours after flooding.

Fig. 23. Gametophytic chromosomes in a dividing cell of the venter. Side view of equatorial plate.

Fig. 24. Same as Fig. 23. Anaphase.

Fig. 25. Polyspermy. Male nuclei as in fourth phase, egg beginning to degenerate. Several more male nuclei appear in adjacent sections. Sixty-seven and a half hours after flooding.

8.

9.

12.

10.

13.

18.

21.

22.

25.

23.

24.

Rickett, H W . 1923. "Fertilization in Sphaerocarpos." *Annals of botany* 37, 225–259. <https://doi.org/10.1093/oxfordjournals.aob.a089844>.

View This Item Online: <https://www.biodiversitylibrary.org/item/270686>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a089844>

Permalink: <https://www.biodiversitylibrary.org/partpdf/319081>

Holding Institution

New York Botanical Garden, LuEsther T. Mertz Library

Sponsored by

BHL-SIL-FEDLINK

Copyright & Reuse

Copyright Status: Public domain. The BHL considers that this work is no longer under copyright protection.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.