The Development of *Thraustotheca*, a Peculiar Water-Mould.¹

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With Plates IV and V and two Figures in the Text.

THE genus *Thraustotheca* comprises at present the single species *T. clavata* (de B.), Humph., and is a rare and imperfectly known representative of the Saprolegniaceae. In the following paper the writer presents certain results of a culture study on the development of this fungus. These investigations were carried on in the Cryptogamic Laboratories of Harvard University under the direction of Dr. Roland Thaxter, for whose helpful criticism and advice the writer wishes to express his gratitude.

HISTORY.

Thraustotheca clavata (de B.), Humph., was first discovered in 1880 by de Bary in a culture from algae collected at Vendenheim near Strassburg, and was named by him *Dictyuchus clavatus*, since its method of spore liberation resembled somewhat that of the genus *Dictyuchus* established by Leitgeb (19) in 1869. Under this name Büsgen (6) published the first description of the species in his study of sporangium formation in this and other Phycomycetes. In 1884 de Bary (3) mentioned the fungus as an example of endogenous cell formation, and in 1887 Rotherth (24) briefly discussed the process in this and other forms. The first full description of the species, with illustrations, was given in 1888 in a posthumous paper of de Bary's (4) edited by Solms-Laubach. The latter suggested that certain peculiarities of the fungus, especially the disintegration of the sporangium membrane, might prove sufficient basis for establishing a new genus; and this was also urged by Fischer (11) in a later discussion of the species. Humphrey (15), although unable to secure *Dictyuchus clavatus* for study, considered it a species sufficiently aberrant to justify separation from the genus. On this species as a type he established the genus *Thraustotheca*, which has since been accepted by Schröter (26) and other systematic writers. A little more than thirty years after the fungus was first discovered

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by de Bary it was again brought to light by von Minden (21) in Germany, and by Coker and Hyman (9) for the first time in the United States. Von Minden made no detailed study of the form, but extended de Bary's description through his own observation of material from an inlet of the Elbe near Hamburg. By Coker and Hyman, however, the fungus, which had been collected from a pool at Chapel Hill, North Carolina, was studied for the first time in pure culture.

In September 1913 a luxuriant growth of *Thraustotheca* suddenly appeared in one of the writer's cultures which contained algae and silt taken from a spring-fed iron watering-trough near Great Barrington, Mass. Since the culture, although under observation for a month, had yielded only two common species of *Achlya*, the fungus was probably derived from an oospore.

METHODS.

The fungus was isolated and grown in cooled, covered battery jars. A little nutrient material was added every two weeks or so: flies or other insects and their larvae or eggs; bits of earthworms or salamanders; as well as the stems, leaf petioles, fruits, and seeds of various plants. By occasionally starting fresh cultures to offset the accumulation of injurious decomposition products, the fungus was thus maintained without difficulty for over eighteen months.

Pure cultures were obtained as follows: Hyphae bearing young sporangia were washed in several changes of sterile water, and allowed to remain in a drop on a slide until spores had been formed. This drop was then added to a few cubic centimetres of sterile water in an atomizer, shaken up, and the resulting suspension of spores sprayed on beef agar in large Petri dishes. After the plates had remained in a cool place for twenty-four to forty-eight hours, they were examined with a binocular microscope, and fragments of the young mycelia that proved to be uncontaminated by bacteria were cut out on small chips of agar, and transferred to fresh nutrient media.

Successful use was also made of the method devised by Klebs (18) and employed with unessential modifications by subsequent investigators, which involves the repeated transference of vegetative mycelium until bacterial contamination has been outgrown. In comparison with spraying the spores, however, this method proved to be unnecessarily tedious.

Cultures were also obtained from single spores under microscopic observation in the following manner: A few zoospores were introduced with a pipette into a drop of water on a slide, and a minute chip of beef agar was placed in the centre of the drop. Almost immediately the zoospores swam to the agar chip and came to rest here and there upon it. Under the microscope single zoospores on minute bits of agar were

isolated by the careful manipulation of a delicate platinum needle, and transferred to nutrient agar. This method proved more simple than the floating cover-glass method of Trow (29), Kasanowsky (16), and others, and quite as efficient. After pure cultures from single zoospores had been thus secured, stock cultures were maintained in flasks on fairly dry cornmeal mush, a medium in which the mycelia retained their vitality especially well.

An abundance of material in the several stages desired for study was secured by the methods of Klebs (18) and his successors, by growing mycelia in a variety of favourable liquid media and transferring them to sterile water or various solutions in which the development of the fungus was followed. Beef extract (1 to 3 per cent.), diluted beef juice, and filtered decoctions of split peas, yellow cornmeal, seed sweet corn, oatmeal, or other cereals were used. To suitable amounts of the medium in Petri dishes several bits of mycelium on agar were transferred; and after three to five days at laboratory temperature the resulting mats of mycelium were of sufficient size for use.

DEVELOPMENT OF THE FUNGUS.

In its development under normal conditions *Thraustotheca clavata* follows a regular cycle, since the mycelium gives rise first to sporangia and later to sexual organs. For convenience, therefore, the successive stages will be considered in the order of their appearance.

Mycelium. In both pure and gross cultures the mycelium consists of a number of main hyphae which grow out in a radial direction, and by branching and rebranching give rise to a thick mat of interwoven filaments. Certain variations in size, shape, structure, and method of branching characterize the hyphae in different nutrient solutions; but since these variations have already been described by Klebs (18), Kauffman (17), Horn (14), and Obel (22), for several Saprolegniaceous species, they need not be discussed here.

In pure cultures the hyphae frequently contain masses of pectin which vary in extent from knobs or bosses on the inner wall surface, and from discoid or cylindric septa (Pl. IV, Fig. 37) to lobed structures occupying a large part of the lumen. Frequently also portions of the hyphal content may separate, become surrounded by a delicate wall, and then resume growth to form endogenous hyphae, which often grow in opposition to each other until one or both may be forced out through the original hyphal wall. Under favourable conditions both the erumpent hyphae and those still *in situ* may form normal sporangia (Fig. 35).

The formation of both pectin masses and endogenous hyphae seems in the case of *Thraustotheca* to result from localized mechanical injuries as well as from the effect of ionized metals or plasmolysis, which Horn (14) found to cause similar phenomena in *Achlya polyandra*, de B.

Sporangia. Typical sporangia of *Thraustotheca* are broadly clavate in shape, and lack papillae of dehiscence. Variations from the type to a fusiform or subspherical shape may occur; and in size the sporangia may show all gradations, from the largest, about 300 by 60 μ , containing several hundred, to small impoverished specimens containing only a few spores. Renewal of the sporangia takes place not only by cymose branching, the only method heretofore noted, but also by successive formation in basipetal series (Fig. 27).

Formation of sporangia by the mycelium follows a diminution of the food supply in accordance with the general law established by Klebs (18). Since certain important details in the process of sporangium formation could not be made clear by serial sections, a study of the living material was made. The following description is derived from observations on a large number of sporangia in hanging-drop cultures under a Zeiss J. water-immersion lens.

The process begins with a gradual accumulation of protoplasm and a proportionate enlargement of the hypha, generally at the tip (Fig. 1). Eventually the accumulating content fills the sporangium initial, leaving at most only a small axial vacuole (Fig. 2). A septum is now formed across the sporangium base. The granular protoplasm in this region suddenly separates, leaving a hyaline zone (Fig. 3). At the bottom of this zone the septum is formed (Fig. 4), while at the periphery the lateral sporangium wall is reinforced, and in a few moments the granular content moves back, obliterating the hyaline area. Because of its reinforcement (cf. Fig. 29) the basal part of the sporangium wall is especially persistent, a peculiarity of the genus noted but not explained by previous investigators. Occasionally the septum shows columella-like outgrowths into the sporangium (Fig. 36); but as a rule the statement of Solms-Laubach and others that the septum is strongly bulged out into the sporangium is not confirmed.

The content now undergoes differentiation into a number of spores. The first step in this process is the separation of the protoplasm into spore initials. Irregular clefts filled with cell-sap (Fig. 6) appear in the centre of the sporangium, and extend (Fig. 7) to form a system of intersecting lacunae (Fig. 8) which divide up the sporangium contents into roughly polygonal spore initials. Since the sporangium content, in spite of this division, contracts as a whole when plasmolysed, we may infer that a thin peripheral layer of protoplasm still remains uncleft.

A rapid change in the appearance of the sporangium now indicates that a second phase in its development has begun. The content suddenly becomes translucent and temporarily vacuolate, while the individual spore initials become less distinct than heretofore (Fig. 9). This is probably the result of a loss of cell-sap with a consequent shrinkage of the sporangium

and contraction or compression of the spores. At any rate, comparative measurements demonstrate that the sporangium has distinctly decreased in size. Since Rothert (24) in *Saprolegnia* saw the clefts extend and rupture the remaining peripheral layer of protoplasm, we may infer that in the present instance also this rupture has taken place, allowing the escape of cell-sap from the clefts with a consequent shrinkage of the sporangium and partial obliteration of the lacunae of demarcation between the spores. The translucent and vacuolate condition of the content is of brief duration, and there seems no need of regarding the vacuolation as marking a separate stage in sporangium development.

In the final phase, which next ensues, the spores, now clearly granular and surrounded individually by delicate membranes, imbibe water and swell, becoming more and more separate (Fig. 10). It is to be noted that the sporangiospores have maintained their identity from the beginning of the process. As Figs. 6-13 show, the completed spores occupy the identical positions of the spore initials when first marked off by the clefts. There is, moreover, no evidence to support the assertion of Büsgen (6) and others that the spore initials fuse to a homogeneous mass.

The foregoing description applies particularly to the densely filled sporangia; but in the less common axially vacuolate sporangia, as well as in certain abnormal sporangia (Fig. 38) which are characterized by large central vacuoles and thin peripheral protoplasm, and which develop at a temperature near the maximum (31-32° C.), spore-formation is also associated with the centrifugal extension of cleavage furrows. If, however, the sporangium wall is punctured just as cleavage begins, both the protoplasm that escapes and that which remains undergo division into irregular spore-masses by an incomplete cleavage from the surface inwards (Figs. 40 and 41).

It is to be noted that the process of sporangiospore formation in *Thraustotheca* presents no evidence of an intersporal substance, but agrees in the main with the descriptions of Rothert (24) and Humphrey (15) for other Saprolegniaceae. The assertion of de Bary (4) and of von Minden (21) that the sporangiospores of *Thraustotheca* are embedded in a 'Zwischen-substanz' is probably based on Büsgen's misinterpretation of the process of spore formation in the species, rather than on individual observation of living materials.

Liberation of the Sporangiospores. The method of spore liberation in *Thraustotheca* differs from that which has been described for any other genus of the Saprolegniaceae. The papillae of dehiscence which characterize most members of the family are lacking, and the non-motile sporangiospores escape by bursting the sporangial wall. At high temperatures (30-31° C.) occasional sporangia develop apical papillae (Fig. 38) which closely resemble the normal escape papillae of other genera. In

these abnormal cases, however, escape of the sporangiospores also takes place by rupture of the wall near the papilla or elsewhere (Fig. 39).

When the individuality of the spores is finally established, they are distinctly polygonal; and the sporangium wall presents a plane, even contour (Fig. 10). Gradually, however, the spores imbibe water, swell, and become spherical; and as their flattened surfaces of mutual contact round out, the spores are separated, and the sporangium wall becomes tightly stretched over their bulging outer surfaces. As this process of swelling and rounding goes on, the restraining wall becomes increasingly

TEXT-FIG. 1. Escape of the sporangiospores. From camera lucida drawings of living material. 1, 2. A sporangium which burst at the side, allowing a large number of spores to escape. Note adherence of the spores. 3, 4. A small sporangium just before and just after bursting at the tip. 5, 6. A short, broad sporangium from which a number of spores were forced out, rupturing the wall near the base.

distended and eventually bursts (Fig. 13). Liberation of the spores does not take place at once. When the membrane is ruptured, the spores in the immediate region of the break are forced out, relieving the pressure. The elastic membrane, however, still restrains the remaining spores, and after a few minutes the distension among these may become sufficient to push out those most favourably situated.

The point at which the bursting of the sporangium membrane takes place varies according to conditions (Text-fig. 1). When the sporangia are long and slender, the longitudinal expansion, augmented by the upward bulging of the sporangial septum, far exceeds the lateral expansion, and the sporangium membrane is generally ruptured at the tip. In broadly clavate

or spherical sporangia, although it is sometimes possible from the arrangement of the spores to predict where they will be set free, the dehiscence is quite irregular. Moreover, the wall often varies in thickness, and the weakest spots are naturally the first to give way. Occasionally, where the thickened basal collar merges into the normal sporangium wall, there appears to be a line of weakness along which rupture takes place. In the case of intercalary sporangia, the thick septa at the ends prevent any longitudinal expansion, and discharge is invariably lateral (Fig. 27).

During sporangium dehiscence, a distinct adhesion and mutual attraction is noticeable among the spores. Frequently sporangiospores remain sticking to hyphae with which they have come in contact when discharged. Microchemical tests fail to demonstrate any intersporal slime such as Swingle (28) found excreted from the spores of *Rhizopus*; and since this adhesive quality is soon lost, it is probably due only to the viscosity of the recently formed cellulose walls. At the moment of discharge, however, the spores seem to exert a mutual attraction; since they do not immediately scatter, but press together, sliding over each other and changing their relative positions, but still keeping in contact. It is of interest to note that the contiguous surfaces of such spores are often decidedly flattened, as if through the exertion of mutual pressure.

The fragility of the sporangium wall has been over-emphasized by previous investigators, probably because they did not observe the actual bursting of the sporangium, and hence inferred that the spores were freed by disintegration of the wall. In pure culture the sporangium walls are as persistent as would be the walls of any other Saprolegniaceae sporangium that had undergone a like distension and rupture.

It seems advisable at this point to call attention to the confusion which often arises from an unrestricted use of the terms 'zoospore' or 'spore' in the case of diplanetic Saprolegniaceae. To avoid any misunderstanding in the case of *Thraustotheca* the term 'sporangiospore' will be used for the non-motile spores formed in the sporangium: the motile spores emerging from these will be called 'zoospores', and the encysted spores into which these in turn metamorphose will be called 'cystospores'.

The liberated sporangiospores (Fig. 13) vary from a rounded polygonal to a practically spherical shape; their average diameter is about 12.5μ ; and the distinct wall is about 0.5μ in thickness. The content consists of a number of microsomes scattered irregularly through a finely granular, almost hyaline matrix, a less densely granular central region indicating the position of the nucleus.

Further History of the Sporangiospores. The sporangiospores may either emit motile zoospores, give rise to hyphae of germination, or form dwarf sporangia, according to certain conditions of the environment. In

pure water, zoospore emergence takes place; in the presence of nutriment, germ tubes are formed; and when, after hypha-formation has begun, the available nutriment is suddenly exhausted or removed, dwarf sporangia are produced. Frequently, also, substances which increase the osmotic pressure, but are without nutrient value, seem to exert the same influence as nutriment in this connexion.

Zoospore emission occurs as follows: The sporangiospore content emerges rapidly through a softened papilla of escape as an amorphous mass (Fig. 15), which assumes the characteristic structure of the motile zoospore. Near the already apparent nucleus (Fig. 16) a contractile vacuole develops, while the surface becomes grooved by a depression (Fig. 17) from which, close to the nucleus, arise two delicate cilia that lash slowly back and forth. This lashing increases in violence until the zoospore finally frees itself with a sudden jerk, and swims actively away (Fig. 18). In the case of sporangiospores retained *in situ*, the emerging zoospores are imprisoned by the enveloping sporangium membrane or by surrounding sporangiospores. As a result, sporangiospores, zoospores, and cystospores may at times be observed within the same sporangium wall (Fig. 29). From retained sporangiospores in contact with the sporangium wall, zoospores often emerge through the wall as in the genus *Dictyuchus* (Fig. 30).

The zoospores are of the laterally biciliate type found in most Saprolegniaceae. Under high magnification the inadequacy of the commonly used terms 'bean'- or 'kidney'-shaped becomes obvious. Precise description is difficult, but the zoospores may be characterized roughly as subovoid and as bearing a longitudinal furrow. Since the customary figures of this type of zoospore are somewhat misleading, an attempt has been made to present the solid form in the accompanying diagrams (Text-fig. 2). In size the zoospores average about 12.5μ in length and 9μ in width. Their locomotion, involving progression and rotation on the long axis, is in an irregular spiral course through the water. The more tapering end is directed forward, and by the tractile action of the shorter anterior cilium the zoospore is drawn rapidly along, while the longer cilium is dragged behind as a more or less passive rudder. Although rotation is generally to the right, it may be reversed or may cease, while the zoospore moves irregularly forward. These changes in motion seem to be dependent upon the activity of the posterior cilium, although the precise method of lashing is not distinguishable. To chemical and other stimuli the zoospores respond much as did the *Saprolegnia* zoospores investigated by Pfeffer (23) and Stange (27). Duration of the motile period is not fixed. A tendency to motility, together with a sensitiveness to those influences of the environment that result in encystment, is inherent in each zoospore. In pure water a zoospore may swim about for 30 or 45 minutes, but at any minute, if

nutrient solutions, or even non-nutrient solutions which increase the osmotic pressure, are allowed to diffuse into the water, encystment immediately occurs. The zoospore becomes quiescent, and contracts to a sphere, while the contractile vacuole disappears and large granules appear in the finely granular content. Usually the cilia, still bending actively into twisted loops (Fig. 19), are dropped off during contraction of the zoospore; but occasionally they seem to deliquesce into minute droplets and disappear.

The cystospore thus formed is, in contrast to the sporangiospore, almost exactly spherical in shape, about 10μ in diameter, and possesses a more sharply defined wall, enclosing a denser content. Germination, which

TEXT-FIG. 2. Zoospores. Diagrams showing the characteristic shape. From camera lucida drawings of living spores. 1. Side view, the active anterior cilium at the left, the trailing posterior cilium at the right. The groove (above) cannot be seen. 2. From above, looking down on the groove, anterior cilium at the left. 3. Oblique, posterior-lateral view. The groove is partly shown. 4. Posterior view showing groove and position of cilia.

may occur at once or after some weeks of inactivity, is invariably by the formation of a hypha (Fig. 20). In hanging-drop cultures the growth of this hypha into a bit of fly muscle, the formation of rhizoids, and the ultimate development of a new plant can easily be observed. Since very little food material is present, the resulting plants are small (about 300–400 μ in length), and bear sporangia containing only a few spores (Fig. 22). Occasionally, also, the hypha of germination may form a dwarf sporangium (Fig. 23) similar to those which arise from the sporangiospores, and will be described later.

The sporangiospores form hyphae of germination in either solid or liquid media. Rapid germination and vigorous growth to a mycelium occur when sporangiospores are transferred to beef decoctions, cornmeal mush, and cylinders of potato or carrot. Under these conditions the spore becomes vacuolate, swells rapidly (Fig. 24), and sends out a tube (Fig. 25)

which eventually forms a mycelium. In the case of sporangiospores retained in unemptied or partially emptied sporangia, the germ-tubes make their way through the enveloping sporangial wall (Fig. 31), a condition presenting close resemblance to the doubtful genus *Aplanes*.

The development of dwarf sporangia from the sporangiospores is shown in Figs. 26, 28, 32, 33, and 34. Within these dwarf sporangia are formed two or three spores somewhat smaller than the normal. Since the total expansion of so few spores is insufficient to rupture the sporangium wall, the spores remain *in situ*, and emit their zoospores through the enveloping membrane (Figs. 26 and 34). These zoospores differ from those already described only in their smaller size and shorter motile period. This phenomenon of dwarf sporangium formation presents an interesting analogy to the production of secondary conidia in the case of certain Entomophthoraceae and Peronosporaceae.

Gemmae. In *Thraustotheca* gemmae are formed when environmental conditions inhibit the development of reproductive organs but permit vegetative growth. By maintaining vigorous mycelia at a temperature (31–32° C.) near the maximum, or in harmless salt solutions at concentrations suppressing the formation of other organs, the formation of gemmae is easily induced. Under these conditions sporangium and oogonium initials, as well as portions of the hyphae, are walled off and become inactive. The gemmae thus formed may be clavate (Pl. V, Fig. 42), spherical (Figs. 43, 46, and 48), cylindrical, or irregular (Fig. 47) in form, and show variations in density of content and thickness of wall. They cannot survive desiccation; and since their metabolic activity, though diminished, still continues, their endurance is proportionate to the amount of protoplasm they contain.

When inhibitory conditions are removed, the gemmae resume their growth and give rise to vegetative hyphae (Fig. 48), sporangia (Figs. 42 and 43), secondary gemmae (Figs. 45 and 46), or sexual organs. In this connexion the initial tendency of the structure from which the gemmae arose has no influence; since any gemma may produce any type of vegetative or reproductive structure—the process being governed by the same rules which apply in the development of these structures from the mycelium.

In view of the conditions under which the gemmae arise, and of the lack of physiological and morphological fixity which characterizes them, Maurizio's (20) opinion that they should be regarded as phylogenetically significant seems hardly justifiable. On the contrary, in the present instance, as in the case of *Saprolegnia mixta* (cf. Klebs, 18), they appear to be 'Hemmungsbildungen' representing only a transient resting-state induced by unfavourable conditions.

The sexual reproduction of *Thraustotheca* closely resembles that of the *prolifera* group of the genus *Achlya*.

Antheridia. The antheridial filaments arise at intervals along the

main hyphae as slender secondary branches (Fig. 53). They are usually gently curving (Figs. 49, 52, and 53), and do not present the irregular crooked appearance which Coker and Hyman (9) regarded as characteristic. As a rule, when the antheridial branches grow through the mycelium, they either come in contact with the developing oogonia or lie in close proximity to them. When this occurs the branches form antheridia (Figs. 49 to 54)—irregular, cylindrical organs, either simple or branched, which curve around the oogonium in close contact with its surface. If they fail to reach the oogonia, however, the filaments often give rise to zoosporangia (Fig. 52). Moreover, antheridia are never formed free, even in cultures containing phosphates or other salts, which Klebs (18) found to stimulate the formation of antheridia on the oogonia. These observations seem to indicate that antheridium formation is a response to some contact or perhaps chemical stimulus furnished by the young oogonia. This stimulus causes no response unless the filaments are very near the oogonia; but usually the extensive branching of the filaments ensures this result. Since the one to several antheridia attached to each oogonium are either of androgynous (Fig. 53) or diclinous (Figs. 49 to 52) origin in cultures from a single spore, the fungus is evidently monoecious.

Oogonia. The oogonia usually arise somewhat later than the antheridial filaments. Although generally borne in a racemose manner on the tips of short lateral branches (Figs. 49 and 53), they may also occupy a terminal or intercalary (Fig. 51) position on the main hypha. In any case, they are formed by the accumulation of contents, swelling of the hypha, and formation of a septum or septa, much as in the case of the sporangium initials. The shape, however, is regularly spherical, although the intercalary oogonia are occasionally barrel-shaped. At an early stage in the development of the oogonia, at times even before the septa have been formed, the antheridial filaments become attached and form antheridia upon them. When this has taken place the coarsely granular content of the latter is modified to innumerable globules embedded in a finely granular matrix. The formation of oosphere initials now takes place (Fig. 54) by a progressive separation of the contents from the centre outward, to form bluntly pyramidal masses which round off and become surrounded by walls (Fig. 55).

The formation of oospheres in an oogonium seems in some way correlated with the attachment of antheridia thereto. If no antheridia are formed on a young oogonium, it does not form oospheres, but gives rise, often repeatedly, to stalked or sessile secondary oogonia by proliferation (Fig. 45), or forms sporangia (Fig. 49). On the contrary, when once an oogonium has acquired antheridia, it does not give rise to sporangia or secondary oogonia, but forms oospheres. Moreover, separation of the oogonial content into oospheres in no case takes place until after attachment

of the antheridia. No exception to the foregoing condition has been noted in a large number of cases ; and the figures of Coker and Hyman (9, Fig. 8) and of Horn (14, Fig. 19) in the case of *Achlya polyandra*, de Bary, strikingly corroborate the writer's observations on this point. The parthenogenetic formation of oospheres would, of course, be a serious objection to this interpretation. No such cases were observed, however, under the usual culture conditions, nor could parthenogenesis be induced by the method Trow (29) found successful in the case of *Achlya americana* var. *cambrica*.

When the oospheres have been formed, the oogonium wall, especially when turned red by chlor-iodide of zinc, is seen to be smooth, thick, and definitely pitted. The character of the pitting varies under different conditions, since, on impoverished mycelia, the oogonia show the weak pitting (Figs. 49, 53, 63, and 64) which has been considered of diagnostic value by previous investigators, while on vigorous mycelia the oogonial walls are marked by large and numerous pits (Fig. 51). It is of interest to note that Horn (14) found a similar variation in the pitting of oogonia in *Achlya polyandra*, de Bary.

After the oospheres are differentiated, they undergo a period of maturation (Figs. 54 to 58), the wall increasing in thickness and the protoplasmic contents separating out as the oil droplets fuse together. Finally the structure of the mature oosphere is attained, characterized by a thick, unroughened wall within which the protoplasm is condensed into a bowl-shaped granular mass partly surrounding a large, slightly flattened oil globule (Fig. 58). This eccentric arrangement of the oospore content may persist for months ; hence, in the case of *Thraustotheca* at least, Maurizio's (20) objection to the term eccentric as representing only a transient condition seems groundless.

Among the maturing oospheres, especially after staining, can be seen slender fertilizing tubes which arise from the antheridia, and enter the oogonia through the pits in the wall (Figs. 50 and 51). In contrast to the thickened antheridial walls, which show the same composition as those of the oogonia, the delicate fertilizing tubes are of cellulose. It is worthy of note that the fertilizing tubes were never seen to penetrate the oospheres, nor was any sudden passage of material down the tubes observed.

Germination of the Oospores. Germination of the oospores of *Thraustotheca* has not been observed by previous investigators. Germination was induced by the writer, however, by placing mature spores in pure water maintained at a temperature optimum for growth (*circa* 25° C.). Under these conditions the protoplasmic content increases in extent, probably at the expense of the oil globule, which becomes irregular (Fig. 59) and gradually disappears. The wall, meanwhile, first swells (Fig. 59), and is resorbed and distended until it becomes comparatively thin (Fig. 60). As a result of these modifications the resting oospore, of about 17 μ in diameter,

becomes transformed into a spherical body 22 to 24 μ in diameter, containing a loose protoplasmic reticulum (Fig. 60). A hypha (Fig. 61) is now sent out, which may, after limited growth, form a sporangium, or may continue to grow into an extensive mycelium depending on the amount of nutriment stored in the spore or present in the surrounding medium. In pure water sporangia are formed, either directly or on short hyphae (Fig. 63); but if nutriment is added, a more or less extensive mycelium arises (Fig. 62). Since the heavy wall of the oogonium persists in pure cultures, the oospores germinate *in situ*; and although in some cases their expansion is sufficient to rupture the enveloping wall, in general the hyphae of germination grow out through the pits, or follow along the empty shells of the persistent antheridia until free (Figs. 62, 63, and 64).

DISCUSSION.

The development of *Thraustotheca* presents several points of interest.

Spore liberation. The method of sporangium dehiscence and spore liberation which characterizes the genus is at present unique among the Saprolegniaceae. In no other member of the family do non-motile sporangiospores escape by bursting the sporangium wall. Moreover, none of the theories advanced in explanation of spore liberation in other genera seem applicable to the process in *Thraustotheca*. The theory that the sporangiospores escape by their own motility is not corroborated, since they are consistently non-motile and without cilia. Even in the better-known genera, however, this theory is not universally supported. In *Saprolegnia*, *Leptolegnia*, and *Pythiopsis* the sporangiospores are undeniably ciliate and motile. Humphrey (15), however, believed that an internal force other than spore motility was involved in emptying the sporangia; and the writer has observed a number of cases which corroborate this point of view. In *Pythiopsis cymosa*, de Bary, for instance, dissolution of the escape-papilla is occasionally delayed until the sporangium becomes so distended by the swelling of the spores that it finally bursts at the tip, expelling the zoospores a considerable distance with such violence that they seem stunned and remain quiet some minutes before swimming away. Moreover, although the sporangiospores of *Aphanomyces* escape from the sporangium and group in a hollow sphere at its mouth, both de Bary (2 and 4) and Rothert (24) found that they lack demonstrable cilia. In the case of *Achlya* certain investigators have asserted that the escaping sporangiospores are ciliate, while others have maintained that they are not. Cornu (10) first claimed that the sporangiospores are ciliate; and this has been corroborated by Hartog (12) for *Achlya polyandra*, Hild., and *A. recurva*, Cornu; by Humphrey (15) for *A. americana*, Humph.; and by Coker (8) for *A. paradoxo*, Coker, and *A. caroliniana*, Coker. On the other hand, Rothert (24), Horn (14), and Coker (7) deny the existence of cilia on the sporangiospores

of *Achlya polyandra*, de Bary. This disagreement may possibly indicate that ciliate sporangiospores are developed only in some species of the genus, or that they are developed in the same species only under certain conditions. In any case, in view of the conditions in *Thraustotheca*, *Aphanomyces*, and *Achlya*, the assumption that automotility of the sporangiospores in the Saprolegniaceae is the prime factor in their escape may well be questioned.

To a second theory, also, that the sporangiospores are forced out of the sporangium by the swelling of some gelatinous substance, no support is given by *Thraustotheca*. No such substance is demonstrated when spore liberation takes place in a suspension of India ink particles or in the presence of various stains. Moreover, in the other genera of the family this theory is not consistently supported. To be sure, de Bary (1), who first promulgated the theory for *Achlya prolifera*, remained unshaken in his conviction throughout his long investigations, and Coker (7) corroborates him in the case of *Achlya de Baryana*, Humph., while Rothert (25) maintains that such an expulsive substance is present also in *Aphanomyces laevis*, de Bary. It must be noted, however, that de Bary and Rothert did not demonstrate this substance, but inferred its existence from the behaviour of the escaping spores; while Coker further maintained that the persistence of the spores in groups showed them to be still embedded in the substance which caused their expulsion, an interpretation which the normal adhesion of the spores by their viscid surfaces leads one to accept with reserve. Furthermore, Hartog (12) and Humphrey (15) were unable by seemingly conclusive tests to demonstrate such a substance in the case of *Achlya*; while Rothert (24) offered somewhat less convincing evidence for *Saprolegnia*. The expulsive material was generally supposed by Büsgen (6) and other investigators to be an intersporal substance derived from the gelatinization of the 'cell plates' which they thought were formed between the spore initials. Walz (30), however, led by his observations on certain algae, maintained that gelatinization of the inner layers of the sporangium wall formed the expansive material which expelled the spores. In the case of *Thraustotheca* no evidence is found to support either of these views.

A third theory was advanced in 1851 by A. Braun (5), who maintained that the contraction of the elastic sporangium wall, which had been distended by the swelling of the zoospores within, was instrumental in emptying the sporangium. With certain modifications this early theory seems applicable to the process of sporangium dehiscence and spore escape in *Thraustotheca*. As has been said, the sporangiospores lack cilia, and no expulsive substance is apparent; but there is no doubt that the sporangiospores swell, and distend the enveloping wall. It is obvious that even the slight increase in the size of the individual spores would, in large masses, produce a considerable aggregate increase in the sporangium volume and a proportionate distension of the wall. On the whole, the swelling of the sporangiospores against the

elastic restraining membrane, combined with the mutual attraction and superficial viscosity of the newly-formed spores, seems to explain quite adequately the sporangium dehiscence and spore liberation that characterizes *Thraustotheca*. Moreover, it is probable that the factors which affect spore liberation in this genus may play at least a contributory part in other cases.

Mutual Attraction of the Sporangiospores. The escaping sporangiospores of *Thraustotheca* show a mutual attraction resembling that which in a more pronounced form causes the aggregation of *Achlya* sporangiospores into a hollow sphere. Hartog (12 and 13) was the first to maintain that this phenomenon in *Achlya* was due, not to the embedding of the spores in a globule of gelatinous material, but to 'adelphotaxy', a form of irritability which he defined as 'the tendency of spontaneously motile cells to assume definite positions with regard to their fellows'. As defined, this term is not applicable to the non-motile sporangiospores of *Thraustotheca*. Since, however, the ciliation and auto-motility of *Achlya* is not indisputably established, and since in *A. paradoxa*, Coker, the escaping sporangiospores show only an irregular aggregation quite comparable to *Thraustotheca*, it seems justifiable to extend the term to include the phenomenon in this genus.

The precise nature of adelphotaxy is at present unknown. It depends seemingly on the life of the spores; since Humphrey (15) found that the spores of *Achlya* fell apart when killed with osmic acid fumes at the precise moment of emergence, and the writer has found a like condition to obtain in *Thraustotheca*. This mutual attraction, moreover, is effective only when the spores are in contact or separated by a very minute space. In *Thraustotheca*, sporangiospores separated during escape by more than one half their diameter remain apart; and in *Achlya* Hartog (12) and Coker (8) have noted that an escaping sporangiospore, if separated from its fellows by its length, fails to take position in the hollow sphere, but moves off. Sharp distinction must be made, however, between adherence of the sporangiospores from mutual attraction and from the glutinous character of the newly-formed walls. In both *Achlya* and *Thraustotheca* the attraction is of brief duration; but the adherence of the hardening walls of the appressed spores holds them firmly even after they have emitted zoospores, and are but empty cysts.

Relationship. Although the exact relationship of *Thraustotheca* is largely a matter of conjecture, certain facts seem of significance in this connexion. A comparison with *Dictyuchus* gives scant basis for the customary taxonomic association of the two genera. The sporangia, both in structure and in method of spore liberation, are distinctly different; while the sexual organs of the three authentic species of *Dictyuchus* are quite unlike those of *Thraustotheca*. To *Achlya*, however, *Thraustotheca*

presents several striking points of similarity. In each case the sporangium consists of an enveloping membrane surrounding a number of discrete spores. Papillae of dehiscence, also, may be induced in *Thraustotheca*; and in *Achlya*, although they are of normal occurrence, may vary greatly, and even become non-functional. Moreover, although the escaping sporangiospores of *Thraustotheca* do not form the hollow sphere characteristic of most species of *Achlya*, they show a degree of adelphotaxy at least comparable to that characterizing *A. paradoxa*, Coker. Furthermore, the sexual organs of *Thraustotheca* very closely resemble those of the *prolifera* group of the genus *Achlya*. These resemblances do not, of course, prove any relationship; but they do seem sufficient to entitle *Thraustotheca* to a tentative systematic position near *Achlya* rather than *Dictyuchus*.

SUMMARY.

1. In the processes of sporangium formation and spore liberation *Thraustotheca* agrees with other members of the family. The intersporal substance mentioned by early investigators cannot be demonstrated.

2. *Thraustotheca* is unique among the Saprolegniaceae in that the non-motile sporangiospores escape by bursting the sporangium wall. The fragility of the sporangium wall has been greatly over-emphasized.

3. The sporangiospores may emit motile zoospores, give rise to hyphae of germination, or form dwarf sporangia, according to conditions of the environment.

4. The zoospores are grooved, laterally biciliate, and of a characteristic shape that cannot be adequately described by the terms 'reniform' or 'bean-shaped', that are generally used in this connexion.

5. Gemmae are formed, but represent merely a transient resting state induced by unfavourable environmental conditions.

6. In the development of the sexual structures certain phenomena seem to justify the assumption that the formation of antheridia is dependent on contact of the antheridial filaments with the oogonia; and that oospore formation is, under normal circumstances, definitely correlated with the presence of antheridia on the oogonia.

7. In germination the oospores send out hyphae which either, after limited growth, form sporangia or give rise to extensive mycelia—the type of development depending on the amount of nutriment available.

8. In its structure and development *Thraustotheca* shows a resemblance to *Achlya* that, in the opinion of the writer, is sufficient to entitle it to a systematic position near the latter genus rather than near *Dictyuchus*.

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EXPLANATION OF PLATES IV AND V.

Illustrating Mr. Weston's paper on the Development of *Thraustotheca*.

The figures were drawn from living material at the level of the stage, with the aid of an Abbé camera lucida. The approximate magnification of the combination of lenses used is given in each case, but applies to the original figures, which have been slightly reduced in reproduction.

PLATE IV.

- Fig. 1. Young sporangium initial becoming filled with protoplasm. $\times 550$.
- Figs. 2-5. Stages in the formation of the septum. Figs. 2-4 $\times 550$; Fig. 5 $\times 1,300$.
- Figs. 6-13. Stages in the development of a small sporangium. $\times 1,400$.
- Figs. 14-17. Emergence of a zoospore from a sporangiospore. $\times 1,400$.
- Fig. 18. Zoospore on the point of swimming away; side view. $\times 1,400$.
- Fig. 19. Cystospore (encysted zoospore) showing discarded cilia. $\times 1,400$.
- Figs. 20, 21. Germination of a cystospore. $\times 1,400$.
- Fig. 22. Small plant resulting from such a germination in the presence of scanty nutriment. $\times 550$.
- Fig. 23. Formation of a dwarf sporangium from a cystospore. $\times 1,400$.
- Figs. 24, 25. Germination of a sporangiospore on nutrient agar. $\times 1,400$.
- Fig. 26. Three-spored dwarf sporangium formed from a sporangiospore. $\times 1,400$.
- Fig. 27. Two successively formed sporangia showing lateral dehiscence and the retention of a large proportion of the spores. $\times 550$.
- Fig. 28. Retained sporangiospores, one of which is forming a dwarf sporangium. $\times 1,400$.
- Fig. 29. Base of a dehiscent sporangium, showing sporangiospores *in situ*, an imprisoned zoospore, a cystospore, and empty sporangiospores. $\times 1,400$.
- Fig. 30. Sporangium which still retains a large number of sporangiospores, some of which have emitted zoospores. $\times 550$.
- Fig. 31. Same sporangium showing development of germ-tubes when nutriment was added. $\times 550$.
- Figs. 32-4. Stages in the development of a bispored dwarf sporangium. $\times 1,400$.
- Fig. 35. Portion of a hypha showing endogenous hyphae which have formed sporangia. $\times 550$.
- Fig. 36. Abnormal columella-like growth of septum into a sporangium. $\times 550$.
- Fig. 37. Portion of a hypha showing thick pectin septum. $\times 550$.
- Fig. 38. Sporangium developed at high temperature showing papilla and large central vacuole. $\times 550$.
- Fig. 39. Similar sporangium after dehiscence. $\times 550$.
- Figs. 40, 41. Incomplete surface cleavage of a punctured sporangium. $\times 550$.

PLATE V.

- Fig. 42. Sporangium formed from a sporangial gemma. $\times 550$.
- Fig. 43. Oogonial gemma which has been transformed into a sporangium. $\times 550$.
- Fig. 44. Part of oogonium wall, showing pits, after treatment with zinc chlor-iodide. $\times 1,400$.
- Fig. 45. Repeated proliferation of oogonial initial with final formation of a sporangium. $\times 550$.
- Fig. 46. Formation of oogonium initial and antheridial branch by an oogonial gemma. $\times 350$.
- Fig. 47. Irregular hyphal gemma. $\times 350$.

Fig. 48. Germination of an oogonial gemma by hyphae. $\times 550$.

Fig. 49. Two adjacent oogonium initials; one, with diclinous antheridium attached, has formed oospores; the other, without antheridia, has formed a sporangium. $\times 550$.

Fig. 50. Formation of oospores in an oogonium which, after proliferating once, has been reached by a diclinous antheridial filament. $\times 550$.

Fig. 51. Intercalary oogonium showing large pits through which fertilizing tubes have entered. $\times 550$.

Fig. 52. Two hyphae of similar origin, one of which formed an antheridium on reaching an oogonium; the other formed a sporangium. $\times 150$.

Fig. 53. Androgynous antheridium on an oogonium. $\times 550$.

Figs. 54-6. Stages in the development of oospores in an oogonium. $\times 550$.

Fig. 57. Later stage in maturation of an oospore. $\times 1,400$.

Fig. 58. Mature oospore. $\times 1,400$.

Figs. 59, 60. Changes in oospore preliminary to germination. $\times 1,400$.

Fig. 61. Formation of germ-tube by free oospore. $\times 1,400$.

Fig. 62. Formation of a mycelium by oospores germinating *in situ*. $\times 350$.

Fig. 63. Formation of a sporangium by an oospore germinating *in situ*. $\times 550$.

Fig. 64. Hyphae of germination from oospores *in situ* growing out through the empty antheridia. $\times 1,400$.

WESTON—THE DEVELOPMENT OF THRAUSTOTHECA, A PECULIAR WATER-MOULD.

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