The Alleged Fertilization in the Saprolegnieae.

BY

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THE question of the 'sexual' reproduction of the Saprolegnieae is one of the oldest still debated in Botany. Pringsheim's first results were contested by Cornu, who, however, was himself led astray by too close an approximation to the Peronosporae; De Bary then took the matter up, and showed from observations on the living Fungus that, despite the formation of male organs, similar in the two groups, those of the Saprolegnieae were functionless. Pringsheim then approached the matter afresh; he alleged new evidence in favour of a true sexual process. This was refuted by De Bary, Marshall Ward, and Zopf from different standpoints. Modern cytological methods enabled me to complete the work of De Bary by means of new facts and new interpretations; and the late Professor Humphrey confirmed my work. Professor Trow has recently attacked the problem anew with the aid of the method of sections, and has published a revival of Pringsheim's views, on grounds which appear to merit a full examination. This is, indeed, the more imperative, as my refutation of his paper of '95 in a short note¹ has remained practically unanswered, even in his recent paper.

¹ Annals of Botany, vol. x, 1896.

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The 'sexual' reproduction in the Saprolegnieae is briefly this:—The hyphae swell into rounded enlargements, which become partitioned off; these are the 'oangia.' The protoplasm in these oangia aggregates into rounded masses, the oospores, which, instead of escaping like the zoospores, round off, become invested with a thick laminated cell-wall within the oangium, and remain for a long time in a resting condition. This is complicated by the fact that in most, but not all the forms, smaller hyphae, which we may call 'male' hyphae, grow into contact with the oangia; and the dilated end of the male hypha is shut off as an 'antheridium' by a septum closely attached to the oangium. On the loss of turgor by the oangium, the antheridium emits tubes which grow from the antheridium into the cavity, and press closely against some or all of the young oospores, without contracting any permanent adhesion to them, as may be clearly seen when the laminated wall is formed round the oospores. On comparison with allied forms the tubes appear clearly identical with the fertilizing tubes of the Peronosporae, and may receive the same name.

We have first to consider the appearances in the maturing oangium. In this a central vacuole is established, if not already present at the moment of closure and septation; and vacuoles appear at fairly regular intervals in the peripheral protoplasm, which disappear by opening into the central cavity, as I showed, so that the layer of plasma is gradually and steadily thinned down. At the commencement it is multinucleate; and as the plasma shrinks the nuclei are brought closer to one another; in *Achlya* they may be seen approximated in files; and their total relative number now steadily decreases. At the same time they show changes of form and structure; many are seen to become ovoid, instead of spherical or lenticular. Again, at first the staining material of the single central nuclein-mass is in the form of a fairly large central body suspended by threads of linin; but as the process goes on its size diminishes and several are seen in each nucleus; in the oval nuclei we

may frequently find these staining masses aggregated in two distinct groups with linin-strands traversing the centre of the nucleus. The nuclear wall is less distinct from the cytoplasm, and the nuclein bodies stain less deeply. Here comes the first point of difference between Trow and myself; I cannot interpret these facts as other than the fusion of nuclei, and the multiple staining bodies as representing the originally separate nuclein-masses of the nuclei that have fused. When there are two groups of granules, each would thus represent the granules of an already complex nucleus at an early, incomplete stage of fusion with a similar one. Trow regards these figures as indicating indirect nuclear division, identifies the several steps with those of ordinary mitosis, and concludes that these are the ordinary gamete-specializing divisions. The reduction in number which goes on steadily from beginning to end of these changes, he ascribes to the digestion of most of the unfortunate nuclei in the cytoplasm.

The reduction in the amount of staining material in a nucleus is, however, not an unexampled phenomenon in the passage from a vegetative to a reproductive cell; the germinal vesicle of the ovarian egg of Vertebrata is a notable case in point. The issue between us is essentially this:—Trow believes that the nuclei divide, and that the greater part of the daughter-nuclei thus formed are bodily digested in the cytoplasm: whilst I infer from my observations that the nuclei fuse two and two at the same time that they shrink in size, while the amount of staining material is simultaneously reduced; and in this way the number of nuclei is so reduced that finally there is but one nucleus left in each oospore. The reduction in staining properties may be regarded as due to a digestive process; but according to my views the digestion would be entirely intra-nuclear. I found that in *Achlya* this reduction is seldom complete at the moment of the resolution into oospores, so that at first each is frequently binucleate; and Humphrey confirmed this: in *Saprolegnia*, on the contrary, each oospore is usually uninucleate at its very beginning. However, I have noted that there are occasional exceptions:—

‘In a few cases, however, I have seen two nuclei of unequal size in the young oospore; and from this I might have inferred a sexual fertilization, had not the oogonium in these very cases happened to be free from all signs of antheridial branches, let alone fertilizing tubes (’95, p. 694, Pl. XXIX, Figs. 25.)’ This observation seems to have been inexplicable to Professor Trow: in his ’95 paper he wrote (p. 638), ‘the fact that Hartog did not find binucleate oospores in *Saprolegnia* may be traced most probably to an examination of apogamous forms like *S. Thureti*.’ But it was just in an apogamous oogonium that I figured such a binucleate oospore, and not in cases where the antheridia and fertilizing tubes were well developed. This fact I recalled in my criticism (’96): ‘I have indeed found and figured a young oospore of *Saprolegnia* with two nuclei, as is so frequently the case in *Achlya*; but in this case there was no antheridium present whatever, while many hundreds of oogonia [sc., of *Saprolegnia*] with attached antheridia and contained antheridial tubes did not supply a second case of a binucleate oospore in this genus’ (p. 99). In his ’99 paper Trow cites the passage in the *oratio obliqua*, and in such a context as to suggest that it applied to *Achlya*. He goes on to say (p. 167): ‘I have never been able to absolutely prove the absence of an antheridium from an oogonium except by the method of following the development throughout on separate hyphae. In the case of oogonia taken haphazard, I have great doubts as to the possibility of demonstrating the complete absence of an antheridium by Hartog’s method.’ There cannot be the least opening for any doubt in examining carefully prepared specimens *in toto* (whether merely floated out, or carefully spread out with needles) as to the presence or absence of antheridial branches, antheridia, or fertilizing tubes. My specimens were laid before, I think, two meetings of the British Association, and were accessible to any one interested to go over them with me, as Mr. Wager did; and I do not admit that Professor Trow has any right to assume either wilful negligence or incapacity on my part, in a matter

of such great importance, merely because my observations contradict the conclusions which he drew from his own.

His account is as follows: 'In *Saprolegnia mixta*, the young oospores have frequently a second nucleus near the point of contact of a fertilizing tube. No opening can be seen in the tube, and it would in any case probably be very difficult to prove its presence even were it there. But a permanent opening does not appear to be necessary for fertilization to take place in such cases. No one, for example, has been able to determine a permanent opening at the end of the pollen-tube of the Angiosperms, where fertilization undoubtedly takes place' (95, p. 635). True, but in Angiosperms (1) the pollen-tube becomes permanently attached to the embryo-sac, as is the case in other siphogamous fertilization; (2) the pollen-tube loses its turgescence. Neither of these is the case in the *Saprolegnieae*; as De Bary, Marshall Ward, Humphrey, and I have seen, the fertilizing tube may grow up to the naked oospore, and even dent it, but it then glides off from its surface and grows past it without losing turgescence. Trow's conclusions appear then to be based, so far as *Saprolegnia* is concerned, on forms in which the final fusion of the last two nuclei left in the oospore-origin is habitually delayed to the same extent that I found, exceptionally, in a form free from male organs.

In *Achlya*, he writes in '99 (p. 159), 'large numbers of sections were examined to try and elucidate the mode of fertilization, but only one section, that reproduced in Fig. 45, appeared to be capable of throwing any light on the actual process. In this case it was possible to trace the fertilization-tube without a break into an egg which was already surrounded with a thin membrane.' The figure as it stands appears, at first sight, to be conclusive. But there are points about it that make me hesitate and finally reject its evidence. The cell-membrane is represented on the oosphere and the antheridium at the surface of the oogangial wall, but the tube itself is represented as a grey homogeneous mass, with neither plasmic network, granules, nor nuclei, and

without any trace of a cell-wall, on its free course to the oospore; this is inexplicable. Now Professor Trow has already told us: 'In the preparations which I have made and used for further study, I have never seen a loose or misplaced section, and have never been at a loss to locate any section of a series. It is fortunate that it is possible to attain certainty in this direction, for there can be no doubt that the oogonia and oospores are too thick and dense for clear pictures of their contents to be obtainable by any other method than one which involves sectioning' (p. 151). But he has omitted to figure the upper and lower sections of the very set to which this solitary case of penetration belongs; he has not noted or explained the absence of the cell-wall from the free part of the fertilizing tube, and the absence of the beautiful cytoplasmic network which is so obvious even in the specimens prepared *in toto*, and which *ex hypothesi* should be so much more clearly seen in sections. For the present, then, it is at least permissible to regard the preparation as unsatisfactory, and to explain the case as one in which the fertilizing tube has passed over the surface of the oospore without penetrating it. Indeed, Professor Trow's observations on the living specimen confirm those of the supporters of the apogamy of *Achlya*: 'Neither in this case nor in that of many others examined, have I been able to trace the entry of the fertilization-tube into the egg; it seems to grow past it, and on the side furthest removed from observation, possibly influenced thereto by the illumination from below' (p. 142). Thus, like the rest of us, he has found that the fertilizing tube passes on, leaving no trace of its futile attempts to perform the ancestral functions. He assumes, however, that a gametoneucleus is transmitted to the oospore by the unseen, temporary penetration of the oosphere; and gives no hint as to what becomes of the part of the tube that he wishes us to believe has penetrated into the egg. There is no trace of it on the wall of the oospore at any time, even in his own sections. The tube bears no branch, reveals no scar; one can therefore only infer that it must be withdrawn after it has

done its work, like the male organ of a Metazoan¹. 'I have satisfied myself, however,' he writes, 'of the presence of two nuclei in the egg *at all times* in this stage, one peripheral and the other central, and the peripheral one always close to the point of attachment on a fertilization-tube' (p. 159). Professor Trow showed his exquisite drawings at the Botanical Section at Bristol; they conveyed clearly to me that this 'peripheral nucleus' was in no way different to other collections of granules—microsomes—lying more or less peripherally in the egg, and his plates are equally convincing: such pseudo-nuclei he has figured in his Fig. 45 (upper oospore) above the entrance of the dotted line. In Fig. 46, the lowest oospore contains two of these in the angle between the two dotted lines to the south of that noted as the 'male nucleus'; and in the right-hand oospore at the north-west of the 'male nucleus,' and separated from it by two vacuoles, is another of these. On the other hand, in Fig. 44 the left-hand egg seems to show two nearly central true nuclei, of which he has only recognized one. The explanation I am inclined to give is that the stain he used, gentian-violet and eosin, does not sufficiently differentiate nuclei and microsomes, and can give no certain results.

I wrote in '95 (p. 698-700): 'The apogamy of Saprolegnieae does not imply a complete absence of processes comparable with fertilization. The essence of ordinary fertilization consists in the union of two cells, cytoplasm to cytoplasm, nucleus to nucleus (and in most cases, archoplasm to archoplasm—referring of course to the views of Fol and Guignard, which have since been somewhat shaken). In this way is formed a new complex cytoplasm, nucleus, &c., which, as such, have never been associated before. In Saprolegnieae the nucleus of the oospore is formed by the fusion of many nuclei, which possibly wandered from different parts of the plant, and which

¹ This is a revival of Pringsheim's erroneous observations and conclusions; De Bary I think compared the passage of the gametoplasm, leaving no trace of exit from the fertilizing tube or entrance into the oospore in the cell-walls of either, to the passage of spiritual mediums like the renowned Mrs. Guppy through brick-walls and closed doors and windows.

are, in any case, essentially different from all those formed by repeated nuclear fission in the previous life-history of the plant. . . . Obviously such endogamous nuclear unions cannot occur in plants with distinct walls partitioning them into distinct cells. But we do find parallel cases in other apocytial plants. In the Dasycladeae the nuclei fuse several together in a gametangium, and round the single fusion-nuclei the protoplasm aggregates to form uninuclear [isogamous] gametes which pair with those of other plants. In *Derbesia*, zoospores with fusion-nuclei formed in precisely the same way have apparently lost the faculty of pairing, and develop directly. . . . In Uredineae the teleutospore is bicellular; in each cell the nucleus divides; and then in the cell the two sister-nuclei fuse again, so that each of the two resting-spores of which the teleutospore is formed has a fusion-nucleus. And in Ustilagineae each spore is primitively binucleate; but becomes uninucleate by the fusion of the nuclei. . . . I refer also to the formation of the large "basidial nucleus" of the Basidiomycetes by the fusion of two or more; from this, by two bipartitions, are formed the nuclei for the basidiospores. In most animals the ovarian egg divides at maturity into a brood of four, three of which are functionless and are termed polar bodies; while the fourth is the functional oosphere which is usually fertilized by the male cell, the spermatozoon. In several cases of so-called parthenogenesis the second cell-division remains incomplete: the cell-nucleus divides as if to give rise to the nuclei of a polar body and an oosphere respectively; but then the former nucleus moves back and fuses, playing the part of a male [I should rather have written 'the same part as the sperm-nucleus'] with its sister, just as we have seen in the Uredineae.' These facts I quoted especially from Boveri and Hertwig in '91; but since then Brauer of Heidelberg confirmed them in the fullest way for *Artemia*; and Wilson's 'Cell in Development and Inheritance' (where they are quoted with figures) should be familiar to every one who, like Professor Trow, proposes to deal with questions of cytology.

To these examples we may add the fusion of the sister-nucleus of the oosphere with one of the basal group in the embryo-sac of Phanerogams when it is in an apocytial condition, and the fusion in the apocytial Heliozoan *Actinosphaerium* of the nuclei, several together, into a much reduced number, as observed by Brauer; and as an intermediate case the formation of the gameto-nucleus of the oosphere in *Sphaeroplea*, which, according to Rauwenhoff's description, resembles the process as 'seen in the formation of the oospheres of *Saprolegnia*' ('91, p. 19). All of these (save *Actinosphaerium* and *Artemia*) were cited in my '95 paper, or in the '91 'Problems of Reproduction,' to which I referred, and which contains the full statement of my views on these subjects. Yet Professor Trow writes, without taking any account of these facts—'The question of wholesale nuclear fusions is one which we expect to see proved conclusively by actual demonstration. It is so improbable in itself, at any stage in the life-history of a plant, that we may be pardoned, in the absence of such demonstration, for unbounded scepticism as to its occurrence. . . . But Hartog's theory, even as a theory, has an unsound foundation. Consider what it means. The nuclei in question, surrounded by their protoplasmic masses—energids, let us say—are female gametes, and very highly developed ones too' ('99, p. 165). Yet I think there can hardly be greater general unanimity on any matter connected with the history of reproduction, than as to the view that regards the conjugating nuclei as *equivalent* nuclei, and free as far as possible from the taint of binary sexual differentiation which is an adaptation of the cytoplasm only. A point on which Weismann, O. Hertwig, and Maupas are agreed is surely worth taking into account.

He proceeds: 'The theory, then, is that of the multiple endogamous union of highly developed female gametes in plants where very dissimilar male gametes are well known. Imagine a botanist broaching as a theory the multiple union of *Fucus* eggs to form a zygote, and the universal incredulity with which it would be justly met, and we have some idea of

the great improbability of Hartog's theory.' Professor Trow is singularly unfortunate in his illustration. Between such widely different groups as the Metazoa, and the Phaeophyceae, it was hardly to be expected that homologies could be found; yet, thanks to his own teacher, Oltmanns, we know that the abortive oospheres of the Fucaceae are absolutely comparable with the polar bodies of the Metazoa.

Professor Trow's careless treatment of facts and records shows everywhere save in his figures. Thus whilst he figures the well-known cellulin-corpuscles of Pringsheim (Fig. 42), he not only fails to recognize them, but volunteers an absolutely impossible explanation of their nature; 'occasionally, as represented in the figure, we have in the large central vacuole what we may regard as skeletons of fat-globules; these skeletons are very delicate spherical shells, almost invisible in some preparations, but generally well seen in those stained with haematoxylin (p. 157) . . . The oil-globules are represented by the cavities in which they lay, and occasionally, in the case of those lying either partly or wholly in the vacuole, by the skeletons we have already described' (p. 158). In '95 I noted that the cellulin-granules become almost invisible in balsam, and what Professor Trow calls shells are certainly (as he figures one in Fig. 43) solid spheres of this substance; that it stains with haematoxylin, was, so far as I remember, one reason for my rejecting that pigment. He throws doubt (p. 166) on the validity of my preparations, for representing in my Figs. 21, 22, oospores of *Achlya*, the outer part occupied by dense protoplasm, 'the central part appears in the form of a big vacuole, the nucleus being suspended in the middle by fine strands of protoplasm'; yet in both these a thin continuous layer of protoplasm is figured surrounding the nucleus. I am far from being a good draughtsman, and my sketches were unfortunately redrawn instead of being printed in their inartistic condition. But in Fig. 25, that of the spore of *Saprolegnia* with two unequal nuclei not yet fused, the larger nucleus especially has a dense investment of cytoplasm, continuous with the reticulum.

Professor Trow says that the fixing solution was 'a saturated aqueous solution of mercuric chloride, and it was applied hot as advised by Hartog and Humphrey' (p. 150). Now my methods have been repeatedly published; in the report of the Congrès de Botanique, of '89, printed in the Bulletin de la Société de Botanique de France, vol. 26 (translated in the Journal of the Royal Microscopical Society); and in the fullest possible detail in the memoir of '95; nowhere do I speak of applying hot sublimate, for the results of the cold solution were good enough; Humphrey however warmed his fixative, and an ambiguous sentence of his possibly explains Professor Trow's error.

I have termed the central deeply staining mass in the nucleus a 'nuclein-mass'; my figures show that I saw it as very frequently containing deeply staining granules embedded in linin; Humphrey calls it a chromatin-mass, evidently in the same sense. Professor Trow is not justified in writing 'we cannot then regard it either as a nucleolus as has been done by Dangeard, or as chromatin or a chromosome as has been done by Humphrey, Hartog, and myself' (p. 152). For this implies that the synonymous terms used by Humphrey and me are also equivalent to his own use of 'chromosome,' than which nothing can be more inexact. I indeed described this nuclein-mass as resolving itself into four chromosomes in division!

Professor Trow writes, 'had Hartog been able to follow the division of the nucleus in the oogonium (as he did in the antheridium)' (p. 164). Here again is an inaccuracy: I expressly state that 'the nuclei multiply by division, *as shown by the small size and large number of those found in the "fertilizing tubes"*' ('95, p. 694). I wish I had been able to 'follow the divisions.' The curious point is that it is a repetition of an error in his '95 paper, to which I expressly called attention in my criticism of '96 (p. 98, note). All these are, singly, small matters; but collectively they count in judging the value of the author's observations and inferences.

Professor Trow's terminology is rather perplexing at times.

The sentence, 'The clear material through which the threads pass we may regard as nuclear sap or nucleo-hyaloplasma, the threads themselves as linin-threads' (p. 152), conveys no clear meaning to my mind as to what he understands by nucleo-hyaloplasma; most botanists would apply the term to the linin-threads themselves minus the microsomes. Again, 'It is difficult to determine the substance which fills these true vacuoles, whether it be hyaloplasm or cell-sap' (p. 154). His conception of hyaloplasma is certainly an unfamiliar one.

With regard to the closing discussion in Professor Trow's paper on nuclear reduction and alternation of generations, I have nothing to say here. A complete and recent statement of my views is to be found in two papers 'The Fundamental Principles of Heredity,' and 'Nuclear Reduction' in 'Natural Science' for Oct.-Nov. '97, and July '98, both translated in the *Biologisches Centralblatt* for 1898. These contain a comparison of the essential relations of gametogeny to other brood-cell formation in Metazoa, Metaphytes, and Protista. A short abstract of my views contributed by me to the discussion on Alternation of generations at the Bristol meeting of the British Association, has appeared in the *Annals of Botany*. It is, I presume, because these are all so accessible, that no reference is made to them in Professor Trow's contribution to the subject.

In conclusion, I have at least to thank Professor Trow for having given me the occasion to go back again to my old specimens, and to find that the lapse of years has in no way damaged their goodness or their legibility, while his exquisite drawings have again convinced me that sections can, in this group at least, demonstrate nothing more than good mounts *in toto*. A little more care in reading his own specimens, as in reading the published observations of others, would have saved me the painful task of criticizing unfavourably the outcome of a difficult and laborious research.

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