The Fertilization of *Batrachospermum*¹.

BY

BRADLEY MOORE DAVIS.

—♦—
With Plates VI and VII.
—♦—

DURING the course of his studies at Harvard University, the writer had the opportunity of examining some very excellent material of what appeared to be a winter-form of *Batrachospermum moniliforme*, Roth. As the conditions were particularly favourable in this plant for the study of the nuclei in the procarps and antherozoids before and after fertilization, the writer began a series of observations upon the subject. The results were so surprising that he supplemented this study with the examination of two other species of the same genus, *B. coerulescens*, Sirdt. and *B. Boryanum*, Sirdt.

Certain peculiar positions of the nuclei in the cells of the procarps and antherozoids at the time of fertilization and after the development of the cystocarp, led the author to think at one time that perhaps the cystocarp developed apogamously. These puzzling conditions were supplemented by the fact that in a certain proportion of the preparations, cystocarps, in various stages of development, were found which bore trichogynes lacking the usual accompanying fused antherozoid. In many cases it was not easy to believe that the antherozoid

¹ Contribution from the Cryptogamic Laboratory of Harvard University, No. 31, prepared under the direction of Dr. W. G. Farlow.

[Annals of Botany, Vol. X. No. XXXVII. March, 1896.]

had been torn off in the manipulation of the specimens. This interesting question of possible apogamy led the writer to try some experiments upon living plants that had best be considered at the outset.

In nature, the filaments of the *Batrachospermum*-plants, being attached to stones and other objects usually in running water, float out in the direction of the current and wave slightly to and fro with the varying movements of the stream. As the plants grow in tufts, the separate filaments necessarily touch and scrape against one another when their positions change in the flowing water: hence it seems quite possible that the antherozoids of one plant or filament might readily be brushed off upon the trichogynes of another as the branches move from side to side. It is difficult to understand how the antherozoids could be brought in such profusion and applied to the trichogynes by swiftly-moving water, and the above explanation of the process of fertilization appears very plausible to the author.

To determine experimentally whether contact of antherozoids with trichogynes is absolutely necessary for the production of fruit, it is necessary to grow plants under such conditions that the possibility of fertilization either by direct contact of the filaments or by currents of water is removed. The normal conditions of environment, temperature, light, &c., must at the same time be strictly adhered to, in order that the experiment may be conclusive. *Batrachospermum Boryanum* is a strictly dioecious species; and one may frequently find plants of *B. coerulescens* which bear very few or no antherozoids: but this special form of *B. moniliforme* is monoecious. It is an easy matter to select female plants of *B. Boryanum* and *B. coerulescens*, and to clear the stone upon which they grow of all male plants. However, it is not easy to grow such female plants isolated in aquaria, because it is so difficult to imitate the normal surroundings of *Batrachospermum*-plants as regards the temperature of the water, for they frequently grow in ice-cold streams: the plants do not thrive when removed from their running brooks: But the writer believes

that he has satisfied the conditions (isolation of female plants and normal environment) necessary to answer the problem stated at the beginning of the paragraph, in the following manner.

Some stones, upon which were female plants of *B. Boryanum* and *B. coerulescens*, carefully freed from antheridial specimens, were placed in large glass jars. The jars were fitted with covers having a few holes punched in them, and were then sunk in the brook in the same situations from which the plants were collected. The conditions as regards light and temperature of the water were therefore the same, for the prepared plants and those growing wild around them. The holes in the covers allowed a constant slow interchange of water, but the swiftness of the current was greatly abated; in fact, the plants were growing in almost perfectly still water. Of course the chances of antherozoids being brought to the trichogynes by the currents of water were thus greatly diminished. Jars containing plants of each of the three species were placed in the stream in the middle of December, and were taken out at different times and examined, the last having been in the stream two and one-half months. The experiments give very positive data. In the first place the plants in the jars appeared perfectly healthy. Two to three centimetres of new growth were added to each branch. The habit was not materially different, except that in some cases the new branches were not so stout as the old growth, the tendency being to produce long thin structures rather than short thick ones. The colour of the plants remained the same, and the cells of the tissue did not differ in any marked manner from those of plants under normal conditions.

An abundance of procarps were produced in the situations peculiar to each species, and seemingly in the usual quantity. Almost all the trichogynes of *B. Boryanum* and *B. coerulescens* were free from antherozoids, and in each case there was no indication of any tendency to produce fruit apogamously. The trichogynes did not wither, but remained attached to the procarps in the usual manner, so that as one examined

a branch one procarp followed another, each identical in appearance with its neighbour. In the case of *B. moniliforme*, the monoecious species, a very fair proportion of the trichogynes were free from antherozoids, perhaps because the quiet water in the jar prevented the filaments of the plants from rubbing together. Those procarps whose trichogynes lacked antherozoids never developed fruit, while neighbouring trichogynes with antherozoids fastened to them were attached to cystocarps in various stages of development, some with ripe spores. The evidence seemed almost conclusive that fusion of an antherozoid with the trichogyne was absolutely necessary for the production of fruit. As the reader proceeds, he will understand why it is desirable to dwell upon this point, even though it be what has always been considered a settled fact.

The tissues of *Batrachospermum* may be most readily studied from crushed-out preparations, which are very easily made because of the flexible character of the filaments and the amount of mucilaginous matter in the frond. It has been the writer's experience that absolute alcohol is the most satisfactory fixing agent, for it preserves the mucilage, which is very helpful in the further manipulation of the material. Chromic acid dissolves the mucilage. It is also possible that chromic acid may weaken the attachment of the antherozoids upon the trichogynes, and allow them to be more easily torn off in manipulation; for it was in material fixed with this reagent that the writer found many specimens of cystocarps whose trichogynes were without these accompanying structures. Of the many stains tried, the haematoxylin formulae gave the best results, and the most satisfactory of these were Mayer's haemalum and acid haemalum, the latter being the more precise stain. Böhmer's haematoxylin is also good. There is little choice between the common fixing agents, and absolute alcohol was employed for the reason before stated, that material killed with this fluid was more easily crushed out because of the mucilage contained in the frond. High powers of the microscope were necessary throughout the investigation, and the writer used the Zeiss 4 mm. apochro-

matic objective with compensation ocular No. 12 (magnification 750 diameters) even in ordinary work, and relied upon a Leitz $\frac{1}{12}$ oil immersion lens for the more minute observations.

The paper is so divided that the accounts of the three species contain but a bare statement of facts; the consideration of the bearing of the results upon the present accepted view of the structure and fertilization of the procarp in the genus being reserved for the concluding portion of the paper.

BATRACHOSPERMUM MONILIFORME, *Roth.*

The plant which we have identified as a winter-form of *B. moniliforme*, grew very abundantly in a quietly-flowing mill-race, covering the stones and dead branches along the sides and bottom of the stream with a thick growth an inch or more in height. The plant matures in mid-winter, and is then almost the sole living occupant of the stream. The material from which the figures are drawn was gathered in February, when the plant was at its best.

The trichogynes are club-shaped structures at the ends of short branches, which extend usually about half the distance from the central axis of the plant to the periphery. At the outset it was immediately apparent that each trichogyne was not merely a swollen prolongation of a cell, the carpogonium¹, which contained the nucleus. There was not only a distinct nucleus in the carpogonium, but also an unmistakable nucleus in the trichogyne, staining with haematoxylin as a dark blue body, quite homogeneous in structure. The

¹ It has been a serious question to the writer what nomenclature of the organs of fructification in *Batrachospermum* he should adopt. The term carpogonium was applied by Schmitz (*Untersuchungen über die Befruchtung der Florideen*) to a particular cell because of its resemblance to the oogonium of certain Chlorophyceae, notably *Coleochaete*. The trichogyne was considered as an extension of this cell specialized for the reception of the antherozoid. The writer's observations have led him to a different view of the structure of the trichogyne, but to avoid confusion he has used the term carpogonium in the strict sense suggested by Schmitz, applying it to the particular cell that bears the trichogyne and gives rise to the fertile filaments, the trichophore in the terminology of Bornet and Thuret.

protoplasm directly around the nucleus usually stains light blue, and the remaining cytoplasm is coloured more or less deeply, depending upon certain structures which will be considered later. The cytoplasm in the carpogonium is often very dense, and is apt to stain deeply, but the very darkly-stained nucleus is usually prominent by virtue of its central position.

The very earliest beginnings of trichogynes cannot be readily distinguished from the tips of ordinary vegetative branches; but a point is soon reached in their development when the form clearly designates the character of the structure. The terminal cell of a branch begins to extend first as a little process, which gradually swells and finally assumes the size and shape of the adult trichogyne. The cell from which the trichogyne springs is of course the carpogonium. It contains a somewhat irregularly lobed chromatophore that lies in the layer of protoplasm next the cell-wall. The chromatophore fills up a large part of the cell, and is very apt to be massed at the end from which the trichogyne arises. When the young trichogyne begins to extend from the carpogonium cell, some of the chromatophore of the latter is apparently drawn up into it. At all events, one finds in the basal portion of young trichogynes, and as a rule also of mature individuals, an irregularly-shaped body that is clearly an extension of the chromatophore of the carpogonium. A glance at Fig. 2, a young trichogyne drawn from living material, will make clearer what has just been described. The shaded portion of the carpogonium (lettered *c*) is the chromatophore, coloured a light green in this species, and all the rest of the cell is quite hyaline but for a few granules scattered here and there, and perhaps one or more vacuoles. It will be seen that the chromatophore of the carpogonium runs up into the trichogyne in the form of an irregular extension. The colour of this extension in the base of the trichogyne is often identical with that of the portion in the carpogonium, but in the upper part of the trichogyne the tint is usually very much lighter. The shapes assumed are very

various, and sometimes the body extends perhaps only a third the length of the trichogyne, sometimes almost to the very end. There are usually one or two rather prominent lobes. Such is the appearance of this body in the young trichogyne, and, the writer, in studying the development of the latter, came to the conclusion that it was truly a portion of the chromatophore of the carpogonium, and deserved to be mentioned as a definite structure in the trichogyne. We have said that the chromatophore in the trichogyne is very variable in its shape, and also in its position, for it lies now at the base of the trichogyne, again along the sides, and sometimes near the top. The boundaries of the chromatophore, which are at first distinct, become less definite as the trichogyne matures and, the outlines grow more irregular. The colour also changes. In the young trichogynes the tint is very similar to the colour of the chromatophore in the carpogonium, that is, a peculiar light green shade that sometimes has a bluish tinge to it. With age the colour fades, and often changes to a yellowish green tint. These outward changes in form and colour are accompanied by modifications in the structure of the protoplasmic body itself. The chromatophore is at first perfectly homogeneous in structure, but with the fading of the colour the body passes into a granular condition, consisting of many small portions of different sizes, each having more or less of the original green tint, and all imbedded in strands of granular protoplasm. This change in structure is illustrated in the upper portion of Fig. 2, where the chromatophore is breaking up and becoming granular in texture. The process above described may be one of degeneration, and the writer has observed at different times very similar appearances in the cells of other Algae. However, it is doubtful if the structure ever entirely disappears from the trichogyne, although it may become so faint as to be noticed only by careful observations, with high powers of the microscope. It is true in general that the remains of this body are distinctly visible in most trichogynes, often with sufficient colour still left in them to make the writer feel confident of their nature

as chromatophore-derivatives. We shall have occasion to speak of the future fate of this body after we have described the process of fertilization.

We may now consider the structure and development of the antherozoids. In this species they are formed in groups at the ends of filaments towards the tips of the branches. Such a group is shown in Fig. 3, and in this case consists of three antherozoids, *a*, *b*, and *c*, in different stages of development. Perhaps the most interesting point in connexion with their development is the fact that the young antherozoids have a very distinctly outlined body in them that has the unmistakable colour of the chromatophore of vegetative cells. The appearance of this body is shown in Fig. 3 *a*. As the antherozoid matures, a change takes place in its chromatophore similar to that in the chromatophore of the trichogyne. The colour fades, the homogeneous structure becomes granular, as is indicated in Fig. 3 *b*, and the chromatophore breaks up into many smaller bodies, which finally become practically colourless, but nevertheless remain in the antherozoids as differentiated masses of protoplasm giving the granulate structure shown in Fig. 3 *c*. These bodies would be considered quite colourless by any one who had not studied their development, though they occasionally show traces of their original green tint. Each antherozoid contains a nucleus that is commonly situated in the layer of protoplasm that lies next the cell-wall (see Fig. 4).

What, now, is the real nature of the trichogyne? It starts as a process from the terminal cell of a branch, but at a very young stage a distinct nucleus is plainly recognizable in the developing structure. Thus in the young stage shown in Fig. 1, a nucleus is present in the swollen extension of the cell *c*. The extension develops into the trichogyne, the cell below (*c*) becoming the carpogonium. The nucleus in the trichogyne usually assumes a position near the middle portion of the structure, lying however in the protoplasm close to the cell-wall. But the position is not a fixed one; sometimes the nucleus is found to be at the top of the trichogyne, and some-

times near the base. It seems to the writer that the trichogyne must be considered as a true cell. There is a nucleus and a body certainly derived from a chromatophore, and which is probably functional, at least in the young trichogyne. These are the two most important structures in the vegetative cells of *Batrachospermum*, and having these, the only differences between the trichogynes and the former are those of shape, position, and mere arrangement and relative proportions of the cell-contents. The last-mentioned difference would seem to be the most important, for in the trichogyne the hyaline cytoplasm is proportionally very large, while the chromatophore is represented in its lowest terms. The trichogyne is united to the carpogonium by a wide strand of protoplasm, but this is not a great distinction, for intercellular communication is characteristic of all the cells of the frond.

Bearing in mind the nuclear and cytological conditions of the procarps, we are now ready to consider the phenomenon of fertilization. The conditions are, a nucleus in the trichogyne, a nucleus in the carpogonium, and the connexion of the two cells by a broad strand of protoplasm. When the antherozoid is applied against the trichogyne, the surfaces of the two cell-walls flatten somewhat and finally become completely united. The masses of protoplasm in the two structures are still separated by a cell-wall of appreciable thickness. The antherozoid then puts forth as a rule a very evident, although small, papilla, that apparently absorbs the cell-wall as it works its way down towards the protoplasm of the trichogyne. Finally, cytoplasmic fusion of the two cells is accomplished; but the point of union is at first very small, so small that it is impossible to determine exactly when the act takes place. The point of fusion then gradually broadens, until in some cases the opening becomes two-thirds the width of the antherozoid: the process goes on rather slowly, and, as will be shown later, the size of the opening is very variable. These points are more readily determined from fixed and stained specimens than from living material, because of the hyaline character of the protoplasm of the

latter. The antherozoids usually attach themselves near the tip of the trichogyne, and commonly at the very end. There seems to be no rule as regards the position of the nucleus in the antherozoid at the time of fertilization. It very frequently lies at the side farthest away from the point of fusion, as in Figs. 6 and 7, or it may be found very near the point of contact. The writer, after examining many specimens in the condition shown in Figs. 6 and 7, has never seen anything to indicate that the nucleus of the antherozoid or any portion of it moves rapidly into the trichogyne. The position of the nucleus of the trichogyne, as has been stated, is somewhat variable; that of the carpogonium usually lies near the base of that structure, but sometimes occupies a position above the middle portion. As it is not difficult to determine these points in this species, the writer does not see how any marked change in the nuclear conditions of the structures could have escaped his examination. There do not seem to be any violent changes in the position of the nuclei effected by the act of fusion. Gradual changes will be spoken of later. Frequently more than one antherozoid may be attached to the trichogyne, but the evidence indicates that only one fuses. The writer has examined this point with considerable care, and has never seen more than one antherozoid of a group in undoubted union with the protoplasm of the trichogyne. It is very interesting to note the manner in which all the antherozoids of a group around a trichogyne extend towards that structure so that their shape is changed from a round outline to an oval. They not only extend towards the trichogyne, but sometimes towards each other.

We will now consider the manner in which the trichogyne is separated from the carpogonium. The experiments tried upon living plants, as well as the writer's observations, seem to prove that the trichogyne never becomes separated from the carpogonium unless fertilized. The strand of protoplasm connecting the two structures is originally rather wide (see Fig. 6); and the first indication that the trichogyne is to become cut off from the carpogonium is shown by the

gradual narrowing of this strand. It is as if the masses of protoplasm in the two structures were slowly contracting; and to judge from appearances this seems to be actually the case. As the strand becomes thinner the space necessarily left between it and the cell-wall at the base of the trichogyne is filled up by a deposit of a substance exactly similar in colour and density to the cell-wall itself. This deposit is shown in Fig. 7, where the connecting strand is much thinner than in Fig. 6. Finally the thin strand is drawn apart and the ends of the two separated masses of protoplasm round themselves off, one becoming the lower end of the separated trichogyne, the other the upper end of the carpogonium. A light streak that is frequently seen running through the deposit between the carpogonium and trichogyne (see Fig. 8), may indicate the presence of the cavity in which the connecting strand originally lay. But this appearance is but transitory; and such a cavity, if it exists at all, is quickly closed up, so that the whole intervening portion of the structure between the protoplasm of the trichogyne and that of the carpogonium consists of this substance exactly similar to the cell-wall.

It will be seen that the deposit between the trichogyne and carpogonium is not exactly of the nature of a plug placed into an opening between the two, but it is an addition on the inside to the thickness of the cell-wall which is deposited as gradually as the separation of the protoplasm takes place, and follows the latter process very closely. That the separation takes place gradually is shown by the great variety of stages which one is able to find without difficulty. The writer has endeavoured to watch the operation in living material, and his attention has never been attracted to any movement of the protoplasm either during the process of fusion of the antherozoid or separation of the trichogyne from the carpogonium.

Concerning the nature of the substance deposited between the trichogyne and carpogonium, the writer can say but little. It did not give a clear cellulose-test with iodine and sulphuric

acid or with chlor-iodide of zinc ; but these reagents do not give very satisfactory results with the walls of the vegetative cells. However, the general resemblance in appearance and behaviour towards certain stains has led the writer to conclude that, if not cellulose, it is at least closely related to that substance.

If it is cytoplasmic communication between the antherozoid and trichogyne which causes the separation of the latter from the carpogonium, it is interesting to note how large must be the point of fusion. In some cases, as for instance the example shown in Fig. 8, the points where the protoplasm of the antherozoids were pressed against the protoplasm of the trichogynes were so small that open communication could not be seen. While the points of fusion in the great majority of specimens were very much larger than in this case, still they are often very small, much smaller than the nuclei of the antherozoids. Fig. 9, which illustrates this point, is also interesting, because it shows the peculiar manner in which the antherozoids tend to lengthen in the direction of the trichogynes. In Fig. 10 the nuclei of the antherozoid and trichogyne both lie very close to the point of fusion. The connecting strand of protoplasm between the two structures was much smaller than the nuclei, and the trichogyne was separated from the carpogonium. The example is unusual, the only one observed, and the writer would not like to attach any particular significance to the peculiar situations of the two nuclei.

We will now consider certain gradual changes which may take place in the position of the nucleus of the antherozoid. It is true that the nucleus of the antherozoid is often found in or near the upper portion of the trichogyne, and such cases are always those in which the fusion-strand is very broad. Fig. 11 illustrates this point, the nucleus lying directly in the opening between the antherozoid and trichogyne. In the example shown in Fig. 12, the nucleus of the antherozoid had passed into the upper portion of the trichogyne, and there was a peculiar arrangement of the cytoplasm before

and behind the nucleus which indicated that the position of the cell-contents had been disturbed by the downward movement. The attention of the reader is called to the fact that the nuclei of the trichogynes in both cases occupy their usual positions in the middle portion of those structures, and that the two trichogynes are entirely separated from the carpogonia. In the specimen illustrated by Fig. 11, the fertile filaments had begun to develop, showing that the carpogonium had been fertilized.

There appears to be no tendency on the part of the nuclei of the antherozoid and trichogyne to approach each other. It would be very unsafe to conclude that the examples in which they lay very close together had any great significance, because the position of the nucleus of the trichogyne is so very variable.

In the majority of cases it is quite evident that the nuclei of the antherozoid and trichogyne remain entirely separate. It is true that one sometimes finds specimens, as Fig. 17, in which there is one large nucleus that might be considered as a fusion-nucleus; but there have been other structures in such cells that have led the writer to believe that such was not the case, and these will be described later.

The carpogonium, soon after its separation from the trichogyne, generally gives rise to several branches of three or four cells each, that grow downward and become pressed against the cell below. A great many short filaments arise from the cells of these primary branches, and they in turn fork and finally terminate in the spores. All the fertile filaments may be traced back to the carpogonium, although they frequently appear to come from the cell situated below that structure. It may be well to emphasize the fact that after the trichogyne is separated from the carpogonium, all communication between the two structures is for ever ended. The communication is never opened again, either by the absorption of the deposit between the two cells or by outgrowths from the trichogyne. The writer mentions this point to guard against any possible

attempt to account for a transfer of the nucleus of the antherozoid to the carpogonium by ooblastema-filaments put out from the trichogyne.

It is characteristic of the trichogynes of *Batrachospermum* that they remain a long time in good condition, only disappearing with the gradual disruption and decay of the whole cystocarp. But several changes often take place in the character of the cell-contents. The nucleus usually increases in size, and often a very interesting process of fragmentation takes place. This latter phenomenon may occur in the antherozoid, in the trichogyne, or in both structures at the same time. It consists of a gradual breaking up of the nucleus into two or more smaller masses, which still take the dark blue stain with haematoxylin. Fig. 13 illustrates a simple case of fragmentation in both antherozoid and trichogyne. In Fig. 14, two large portions of the nucleus in the trichogyne lie one at the base and the other near the middle of that structure. The nucleus of the antherozoid is very granular, and perhaps is about to split up into a great many small fragments of the character shown in the antherozoid of Fig. 15. Fig. 16 illustrates an interesting case in which there were two well-defined nuclei in the antherozoid, and the nucleus of the trichogyne lay at the top of that cell. The phenomenon of fragmentation often gives rise to appearances which might be incorrectly interpreted if one were not familiar with the process. In Fig. 17 there is shown a large prominent nucleus in the upper portion of the trichogyne that might be considered as a fusion-nucleus; but the protoplasm in the central portion of the trichogyne was very dark, and gave indications of organization, and the question might well be asked whether it was not the remains of the nucleus of the trichogyne. Again, the cell-contents of the antherozoid may be almost hyaline but for a few scattered dark granules, and there may be one large nucleus in the trichogyne; but it is quite possible that fragments of a divided nucleus as small as those shown in Fig. 15 might readily be quite lost when scattered in the cytoplasm. If the nucleus of the antherozoid

ever fuses with that of the trichogyne, indications of the process ought to appear in the younger specimens, and it is very unsafe to draw conclusions from old examples. The nuclei in these structures often do not undergo any material change: Fig. 18 illustrates such a case, the example being part of a crushed-out specimen of an adult cystocarp with fertile filaments and ripe spores (lettered *s*).

In living specimens of old trichogynes certain portions of the cell-contents frequently appear to have a greenish tinge; and not only the trichogynes but also the antherozoids contain these peculiarly coloured portions of the protoplasm. The writer has observed some instances where the colour was so distinctly green and the outline of the body so well defined, that it seemed reasonable to suppose it to be really of the nature of a chromatophore. One sometimes finds antherozoids which have not fused with the trichogyne that are quite green in colour. Fig. 5 shows the arrangement of this chromatophore-like body in one case, but there is no regularity in its form or position. The body takes a slightly deeper stain with haematoxylin than the rest of the cytoplasm, perhaps because of its granular character, and the outline is sometimes well shown in preserved and stained specimens (see Figs. 8, 9, 10, and 11). Remembering how long the trichogynes and the antherozoids remain intact, it does not seem unreasonable to suppose that the trace of colour may be true chlorophyll with some functional value. From the appearance of specimens which the writer has observed, it seems to him that the colour deepens in tint after fertilization, and that the chromatophores become larger.

The writer realizes that the process which he has called fertilization lacks the characteristics of that phenomenon as considered by biologists, in that there is no fusion of sexual nuclei. Nevertheless, it is the act of cytoplasmic fusion with the antherozoid that gives the stimulus necessary for the development of the cystocarp, and as such the term is perhaps not misapplied.

BATRACHOSPERMUM COERULESCENS, *Sirdt.*

This plant, which agrees more closely with *B. coerulescens*, as Sirodot¹ has described it, than with any other species, was found in its best condition in the late fall, but it grows all through the winter and spring. The types of trichogyne and cystocarp are of such an opposite character from *B. moniliforme* that a careful comparison of the two species as regards the process of fertilization is very desirable.

We shall take up the subject in much the same order as in *B. moniliforme*, so that the comparison may be easily followed.

The procarpic branch is very short, lying near the axis of the main branches, and bears a very narrow long trichogyne that arises from the terminal cell (the carpogonium). There are curious short filaments attached to the cells of the procarpic branch below the carpogonium, but they are of no great significance.

The mature trichogyne contains a well-defined nucleus, as does also the carpogonium, but the most interesting feature about the contents of the trichogyne is the particularly prominent chromatophore-derivative. In very young trichogynes, when the process from the carpogonium is quite small (see Fig. 19), a homogeneous body of irregular outline may be clearly seen closely pressed against the cell-wall and extending usually to the very top of the cell. Its colour is distinctly green, and its outline may be followed without difficulty, as it runs into the carpogonium and there becomes the chromatophore of that structure. As the process, which represents the early stage of a trichogyne, gradually lengthens and swells into the shape more nearly like the mature structure, the chromatophore, which lay at first closely pressed against the cell-wall, is commonly left behind in the lower portion of the structure (see Fig. 20). The shape finally assumed by the chromatophore-derivative is very variable, and frequently lobes of the structure become detached (Figs.

¹ Sirodot, *Les Batrachospermes*, Paris, 1884.

20 and 21). Eventually the chromatophore of the trichogyne becomes separated from that of the carpogonium, as in Fig. 21, an example of a mature trichogyne in which this body is particularly well developed. As the trichogyne grows old, the colour of the chromatophore gradually changes from the peculiar somewhat bluish-green tint to a green with a trace of yellow in it, becoming at the same time somewhat fainter in colour. With the change in tint comes the same difference in the character of the protoplasm which we have considered in the case of *B. moniliforme*, namely, a change from a homogeneous appearance to a granular structure with a less definite outline.

The writer found several instances of an abnormal development of the trichogyne that are worth noticing. The terminal cell of a procarpic branch had given rise to a process, but the latter, instead of developing directly into the trichogyne, had grown out and divided into two cells, thus continuing the growth of the branch (see Fig. 22). This exhibition of vegetative character in the young trichogyne is exceptional and interesting, but quite in keeping with its structure as a nucleated cell.

The phenomenon of fertilization may be studied very advantageously here, because the antherozoid sends out a very distinct process. After the act of protoplasmic fusion, the long delicate strand of cytoplasm between the trichogyne and carpogonium (see Fig. 23) becomes thinner, and finally breaks. The separated ends contract, but do not round themselves off quickly. Little extensions from the upper portion of the carpogonium and lower portion of the trichogyne are frequently found when these structures must have been long separated from each other. Between the separated portions of protoplasm is deposited a substance apparently of the same nature as the cell-wall. The nuclei of this species are very small, but they stain very deeply indeed. That of the trichogyne usually lies near the middle portion of the cell, but occasionally at the tip (see Fig. 25). The nucleus of the antherozoid is very irregular in its situation, and

frequently occupies a position farthest away from the point of fusion (see Figs. 24, 25, and 28). It will be noticed from the figures that the point of fusion is at first very small, and it sometimes remains so for a long time, although as a rule it gradually widens. However, the process of widening takes place particularly slowly in this species, and the tendency on the part of the nucleus of the antherozoid to pass into the trichogyne is much less noticeable than in *B. moniliforme*. In fact, cases in which the nucleus of the antherozoid had left that organ, as in Fig. 26, were rather infrequent. We must make special mention of one very interesting specimen (Fig. 27). Here there was no nucleus in the trichogyne proper; but just inside the antherozoid, near the point where the two structures were united, was one nucleus, and a second lay in the antherozoid at the side farthest away from the point of fusion. Apparently the nucleus of the trichogyne originally lay near the tip of that structure, as in Fig. 25, and when fusion took place the movement of the cytoplasm was into the antherozoid, and the surge carried the nucleus of the trichogyne with it. Certain appearances of the protoplasm around the nucleus of the trichogyne tended to support this explanation.

Although more than one antherozoid may be frequently found clinging to the trichogyne, as in Fig. 28, only one ever fuses, and this plant was particularly favourable for the study of this point. Specimens were occasionally observed in which antherozoids were attached to the base of the trichogyne (Fig. 29), but there never appeared to be protoplasmic fusion under such conditions.

The interesting phenomenon of nuclear fragmentation is very characteristic of this species. The process does not, as a rule, begin until the trichogyne is cut off from the carpogonium as an independent cell. However, in the example shown in Fig. 23, a specimen in which the trichogyne was still united to the carpogonium, the nucleus of the trichogyne was very large and irregularly elliptical in its outline, as though it were about to divide. Two fragments of the

nucleus of the trichogyne are shown near together in Fig. 28, while a similar condition in the antherozoid is indicated in Fig. 30; and in the specimen from which Fig. 31 was drawn, fragmentation had taken place in both structures at the same time. The nuclear fragments may finally be quite widely scattered in the cytoplasm (Fig. 29 and 32, *t*), and the process of division is sometimes carried on until several derivatives of the primary nucleus exist in the cell.

The essentials of the structure and development of the cystocarp are precisely the same as in *B. moniliforme*, although there are minor differences in the length and arrangement of the fertile filaments. The appearance of a crushed-out developing cystocarp is shown in Fig. 32, where *t* = trichogyne, *c* = carpogonium, and *s* = sterile filaments from the cell below the carpogonium.

Batrachospermum coerulescens therefore agrees with *B. moniliforme* in all the important characteristics of the phenomenon of fertilization. Certain structures of the trichogyne and antherozoids are illustrated much better, as for example the presence of the chromatophore-derivative in the trichogyne before and after the fertilization of the carpogonium; in fact, it was here that the attention of the writer was first called to this curious cytological condition.

BATRACHOSPERMUM BORYANUM, *Sirdt.*

The trichogyne of *B. Boryanum* is very variable in size and shape, ranging from an almost globular structure to a lengthened form in which the end is sometimes very much prolonged. The general type of procarp is, however, somewhat similar to *B. moniliforme*. The cells are smaller than in the two species previously described, and consequently it is a less satisfactory form to study; but the writer made as careful an examination of the cytological conditions here as in the other species.

There is a nucleus in the trichogyne as well as in the carpogonium; and in the former there is also a more or

less clearly differentiated portion of the protoplasm, having a greenish tint: in young specimens this body may be readily traced back into the carpogonium, where it joins the chromatophore of that structure. The antherozoids of this plant are unusually large, and are somewhat peculiar in that the protoplasm is frequently entirely confined to a rather thin layer next the cell-wall, the central portion being occupied by a large cavity or vacuole. Half-developed antherozoids contain a very distinct chromatophore. The colour and structure of this body gradually change as the antherozoid matures, in a manner precisely similar to the antherozoids of *B. moniliforme*; i. e. the green tint becomes fainter and the structure granular and less definite in outline.

The separation of the contents of the trichogyne from the carpogonium takes place in exactly the same manner as in the two other plants. The area of the point of fusion between the antherozoid and trichogyne may be very small to accomplish the fertilization of the carpogonium (see Figs. 35, 36, and 37). The position of the nucleus in the trichogyne is apparently not materially affected by the process of fusion. The nucleus of the antherozoid is very irregular in its situation, and one finds many instances where its position is remote from the point of fusion, as in Fig. 33; but it is often very near this point (Fig. 34). The nucleus of the antherozoid frequently passes into the trichogyne, but no indications were observed that it ever moved far down into that structure. Fig. 33 shows a particularly interesting case in which, if one may judge by the position of the granular protoplasm in the upper portion of the trichogyne, the movement of the cytoplasm was into the antherozoid rather than from it. Fig. 35 illustrates a type of trichogyne with a prolonged upper portion in which the nucleus was situated: but one also finds examples in which the nucleus lies below the middle portion of the cell.

Nuclear fragmentation takes place in the cells of the antherozoid and trichogyne as these structures grow old. Some of the figures illustrate this phenomenon: thus Figs. 35 and 36 show fragmentation in the antherozoid, and

Fig. 37 is an example in which it has begun in both structures. In the specimen shown in Fig. 38 there was no prominent nucleus in the fused antherozoid, whilst there were two well-marked nuclei in the lower portion of the trichogyne; but the presence of a number of darkly staining bodies in the antherozoid suggests the possibility of extensive division of its nucleus, while the basal position of the two nuclei in the trichogyne seems to be good evidence that they were once portions of the same structure. In this species also the old trichogynes and antherozoids attached to developing cystocarps frequently contained differentiated portions of the protoplasm that had a faint but definite greenish colour. The writer is compelled to consider them, at least morphologically, as true chromatophores, for they appear to be the remains of the chromatophores contained in the young trichogynes and antherozoids.

SUMMARY AND CONCLUSIONS.

The writer has reserved this portion of the paper for a consideration of the bearing of the observation just described upon the accepted idea of the structure of the procarps and of the process of fertilization in the genus. The problems involved in a satisfactory explanation of the process of fertilization are of great interest but very puzzling: we cannot, indeed, expect a solution of the questions until many more species have been studied in this genus and related forms. The number of species that the writer has examined is of course small, and they all come from the same locality. However, there is this to be said to their advantage, that each species represents a different sub-division of the genus as it has been considered by Sirodot. The sub-division **Moniliformes** is represented by *B. moniliforme*, that of the **Helminthoides** by *B. Boryanum*, and the **Verts** by *B. coerulescens*. The two extremes of the genus are thus represented: for *B. moniliforme* and *B. coerulescens* are types in which the procarps present the greatest differences in the size and form of the cells.

The following *résumé* of the principal points of the paper had best precede the consideration of the literature, when each topic may be discussed in turn.

I. *The trichogyne* is a cell, possessing a well-defined nucleus, and when young, a body that must be considered, at least morphologically, as a chromatophore. The chromatophore becomes less clear in outline and fainter in colour as the trichogyne develops, but traces of it may usually be found in the mature structure.

II. *The carpogonium* is the cell situated directly below the trichogyne and connected with it by a strand of protoplasm. It contains a centrally-placed nucleus.

III. The *antherozoids* in an early stage of their development contain a body certainly derived from the chromatophore of the vegetative cells, and having a distinct green colour. As an antherozoid matures, this chromatophore-derivative changes greatly in appearance, its structure becoming granular and the colour fading until the antherozoid is practically colourless. The nucleus is usually situated in the layer of protoplasm near the cell-wall, the central portion as a rule containing a large vacuole.

IV. *The fertilization* of the procarp is accomplished when the trichogyne becomes separated from the carpogonium. The process of separation consists of a gradual drawing apart of the cell-contents of the two structures until the connecting-strand becomes so thin that it breaks. The cavity left by the separation of the two masses of protoplasm is filled in by a deposit of a substance similar in character to the cell-wall. The exciting cause of the process of fertilization is the *cytoplasmic* fusion of one antherozoid with the contents of the trichogyne. The evidence to support this statement is of two kinds. First, as shown by isolation-experiments on living plants, the fusion of an antherozoid is necessary for the further development of the carpogonium. Second, it appears that the process of separation may take place when the point of union between antherozoid and trichogyne is very small,

and that the nucleus of the antherozoid need not even enter the trichogyne to accomplish this act of fertilization.

V. *The nucleus of the antherozoid* has no fixed position in that structure at the time of fertilization, and frequently is situated at the side farthest removed from the point of fusion. The nucleus may never leave the antherozoid, but if the opening is large it may pass down into the upper portion of the trichogyne. Although it frequently enters the trichogyne, there is no particular tendency on its part to move down that structure, and the position of the cell-contents of the latter does not appear to be materially affected by its entrance.

VI. *A process of fragmentation* of the nuclei of both antherozoid and trichogyne is very apt to begin soon after the fertilization of the carpogonium.

VII. *The cystocarp* consists of many fertile filaments, all of which may be traced back to the carpogonium. When the communication between the trichogyne and carpogonium is severed, all protoplasmic union is for ever ended between the two structures.

VIII. *In the old trichogynes and antherozoids* one may find differentiated portions of the protoplasm having a faint green tint. These are probably the remains of the chromatophore-derivatives of the young antherozoids and trichogynes. Having a green colour, it is possible that they are functional, and that the long life of the trichogynes may be attributed to their presence.

The idea of the trichogyne of *Batrachospermum* being a distinct cell with its own nucleus and also a chromatophore-derivative is quite new. From the study of certain other genera of Florideae, the writers upon the subject, Bornet and Thuret¹, Janczewski², and Schmitz³, have considered the

¹ Bornet et Thuret, Recherches sur la Fécondation des Floridées. Ann. d. Sci. Nat., Bot., 5^e série, t. vii, 1867.

² Janczewski, Notes sur le Développement du Cystocarbe dans les Floridées. Mém. d. la Soc. Nat. d. Sci. Natur. d. Chérbourg, t. xx, 1877.

³ Schmitz, Untersuchungen über die Befruchtung der Florideen, Sitzgb. d. k. Akad. d. Wiss. z. Berlin, 1883.

trichogyne to be always a prolongation of a female cell, specialized to receive the antherozoid. This conception is well illustrated in the case of *Nemalion*, for there the trichogyne is a mere extension of the cytoplasm from the carpogonium, and of course has no independence of character as a cell. The female nucleus is supposed by all these writers to lie in the swollen portion of the cell below the trichogyne, which is now pretty generally spoken of as the carpogonium. The general tendency of thought is then to consider the carpogonium as homologous with the oogonium of certain chlorophyllaceous Algae, and the resemblance between the typical carpogonium with its trichogyne and the peculiar oogonium of members of the Coleochaetaceae has often been noticed. However well such a conception may apply in many genera of the Florideae, the writer does not think that *Batrachospermum* can be considered in such a light. Its trichogyne, with a nucleus and a body morphologically a chromatophore, is not a mere prolongation of a cell.

It may be mentioned that Schmitz¹ states, with an accompanying figure of *B. moniliforme*, that he has observed fragments of a substance, staining as chromatin reacts, in the trichogyne after it has been cut off from the carpogonium. He regarded these nuclear fragments as being evidence of a reduction-process, whereby some of the chromatin of the female cell is discarded. It is of interest to confirm his observations upon *Batrachospermum*, although the writer cannot put the same interpretation upon the facts; for the nuclear fragments are probably derived from the nucleus of the trichogyne.

The writer, while using the term fertilization throughout this paper, has realized that the process which he has described would not be called a sexual process by most biologists. From the observations here recorded it does not appear that the nucleus of the antherozoid, or any portion of it, ever reaches the carpogonium. Even when the nucleus left the

¹ Schmitz, l. c., p. 13.

antherozoid, nothing more remarkable ever happened than its passage into the upper portion of the trichogyne. The cytoplasmic fusion of an antherozoid with the trichogyne cannot be considered as a sexual process in the usually accepted meaning of the expression, for the essential characteristic of the process is considered to be the intimate union of the substances of sexual nuclei. However, the isolation-experiments and all observations seem to prove that the union of an antherozoid with the trichogyne is necessary for all farther development of the procarp, and also that it is cytoplasmic fusion between the two which is the exciting cause of the separation of the trichogyne from the carpogonium. It is hard to suggest what other term than fertilization can be used to describe this act, because all the subsequent changes are apparently the direct sequence of the influence of an antherozoid upon the trichogyne.

What then can be the meaning of these curious conditions? There seem to the writer only two possibilities open:—
(1) Perhaps the conditions of sexual reproduction among the lower plants are such that the fusion of the nuclei of the two sexual cells is not absolutely necessary: possibly a stimulus of some sort, the result of cytoplasmic fusion, is all that is required. Such a stimulus could readily be conducted from one cell to the other by the wide strand of protoplasm between the trichogyne and carpogonium.
(2) The second possibility is that the present condition is a much modified form of what was once a true sexual process, the plant having lost the most important feature of the process (the union of sexual nuclei), but the stimulus of cytoplasmic fusion being still required: that is, the plant is tending towards a condition of apogamy. The present trichogyne may then be a modification of some ancestral type of structure when the procarp resembled that of *Nemalion* at the present time.

As we know very little about the fate of nuclei in the sexual cells of the lower Cryptogams during the process of fertilization, it is quite impossible to draw any analogies.

From the present trend of theories of heredity one would suppose the first suggested possibility very improbable. But the second condition would be also very remarkable indeed. It will perhaps be safest to defer judgement until we have more detailed information about the process of fertilization in allied forms of the Florideae and other Algae.

UNIVERSITY OF CHICAGO,
October 1, 1895.

EXPLANATION OF FIGURES IN PLATES VI AND VII.

Illustrating Mr. Davis' paper on *Batrachospermum*.

All figures sketched with the Camera lucida.

PLATE VI.

Batrachospermum moniliforme, Roth.

Figs. 6-18. Drawn from specimens fixed in absolute alcohol and stained with Mayer's acid haemalum, glycerine preparations. $\times 2400$.

Fig. 1. Procarpic branch with very young trichogyne developing from carpogonium-cell (*c*), stained with Mayer's acid haemalum. $\times 2400$.

Fig. 2. A trichogyne drawn from life, showing distribution of chromatophore-derivative. $\times 1600$.

Fig. 3. A group of antherozoids (lettered *a*, *b*, and *c*) in different stages of development, illustrating the gradual change that takes place in the chromatophore as the structure matures. Drawn from life. $\times 2400$.

Fig. 4. A group of developing antherozoids, stained with Mayer's acid haemalum. $\times 1600$.

Fig. 5. An old trichogyne, showing the chromatophore-derivative as it appears in this structure and in the two antherozoids at the right and left. $\times 2400$.

Fig. 6. Trichogyne united to carpogonium; nucleus of antherozoid at further side of that structure.

Fig. 7. Antherozoid at the left fused with the trichogyne; that at right merely applied against the cell-wall; cell-contents of trichogyne about to separate from the carpogonium.

Davis.—The Fertilization of Batrachospermum. 75

- Fig. 8. Illustrates an instance where the point of fusion is very small.
Fig. 9. Fertile filaments beginning to develop from the carpogonium.
Fig. 10. Shows peculiar and very exceptional position of the nuclei of the trichogyne and antherozoid.
Fig. 11. Nucleus of antherozoid near point of fusion.
Fig. 12. Nucleus of antherozoid having passed into the upper portion of the trichogyne.
Fig. 13. Illustrates fragmentation of nucleus in trichogyne and antherozoid.
Fig. 14. Nucleus of antherozoid breaking up into many small fragments.
Fig. 15. Nucleus of antherozoid in many small fragments, probably derived from such a condition as that shown in Fig. 14.
Fig. 16. Two nuclei in fused antherozoid.
Fig. 17. Shows manner in which fertile filaments grow downward from the carpogonium (*c*); prominent nucleus from antherozoid in upper portion of the trichogyne.
Fig. 18. Portion of a mature cystocarp showing trichogyne (*t*) and antherozoid; each containing its original nucleus; *s*, developed spores.

Batrachospermum coerulescens, Sirdt.

Figs. 19–23 drawn from living specimens.

- Fig. 19. Carpogonium with very young stage of trichogyne; illustrates distribution of chromatophore in that structure. $\times 2400$.
Fig. 20. Half-developed trichogyne with chromatophore extending from the carpogonium. $\times 1600$.

PLATE VII.

Figs. 23–32 drawn from glycerine preparations of specimens fixed with absolute alcohol and stained with Mayer's acid haemalum, magnification 1600 diameters.

- Fig. 21. Mature trichogyne with chromatophore-derivative. $\times 1100$.
Fig. 22. A peculiar instance where the trichogyne-cell has continued to grow forward and has divided into two cells. $\times 1600$.
Fig. 23. Trichogyne still connected with carpogonium.
Fig. 24. Nucleus of antherozoid lies in a position farthest removed from point of fusion.
Fig. 25. Nucleus of trichogyne lying very near the top of that structure.
Fig. 26. Nucleus of antherozoid having passed into the trichogyne.
Fig. 27. Nucleus of trichogyne having passed into the antherozoid, probably derived from a condition of affairs such as is shown in Fig. 25.
Fig. 28. Illustrates fragmentation of nucleus of the trichogyne.
Fig. 29. A condition similar to Fig. 28, except that the nuclear fragments in the trichogyne are widely separated; antherozoid near base of trichogyne.
Fig. 30. Shows fragmentation of nucleus of the antherozoid.
Fig. 31. Fragmentation in both trichogyne and antherozoid.
Fig. 32. A half-developed cystocarp; *t*, trichogyne with nuclear fragments; *c*, carpogonium; *s*, short sterile branches.

76 Davis.—*The Fertilization of Batrachospermum.*

Batrachospermum Boryanum, Sirdt.

All figures drawn from specimens fixed in absolute alcohol, glycerine preparations, and stained with Mayer's acid haemalum ; $\times 2400$ diameters.

Fig. 33. A very short type of trichogyne, apparently the surge of the protoplasm during the process of fusion was into the antherozoid.

Fig. 34. Nucleus of antherozoid near the point of fusion.

Fig. 35. An elongated type of trichogyne with nucleus near the tip ; illustrating fragmentation of nucleus of the antherozoid.

Fig. 36. Fragmentation of nucleus of the antherozoid.

Fig. 37. Nuclear fragmentation in both antherozoid and trichogyne.

Fig. 38. Two large fragments of nuclei at the base of the trichogyne ; nucleus of antherozoid probably very much divided and scattered in the cytoplasm.

Davis del.

Davis del.

Davis del.

DAVIS. — BATRACHOSPERMUM.

University Press, Oxford.

Davis, Bradley M. 1896. "The fertilization of *Batrachospermum*." *Annals of botany* 10, 49–76. <https://doi.org/10.1093/oxfordjournals.aob.a088599>.

View This Item Online: <https://www.biodiversitylibrary.org/item/235772>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a088599>

Permalink: <https://www.biodiversitylibrary.org/partpdf/318408>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

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>.