A STUDY OF SOME HOMOTHALLIC AND HETEROTHALLIC ASCOMYCETES

Thesis for the Degree of M. S.

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THESIS

A Study of Some Homothallic and Heterothallic Ascomycetes.

A thesis submitted in partial fulfillment
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THESIS

This thesis is hereby

approved for recommendation

to the Graduate Council.

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INTRODUCTION. 1/

The discovery of sexuality in the Mucors by Blakeslee (3) has stimulated other workers to investigate this highly interesting problem of sex in the Fungi, as well as to go over the work already done on the Mucors.

The work presented in this paper on some of the Ascomycetes was stimulated, both indirectly and directly, by the ideas of sexuality as brought out in Blakeslee's work.

The number of species in this paper includes only a part of the fungi on which work is being done. It is the plan of the writer to complete many of this group at a future time.

1/ The present work was suggested by Dr. Ernst A. Bessey of Michigan State College, under whose guidance and direction it has been carried on.

HISTORY.

Heterothallism and homothallism were described by Blakeslee (3) in the Mucorales. Since then additional work has revealed that the greater number of the Mucorales belong to the heterothallic group. In the aquatic Phycomycetes Couch (5) has reported heterothallism for Dictyuchus, a genus of the Water Molds. Numerous investigations have also been carried on in some of the higher fungi.

Craigie (6) reports that the sporidia of the Wheat rust and Sunflower rust are of two distinct sexes which must unite before the aecial cups will be formed. Similar sexual differentiation for corn smut was reported by Hanna at the 1928 meeting of the American Phytopathological Society at New York.

In 1918 further light was thrown on the problem of sexuality of fungi by the researches of Mlle. Bensaude (1) in which she showed that Coprinus fimetarius, one of the Basidiomycetes, resembles certain of the Mucorales investigated by Blakeslee in that it is heterothallic. When sub-cultures were made from two single spore cultures and grown separately, they remained for eight months in the primary condition, in which the mycelium branched irregularly, produced no clamp connections, and consisted of uninucleate cells; furthermore the mycelia were completely sterile, producing no fruit bodies. However, when two mycelia of opposite sex were brought together and allowed to fuse, they soon developed a secondary mycelium which was characterized by regular branching, the presence of clamp-connections, dicaryon cells, and the production of fruit bodies.

Kniep (15) has shown that Schizophyllum commune is a hetero-

thallic species, but that in this species fruit bodies are sometimes produced from monosporous mycelia. Thus it is seen that the phenomenon of heterothallism is not necessarily associated with the sterility of monosporous mycelia. Kniep found that spores originating from such monosporous fruit bodies were all of the same sex, and that when sown in polysporous cultures the mycelium to which they gave rise never produced clamp-connections. Thus in a heterothallic species fruit bodies may develop from monosporous mycelia; but such fruit bodies produce spores which are all of one sex.

Both Mlle. Bensaude (1) and Kniep (14) have found that the presence of clamp-connections is invariably associated with the conjugate division of the nuclei. It is thought, because of this then, that a reliable criterion is thus established for determining whether a given species of Basidiomycete is homothallic or heterothallic. If on monosporous mycelia clamp-connections appear regularly, the species must be homothallic; while if on monosporous mycelia no clamp-connections or fruit bodies appear until two mycelia of opposite sex fuse together, the species must be heterothallic.

Miss Mounce (17 & 18) has shown that Coprinus sterquilinus and C. stercorarius are homothallic, and that C. lagopus and C. niveus are heterothallic. Thus in a single genus there exist homothallic and heterothallic species.

Hanna (12) carried the work on Coprinus lagopus further and showed that sex in this fungus is determined by factors which segregate according to Mendelian principles. Newton (19) duplicated Hanna's work and obtained similar results.

A study of the sexual differentiation on coprophilous Pyrenomycetes has been reported by the Marchal Brothers (16) which will be referred to later. Edgerton (9) reported the occurrence of plus and minus strains in the genus Glomerella which is interesting because it is hetero-homothallic. Reference will be made to this later. Dodge (8) showed that two separate strains are necessary for the complete formation of fruit bodies in Ascobolus magnificus. Betts (2) reports heterothallism for Ascobolus carbonarius. Miss Wineland (25) caused the production of mature perithecia of a species of Gibberella when she placed certain strains together. Kirby (13) reports the occurrence of two strains in Ophiobolus cariceti. Shear and Dodge (21) have demonstrated the heterothallic nature of Neurospora sitophila. Dodge (7) gives the cytological explanation for this behavior in Neurospora. Wehmeyer (23) gives a historical summary of sexuality in the Ascomycetes.

With the background so well outlined the present work was begun with the hope of adding a little more to the already available data.

PRESENT WORK.

Methods and Results

Single spore cultures were made of all the species listed below. The single spore cultures were obtained by the dry needle method as described by Hanna (11). All species of Chaetomium were cultivated on potato dextrose agar except C. murorum which was grown on agar made from horse manure water. Fimetaria fimicola was also grown on potato dextrose agar. The remaining species, which includes species of Pleurage, Ascobolus, and an unidentified Pyrenomycete, were grown on horse manure agar.

These experiments were repeated several times always using single spore cultures.

The results of the experiments are as follows:

All species of Chaetomium studied were homothallic.

All species of Pleurage studied were homothallic.

Fimetaria fimicola was found to be homothallic.

Ascobolus stercorarius was found to be hetero-homothallic.

Ascobolus immersus gave no results that are satisfactory.

One species of an unidentified Pyrenomycete was proven to be heterothallic.

LIST OF FUNGI USED.

1. Chaetomium globosum Kunze.
2. C. . . . . elatum Kunze & Schmidt.
3. C. . . . . funicolum Cook.
4. C. . . . . spirale Zopf.
5. C. . . . . aureum Chivers.
6. C. . . . . trilaterale Chivers.
7. C. . . . . cochliodes Pallister.
8. C. . . . . angustum Chivers.
9. C. . . . . aterrimum Ellis & Everhart.
10. C. . . . . murorum Corda.
11. C. . . . . sp. 1/
12. C. . . . . sp.
13. C. . . . . sp.
14. Fimetaria fimicola (Roberge) Griffiths & Seaver.
15. Pleurage anomala D. Griff.
16. P. . . . arizonensis D. Griff.
17. P. . . . anserina (Ces.) Kuntze.
18. P. . . . decipiens (Wint.) Kuntze.
19. P. . . . minuta (Fuckel) Kuntze.
20. Ascobolus immersus Pers.
21. A. . . . stercorarius (Bull.) Schrot.
22. Pyrenomycete of unknown genus and species.

1/ Species No. 11, 12, and 13 of Chaetomium are as yet unidentified. They are not included in Chivers' (4) monograph. These C. species are all homothallic.

CULTURAL NOTES ON SPECIES.

Chaetomium globosum Kunze. 1/

Origin: This was given to me by Mr. F. C. Strong, of Michigan State College, who found it on some straw which came from Maryland.

Single spore cultures 2/ on potato dextrose agar gave rise to mature perithecia after a period of two and a half weeks. Growth is very rapid. Perithecia appear in great numbers. The union of mycelia from single spore cultures gives no evidence of increased fructification.

Chaetomium elatum Kunze and Schmidt.

Origin: Found on straw from horse barn at Michigan State College.

Single spore cultures on potato dextrose agar grew very rapidly, producing mature perithecia after a period of two and a half weeks. There is no evidence of increased fruiting by the union of single spore cultures.

Chaetomium funicolum Cooke.

Origin: Found on straw from horse barn. 3/

Single spore cultures on potato dextrose agar gave rise to

1/ All species of Chaetomium studied had 8 spores per ascus.

2/ In no case were there less than thirty single spore cultures of each species of Chaetomium grown.

3/ When not otherwise stated the origin is at Michigan State College or immediate vicinity.

mature perithecia after a period of three weeks. Perithecia occur in great numbers. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium spirale Zopf.

Origin: Found on straw from horse barn.

Single spore cultures on potato dextrose agar gave rise to mature perithecia after a period of three weeks. Perithecia numerous but not crowded. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium aureum Chivers.

Origin: This was sent to me by Mr. Edgar C. Tullis of the University of Arkansas. This fungus was found on the hulls of rice.

Single spore cultures on potato dextrose agar gave rise to mature perithecia after a period of three and a half weeks. The perithecia are numerous and closely compacted, forming a dense mat. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium trilaterale Chivers.

Origin: This was given to me by Mr. Stanley Johnston of South Haven, Michigan. This fungus appeared in cultures made by him from roots of blueberry plants (Vaccinium sp.)

Single spore cultures on potato dextrose agar gave rise to mature perithecia after a period of three weeks. Perithecia formed

abundantly. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium cochliodes Pallister.

Origin: Found on paper which was in contact with straw and horse dung.

Single spore cultures on potato dextrose agar grew rapidly, producing mature perithecia after a period of two and a half weeks. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium angustum Chivers.

Origin: Found on straw from horse barn.

Single spore cultures on potato dextrose agar gave rise to mature perithecia after a period of three weeks. Perithecia formed abundantly. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium aterrimum Ellis and Everhart.

Origin: Found on wheat straw from horse barn.

Single spore cultures on potato dextrose agar gave rise to mature perithecia after a period of three and a half weeks. Growth is much slower than in C. globosum, and the perithecia are moderately abundant. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Chaetomium murorum Corda.

Origin: Found on horse dung.

Single spore cultures on agar made from horse manure water, gave rise to mature perithecia after a period of three and a half weeks. Mycelial growth not conspicuous, but soon covers entire surface of agar in petri dish. Perithecia few and scattered. The union of mycelia from single spore cultures gives no evidence of increased fruiting.

Fimetaria fimicola (Roberge) Griffiths and Seaver.

Origin: Found on horse dung.

Single spore cultures on dung agar gave rise to mature perithecia after a period of three weeks. Mycelial growth is rapid. Perithecia form in abundance over entire surface, ranging from sunken to superficial. The union of mycelia from single spore cultures gives no evidence of increased fruiting. Ascus 8 spored.

Pleurage anomala D. Griff.

Origin: Found on horse dung.

Single spore cultures grown on horse dung agar gave rise to mature perithecia after a period of three and a half weeks. Mycelial growth scanty, perithecia few and scattered, mostly sunken or partly imbedded in substratum. Mycelial union from single spore cultures gives no evidence of increased fruiting. Asci 4 spored.

Pleurage arizonensis D. Griff.

Origin: Found on horse dung.

Single spore cultures grown on horse dung agar gave rise to mature perithecia after nearly four weeks. Mycelial growth scanty, perithecia few and scattered, partially sunken in the substratum. The union of mycelia from single spore cultures gives no evidence of increased fruiting. Asci 4 spored.

Pleurage anserina (Ces.) Kuntze.

Origin: Found on horse dung.

Single spore cultures on horse dung agar gave rise to mature perithecia after a period of three and a half weeks. Mycelium spreads quite rapidly over surface of agar, but is scanty; perithecia few and scattered, partly sunken in the substratum. The union of mycelia from single spore cultures gives no indication of increased fruiting. Asci 4 spored.

Pleurage decipiens (Wint.) Kuntze.

Origin: Collected on horse dung.

Single spore cultures grown on horse dung agar gave rise to mature perithecia after a period of three and a half weeks. Mycelial growth scanty, perithecia few and sunken in the substratum. The union of mycelia from single spore cultures gives no evidence of increased fruiting. Asci 8 spored.

Pleurage minuta (Fuckel) Kuntze.

Origin: Found on horse dung.

Single spore cultures grown on horse dung agar gave rise to mature perithecia after three and a half weeks. Mycelial growth scanty, perithecia few and scattered, superficial or with base slightly sunken in the substratum. The union of mycelia from single spore cultures gives no evidence of increased fruiting. Asci 8 spored.

Ascobolus immersus Pers.[1/]

Origin: Found on weathered horse dung.

Two single spore cultures were obtained after many attempts to germinate the spores. These two single spore cultures produced no fruit bodies. Mycelial matings of these two cultures produced no fruit bodies. Ramlow (20) explains that plain manure agar gives very weak mycelial growth and almost never the fruit bodies of Ascobolus immersus, but if filter paper is added to the manure agar the spores produce vigorous mycelia and abundant apothecia.

No conclusion can be made from my work.

Ascobolus stercorarius (Bull.) Schrot.

Origin: Found on horse dung.

Single spore cultures gave rise to a very few scattered apothecia after a period of four to five weeks. Of more than twenty single spore matings, approximately fifty per cent gave rise to abundant apothecia along the margin where the mycelia of the two

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1/ Species of Ascobolus were identified according to Seaver's (22) recent work.

strains met. In the other cultures no fruiting resulted from such union of mycelia. It is concluded from the data obtained that this species is hetero-homothallic.

Spherical Pyrenomycete.

Genus? sp.?

Origin: Found on old cultures of horse dung.

Single spore cultures produced no fruit bodies. Mycelial matings from nine single spore cultures produced perithecia in approximately fifty per cent of the matings along the margin where the two mycelia met. The remaining matings produced no perithecia. The spores of this fungus were induced to grow only after being treated in dilute HCl (1/10 of 1%) and incubated in a hot air oven for twenty minutes up to 70°C. The experimental data indicate that this fungus is heterothallic.

Perithecia scattered or thinly gregarious, superficial, spherical, non-ostiolate, covered on all sides when young with long ribbon-like cream colored appendages of uniform dimensions throughout their length. As the perithecium matures the appendages disappear, leaving it naked. Perithecia reach diameter of 600μ. Asci club-shaped, 75-90 x 20μ, 8 spored, soon dissolving leaving ascospores loose in perithecia. Spores lemon-shaped, apiculate at both ends 12-15.5 x 8-11μ. Ascospores escape by the decaying of the perithecia.

DISCUSSION AND COMPARISON.

The work of Marchal (16) on Chaetomium elatum Kunze and Hypocopra fimicola Sacc. (Fimetaria fimicola (Roberge) Griffiths and Seaver) is substantiated by the present work, in which like that of Marchal a cytological investigation of the nuclear behavior was not included.

Dodge (7) in his study of Neurospora found an interesting phase of spore formation in the species N. tetrasperma. The ascus in this species normally contains four spores. If several perithecia are crushed in which spores are maturing there will probably be found one or two asci with an abnormal number of spores such as three large and two small spores, or even in an extreme case only one giant spore. Shear and Dodge (21) found that monosporous mycelia from such small spores bore only sclerotia or bodies which resembled aborted perithecia. No ascospores developed in these structures. By properly mating these cultures, normal perithecia are formed containing asci which normally have only four spores. The larger normal spores have two nuclei when first delimited and thus contain nuclei of both sexes. One might conceive from the above data that in a genus which contains both four and eight spored species he would find them to be homothallic and heterothallic respectively.

Wilcox (24) found , in the heterothallic species Neurospora sitophila which has 8 spored asci, that the spore haplonts may be arranged in the ascus in either one of the four following combinations, AA, BB, AA, BB; BB, AA, BB, AA; AA, BB, BB, AA; or BB, AA, AA, BB, where A and B are reciprocal as to their sexuality.

In my work I found that the four and eight spored species of the genus Pleurage were both homothallic. The nuclear condition in these species has not been studied. However, if it is assumed that the four spored species has two nuclei per spore and that the eight spored species has one nucleus per spore it must be granted, at least for the eight spored species, that the sex determiners are carried in one nucleus. It is therefore clear that in a genus in which the number of spores varies with the species no prediction can be made that one of the species will be homothallic and the other heterothallic.

Edgerton (9) found in the genus Glomerella a condition which approaches that found in the Mucorales. The peculiar trait of producing abundant perithecia at one time and a scarcity at another time in nature gave grounds for presupposing sexual differentiation. Single spore isolations yielded two strains, a "plus" strain of floccose growth and abundant aerial mycelium, and a "minus" strain with scarcely any aerial mycelium. However, single spore cultures of each strain produced abundant perithecia. When both strains were grown in one petri dish there appeared a great mass of perithecia along the margin where the mycelia of the two colonies met. To determine whether this was truly sexual stimulation or due to chemical and food conditions some of the perithecia on the border were crushed and separate asci were removed and planted separately in petri dishes. Edgerton found that segregation took place giving the two characteristic strains. This segregation of strains gives definite proof that there was sexual stimulation and therefore that there is sexual differentiation.

A similar condition was found in my work with Ascobolus

stercorarius. Single spore cultures of this species gave rise to a few apothecia. By mating single spore cultures it was found that approximately 50% of the matings stimulated a great abundance of apothecia along the margin where the mycelia of the two strains met. In the other cases there was no such stimulation. This shows clearly that whereas single spore cultures may produce a few apothecia there is, however, a definite sexual differentiation. Such a species can be designated as a hetero-homothallic form. Further work is in progress to discover whether the antherids are functional in all cases or whether parthenogamy may occur to explain the production of the occasional apothecia on single spore cultures. The two single spore cultures of Ascobolus immersus produced no fruit bodies. These two mated together also produced no fruit bodies. Difficulty was encountered in germinating these spores. I have not done enough with this species to venture even a guess as to its sexuality. However, Ramlow (20), who studied this species morphologically and cytologically, has demonstrated that this species is parthenogamic (i.e. there is no distinct antherid and the paired nuclei in the ascogonial cells and ascogenous hyphae are all of ascogonial origin). Such a species would necessarily be homothallic unless antherids are formed when two sexes of mycelia are present, and no antherids if only one is present. He further found that A. immersus fruited well when a portion of filter paper is partly immersed in the manure agar. The manure agar alone produced a weak mycelium and almost never the fruit bodies. In my culture media of manure agar no filter paper was added; this may explain the weakness of growth and the non-production of fruit bodies.

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