A Contribution to the Physiology of the Saprolegniaceae, with special reference to the variations of the sexual organs.¹

BY

C. H. KAUFFMAN.

University of Michigan.

With Plate XXIII.

THE Saprolegniaceae have been a favourite subject for investigation by some of the most prominent botanists of the last century. Unger ('43), Schleiden ('42), and Naegeli ('47) were among the first contributors towards a knowledge of these fungi, while Pringsheim ('57, '60, and '74) and De Bary ('81) gave them the first extended morphological and systematic study. In 1872 Cornu began a monograph which he never finished. De Bary gave them special attention up to the last years of his life, and his accurate observations and experiments were reported posthumously in 1888 by Solms-Laubach. In this country Humphrey ('93) extended our knowledge of American forms and summed up the taxonomic facts in a monograph. In more recent years investigations have taken new directions: Trow ('95, '99 and '04), Hartog ('95 and '99), and Davis ('04) have attacked the problems of fertilization and of nuclear phenomena by means of modern cytological technique, while Klebs ('99 and '00) has obtained important experimental results towards a theory of reproduction.

It is upon the foundation laid by Klebs that the present investigation has attempted to build; and the following paragraph may be considered as presenting the lines along which advance seemed possible:—

(a) The method which Klebs applied to the study of a single species has been used for a comparative study of a number of species of the same genus. (b) The special problem of sex determination or at least of antheridial development has been approached with a species whose reproductive organs are more suitable for such study than those of *Saprolegnia mixta*. (c) An effort has been made to learn something of the taxonomic values of those characters which have been generally used in the genus

¹ Contribution ninety-three from the Botanical Laboratory of the University of Michigan.

Saprolegnia for purposes of classification. (d) The details of technique are not especially emphasized by Klebs, and this made it difficult to repeat his experiments with ease and rapidity. It was therefore found to be necessary to work out in detail the technique used, and it seems advisable to report the same, and so make pure cultures of the fungus more available for class use or for purposes of research.

Most of the experiments were done during the winter months of the last five years in the intervals of other work. I am indebted to Professor R. A. Harper, of the University of Wisconsin, who first suggested to me the line of work which has culminated in this paper. My thanks are also due to Professor G. F. Atkinson, of Cornell University, and to Professor F. C. Newcombe, of this laboratory, for their continued interest and assistance.

TECHNIQUE.

The methods of culture used by De Bary, Humphrey, and others usually afford material for study which is fairly reliable, as it can be obtained approximately free from bacteria and other fungi. No cultures had, however, been made from a single isolated zoospore or gemma up to the time of Klebs's paper, and no method has as yet been outlined for cultures with single zoospores. In only one instance have I come across a reference to a possible method of zoospore culture. Trow ('95) isolated a single zoospore of *Aphanomyces* by means of cover-glasses on which white of egg had coagulated in thin coatings, and which had been floated on water containing *Saprolegnia*, &c. A single culture resulted. On the other hand, cultures have been obtained from masses of zoospores presumably originating from a single sporangium (Maurizio, '96); but in all such cases the possibility of contamination with zoospores from other species is great.

Maurizio ('94) isolated the various species by snipping off the ends under a lens before the formation of sporangia. From a bundle of hyphae cut off in this way and floated in water a single hypha was removed by drawing up into a pipette and transferring to a fly's leg on a slide. The water on the slide was changed every day by the use of filter paper. If this did not yield a single species the process was repeated. His substratum during this study were meal-worms, flies, and cress-seedlings. In 1896 he made a considerable variety of cultures on peptone, cane sugar, milk sugar, egg albumen, Liebig's extract, beef broth, and glycerine, all of which flourished. Boric and salicylic acid were used to keep off the bacteria, and this indicates that his work was not always done under sterile conditions. Trow ('95) used the same method of isolating hyphae, usually selecting such as had oogonia on them.

Klebs ('99) isolated a single gemma of *Saprolegnia mixta* and under entirely sterile conditions succeeded in studying the development of a single

species in great detail. He found it grew very luxuriantly on the various substances which Maurizio had used, and evidently had no trouble in keeping it sterile, once he had isolated the gemma.

Davis ('04) does not give any details as to how he isolated his material. He does not seem to insist on its having been entirely sterile at all times.

My own method was gradually developed from the hints thrown out by preceding investigators, and although far from meeting all the requirements of the whole family, it has made it possible to isolate the species of the genus *Saprolegnia* from both bacteria and other species and genera of the water moulds with great ease and certainty.

The fungi were obtained, as is usual, from water brought in from as many habitats as possible—river, ponds, lakes, ditches, brooks, and springs—and contained algae or other aquatic plants or merely decaying vegetable matter. The separate collections were placed in jars in the laboratory which were then filled up with distilled water. The tap water is well known to be deleterious to many algae, and they do much better in their native water or in distilled water. The jars were all labelled, and into each was thrown a fly or a small piece of dry beef; the former is better, as bacteria attack the beef too quickly. After being exposed twenty-four hours this bait was usually found to be inoculated, especially if the jars, which were kept in a cool window, had been warmed up somewhat by the sunshine. It is well to expose the bait as short a time as possible. The fly or meat was then transferred to sterile glass capsules containing distilled water, and in a few days was covered by the characteristic zone of the fungus. To remove the bacteria, a piece of the mycelium was cut off with scissors and transferred to a Petrie dish containing beef-gelatine.

As satisfactory results are only obtained from the right sort of beef-gelatine, the method of preparation may be given at this place. About half a kilogram of fresh lean beef is chopped fine and placed in a glass jar and one liter of distilled water poured over it. After standing twenty-four to thirty-six hours in a cool place the water is siphoned off and to every 500 cc. is added 52 to 55 grams of sheet-gelatine. The mixture is then heated on the water bath and stirred till all the gelatine is dissolved. After allowing it to cool to between 45° and 50° C., white of egg is added and the mixture thoroughly shaken to a froth, when it is placed in a steam sterilizer and boiled thirty minutes. It is then filtered through several layers of filter paper into Erlenmeyer flasks as stock material. The gelatine should be perfectly clear and become hard when set out into the ordinary room temperature. Sterilization is repeated on the two following days. From the stock material a thin layer is poured into Petrie dishes from time to time, and these are used to isolate the fungus.

The Petrie dish containing the impure mycelium is now placed on

a cold window-sill or in a refrigerator at a temperature between 5° and 10° C. or lower. The mycelium immediately begins to grow vigorously, while the bacteria multiply slowly, so that in from twelve to eighteen hours the margin of the mycelial mass outstrips the bacteria by as much as 4–6 mm. From the outer zone a piece of gelatine containing the ends of hyphae is cut out with a slender scalpel, and transferred to a clean dish, where it will again grow rapidly. Usually this is sufficient to separate the bacteria; if there are still some present the process is repeated. The new growth may appear to contain bacteria because of the action of proteolytic enzymes which soften the gelatine around the new culture; this appearance is still more exaggerated by the use of some commercial extracts of beef whose transformations produce a turbid liquid. It may be added that no lactic acid or other antiseptic was used, although the mycelium grows in acidified solutions; it seemed desirable that no stimuli of such a kind should be present before the regular experiments were begun.

If now there is only one species present, it is easy to obtain a culture from a single zoospore, and even if several species are present one of the number can be easily separated. A piece from the margin of the Petrie dish culture which is free from bacteria is transferred into sterile water in a small glass capsule, where, after twenty-four hours, it will be found to have produced sporanges and zoospores abundantly. The spores are more active and escape more readily from the sporange if the capsule is placed in a warm place a short time before using. The water is found to be swarming with spores and can be taken up in a sterile pipette and a drop of it mixed with 20 cc. of sterile water; from this diluted fluid a pipetteful is again taken and squirted over the surface of a clean gelatine plate in as fine droplets as possible. After another twenty-four hours or less one can find germinated spores lying far from any other spores, and these are then cut out with a very narrow and pointed scalpel and transferred to a gelatine dish as the final step in separation. We now have a culture from a single zoospore, and all further experiments can be made on mycelium obtained from it.

As all the species which were studied produced so-called gemmae, these may be used in the manner of Klebs and Trow as the starting-point of cultures. Their method, however, requires more skill and is to be preferred only where it seems difficult to get zoospores. It can also be used where it is desired to get a definite species from a mixture of several species. As a last resort, the method outlined by Maurizio is available.

The results of the present paper were obtained from cultures which were started from single spores. After the isolation was accomplished, the work was done with scrupulous care as regards sterilizing the instruments, glassware, and solutions used. It is very easy to transfer accidentally spores or bits of mycelium from one set of cultures to another, and hence mix up

the different species. Whenever cultures became contaminated in any way they were immediately discarded. Contamination from other species of *Saprolegnia*, however, never occurred, since the instruments, &c., were always heated between using. Only in this way can permanent results be obtained.

EXPERIMENTAL.

All the cultures started were labelled by a letter. The following letters represent pure cultures from a single spore: C, F, H, I, K, L, M. After the development of fruiting bodies an attempt was made to determine the species; it resulted as follows:—

- C. *Saprolegnia hypogyna*, Prings.
- H. *Saprolegnia mixta*, De Bary . . . a form!
- F. ditto ditto
- I, L. *Saprolegnia*, sp.
- K. *Pithyopsis*, sp.?
- M. *Aphanomyces laevis*.

C. *Saprolegnia hypogyna* was collected, October 25, on Pontederia at Cedar Lake, west of Chelsea, Mich. The culture was made from this material January 9.

In this species, which has not hitherto been reported for America, there is a short cell cut off by a cross-wall immediately below the oogonia. Maurizio ('94) studied this species, and also what he designated as five varieties, from a taxonomic point of view. After he had isolated a pure culture from the original, he found it so variable that he was led to begin the 'isoliren' anew, and obtained three forms closely related to *S. hypogyna*. This illustrates the shortcomings of his method. Later, he found other varieties from other sources. Only Pringsheim and De Bary had described the species before him, Pringsheim with a few figures as a variety of *S. ferax*, De Bary without figures but as a distinct species.

Cultures were immediately made to study its behaviour. A transfer was first made to a capsule of pea-broth, as described by Klebs, by sterilizing three or four peas in 50 cc. of water. After three days the mass of resultant mycelium was taken out, washed in sterile water, and divided into equal parts, which were then transferred to the various solutions or substrata.

For the sake of comparison with the work of other investigators, flies, wasps, and dry beef were also used in some cultures. On flies and wasps, *Saprolegnia hypogyna* formed sporanges and zoospores in great abundance after three or four days, of the characteristic *Saprolegnia* form and the method of emptying. In six to ten days the culture was full of oogonia. After a trial series of various substances, solutions of those substances were used which seemed to effect the production of sexual organs. The best substances, as found also by Klebs, were haemoglobin and leucin.

Solutions were made by mixing a .05 per cent. haemoglobin solution and a .2 per cent. solution of the inorganic salt desired, using half of each solution; this gave a solution containing in each case .025 per cent. haemoglobin and .1 per cent. of the salt. The following salts were tried: K_3PO_4 , xKH_2PO_4 , xNa_2HPO_4 , $Ca(NO_3)_2$, xNH_4NO_3 , KNO_3 , $x(NH_4)PO_4$, $(Ca)_3(PO_4)_2$, $xNaH_2PO_4$, K_2SO_4 , $xZnCl_2$. Also peptone in .02 per cent. solution. Those marked with an x showed few if any oogonia. The results are based on the first eight days' growth:—

(a) Solutions with .05 per cent. haemoglobin and no salts were tried first. Here the oogonia were abundant, with many oospores in each oogonium; the hypogynous cells were present but with hardly any side-branch.

(b) Haemoglobin and peptone. Oogonia very abundant with 2–10 oospores in each oogonium; the hypogynous cell beneath each oogonium had no side-branches, but there were projections into the oogonium.

(c) Haemoglobin and potassium phosphate. Oogonia abundant, but the oospores did not always mature; in such cases there was always a great abundance of antheridial filaments, which arose either from the hypogynous cell or from the neighbouring mycelium; hypogynous cell was either absent or present.

(d) Haemoglobin and calcium nitrate. Oogonia medium abundant; hypogynous cell was present; side-branches short or wanting, not developing into antheridia.

(e) Haemoglobin and potassium nitrate. Oogonia abundant with 1–10 oospores; hypogynous cell either present or absent; antheridial branches present, often luxuriant around the disintegrating oogonia.

(f) Haemoglobin and calcium phosphate. Oogonia abundant, with one to many well-formed oospores; hypogynous cell always present, sometimes several; no antheridial branches.

Another set of cultures contained .05 per cent. haemoglobin, and the same series of salts as before, including ammonium tartrate and rubidium hydroxide. The results noted were obtained during the first eleven days of development:—

(a) Solutions with .1 per cent. haemoglobin were used, containing no salts. Oogonia very abundant with 3–15 oospores in each oogonium; always with a well-formed hypogynal cell which is only rarely branched, and then the side-branch is short. A few cases of additional hypogynous cells were seen.

(b) Haemoglobin and .1 per cent. peptone. Oogonia abundant, with 3–10 well-formed oospores; hypogynous cell always present, sometimes two cells; no antheridial branches.

(c) Haemoglobin and .05 per cent. potassium phosphate. Oogonia abundant, with three somewhat disintegrated oospheres, but with antheridial filaments in great profusion around the oogonia.

Also, haemoglobin and .1 per cent. potassium phosphate and .1 per cent. sodium chloride; result as in the preceding, except more marked; about 75 per cent. of the oogonia have profuse antheridial filaments.

(d) Haemoglobin and .1 per cent. calcium nitrate. Oogonia very abundant, with well-developed oospores; hypogynous cell always present, rarely a second cell; about 1 per cent. with short antheridial branches.

(e) Haemoglobin and .1 per cent. potassium nitrate. Oogonia abundant, oospheres often poorly developed where the antheridial filaments are profuse; antheridial side-branch at times normally developed, with a well-formed antheridium.

(f) Haemoglobin and .05 per cent. calcium phosphate. Oogonia abundant, but rather small, with only 1-5 oospores; hypogynous cell always present, rarely a second one; only 1 per cent. have short antheridial branches, with, however, no antheridia.

(g) Haemoglobin and .1 per cent. potassium sulphate. Oogonia moderately abundant with well-formed oospores; hypogynous cell always present, rarely a second; only 1 per cent. with short antheridial branches.

The rubidium allowed only vegetative growth, which was moderately luxuriant. In the ammonium tartrate solution the oogonia were developed poorly.

For a special study of the effects of calcium and potassium sulphate, the following sets of cultures were made:—

(c) .05 per cent. haemoglobin, .1 per cent. potassium phosphate, and .1 per cent. potassium sulphate. Oogonia fairly abundant, always with an hypogynous cell, and always with an antheridial side-branch which bears antheridia; often these antheridia are diclinous and surround the oogonia profusely.

(d) .05 per cent. haemoglobin and .1 per cent. calcium nitrate. Oogonia abundant with well-formed numerous oospores up to twenty in number; hypogynous cell either present or absent; antheridial side-branches few, short, and undeveloped.

(f) .05 per cent. haemoglobin, .05 per cent. monohydrogen calcium phosphate. Oogonia moderately numerous, from 1-15 oospores, oftener only a few; hypogynous cell with or without a side-branch, which, however, is never fully developed.

(h) .05 per cent. haemoglobin, .1 per cent. disodium hydrogen phosphate, and .1 per cent. potassium sulphate. Oogonia medium numerous, with hypogynous cells; antheridial side-branches are present, and diclinous filaments with antheridia surround the oogonia as in (c).

H. Saprolegnia mixta, De Bary. This is apparently a form of the plant described by De Bary, differing in fly cultures mainly in having antheridia on 75 per cent. or more of its oogonia. A full description of the plant as it appeared in fly culture is herewith given.

Hyphae rather slender ; zoosporangia nearly cylindrical. Oogonia with rather thick walls, terminal, intercalary or lateral, flask-shaped, rarely spherical, the lateral on short oogonial branches ; pits medium to large, rather numerous but not easily seen ; antheridial branches usually androgynous when the oogonium is lateral, diclinous when the oogonia are terminal or intercalary, long and slender when diclinous, short and slender and not coiled when androgynous ; antheridia on 75 per cent. to 90 per cent. of the oogonia, long, subcylindrical, not very profuse on a single oogonium, often only one present. Oospores up to 15 and 20 in an oogonium, average diameter 24 microns, with rather thick walls.

The culture was obtained from a jar of *Cladophora* collected November 9, 1905, in the Huron River at Ann Arbor. The final culture from a single zoospore was made January 1, 1906.

This form or one of its near relatives was studied by Klebs, and the results published in the paper which instigated this one. My own results on this form corroborate most of his essential observations, although the most attention was paid to the production of antheridia with inorganic salts.

Cultures were made with solutions containing .05 per cent. leucin, and .1 per cent. inorganic salts. The observations include the first nine days of growth:—

(a) A 1 per cent. solution of leucin was first tried without any salts. Oogonia were abundant, and about 75 per cent. of them were provided with antheridia.

(c) Leucin and potassium phosphate. Oogonial initials were formed, but did not develop into maturity ; gemmae formed in considerable numbers.

(d) Leucin and calcium nitrate. Oogonia fairly abundant ; about 80 per cent. to 90 per cent. of them had antheridia ; oospores numerous up to 15 and 18.

(e) Leucin and potassium nitrate. Oogonia abundant, antheridia on 70 per cent. of the oogonia ; oospores numerous up to 25.

(f) Leucin and calcium phosphate. Oogonia abundant ; antheridia on 90 per cent. or more, the oogonia densely covered by the antheridia in many cases ; oospores not very well developed.

(g) Leucin and potassium sulphate. Oogonial initials present ; antheridia not developed.

(h) Leucin and sodium chloride. Oogonia fairly abundant, with antheridia on 80 per cent. of them ; oospores as many as 25 in each oogonium.

(i) Leucin and magnesium sulphate. Antheridia on 95 per cent. or more of the oogonia ; oospores 10–20 in each oogonium.

Cultures with Na_2HPO_4 , $(\text{NH}_4)_3\text{PO}_4$, NaH_2PO_4 , and NH_4NO_3 had no oogonia ; in the first three, gemmae formed in abundance. With ZnCl_2 in .1 per cent. solution death resulted.

No haemoglobin solutions were carried through satisfactorily, so that the results on these are omitted.

F. Saprolegnia mixta, De Bary (form). This can also be considered as a form of De Bary's plant, differing from it on fly cultures by the large but comparatively few oogonia with many oospores, and by not more than 1-2 per cent. of its oogonia having antheridia, as well as in the somewhat different appearance of the vegetative zone.

The original culture came from a jar of *Elodea* obtained in a pool near the Huron River, Ann Arbor, November 9, 1905. The culture from a single zoospore was made January 1. The cultures were made in the following solutions, containing .05 per cent. leucin and .1 per cent. of the inorganic salts. Examinations were made during the first ten days:—

(a) Leucin .1 per cent. solution, without salts. Only a few oogonia were found, and these had no antheridia.

(c) Leucin and potassium phosphate. Oogonial initials were present, but no oospores.

(d) Leucin and calcium nitrate. Several oogonia only were seen, with no antheridia.

(e) Leucin and potassium nitrate. Oogonia scattered, with many oospores each, a few with antheridial filaments.

(f) Leucin and calcium phosphate. No oogonia formed; gemmae finally appeared.

(g) Leucin and potassium sulphate. Oogonia scattered, large; about 25 per cent. with antheridial filaments both androgynous and diclinous.

(h) Leucin and sodium chloride. Several oogonia and a few antheridial filaments.

(i) Leucin and magnesium sulphate. A few large oogonia with many spores, some with antheridia.

Cultures with NaH_2PO_4 , Na_2HPO_4 , and $(\text{NH}_4)_3\text{PO}_4$ formed gemmae only; with NH_4NO_3 and $\text{Rb}(\text{OH})_2$ they remained vegetative altogether.

A few cultures were also made with haemoglobin, using a .1 per cent. solution, the salts as before:—

(a) Haemoglobin and peptone. Oogonia scattered, no antheridia.

(d) Haemoglobin and calcium nitrate. Oogonia abundant, no antheridia.

(e) Haemoglobin and potassium nitrate. Oogonia abundant; about 25 per cent. with antheridia, both androgynous and diclinous, sometimes from the oogonial stalk directly under the oogonium.

L. Saprolegnia, sp. As no oogonia were obtained in the cultures of this species, it was impossible to determine it. Under all the conditions which influenced the production of sexual organs in the species so far mentioned, this one remained either vegetative or produced abundant gemmae. In very few instances were sporanges or zoospores seen, the

former being usually transformed gemmae. Both leucin and haemoglobin series of cultures were tried. The sporanges were of the *Saprolegnia* type and emptied in the same manner.

The plant was obtained from mosses collected in a ditch near a peat bog at Chelsea, Mich., November 11. Since the mycelium did not respond readily when transferred to pure water in the formation of zoospores, the pure culture was made by transferring a single gemma as Klebs had done. This germinated by sending out from its anterior end numerous branches, while the hyphae at the end where it had been attached shrivelled up.

Gemmae were formed in greater or less numbers in nearly all the solutions of the series of haemoglobin. The solutions tried contained .025 per cent. haemoglobin with .1 per cent. peptone, laevulose, glucose, K_3PO_4 , KNO_3 , $CaHPO_4$, $Ca(NO_3)_2$, K_2SO_4 , $NaCl$, $Cs(OH)_2$ respectively. Various combinations of these were also tried, but without success. With $Cs(OH)_2$ no gemmae were formed, while the vegetative growth was good.

Leucin was used with $Ca_3(PO_4)_2$, $Ca(NO_3)_2$, KNO_3 , K_3PO_4 , and $MgSO_4$. In all these leucin solutions, only gemmae were formed, often in great abundance. In shape the oogonia were often globular, as if they were oogonial initials, especially in the haemoglobin-laevulose and potassium phosphate and potassium sulphate solutions. The clusters of gemmae are short and much branched in many cases.

I. *Saprolegnia*, sp. This species reacted similarly to L, except that in one culture oogonia were seen. This occurred on a pure fly culture, but they remained immature and disintegrated, and no determination was made as to the species. The oogonia which were noticed were densely covered by antheridia, even in the rather young condition, and this fact makes it very unlikely that we had another specimen of *S. mixta*. The antheridia were both diclinous and androgynous.

The material was obtained from a jar containing Elodea, and brought from the Huron river in September. A culture from a single zoospore was obtained January 10. The series of cultures were similar to those used with the preceding species. Haemoglobin with the various salts always produced abundant gemmae, which were usually large, fusiform or spherical in shape. With rubidium hydroxide no gemmae appeared; only a luxuriant vegetative growth seemed possible. With leucin and the series of salts the results were practically the same as in the haemoglobin cultures.

K. In contrast to the two preceding forms, this species produced sporangia in abundance. These sporanges were often clavate to narrowly ovate, and were arranged irregularly in acropetal series. This indicates a *Pithyopsis*. No oogonia were seen in any of the cultures, although entire series of both haemoglobin and leucin cultures were carried through. As in the case of the two preceding forms, gemmae were present in all of the cultures except in that of rubidium hydroxide; in the latter

the vegetative appearance was excellent. The gemmae in these cultures often passed over into zoospore formation in great numbers. Their shape approximated furthermore closer to sporanges than to oogonial initials.

M. *Aphanomyces laevis* was also obtained in pure culture from a single zoospore, but only a few cultures could be made at the time.

GENERAL DISCUSSION.

The different phases of the investigation may be divided into the following headings:—

- I. Relation of growth and reproduction.
- II. The development of the sexual organs.
- III. Physiological varieties.
- IV. The problem of species.

I.

All the species studied above showed the same general reaction on the various substrata as *Saprolegnia mixta*. They all grew rapidly on beef-gelatine, meat, and pea-broth. There is, however, a decided difference in the rate of growth; and on this point some observations were made with beef-gelatine cultures in Petrie dishes. Cultures of *S. mixta* (H), *S. hypogyna* (C), *S. mixta* (F), of the three unknown species (I), (K), and (L), and of two species of *Achlya*, were made on beef-gelatine by inoculating the centre of the dishes with them. The cultures were started February 13, and on the 19th (I), (K), and (L) had grown to the margin of the dishes, while (H) and (F) and the two *Achlyas* were just half way; (C) had reached a point about half way between the other two lots. The dishes were all kept under the same conditions of temperature, &c. Other incidental observations had all along pointed to this result. This of course does not indicate the absolute rate of growth. Under favourable conditions of temperature and nutrition they grow to the margin in a very much shorter time. The few trials made indicate a rate of .2 mm. per minute, or even less than a minute.

It is to be noted that the two rates of growth are correlative with the two sets of species as regards their tendency to produce reproductive organs. (I), (K), and (L) do not produce oogonia under the conditions found by Klebs for *S. mixta*, or under the same conditions as found by myself for *S. hypogyna* and (H) and (F). This indicates a close physiological relationship between these latter species. It is quite clear that (I), (K), and (L) are different species, and if they are Saprolegnias, that they are possibly of close relationship among themselves. On the other hand (H), (F), and (C) seem to be also closely related physiologically, and a study of the nutrition relations of other species like *S. monoica*, *S. ferax*, and *S. torulosa* may show that these, too, act like *S. mixta*; such results would give us not only a morphological but also a physiological basis for classing them

together as the 'ferax' group. Maurizio also found that many species of *Saprolegnia* which he met produced no oogonia, but he only tested them with ordinary substrata.

(H), (F), and (C) were kept vegetative for six months at a time by continued cultivation on beef-gelatine. It was found best to transfer from the old to the fresh plate as soon as the mycelium had reached the margin of the dish, as it was noticed that when the culture was allowed to get too old the transferred hyphae no longer grew. This was either due to the accumulation of waste toxic substances produced by metabolism, or to famishing on account of the inability of the hyphae to get at the gelatine, the substratum immediately surrounding them being rapidly dissolved by the proteolytic enzyme secreted by the fungus. In order to determine this point a culture of *S. hypogyna* was made in a flask containing 500 cc. of a 5 per cent. maltose and a 0.1 per cent. peptone solution. The mycelium rapidly filled the liquid, forming a thick mat near the surface and remaining in a vegetative condition for thirteen months. The mass was then taken out, carefully examined, and a new culture made from it on beef-gelatine. After a time it was tested and found to produce the same reactions of *S. hypogyna* already recorded for that species. The mycelium from the thirteen-month culture was found to be in part disintegrated, in part filled with a very thin protoplasmic content somewhat granular in appearance, while a number of the end hyphae appeared normal. These apparently still retained the necessary vigour for further growth on a favourable substratum. No reproductive hyphae could be found either in the loosely webbed peripheral zone of the mass nor in the interior. Vegetation therefore took place for thirteen months without the renewal of the substratum and in spite of the chemical substances excreted during the metabolic activities of growth. This is possible because, as Klebs has shown, the metabolic products of a carbohydrate substratum are much less harmful than those of a proteid substratum. The latter is always found unfavourable for the continued existence of the fungus because of the toxic character of the waste products excreted. The small amount of peptone used supplied the necessary nitrogen, without interfering with the life of the fungus.

The proposition of Klebs, now pretty well accepted, that the fungus can be kept indefinitely in a vegetative state, seems to be well founded. Klebs himself kept *S. mixta* in a vegetative condition for six years, and all the species studied by me were kept at least six months without producing zoospores, oogonia, or gemmae of any kind; and, as just seen, *S. hypogyna*, which can be made to reproduce most abundantly within a few hours or days, was kept from doing so for thirteen months; at the end of that time it was able to produce oogonia in three days.

Although it seemed superfluous to repeat all of Klebs's experiments, a set of cultures was prepared to study the effect of the different concentra-

tions in order to find the optimum concentration for the production of oospores. The substances selected were haemoglobin, peptone, glycoll, urea, dextrose, and fructose. The results obtained, both as to amount of growth under different concentrations and the time required for the formation of the reproductive organs of both kinds, approximate so closely to his results that it was decided to use his optimum concentrations for the further studies.

II.

The effect of nutrition on the development of the sexual organs was first effectively studied by Klebs. He also pointed out a significant variation in the number of antheridia present under different conditions. *Saprolegnia mixta*, studied by him, was said by De Bary to have normally about 50 per cent. antheridia, which according to Trow are functional male organs. Klebs found that in a pure leucin culture—apparently 0.1 per cent. solution—no antheridia appeared; but on the addition of Knopp's solution of inorganic salts, or of a mixture containing K_3PO_4 , KNO_3 , and $MgSO_4$, the antheridia were formed in considerable numbers. He then tried various inorganic salts with 0.1 per cent. leucin, and found that K_3PO_4 , K_2HPO_4 , and KH_2PO_4 caused the number of antheridia to be increased, potassium phosphate being the most effective and producing them on 50 per cent. of the oogonia.

The form of *S. mixta* used by me seems to deviate in some of these respects. The normal fly culture of (H) has as high as 75 per cent. antheridia on its oogonia. This is also the case in a 0.1 per cent. leucin solution. It is also seen that in my cultures $Ca(NO_3)_2$, $Ca_3(PO_4)_2$, and $MgSO_4$ acted so favourably on antheridial formation as to produce a yield of as high as 90 per cent. or even more antheridia-bearing oogonia. Not only were there no antheridia found in the sodium and ammonium phosphate solutions, but even the oogonia were lacking. These latter salts, then, act as did those in Klebs's solutions, and inhibit in some way under the conditions the formation of oogonia.

If we now compare the results obtained in *S. hypogyna*, the effects of inorganic salts in inducing antheridial growth is shown markedly. Pringsheim, as a result of his belief that parthenogenesis is brought about by changes in the external conditions, considered the plant in question as merely a variety of *S. ferax*. De Bary, however, considered it a valid species, on the basis of a culture which he kept for three years, and in which, he says, it remained constant, not only with reference to the hypogynous antheridial cell so characteristic of the species, but also as to the persistent absence of antheridial filaments.

It was my original intention to study *Saprolegnia ferax*, which is said to lack the antheridia entirely; whether it does so or not has to be settled by a culture from a single spore. As this species did not appear in my cultures

at the time when I needed it, the study has not been made. There appeared, on the other hand, a *Saprolegnia* which I discovered to be *S. hypogyna* as detailed above. As this species seemed to offer as great, if not greater, possibilities than even *S. ferax*, it was decided to study it instead.

A pure culture was transferred to a sterilized wasp as offering the ordinary insect culture-media in general use. As in *S. mixta*, sporanges are formed and emptied in great numbers within a few days and following a luxurious growth of hyphae. On the ninth day oogonia were abundant, and a few days later very abundant, containing from one to ten oospores. In this culture not a single antheridial filament was found, although it was diligently sought; also the hypogynous cell was always present, and all the other chief characters of the plant agreed with the description by De Bary of *S. hypogyna*. If, therefore, Pringsheim and De Bary had the same species which I had, and it would seem that they had at least one of the forms, then, as long as they used flies or similar substrata, they would be sure to find only the hypogynous cell and no antheridial side-branches.

Now if *S. hypogyna* is grown in .05 per cent. haemoglobin solution, its appearance is nearly like that on the wasp, except that a very few hypogynous cells have a short protuberance—an abortive antheridial filament. If peptone is added to the haemoglobin solution no protuberances appear at all. In haemoglobin solutions Klebs found that K_3PO_4 , Na_2HPO_4 , and $Ca_3(PO_4)_2$ were the only salts which induced antheridial formation in *S. mixta*. As noted in another part of this paper, not only these but other salts also induced antheridial formation in the case of *S. hypogyna*. These different inorganic salts act with very different degrees of effectiveness; in fact it is possible to select a regularly graded series of cultures showing the relative value for antheridial formation of the several salts used. Such a series is shown in Plate XXIII. The haemoglobin cannot be sterilized, but as the cultures were made in winter and kept continuously at a temperature of about 15° C., no trouble was experienced in this respect. The haemoglobin used (Merck's) was very clean itself, and if the paper, dishes, &c., used in weighing and transferring were sterilized, very seldom did any bacterial contamination appear in the comparatively short time necessary for the experiments. The following salts, decreasing in effectiveness for the production of antheridial side-branches, were used: K_3PO_4 , KNO_3 , Na_2HPO_4 , K_2SO_4 , $(Ca_3PO_4)_2$, and $Ca(NO_3)_2$, and these were added to a haemoglobin solution. It can be seen that *S. hypogyna* is very well adapted to show off the action of the various salts. KNO_3 and K_2SO_4 were especially favourable and produced antheridial branches on as high as 40 per cent. of the oogonia. This result differs somewhat from that of Klebs, and the question of impurities in the salts used would naturally arise. It must be remembered, however, that minute amounts, such as might be present in the salts used, do not affect the production of oogonia and antheridia, at least not to such a

striking extent. The salts were obtained from Kahlbaum and had the C.P. stamp. As to the rest, the cultures were always made in series, so that the checks would show any outside influence. With leucin, as noted, $(\text{Ca}_3\text{PO}_4)_2$ and MgSO_4 also caused the appearance of antheridial side-branches, and it might seem from the action of so many salts that it was due to the effect of inorganic salts in general. To get some light as to the possible effect of concentration, the following was tried: $\text{Ca}_3(\text{PO}_4)_2$, in the concentration of 0.1 per cent., yielded antheridial side-branches very rarely in haemoglobin solutions. Hence a concentration half as great was used, but with no marked result. There is room, however, for further experimentation along this line.

If we now examine the effect of the different salts in detail, we find that certain differences stand out in a pronounced manner. In a normal culture on insects or haemoglobin the usually terminal oogonium is globular, sometimes flask-shaped, the smooth wall rather firm and with rather large pits which do not project above the surface (Figs. 3 and 12). The oospores are from one to ten in number, sometimes more, and always centric in structure. Attached to the base of the oogonium is the hypogynous cell, cut off from the rest of the oogonial filament by a cross-wall, and in length usually from half to once the diameter of the oogonium. Normally this cell is filled with protoplasm, and finger-like projections often extend from the upper end into the oogonium; at times there is merely an upward bulging of the wall separating oogonium and hypogynous cell.

In cultures of haemoglobin and peptone (Figs. 1, 2, and 3) the pits become quite prominent at times, as if the turgor of the maturing oogonium had pushed out the thin-walled pits.

In calcium nitrate solutions, the cross-wall separating the hypogynous cell from the oogonial stalk may be entirely lacking; when present it occasionally produces short side-branches, but these never differentiate any antheridia. Oogonia are formed in great abundance with a great many oospores, and the whole organ has a very healthy appearance, while the oospores matured in every oogonium during the period of examination. The pits vary and may be large or small.

In calcium phosphate cultures (Figs. 8, 9, and 10), there is produced in the oogonial filament more than one cross-wall, at times three cells being cut off in this way. This was also observed in the cultures with calcium nitrate, as well as in some of the potassium nitrate cultures. Just what the stimulus is in the formation of these extra cross-walls is not very clear. Molisch ('95) found calcium necessary for wall-formation in *Spirogyra*, and this may also be a factor here. It is well known, on the other hand, that the mycelium of some Saprolegniaceae can form cross-walls under poor nutrient conditions, but this never occurred as far as my observations went in this species. Maurizio ('95) considered this doubling and tripling of cross-

walls as a character to distinguish, along with the size of the oospore, one of his varieties.

The most potent solutions for inducing antheridial formation are the solutions containing potassium phosphate and disodium phosphate (Figs. 11, 13, 14, 15, 16). In solutions with potassium phosphate the antheridial filaments develop completely, i. e. the antheridium is differentiated at the apex of the side-branch, and is applied in the normal manner to the oogonium, and fertilizing tubes are put forth and touch the oospheres. Such a case is seen in Figs. 16 and 13. We have here, then, a combination of conditions much more favourable than in the preceding cases. It is seen, too, that there is considerable variation under these conditions with reference to the source of the antheridial side-branch. If the hypogynous cell is considered as a potential antheridium, we would expect that the side-branch would naturally arise from it. This is not always the case; instead, the side-branch may arise from below the hypogynous cell, as seen in Figs. 11 and 13; or it may arise from the oogonial branch immediately below the oogonium in the absence of the hypogynous cell, as in Fig. 14 *a*. And, finally, as shown in the same figure, *b*, it may arise from a different filament in the usual manner of antheridia of diclinous origin. No cases were seen where the antheridial filament or branch grew out of the oogonial wall as in *Achlya racemosa*.

The question of whether the tendency of antheridial filaments to arise from the oogonial stalk or from some other branch of the fungus is of sufficient constancy to warrant its use as a specific character seems to be answered here (Fig. 14). There is no doubt that the tendency of the male filament to arise at a definite point is to a large extent a matter of conditions. In *S. mixta* (H), which I studied, the oogonia were located either apically on short branches, or on the ends of long filaments, or intercalary. On short-stalked oogonia, the antheridial filaments grew from both the same and other filaments, i. e. were both androgynous or diclinal; the oogonia on the ends of long filaments usually were supplied by diclinal antheridia, and the intercalary entirely by the latter. Evidently the position of the oogonia on the plant conditioned here in some way the origin of the antheridial stalk. In *S. hypogyna* this was brought out strikingly in the cultures with K_3PO_4 , and less so in those with KNO_3 and Na_2HPO_4 . Here numerous oogonia occur in which the usual hypogynous cell is present, but there are no antheridial filaments developed from any of them, nor from the oogonial stalk anywhere; on the other hand, the oogonia are surrounded often very profusely by antheridial filaments with well differentiated antheridia, as shown in Fig. 18. Here, then, all the antheridia are of diclinous origin, and the fact that my cultures were made from a single zoospore goes far to establish the notion that the place of origin of the antheridial filament cannot be retained as a good specific character, at

least in ordinary variable cultures. It is also to be noted, that in all the species of *Saprolegnia* which I studied from a single zoospore, and in which oogonia and antheridia formed at all, the antheridial filaments were of both androgynous and diclinal origin, or would become so under the varying conditions of culture. Whether this variability extends to all the species of *Saprolegnia* and of *Achlya* also, cannot be determined in the absence of experimental evidence.

The results so far indicated show *that it is possible to produce, where hitherto they were believed to be absent, antheridial branches of the normal type known in other species of the family, and that their production is conditioned by the presence of definite inorganic salts*. It seems clear, also, that more radicals of salts subserve this function than was found to be the case in the *S. mixta* studied by Klebs. At first sight, calcium might also seem to be necessary; but no results were obtained with any other calcium salts than $\text{Ca}(\text{NO}_3)_2$, $\text{Ca}_3(\text{PO}_4)_2$, and CaHPO_4 , and this would seem to indicate that nitrogen and phosphorus are the active elements. This view is strengthened by the fact that the most active salts are K_3PO_4 and KNO_3 . But we have seen that K_2SO_4 also belongs to the list. From the data so far at hand it is likely that potassium is therefore also an active element. This is also apparent in the cultures where K_2SO_4 was added to the solution of Na_2HPO_4 . When the latter alone was used with haemoglobin the development of reproductive organs was not near as definite as with K_2SO_4 . In any case side-branches were produced and developed to a greater or less extent in all these salts when used separately with haemoglobin.

Just what we mean when we say that one element is more active than another is not yet clear. Our knowledge of the process or sets of processes which start the mechanism of antheridial formation going is practically nil. We can with De Bary ('81) take the view that a chemical compound is excreted by the oogonia which induces the formation of antheridial filaments, and that the different salts offer the conditions which make the secretion of this active principle possible. Klebs found only the phosphates especially effective for the induction of antheridia; but if it is true that nitrates and potassium are also active, then the problem becomes still more complicated. If it depends in its entirety on the improved condition of nutrition, as Klebs believes, then the effects of phosphates, nitrates, and potassium is more easily understood, although it still fails to afford any explanation of the actual relations of antheridial filament and mycelium. That the relations are chemical in nature is made plausible by the researches of Pfeffer ('84) and Miyoshi. It would be conceivable that some constituent of the salts used combines within the cell in such a way with the proteids as to set free an enzyme, and that this is the active principle. The origin at so many different points indicates that it is largely a matter of equilibrium of the conditions which do take part, and that the equilibrium, or perhaps lack of

equilibrium, has occurred at the point where the antheridial filament originates.

In this connexion attention should be called to Figs. 17 and 18, which show a condition apparently seen in part by Klebs ('99, p. 566). Here the antheridial filaments, poorly developed on the oogonium of Fig. 17, but with well-developed antheridia in Fig. 18, grow profusely around the oogonia. In all these cases, however, the oospheres seldom mature, but disintegrate as if absorbed. Klebs indeed states that antheridia-like filaments form in profusion, but, in his opinion, instead of fertilizing the oospheres, consume them. From his brief statement it seems that he did not observe definite antheridia at the apices of the filaments in his *S. mixta*. As shown in my figure, these are very abundant and definite in such cultures of *S. hypogyna*. That these are true antheridial branches is shown by these antheridia at the apices as well as by the presence of intermediate forms where the short side-branch has developed to a greater or less extent in the direction of the extreme stage. They are mostly without fertilizing tubes and lie only on the surface of the oogonium; the figure lacks perspective, and the antheridia, which seem to have penetrated the oogonial wall, lie on its hither surface. The idea that the antheridia devour the contents of the oogonium is hardly applicable in this case; and yet there seems to be a close connexion between the disintegrating process and the presence of many antheridia. In Fig. 17 two oospores were formed before the blighting effect of the male filaments reached them, while the remaining oospheres disintegrate. It may be that this phenomenon is explicable in another way than by assuming a direct evil effect due to contact of antheridial filaments, viz. that the nutrition the plant as a whole is directing into these filaments is taken from the amount which would normally supply the oogonial filaments, and hence the latter are famished. There is, however, the difficulty that one finds in the same cultures with these extreme forms, oogonia with male branches well developed, and which have the normal structure as in Fig. 16.

As already stated, not only was the effect of phosphates very marked, but also that of nitrates, and even potassium sulphate and magnesium sulphate showed a favourable increase in the number of oogonia as well as of antheridia. To test the stimulative effect of salts whose nutritive value is known to be of no importance in certain other fungi, sodium chloride was added to a number of haemoglobin solutions along with potassium phosphate, and compared with those containing phosphate and haemoglobin only. There was a decided improvement in the appearance of the oogonia, as well as in the number of antheridia formed. Fig. 18 was drawn from an example of such a culture. A set of cultures with potassium sulphate added to the other ingredients seemed also to have a favourable effect, perhaps neutralizing the disintegrating effect of K_3PO_4 , and allowing the oogonia

to reach maturity by inhibiting the production of profuse antheridial filaments.

A further result that requires notice is the effect of rubidium and caesium hydroxides. These were added to the haemoglobin solutions, the same as the other salts. The results in all the cultures was a good vegetative growth and a complete inhibition of sexual organs; and not only these, but the sporanges, and even the gemmae, were almost entirely prevented forming. Even after two weeks in a 0.1 per cent. solution with haemoglobin only vegetative hyphae were present. Since rubidium was found by Benecke ('95) to be able to replace potassium as an essential element to growth, it was to be expected that it could replace it in the rôle of an aid to the formation of sexual organs. Of course it may be that potassium does not, as such, affect the production of oogonia to any great extent. The rubidium was not tried in the form of a nitrate. It is possible, too, that, like sodium, potassium merely performs the function of accelerating the process of reproduction; but, if so, one might still expect the same property in rubidium. The view that the sexual organs are produced only after a good growth of mycelium would seem at first thought to find an exception in the case of rubidium, and in order to avoid that conclusion one must consider it as acting inhibitive in such cultures.

III.

The observation that certain species of the *Saprolegniaceae* never or seldom develop sexual organs has been made before. The subfamily Leptomitae, as used by Humphrey, includes only one species of *Apodachlya* which develops sexual organs, a new species described by Humphrey himself. Maurizio ('96) found 'many species of *Saprolegnia* which yielded no oogonia even after three to six months of culture'. I obtained from five different sources, two of which are not mentioned in the introduction, species from single zoospores which either formed no oogonia at all, or, in one case (I), only a few. One of these was presumably a *Pithyopsis*, although no final decision was reached as to its position. No oogonia were seen at all, either on this or on (L), in any of the cultures which were set up especially to determine this point. These species vegetated very luxuriantly, and just whether they were all distinct species or not is uncertain; the same rate of growth and the sporanges are the only evidence to go by. We have here, however, a distinct physiological character by which these species of *Saprolegnia* are separable from those that produce oogonia so readily, like *S. ferax*, *S. mixta*, and *S. hypogyna*. The question here naturally arises whether these plants ever produce oogonia. I am inclined to think that they do, and that the conditions necessary are merely of a different kind than those so far tried on them.

Klebs found that different species of *Oedogonium* behaved very differently to a change from light to darkness in the effect this change had on the production of zoospores. I have been able to verify this for several species of *Oedogonium* in our laboratory. It is therefore not surprising to find similar differences of a deep-seated nature present in the various species of *Saprolegnia*. We need further experiments with these forms in order to determine the conditions.

The two forms of *Saprolegnia* which I have denominated (H) and (F) need further discussion. In all the essential characters except the number of antheridia these two forms would come under *Saprolegnia mixta*. (H) comes more nearly to it except that about 70 per cent. oogonia have normally antheridia, and this can be increased to over 90 per cent. This form approaches *S. monoica* in its many antheridia, and it is very probable that under the most advantageous conditions all the oogonia would bear antheridia. But *S. monoica* has been described as having only androgynous antheridia, larger than those of *S. mixta*, and with long and stout vegetative hyphae. On the basis of what we have learned concerning the variation of antheridial filaments of *S. hypogyna*, it would seem that the androgynous origin of antheridia was not likely to be constant enough to warrant keeping two species separate on its account. It seems to me, however, that this is too large a conclusion. For, although no genuine *S. monoica* was found in my cultures, it seems likely from Humphrey's careful observations that the size of the hyphae and antheridia, as well as the usually 'indescribable differences', make this an easily recognizable species; Humphrey also points out that it is comparatively rare. I can, therefore, not subscribe to the suggestion made by Davis ('04) that *S. mixta* is only a cultural form of *S. monoica* or vice versa. But neither does my plant (H) seem to be exactly the form described by De Bary, since both he and Klebs found that 50 per cent. was the highest number of oogonia which were supplied with antheridia.

This would hardly be a point on which to insist, if the results from my study of (F) did not also warrant it. The cultures of (H) and (F) were made side by side, and constitute checks on each other with reference to temperature, &c., so that the uniform differences must be considered constant. Each was derived from a single zoospore, and the cultures were kept from becoming contaminated. When the cultures were made on wasps, (F) had from 1 to 2 per cent. antheridial oogonia, while those of (H) amounted to 75 per cent. or more; in leucin solutions those of (F) gave a maximum of 25 per cent., while those of (H) yielded as high as 95 per cent. of antheridial-bearing oogonia. It seems to me that we must consider these as separate entities which are closely related to the *Saprolegnia mixta* of De Bary, and that they will remain distinct along with that species when all are placed under the same conditions of culture. Nor does it seem to me

that the results of Klebs, Davis, and myself on the variability of *Saprolegnia mixta* or of any of its forms, in obtaining cultures which produce no antheridia, warrant the view that this condition represents *Saprolegnia ferax*. This species, it is true, is said to have no antheridia; but any one who has seen the true *S. ferax* (*S. Thureti*) will remember some very essential characters which always stand out in fly cultures of this species. One of these is the presence of large cylindrical oogonia with the oospores in a single row. Humphrey has expressed himself forcibly on this point: 'The rather common occurrence of cylindrical oogonia and the very conspicuous pitting of the oogonia mark this species unmistakably.'

With reference to the variability of *Saprolegnia hypogyna* there are several points that need attention here. I have already fully indicated some lines of variation. Now if these variations could be fixed, the resulting forms would be similar to the varieties of *S. hypogyna* described and figured by Maurizio ('94). According to him, all his varieties agree with De Bary's *S. hypogyna* in the following characters: firstly, the hypogynous antheridial cell; secondly, the centric oospheres; thirdly, the flask-shaped oogonia. The oogonia of my plant were globular, which according to De Bary's original description ('88), is one of the shapes. In his 'variety I' the antheridial cross-walls are nearly always wanting—a phenomenon also noticed in some of my cultures—the oogonial stalk is variously bent, and the pits project somewhat above the surface of the oogonium—also noted in my cultures. In his 'variety II' the characters are: many pits, straight stalk, and additional cross-walls on the oogonial stalk. In my cultures the pits were found to vary in number and size, and additional cross-walls on the oogonial branch were common in calcium phosphate solution, as shown in Fig. 9. His 'variety III' is like his II except the additional cross-walls, and in its short protuberances on the side of the hypogynous cell.

Here, then, is a form found in nature with all the characters of those forms which I succeeded in getting in cultures with CaHPO_4 , as shown in Figs. 5, 6, and 7 (compare also Fig. 20, Pls. IV and V, Maurizio, '94). In both the side-branches have attained only partial development, as no antheridia are cut off from them. The hypogynous cell is usually empty, as is also the tube which pushes up into the oogonial wall in some cases; in fact so much alike are his 'variety III' and my cultural form that it is difficult to select differences, the number and size of pits seeming to be the only differing character.

Maurizio's 'varieties IV and V' are characterized by the few pits; the former also by the thin oogonial wall, and the latter by the presence of definite protoplasm within the projection of the hypogynous cell where it pushes into the oogone; both lack side-branches. No especially thin walls were noticed in the oogonia of my cultures. The presence or absence of a healthy-looking protoplasmic content of the projection certainly varied

in my cultures to the full extent of what Maurizio observed. Sometimes the hypogynous cell itself is transparent; again, they may be filled with protoplasm; the last condition is especially marked in calcium nitrate cultures.

The comparisons just made serve to bring out clearly the possibility of inducing a variation which overlaps or imitates a character in a form which is apparently constant in nature; and this fact naturally leads to a consideration as to the meaning and values of varieties and species, a theoretical question that may better be discussed in a separate section.

The significance of the hypogynous cell has been fully discussed by Maurizio (loc. cit., p. 149), and my own results seem to bear out his contention that the projection into the oogonium is not a fertilization tube, but represents merely a tendency in this genus to produce secondary growth, as in sporanges. It was often noticed that, even where a side-branch was fully developed and bore an antheridium of its own, these projections appeared to push up to a greater or less extent. Probably the hypogynous cell itself is no true antheridium, but merely an aborted or latent cell which has either lost its sexual function under ordinary conditions or had never attained it; in either case we would hardly expect that its function could be so easily called forth as to form these projections as fertilizing tubes. Furthermore, it is not rarely absent altogether, and then the ordinary vegetative filament is the starting-point of the antheridial stalk.

IV.

There are several theoretical questions which this investigation touches and which may be briefly discussed. The first of these is the question of the constancy of species. As De Vries has pointed out, experimental evidence on this point is not very extensive. The expression has been used for a long time to indicate a constancy of characters during culture, viz. that if the individual remained constant in its specific characters it proved the autonomy of the species, while if it varied in a certain direction it belonged to another species, and the direction of variation indicated the species to which it belonged. Such cultures were usually made under so-called natural conditions. If, for example, a specimen of *Saprolegnia* was grown on a fly for three years and showed no change of a retrogressive or other nature in the characters to be tested, it was declared a definite species. If, on the other hand, the new characters disappeared at times, or were changed during the course of the cultivation to a character already known, the new form was considered merely a variety.

This is the attitude at present exemplified by mycologists who describe new species of the higher fungi. Often a third name is added to the binomial, and this implies that the new characters of the plant in question

are so few or of such a grade of importance as to leave open the question of its rank. Later specimens of what seem the same fungus are received from other sources, and if all the new characters are also present in these, the evidence is considered sufficient to raise the fungus in question from its varietal position to the rank of a new species.

This attitude fails to take into account one important consideration, viz. the question of the Constancy of Conditions under which these different individuals grew, or under which their constancy of character was tested. If, as has been brought out by recent investigators (Klebs, '06), the same conditions of nutrition, temperature, &c., produce always the same result, then the mere cultivation for a long period of time in and of itself is no test of species in the sense in which the term has been used. The problems of constancy include a trial of all the conditions. It would seem that we are driven to the same resources for accuracy in biological studies which are fundamental in physics and chemistry, namely, that of standards. Each species is recognizable, not only by certain prescribed or described characters, but in addition by these characters only when present under certain definite prescribed conditions. For example, *Saprolegnia hypogyna* is known by the hypogynous cell on the oogonial stalk and by certain other less important characters, all of which are present under the conditions of culture on a fresh fly, or more precisely in a definite per cent. leucin or haemoglobin solution, at a temperature, say, of 20° C., &c. Differently stated, this implies that the characters acquired in a new environment are persistent as long as the environment is persistent. It would seem necessary, then, in monographing such a family as the *Saprolegniaceae* to refer all the species to definite conditions, which should be uniform for all, and to determine in each case the variability to the extreme attainable limits; we should then have attached to each description of a species an account of its variabilities. This view demands that comparisons with old descriptions should take into account the exact condition under which the plants were raised; a procedure fraught, no doubt, with difficulties. Such a physiological basis for species seems to me the only starting-point in a revision of the relationship of the species of any family of plants. Whether it is practicable in all cases is another matter which cannot be discussed here.

Another question which always arises in this connexion is the one as to whether species are distinct or whether there is a gradual blending of one species into another by means of intermediate shadings. In other words, whether we have new species formed by mutation or by continuous variation. It is not my intention to take sides. I merely wish to point out in what way my results touch upon the problem. As has been seen, some of the forms of *Saprolegnia* are very close to each other morphologically, and yet are physiologically distinct. This appears very strikingly in such forms of *S. mixta* as (H) and (F), along with the one that Klebs

studied. Although the number of antheridia-bearing oogonia seem the chief constant distinctions, other minor and less noticeable differences are present. Maurizio made out a series of six or more forms, with constant differences, of *S. hypogyna*. It is not certain that his conditions of culture were uniform for all his studies, for Maurizio worked under the assumption that time was the principal factor in the test for constancy. The fact that under certain conditions I was able to obtain various characters which distinguished his varieties, seems to indicate the method by which Maurizio's varieties may have been derived; namely, by mutations from the simplest *S. hypogyna*. It is necessary to distinguish clearly between physiological variation and mutation, but this need not preclude the possibility of a mutation taking place in a direction such as a physiological variation takes. Of course the whole problem becomes deeply involved because of our complete ignorance as to what conditions a mutation.

It seems to me conceivable that the variations of any individual may extend far in any one direction; and also that the variations may be such as to produce structures which are present in other bona fide species, and yet the two species need not necessarily be phylogenetically related. In this way we would expect to find overlapping of forms, and it is on this supposition that the possibility rests that Maurizio's plant is not the same as that obtained by me in CaHPO_4 solutions and apparently having all the characters of his variety.

What shall we call all these closely related forms? De Vries has offered to solve the problem for us in the term 'elementary species', with all that it implies. Because of the presence of parthenogenesis in our plants, and in the absence of cultures carried through generation after generation from oogonia, no evidence in this paper can be offered to substantiate or to refute De Vries's view. But indirect application of his theory to the facts obtained in the foregoing pages is possible, and to that extent only has it been applied in the foregoing pages. Klebs's notion of variations, on the other hand, is strictly substantiated in so far as it applies to the individuals studied. Furthermore, his idea that we can produce De Vries's retrogressive and degressive mutations by the change in conditions seems also borne out by the results. Shall we say, then, that the only mutation which exists is progressive mutation, and that the many forms found, and which remain constant from each other under the same conditions, were developed from some common ancestor by this method? In so far as the matter of this paper is concerned, this would be an apparent explanation of the facts. Whether it is the real one cannot be determined by such few and narrowly restricted experiments.

SUMMARY OF RESULTS.

(1) It is possible, by the method of culture outlined, to isolate species of *Saprolegnia* relatively quickly by means of a single zoospore.

(2) The study of the various species of *Saprolegnia* verifies the work of Klebs on *S. mixta* as to the effect of nutrition on the differentiation of the reproductive and vegetative processes.

(3) Not all the species of *Saprolegnia* produce sexual organs under the conditions favourable for those of *S. mixta* and *S. hypogyna* and are therefore physiologically as well as morphologically distinct.

(4) *Saprolegnia hypogyna*, which has morphologically no true antheridia, can be made to develop such under proper nutrient conditions; K_3PO_4 , KNO_3 , Na_2HPO_4 , $Ca_3(PO_4)_2$, and $Ca(NO_3)_2$, added to haemoglobin solutions, are, in different degree, effective to this end.

(5) The antheridial filaments arise in either a diclinous or an androgynous manner in *all* the species examined under varying culture conditions, and hence their origin is of no special importance as a diagnostic character. No sign of heterothallic species was detected, unless the absence of sexual organs in some species points that way. It is believed that these species have not yet been placed under the proper conditions for the formation of sexual organs.

(6) The variations were so extensive that nearly all the characters used for diagnostic purposes in this genus were affected. At first thought this seems to indicate that no separation can be made of the different species of *Saprolegnia* by means of constant characters. We can, however, use the resources of the chemist and physicist, and, by stating the exact conditions of culture, establish a standard to which all forms may be referred. A species thus defined can be identified by placing it in these conditions. The assumption here needed seems to be perfectly justified; for it has not been proved as yet that characters which have been developed under cultivation will be retained by the plant when it is returned to the original environment.

(7) In spite of the great similarities of certain very constant forms and their overlapping under different conditions, it is concluded that they are entirely distinct forms, at least physiologically, if not always morphologically. This result, combined with what Maurizio and De Bary found in this family of fungi, forces one to assume that there are a great many simple forms—elementary species in the sense of De Vries—within the genus *Saprolegnia*.

In conclusion, I may say that this paper adds something more of evidence towards the doctrine that sex in plants is determinable by external conditions. It is admitted that the evidence is not conclusive; for the structure and the development of the plants studied are of such a nature as

to preclude any final results on this point. If we could start with a dioecious form of *Saprolegnia* and obtain the results here obtained, the outlook would be more encouraging. Of course if we were to consider that the normal condition of *Saprolegnia hypogyna* is one in which the antheridium is simply suppressed, and that the new conditions have merely removed the obstacle which had hitherto prevented its development, sex-determination would not be touched by the reactions detailed in this paper. But I am not at all sure that the hypogynous cell must be so considered. At any rate, the question of sex-determination remains one of the most attractive problems of biology.

LITERATURE CITED.

The literature is fully reported by Humphrey up to 1893, and by Klebs up to 1900. The following are referred to in the text.

- BARY, A. DE ('81): Untersuchungen über Peronosporéen und Saprolegnieen. Beiträge zur Morph. und Phys. der Pilze, Reihe IV.
 ——— ('88): Species der Saprolegnieen. Bot. Zeit., 1888, xlv, pp. 597, 613, 629, und 645.
 BENECKE, W. ('95): Die zur Ernährung der Schimmelpilze notwendigen Metalle. Jahrb. f. w. Bot., 1895, xxviii, p. 485.
 CORNU, M. ('72): Monographie des Saprolegniées. Ann. Sci. Nat., Bot., sér. 5, tome xv, 1872, p. 5.
 DAVIS, B. M. ('04): Oogenesis in *Saprolegnia*. Bot. Gaz., 1904, xxxv, p. 347.
 HARTOG, M. M. ('95): On the Cytology of the Vegetative and Reproductive Organs of the Saprolegnieae. Trans. Roy. Irish Acad., 1895, xxx.
 ——— ('99): The alleged Fertilization in the Saprolegnieae. Ann. Bot., 1899, xiii.
 HUMPHREY, J. E. ('93): The Saprolegniaceae of the United States. Trans. Amer. Phil. Soc. N.S., xvii, 1893, p. 63.
 KLEBS, G. ('99 and '00): Zur Physiologie der Fortpflanzung einiger Pilze. II. Jahrb. f. wiss. Bot., 1899, xxxiii, p. 513. III. Jahrb. f. wiss. Bot., 1900, xxxv, p. 80.
 ——— ('96): Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.
 ——— ('03): Willkürliche Entwicklungsänderungen bei Pflanzen. Jena, 1903.
 ——— ('06): Über Variationen der Blüten. Jahrb. f. wiss. Bot., 1906, xlii, p. 155.
 MAURIZIO, A. ('94): Zur Entwicklungsgeschichte und Systematik der Saprolegnieen. Flora, 1894, lxxix, p. 109.
 ——— ('96): Studien über Saprolegnieen. Flora, 1896, xxxii, p. 14.
 MOLISCH, H. ('95): Bericht. d. Wien. Akad., 1895, civ, p. 795 (discussed by Pfeffer in Plant Physiol., Eng. Ed.)
 NAEGELI, C. ('47): Zeitschrift für wiss. Bot., 1847, Hefte 1, 3, und 4.
 PFEFFER, W. ('84): Locomotorische Richtungsbewegungen durch chemische Reize. Unters. aus dem Bot. Inst. zu Tübingen, 1884, i, p. 363.
 SCHLEIDEN, M. J. ('42): Grundzüge der Bot., 1. Aufl., Leipzig, 1842, p. 264.
 TROW, A. H. ('95): The Karyology of *Saprolegnia*. Ann. Bot., 1895, ix, p. 609.
 ——— ('99): Observations on the Biology and Cytology of a new variety of *Achlya americana*. Ann. Bot., 1899, xiii, p. 131.
 ——— ('04): On Fertilization in the Saprolegnieae. Ann. Bot., 1904, xviii, p. 541.
 UNGER, F. ('43): Einiges zur Lebensgeschichte der *Achlya prolifera*. Linnaea, 1843, xvii, p. 127.
 VRIES, H. DE ('05): Species and Varieties, their Origin and Mutation. Chicago, 1905.

EXPLANATION OF FIGURES IN PLATE XXIII.

Illustrating Dr. Kauffman's Paper on Saprolegniaceae.

All the figures were drawn with camera lucida. They are all taken from specimens of *Saprolegnia hypogyna*. The magnification is uniform for all the figures.

Figs. 1-3. From cultures in haemoglobin and peptone.

Figs. 4-7. From cultures in haemoglobin and CaHPO_4 .

Figs. 8-10. From cultures in haemoglobin and $\text{Ca}_3(\text{PO}_4)_2$.

Fig. 11. From cultures in haemoglobin and KNO_3 .

Figs. 12-15. From cultures in haemoglobin and K_3PO_4 .

Figs. 16-18. From a culture in haemoglobin and K_3PO_4 , with an addition of NaCl .

UNIVERSITY OF MICHIGAN.

May, 1907.

Huth, lith. et imp.

Kauffman, C. H. 1908. "A contribution to the physiology of the Saprolegniaceae, with special reference to the variations of the sexual organs." *Annals of botany* 22, 361–387.

<https://doi.org/10.1093/oxfordjournals.aob.a089178>.

View This Item Online: <https://www.biodiversitylibrary.org/item/232525>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a089178>

Permalink: <https://www.biodiversitylibrary.org/partpdf/318914>

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>.