NOTES.

ON FERTILISATION, AND THE SEGMENTATION OF THE SPORE IN FUCUS[1]. By J. Bretland Farmer, M.A., Professor of Botany at the Royal College of Science, and J. Ll. Williams, Marshall Scholar at the Royal College of Science, London.—The object of the present communication is to give an account of the chief results of an investigation into the processes connected with the formation and fertilisation of the oospheres and the germination of the spore in *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Fucus platycarpus*. The more obvious details of development have been especially studied by Thuret, and later by Oltmanns. But neither of these writers paid any special attention to the behaviour of the cell-nuclei, nor did they succeed in observing the actual process of fertilisation. Behrens has communicated an account (Ber. d. Deutschen Bot. Gesel., Bd. IV) of some researches made by himself on the fertilisation of the oospheres, but we are unable to accept his conclusions for reasons shortly to be recounted.

The material for these investigations was obtained in London from Bangor, Plymouth, and Jersey, but it was compared with other material collected, and fixed at the seaside at Bangor, Weymouth, and Criccieth. Furthermore, all the growing apices and conceptacles for sectioning were collected by one of us directly at the three last-named places. Some samples were gathered between the tides, and fixed at once, others were first kept for a time in salt water; the best results, however, were obtained from plants collected in a boat about two or three hours after the tide had reached the plant, and also from other plants taken a short time before they were left exposed by the ebb tide.

In order to study the stages of fertilisation and germination, male

[1] Paper read before the Royal Society, June 18, 1896.

and female plants were kept in separate dishes, and were covered over so as to prevent drying up. This method gave far better results than those more usually advocated. On the appearance of the extruded sexual products, the female receptacles were placed in sea-water, and after the complete liberation of the oospheres, a few male branches with ripe antherozoids were first placed in a capsule of sea-water until it became turbid owing to their number. If on examination the antherozoids proved to be active, small quantities were added to the vessels containing the oospheres. The latter were then fixed at intervals of five minutes during the first hour, and then at intervals of fifteen minutes, up to six hours after the addition of the antherozoids. After that, samples were killed at longer intervals up to three days, and this was continued till we had material fixed at all stages for the first fortnight. At first we used sea-water in which to keep the embryos growing, but a proper solution of Tidman's sea-salt was found to answer quite as well.

For fixing, we tried the following reagents—chrome-alum, picric-alum, Mann's picro-corrosive, corrosive sublimate, and acetic acid; these were all dissolved in sea-water; absolute alcohol, Flemming's and Hermann's solutions, and the vapour of osmic and formic acids. The Flemming's (strong formula) and Hermann's solutions were diluted with equal parts of sea-water. The first three fixatives were unsuccessful, acetic-corrosive yielded fair nuclear figures, but the material proved very brittle, and the spores were somewhat distorted. A portion of the cytoplasm was disorganised and the polar radiations were not preserved. Absolute alcohol fixed the oospheres and newly fertilised spores without distortion, but was useless for all other stages. Vapour fixing with osmic acid succeeded better than any of the preceding reagents, but was greatly inferior to either Hermann's or Flemming's solutions in preserving the protoplasmic structure in an unaltered state.

After the material had been fixed it was dehydrated and passed in the usual way into paraffin, the temperature of which was not allowed to exceed 50°C., and it was then cut with the microtome. The sections were stained with Heidenhain's iron-hæmatoxylin, with Flemming's triple stain, and a large number of other dyes. The results, which were compared carefully, led us to rely chiefly on the two staining processes mentioned, but at the same time we often obtained valuable preparations with other staining reagents as well.

In spite of repeated attempts, we have not succeeded in observing the first nuclear division in the oogonium, but the later ones have been seen both in *Fucus vesiculosus* and in *F. platycarpus*, in which eight oospheres are formed. Oltmanns asserts that in *Ascophyllum*, in which only four oospheres are commonly formed, eight free nuclei occur at an earlier stage, but that four of these ultimately abort, and do not become centres of cell formation. Our observations tend to confirm him in this respect, but we found that in some cases a fifth oosphere, smaller than the rest, was occasionally differentiated, and that when freed from the oogonium it exerted an attraction on the antherozoids just like its larger sister oospheres.

When an oogonial nucleus is about to divide, it first becomes slightly, then very much, elongated so as to resemble an ellipse. Fine radiations are seen to extend from the two ends into the surrounding cytoplasm. The latter is at first tolerably uniformly granular, but as the radiations around the polar areas increase, these regions become cleared altogether of the granules which then become massed outside them. The nucleus rapidly becomes more spindle-shaped, and its chromatic elements are chiefly grouped near each pole, leaving a clear space about the equator in which the nucleolus is situated. In this respect the nuclei of *Fucus* offer a striking contrast to those of *Pellia epiphylla* already described (Annals of Botany, vol. viii. p. 221) by one of us. In the latter plant the chromatic portion of the nucleus assumes an equatorial position at the corresponding stage in division, while the polar regions are clear.

The polar radiations continue to increase and the nucleus to lengthen, until the entire structure recalls the figure of a dumb-bell, in which the nucleus answers to the handle, and the radiation areas to the knobs. If the radii be traced outwardly, they are seen to terminate either in the frothy protoplasm, on the angles where the foam walls meet, or on the large granules which surround the cleared areas and are embedded in the foam. This point is one of considerable importance, and we shall revert to it further on. No structures were seen which could *certainly* be identified as centrosomes, although bodies suggestive of them were often observed; but these proved to be so variable in size and position, as well as in number, that we feel unable to attach any special significance to them.

The next stage in the mitosis is that in which the interpolar spindle arises, with the chromosomes disposed upon its equator. The spindle

is very remarkable inasmuch as it is entirely intranuclear, somewhat resembling that described by Fairchild for *Valonia*, or by Harper for *Peziza*. The nuclear wall can be distinguished until quite late in karyokinesis, and it is possible that no complete mingling of the cytoplasm with the contents of the nucleus takes place here. The spindle is extremely clear, and in several preparations, owing to a fortunate contraction during manipulation, the ends of the nuclear part of the spindle also had broken away from the cytoplasmic poles, and were visible as clean conical structures forming the poles of the nuclear spindle. The chromosomes were too minute to admit of their development being satisfactorily studied, but in *all the oogonial spindles* their number was estimated at *ten* when seen arrayed on the spindle equator. They were only seen in profile, and consequently it was difficult to be sure whether there were really ten or twelve, but the absolute number is not of importance as all the nuclei were compared from the same aspect. Remains, more or less preserving the original form, of the nucleolus were sometimes visible at this and even in a later stage. No division-planes are formed in the oogonium until the full complement of nuclei are produced; after this the positions which they will ultimately occupy are indicated by the heaping up into lines (or rather plates) of the cytoplasmic granules above referred to. These seem to be repelled equally from all the nuclei, thus effecting a symmetrical division of the entire oogonium.

After the complete delimitation of the oospheres within the oogonium, we observed, as an occasional circumstance, that one of the oospheres might contain two, or even three, nuclei, a fact also noticed by Oltmanns. When the oospheres are extruded, and come to lie free in the water, they grow in size, and are turbid with granules, which are very abundant in the cytoplasm. The chromatophores early become distinguishable from the other constituents of the cell, and the nucleus occupies a central position. It is itself surrounded by a dense layer of cytoplasm, which later on becomes very strongly marked. About five minutes after the mixing of the sexual cells, the antherozoids are found to have slipped into many of the oospheres. We failed to observe the act of penetration, but found a number of cases in which the antherozoid could be recognised within the oosphere, before its final fusion with the nucleus of the latter. It is a roundish, densely staining body, and, unlike the majority of animal sperm-cells as yet described, it imports into the egg no system of radiations along

with it. Judging from the short period of time elapsing between its penetration of the surface of the oosphere and its arrival at the exterior of the female nucleus, it must pass through the intervening cytoplasm with great rapidity. It then becomes closely appressed to the nucleus, and is about as large as the nucleolus of the latter. It rapidly spreads over a part of the female nucleus as a cap, and it presents a less homogeneous aspect than before. Both it and the female nucleus assume a granular condition, which is probably to be interpreted as representing a coiling and looping of the linin of the respective nuclei. Finally the two nuclei coalesce, and the original components can no longer be distinguished. Complete fusion may be effected in less than ten minutes after addition of the antherozoids to the water. The results are in striking accordance with those described by Wilson in connexion with the fertilisation of the eggs of Echinoderms in his recent Atlas of Fertilisation.

A delicate pellicle is meanwhile formed around the periphery of the oosphere, which is thus easily distinguished from the unfertilised oospheres, in which such a membrane in wanting. The texture of the cytoplasm also changes, and tends to assume a more definitely radiating character, the lines starting from the nucleus as a centre.

We observed, not unfrequently, rather large cells in which two nuclei of equal size were lying in close juxtaposition. These cells, with their nuclei, answer exactly to the description given by Behrens of the fertilisation stage in plants examined by him. We are unable however to accept his interpretation, for, in the first place, the series of fertilisation stages which we have observed, and have briefly described above, in no way correspond with the appearances described by him, and secondly, because these large cells (Behrens himself emphasises their size) are seen in material to which no antherozoids have had access. Furthermore, the average size of the young oospores is *not* obviously greater than that of the oospheres themselves. We regard the bodies in question as representing abnormal developments of oogonial cells, and not as being in any way concerned with fertilisation. Moreover, we have occasionally observed one cell in the divided oogonium much larger than the rest, to contain two, or even sometimes three, nuclei, and these nuclei are then always close together. These facts have led us to reject Behrens' account of the process.

A very large number of experiments were made, in order to determine, if possible, the time which elapsed between the addition of

the antherozoids to the oospheres and the first division of the spore. A short summary of different sets of observations on *Ascophyllum* is given in the subjoined tables.

SERIES I.—*Observations on* Ascophyllum *conducted at the Seaside.*

(*a*) The antherozoids were added to the oospheres at 10 o'clock A.M.

Lot 1.	Fixed 23 hours	after the addition	of antherozoids.		Nucleus preparing for division.
„ 2.	„ 24	„	„	„	Nucleus divided, rhizoid-rudiment present, no dividing wall.
„ 3 & 4	„ 32	„	„	„	Nucleus divided, no rhizoid, dividing wall present.
„ 5.	„ 36	„	„	„	Spore divided into about six cells.

(*b*) The antherozoids added between 11 and 12 P.M.

Lot. 1.	Fixed 24 hours	after the addition	of antherozoids.		Nucleus divided, a few with rhizoid-rudiments and division wall.
„ 2.	„ 25	„	„	„	Same result.
„ 3.	„ 25	„	„	„	Not beyond spindle stage.
„ 4.	„ 28	„	„	„	Nucleus divided, no rhizoid or dividing wall.

SERIES II.—*Observations on* Ascophyllum *carried on in the Laboratory.*

Antherozoids added between 5 and 7 P.M.

Lot 1.	Fixed $22\frac{1}{2}$ hours	after the addition	of antherozoids.		Nucleus divided, no rhizoid or dividing wall.
„ 2.	„ 23	„	„	„	Nucleus preparing for division.
„ 3.	„ 23	„	„	„	Same as 1.
„ 4.	„ $24\frac{3}{4}$	„	„	„	Nucleus divided, rhizoid present, no dividing wall.

The above observations prove that there is no essential difference between the behaviour of material examined in London and at the seaside respectively.

After fertilisation, the cells rest for a long interval of time—commonly about twenty-four hours, as shown in the foregoing table—

before they begin to segment. The principal changes which occur during the interval are, first, in the rapid increase in the thickness of the peripheral cell wall, and, secondly, in the more regular arrangement of structure exhibited by the protoplasm. The alveolar, or foam character is extremely clear, and the chromatophores, which by this time have become very prominent, are noticed to be situated in the angles formed by the convergence of the foam walls; they are often bent and otherwise distorted, and so accommodate themselves to the structural condition of the foam. Other granules, which stain deeply, and probably represent food reserve of a proteid nature, are also abundantly scattered through the cytoplasm.

The first segmentation-division resembles, in a general way, the oogonial nuclear divisions already described, and the polar areas become similarly cleared of granules. The achromatic threads forming the polar radiations are very clearly seen to be attached to the foam-like structure of the cytoplasm, and indeed, in some cases, insensibly to pass into it. At other times fibrils end on granules (or, perhaps, on the protoplasmic lining of the granules), and sometimes again a fibril may fork, and its branches end either on granules or on the foam angles. The inference to be drawn from these facts seems to be that the radiations are the result of a change—a differentiation—in the protoplasm as it already exists, and that they do not owe their origin to the presence of any special 'spindle-forming substance,' by virtue of which they may be supposed to develop and 'grow' as new structures in the cell. We propose, however, to discuss the general bearings of our observations on this and on other questions of theoretical interest in a future memoir, in which the evidence for our views will be set forth in detail.

When the achromatic nuclear spindle appears, it also, as in the oogonial mitoses, is intranuclear, and it is often separated from the well-defined persistent nuclear wall by a clear space. The chromosomes, when assembled on the spindle, at the equator, are seen to be *twice as numerous* as in the oogonial nuclei, *i. e.* seen in profile we counted them as *twenty* in number. We were unable to distinguish any such grouping of the chromosomes as would lead to the conclusion that the chromosomes of the male and female nuclei respectively had so far preserved their original identity as to appear in the form of two separate groups. The long interval of time which, in *Fucus*, elapses between fertilisation and the first nuclear division possibly

may admit of a more thorough mingling or confusion of the parental chromosomes than would seem to be the case in some animals, *e.g.* the Copepoda as described by Rückert and by Häcker.

During the diaster stage the connecting achromatic fibres are at first very distinct, but they soon become fainter, and no cell-plate is formed across them. The two daughter nuclei generally pass into the state of rest, each being first hemispherical, with crenate projections on the flattened side turned towards its sister nucleus. Only after nuclear division is complete does the first cell wall appear. The cell is sometimes spherical when this happens, and then it is divided into two similar hemispheres. Further divisions may then appear, whilst the general contour of the embryo still remains more or less spherical. These cases occurred most frequently when the germinating spores were illuminated on all sides. But most commonly the first cell wall cuts the spore into two dissimilar halves, one of which grows out and forms a rhizoid. Often this projection is already apparent even before the first nuclear division occurs, and in any case one of the two daughter nuclei always passes down into the protuberance.

The immediately succeeding divisions have been sufficiently described by Thuret and others, but we may remark that the division of the nuclei in all cases precedes the formation of a cell plate, which is not formed in connexion with the achromatic connecting fibrils as in the higher plants.

The doubled number of the chromosomes is retained during the vegetative divisions of the thallus, and is constant throughout the somatic cells of the mature *Fucus* plant. Hence it follows that the reduction in the number of the chromosomes (in the female plants) is associated with the differentiation of the oogonium—the mother cell of the sexual products. Thus *Fucus*, in this respect, approximates more closely to the type of animal oogenesis than to that which obtains in those higher plants in which the details of chromosome reduction has been followed out.

Regarded from the standpoint of the number of its chromosomes, the *Fucus*-plant resembles the *sporophyte* of the higher plants, whilst the gametophyte of the latter, with its reduced number of chromosomes, finds its analogue merely in the maturing sexual cells of *Fucus*. But until we know more of the nuclear changes as they occur in other Algæ, and especially in the more primitive forms, it seems unadvisable

to go further than to indicate the possibility that we may require to revise our present ideas on the comparative morphology of the higher and lower groups of the vegetable kingdom. Even if we regard the reduction in the number of the chromosomes as a fact which is primarily of physiological importance, we may safely conclude, from the universality of its occurrence, that it is also intimately connected with the phylogenetic development of living forms, and hence it must meet with due recognition on the part of the morphologist who is engaged in comparing the life-history of one group of organisms with that of others.