CULTURAL AND GENETICAL STUDIES OF CERTAIN AGARICS

by

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INTRODUCTION

During the past two decades there has been an increasing amount of experimental study of the Agaricaceae. Some of this has dealt with the development of the fruiting-body and the cytological phenomena that accompany the growth and maturation of the sporophore. Another part of it deals with the more careful study of the fruiting-body as an aid in the preparation of a system of classification that would show the more probable relationships of the mushrooms. There have been a few papers dealing with the culturing of Agarics for the production of fruiting-bodies, but most of this type of study has been incidental to other problems. A very few workers have been engaged in the study of the physiology of members of the Agaricaceae.

The subject of greatest interest to students of this group of fungi in the past few years, however, is what might be called "sexuality." It was known for a long time that the mycelium of some mushrooms possessed clamp-connections. Kniep (42, 43, 44) was studying the formation of these structures and the conjugate division of the nuclei in the cells when Bensaude (5) reported in Coprinus fimetarius Fr. the existence of what has been called "heterothallism." Bensaude found that the mycelium derived from the germination of a basidiospore was characterized by the presence of only one nucleus in each cell and by the absence of clamp-connections. However, when certain spores were allowed to germinate and the mycelia from these grown together, then the resultant mycelium was dicaryon and had clamp-connections. As a result of the work of numerous investigators it has been found that there is a wide range of phenomena involved in the "sexuality" (which includes heterothallism) of the Hymenomyceteae.

The present investigation was begun with the hope that some contribution could be made to the study of the inheritance of characters in the Agarics, a problem that has received little attention to date. Before much progress could be made it was necessary to find suitable media, artificial, if possible, for the development of fruiting-bodies of the fungi studied and to determine the type of "sexuality" possessed by each species.

This paper, therefore, consists of two main parts, which are as follows: a study of many kinds of culture media for the production of fruiting-bodies and a study of the types of "sexuality" in the various fungi. The results of the genetical study were meager and are discussed only briefly in this paper.

CULTURAL STUDIES

Review of literature

In most of the experiments dealing with the production of fruiting-bodies of mushrooms in pure culture the investigators have used some substance or substances upon which the particular fungi normally grow. For example, sterile horse-dung has been used by Bohn (7), Borriss (8, 9), Brunswik (15), Buller (17, 18), Hanna (33, 34), Lakon (46), Oort (56) and Sass (63) for growing species of Coprinus. Rabbit-dung was used by Gilmore (32) in her work with Psilocybe coprophila Fr. Several workers, Hanna (35), Humphrey (38) and Mounce (50), have used horse-dung mixed with sawdust or soil or horse-dung placed on or above a layer of soil or used as a spawn to be placed in the soil. In some experiments horse-dung was used with some enriching material such as malt extract.

Poole (57) found that a mixture of the dead roots and canes of the dewberry plus prune juice was a good medium for the production of fruiting-bodies of Collybia dryophila Fr. It was discovered by Arnold (2) that C. cirrata Fr., C. cirrata var. Cookei Bres. and C. tuberosa Fr. formed typical sporophores when grown on the caps of Boletus luteus. This was true when the caps were placed on the surface of wet soil and the caps and soil sterilized by steam under pressure. However, when the soil was first sterilized by means of dry heat, the fungi would not grow. This same investigator found that Marasmius elongatipes Pk. would fruit when it was grown on a medium consisting of powdered oak wood, malt extract solution and peptone.

Thompson (68) was able to secure fruiting-bodies of Nyctalis asterophora Fr. and N. parasitica (Bull.) Fr. on corn-meal and oat-meal agar.

Etter (25) secured good results with several species by the use of a medium consisting of corn-starch, corn-meal, sawdust from some wood that the fungus normally grew upon and a strong solution of malt extract that was added to the medium at the proper time. Kaufert (40) obtained fruiting-bodies of Pleurotus corticatus Fr. by the use of sawdust of the basswood tree plus four per-cent malt extract solution.

Apparently very few attempts have been made to use vegetable material itself for the production of fruiting-bodies. Long and Harsch (47) found that the few species of Agarics that they tried as well as more than thirty species of wood-rotting fungi developed fruiting-bodies on a number of vegetables and fruits as well as on corn-meal and malt. Of the vegetables the parsnips and prunes were especially good; beans, celery, beets, potatoes and alfalfa were useful to some extent.

Not many workers have successfully used artificial media. Gilmore (32) used a medium of glucose plus certain salts and peptone for Psilocybe coprophila Fr. Dickson (22, 23, 24) found that malt extract was suitable for Coprinus sphaerosporus (new species), and Sass (63) also found this medium to be useful in his work with certain species of Coprinus.

The writer did not find in the literature any record of an attempt to discover purely artificial media that would permit the production of fruiting-bodies. A medium that could be made up in exactly the same manner every time and which would contain exactly the same materials would be valuable for cultural and genetical studies. With this in mind, the writer tried to find such a medium for at least some fungi. Lack of notable success led him to try other types of media.

Careful study of the physical factors involved in the production of sporophores has been almost entirely neglected. Almost all of the

workers referred to in the preceeding paragraphs have stated that diffuse sunlight is necessary for the formation of fruiting-bodies; Dickson (22), however, found that normal sporophores of Coprinus sphaerosporus would develop in the dark. Long and Harsch (47) say that light is essential and that the character of the substrate is of secondary importance. Most of their work was done with such wood-rotting fungi as Polyporus, Fomes, etc.

Borriss (8, 9), working with Coprinus lagopus Fr., reported that certain rays of spectrum (400-500 m u) are most effective in regard to the production of fruiting-bodies and that rays longer than 640 m u are of no value. The greatest results were obtained by the use of rays between 440-450 m u. Apparently of almost equal importance is mechanical stimulation of the fruit-body primordium. Temperature and humidity were much less important.

Several workers, Etter (25), Hanna (35) and Kaufert (40), have pointed out that the various colorings or hairs on the pileus or lines on the stipe are apparently influenced by the relative humidity of the atmosphere. Freedom for the cap to expand outside of the container also is necessary for the typical markings to develop.

Etter (25) and Kaufert (40) have emphasized that the medium must be porous and that there must be an abundant supply of readily-available food materials.

In general, rather moderate temperatures (22°-27°C.) are best for the growth of the mycelium and production of fruiting-bodies.

Another possible factor which has been hinted at in some reports, Lakon (46) and Gilmore (32), is indicated by the fact that certain fungi have produced numerous typical fruiting-bodies during the summer months,

but during the winter months these same fungi, under seemingly similar conditions, form atypical sporophores or none at all. The cause of this is unknown.

The relation of the genetical constitution of the fungus to its ability to form fruiting-bodies has been studied only within the last few years. At first it was thought that only the dicaryon mycelia would form sporophores, and the appearance of the fruiting-bodies was suggested as a criterion for judging whether or not a mycelium was monocaryon or dicaryon. It was soon pointed out, however, that the monocaryon mycelia of certain Agarics would produce fruiting-bodies. A criticism that might have been more pertinent, however, would be that, since so little was known concerning the factors operative in the formation of fruiting-bodies, their absence should not be interpreted as indicating that the hyphae were monocaryon.

Zattler (90) found in Schizophyllum commune Fr. that certain monosporous strains when grown together would produce typical fruiting-bodies, while certain other strains when grown together would produce abortive rudiments of sporophores. This was interpreted as being due to a gene, g, which is recessive and which operates toward the production of a rudimentary fruit-body.

Gilmore (32) found evidence in Psilocybe coprophila that the production of irregular fruiting-bodies by that fungus was controlled, in part, by a gene that was neither dominant nor recessive.

Dickson (24) found that abnormal fruiting-bodies were produced by Coprinus macrorhizus Rhea. His interpretation of results was that typical fruiting-bodies would form only when the monosporous strains involved had a full normal complement and that the abnormal sporophores were the result of the presence in at least one of the mycelia of a number of recessive genes and/or such chromosome aberrations as translocations and deletions.

Materials and methods

Tissue cultures of a number of Agarics were used in this study. The cultures were secured by aseptically removing from the interior of the stipe of a freshly-collected mushroom a small piece of tissue and transferring it to acidified potato malt agar that had been poured into a sterile Petri dish and allowed to harden. If any mycelium developed, it was transferred to slanted tubes of potato malt agar. From these tubes transfers were made to the various media that were to be tested.

Nearly all of the mushrooms that were studied were secured in culture during the fall of 1936 and during the growing seasons of 1937 and 1938. With the exception of Coprinus radians Fr., which was cultured from a sporophore that appeared on the campus of Northwestern University, Evanston, Illinois, in 1935, all of the fungi were collected either in the city of East Lansing, Michigan, or in the fields or woods nearby. Since not all of the fungi were available from the start of the study and because of difficulties in setting up large numbers of some of the experiments, some of the species were grown on only a few of the media while others were tested on all of them.

The species that were identified, cultured and tested on different media are listed. After each name is the approximate date upon which the species was cultured. They are as follows[1]: Coprinus atramentarius Fr. (October, 1936), C. comatus Fr. (October, 1936), C. fimetarius

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[1] The plants described by Peck and Longyear as well as Coprinus fimetarius macrorhizus Fr. are listed as they are given by Kauffman (41). The author citations for the other species have been determined in accordance with the rules of nomenclature as formulated by the 1930 meeting of the International Botanical Congress. In the determination of the citation of author the works of Briquet (11), Fries (26, 27, 28, 29, 30), Gillet (31) and Quelet (58) were consulted.

macrorhizus Fr. (June, 1937), C. micaceus Fr. (September, 1936; August, 1937), C. plicatilis Fr. (August, 1937), C. quadrifidus Peck (June, 1937), C. radians Fr. (June, 1935), C. radiatus Fr. (November, 1937), C. semilantanus Peck (August, 1936), Galera crispa Longyear (July, 1937), G. tenera (Fr.) Quelét (July, 1937), Panaeolus campanulatus (Fr.) Quelét (September, 1936), P. retirugis (Fr.) Quelét (September, 1936), P. solidipes Peck (September, 1936), Psathyrella disseminata (Fr.) Quelét (June, 1937), Psilocybe subviscida Peck (June, 1938), Stropharia semiglobata (Fr.) Quelét (October, 1936).

During the first eight months of testing of the media and fungi the cultures were grown at room temperature (about 21°C.) in the laboratory. Additional light was supplied at night by means of a 60 watt electric light bulb that was suspended about three feet above the cultures. During the next four months the temperature varied from about 24° to 35°C., and no additional light was supplied. After that the temperature was again near 21°C. for eight months; during this period, also, no supplementary illumination was used.

In trying to find media upon which the fungi would form fruiting-bodies several types of media were tried. The formulae and methods of preparation of the media that were tested are listed below in several groups. Each group consists of media of the same general type.

The agar, malt extract and yeast extract were Difco products. Unless there is a statement to the contrary, it is to be understood that the medium was tubed, sterilized at 15 pounds pressure for 30 minutes and then slanted.

After the name of each medium there is a date to signify when that medium was used in the tests. Since all of this phase of the study was

simply a search for a medium upon which typical sporophores would form, the experiments were not duplicated.

A. Natural media (with or without other materials added)

(1) Sterile horse-dung (June, 1937)
Fresh horse-dung was placed in 250 ml. or 500 ml. Erlenmeyer flasks, moistened with distilled water and sterilized at 15 pounds for one and one-half hours.

(2) Grass (June, 1937)
Grass from a lawn was dried and cut into small pieces. Some of this was placed in Erlenmeyer flasks and enough water added to saturate it. The material was sterilized for one and one-half hours at 15 pounds pressure.

(3) Decayed wood (June, 1937)
Wood from a much-decayed log of Fraxinus americana was dried at 100°-140°C. and then powdered. 125 ml. of this were placed in a 250 ml. or 500 ml. Erlenmeyer flask, and 75 ml. of distilled water were added. After the wood had become saturated the excess water was poured off and the wood sterilized at 15 pounds pressure for one and one-half hours.

(4) Sawdust (June, 1937)
125 ml. of fine sawdust from several deciduous trees were soaked in distilled water for one hour. The excess water was poured off and the contents of the flask were sterilized at 15 pounds pressure for one and one-half hours.

(5) Horse-dung straw (November, 1937)
A mixture of horse-dung and two-year old wheat straw was placed in 250 ml. or 500 ml. Erlenmeyer flasks, moistened, then sterilized at 15 pounds for one and one-half hours.

(6) Sand horse-dung decayed cottonwood (38) (November, 1937)
Into 100 ml. and 250 ml. beakers was put enough sand to make a layer three cm. deep. To the 100 ml. beakers were added about 100 ml. of a mixture of fresh horse-dung and small pieces of partially decayed cottonwood. In the larger beakers only horse-dung was added. The materials were saturated with distilled water and the excess poured off. The beakers were covered with several pieces of cheesecloth and were then sterilized at 15 pounds for one and one-half hours.

(7) Horse-dung agar (May, 1937)
Fresh horse-dung was extracted in distilled water for a few hours, filtered through cheesecloth and cleared by means of an egg white. To this were added 15 gm. agar.

(8) Straw (June, 1937)
Two-year old wheat straw was cut into small pieces and placed in 250 ml. or 500 ml. Erlenmeyer flasks. The straw was saturated with distilled water, the excess water was poured off and the contents of the flask sterilized at 15 pounds for one and one-half hours.

(9) Straw malt-extract (July, 1938)
This was prepared in the same manner as the straw medium except that a solution of 30 gm. of malt-extract per liter of distilled water was used in place of the water alone.

(10) Straw potato malt-extract (July, 1937)
This was prepared in the same manner as the straw medium except that a mixture of the extract of 200 gm. of potatoes per liter of distilled water plus 15 gm. of malt-extract per liter was used.

(11) Potatoes (July, 1937)
Potatoes that had been extracted in distilled water were placed in Erlenmeyer flasks and sterilized at 15 pounds for one-half hour.

(12) Bread (August, 1937)
Small pieces of whole wheat and white bread were placed in Erlenmeyer flasks and saturated with distilled water. The contents were then sterilized.

(13) Milk malt-extract agar (November, 1937)
Malt extract ---------- 15 gm.
Agar ---------- 15 gm.
Pasteurized milk ---------- 1 liter

B. Artificial media (including some natural nutrients)

(14) Xylose potato agar (April, 1938)
Xylose ---------- 12.5 gm.
Agar ---------- 15.0 gm.
Potato extract (200 gm. potatoes
in 1 liter distilled water ------ 1 liter

(15) Glucose agar (October, 1936; February, 1937)
Glucose ---------- 10 gm.
Agar ---------- 15 gm.
Distilled water ---------- 1 liter

(16) Potato glucose agar (October, 1936; February, 1937)
Glucose ---------- 20 gm.
Agar ---------- 15 gm.
Potato extract (200 gm. potatoes in
1 liter distilled water) -------- 1 liter

(17) Stronger potato glucose agar (October, 1936; February, 1937)

Glucose	10 gm.
Agar	15 gm.
Potato extract (400 gm. potatoes in 1 liter distilled water)	1 liter

(18) Peptone potato glucose agar (October, 1936; February, 1937)

Glucose	20 gm.
Peptone	2 gm.
Agar	15 gm.
Potato extract (200 gm. potatoes in 1 liter distilled water)	1 liter

(19) Modification of Piefer, Humphrey and Acree's Medium (61) for Wood-destroying Fungi (May, 1937)

Glucose	40.00 gm.
KH_2PO_4	4.00 gm.
Asparagine	4.00 gm.
$(NH_4)_2HPO_4$	2.00 gm.
$MgSO_4 \cdot 7H_2O$	2.00 gm.
$CaCO_3$	0.25 gm.
$CaCL_2$	0.10 gm.
Agar	15.00 gm.
Distilled water	1 liter

(20) Modification of Leonian's (61) Agar Medium (May, 1937)

Glucose	6.0 gm.
Malt extract	6.0 gm.
KH_2PO_4	1.2 gm.
$MgSO_4 \cdot 7H_2O$	0.6 gm.
Agar	16.0 gm.
Distilled water	1 liter

(21) Glucose corn-meal agar (October, 1936; February, 1937)

Glucose	10.0 gm.
Corn-meal	20.0 gm.
Agar	15.0 gm.
Distilled water	1 liter

(22) Modification of Barnes's (61) Medium (June, 1937)

Glucose	4.0 gm.
K_2PO_4	1.0 gm.
NH_4NO_3	1.0 gm.
KNO_3	1.0 gm.
Agar	25.0 gm.
Distilled water	1 liter

(23) Potato mannose agar (June, 1937)

Mannose	1.4 gm.
Agar	16.0 gm.
Potato extract (200 gm. potatoes in 1 liter of distilled water)	1 liter

(24) Standard strength potato malt agar (throughout the experiment)
Malt extract ---------- 15.0 gm.
Agar ---------- 15.0 gm.
Potato extract (200 gm. potatoes in 1 liter of distilled water) ------- 1 liter

(25) Twice standard potato malt agar (October, 1936; February, 1937)
Prepared as standard potato malt agar except that 400 gm. of potatoes were used in preparing the extract of potatoes rather than 200 gm.

(26) Half standard potato malt agar (July, 1937)
To 100 ml. of the twice standard potato malt extract were added 300 ml. of distilled water. To the resultant 400 ml. were added 6.5 gm. of agar.

(27) Soft potato malt agar (June, 1937)
Malt extract ---------- 15 gm.
Agar ---------- 5 gm.
Potato extract (400 gm. potatoes in 1 liter of distilled water)--------- 1 liter

(28) Potato malt agar - Czapek (61) (May, 1937)
$NaNO_3$ ---------- 1.00 gm.
KH_2PO_4 ---------- 0.50 gm.
KCl ---------- 0.25 gm.
$MgSO_4.7H_2O$ ---------- 0.25 gm.
$FeSO_4.7H_2O$ ---------- trace
Malt extract ---------- 5.00 gm.
Agar ---------- 5.00 gm.
Potato extract (400 gm. potatoes in 1 liter of distilled water) -------- 1 liter

(29) Malt peptone agar (52) (June, 1937)
Malt extract ---------- 70.0 gm.
Peptone ---------- 10.0 gm.
Agar ---------- 20.0 gm.
Distilled water ---------- 1 liter

(30) Modification of mineral malt peptone agar (from Naumov (52). (June, 1937)
$(NH_4)_2HPO_4$ ---------- 2.0 gm.
K_2HPO_4 ---------- 1.0 gm.
$ZnSO_4$ ---------- 4.0 gm.
$MgSO_4.7H_2O$ ---------- 0.5 gm.
Malt extract ---------- 25.0 gm.
Peptone ---------- 10.0 gm.
Agar ---------- 20.0 gm.
Distilled water ---------- 1 liter

(31) Mineral Potato malt agar (November, 1937)

KH_2PO_4	1.000 gm.
$CaCl_2$	0.100 gm.
$MgSO_4.7H_2O$	0.600 gm.
$NaNO_3$	1.000 gm.
$FeCl_3$	0.020 gm.
H_3BO_3	0.040 gm.
$BaCl_2$	0.100 gm.
$MnSO_4$	0.100 gm.
$CuSO_4$	0.004 gm.
$ZnSO_4$	0.080 gm.
$LiCl_3$	0.004 gm.
Na_2SiO_3	0.006 gm.
$Co(NO_3)_2$	0.010 gm.
$Ni(NO_3)_2$	0.006 gm.
$AlCl_3$	0.010 gm.

These materials were added to 50 ml. distilled water, dissolved and then added to 1 liter of standard strength potato malt agar.

(32) Dung extract malt agar (June, 1937)

Malt extract	15.0 gm.
Agar	15.0 gm.
Horse-dung extract (in distilled water)	1 liter

(33) Dung potato malt agar (May, 1937)
Fresh horse-dung was extracted 24 hours in 300 ml. of distilled water. The 200 ml. filtrate was diluted to 1 liter. 500 ml. of this mixture were added to 500 ml. of an extract of 400 gm. potatoes in 1 liter of water. To this liter of material were added the following:

Malt extract	15.0 gm.
Agar	15.0 gm.

(34) Soil extract potato malt agar (October, 1936; February, 1937)
Soil from the greenhouse was extracted 24 hours in 500 ml. distilled water. The 250 ml. filtrate from this was added to 250 ml. of an extract of 400 gm. of potatoes in 1 liter of distilled water. To this were added the following:

Malt extract	15.0 gm.
Agar	15.0 gm.

(35) Soil dung potato malt agar (May, 1937)
Soil was extracted in distilled water and the filtrate diluted to 1 liter. To this liter was added horse-dung which was extracted for 24 hours. 300 ml. of this extract were added to 200 ml. of an extract of 400 gm. of potatoes in 1 liter of distilled water. To this

mixture were added the following:
Malt extract ---------- 7.5 gm.
Agar ---------- 7.5 gm.

(36) Grass malt agar (May, 1937)
Grass from a lawn was cut into small pieces that were soaked for 18 hours in distilled water. The filtrate was diluted to 1 liter and to it were added the following:
Malt extract ---------- 15.0 gm.
Agar ---------- 16.0 gm.

(37) Leaf mold potato malt agar (October, 1936; February, 1937)
40 gm. of partly decayed leaves of beech and maple trees were extracted 24 hours in 700 ml. of distilled water. To the 500 ml. extract were added 200 gm. potatoes. This was steamed for 1 hour. The filtrate was restored to 500 ml. volume with distilled water and to it were added the following:
Malt extract ---------- 7.5 gm.
Agar ---------- 8.0 gm.

(38) Yeast potato malt agar (May, 1937)
Malt extract ---------- 15.0 gm.
Agar ---------- 15.0 gm.
Fleischmann's yeast ---------- 2 cakes
Twice standard potato extract
(see medium 24) ---------- 1 liter

(39) Yeast potato malt agar - Czapek (61) (May, 1937)
To 1 liter of the medium prepared as in medium number (27) were added 4 cakes of Fleischmann's Yeast.

(40) Soil dung yeast potato malt agar (May, 1937)
To 300 ml. of soil dung potato malt agar (medium number 34) were added 2 cakes of Fleischmann's Yeast.

(41) Yeast grass malt agar (May, 1937)
To 1 liter of the grass malt agar (medium number 35) were added 4 cakes of Fleischmann's Yeast.

(42) Yeast extract potato malt agar (August, 1937)
Yeast extract ---------- 10.0 gm.
Standard potato malt agar (medium 23)--1 liter

(43) Live yeast potato malt agar (June, 1937)
To each slant of standard potato malt agar was added a suspension of vigorously growing cells of Saccharomyces cerevisiae. After the yeast cells had grown for three days the various fungi were planted on the slants.

(44) Modification of Czapek's (61) Medium (October, 1936; February, 1937)

$NaNO_3$	2.0 gm.
KH_2PO_4	1.0 gm.
KCl	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
$FeSO_4 \cdot 7H_2O$	0.1 gm.
Sucrose	20.0 gm.
Agar	15.0 gm.
Distilled water	1 liter

(45) Duggar's Medium (37).(August, 1937)

NH_4NO_3	1.0 gm.
KH_2PO_4	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.25 gm.
$FeCl_3$	trace
Sucrose	5.0 gm.
Agar	5.0 gm.
Distilled water	1 liter

(46) Sucrose corn-starch agar (January, 1938)

Sucrose	15.0 gm.
Soluble corn-starch	10.0 gm.
Agar	16.0 gm.
Distilled water	1 liter

(47) Lactose milk agar (February, 1938)

Lactose	15.0 gm.
Agar	15.0 gm.
Pasteurized milk	1 liter

(48) Cellobiose potato agar (April, 1938)

Cellobiose	35.0 gm.
Agar	15.0 gm.
Potato extract (200 gm. potatoes in 1 liter of distilled water)	1 liter

(49) Raffinose potato agar (June, 1937)

Raffinose	1.6 gm.
Agar	16.0 gm.
Potato extract (200 gm. potatoes in 1 liter distilled water)	1 liter

(50) Mannitol potato agar (April, 1938)

Mannitol	15.0 gm.
Agar	15.0 gm.
Potato extract (200 gm. potatoes in 1 liter of distilled water)	1 liter

(51) Modification of Claussen's (61) Medium (May, 1937)

KH_2PO_4	0.050 gm.
NH_4NO_3	0.050 gm.
$MgSO_4 \cdot 7H_2O$	0.020 gm.
$Fe_3(PO_4)_2$	0.001 gm.
Inulin	2.000 gm.
Agar	15.000 gm.
Distilled water	1 liter

(52) Czapek's Glycerine Agar (61) (July, 1938)

Glycerine	30.0 gm.
$NaNO_3$	2.0 gm.
K_2HPO_4	1.0 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
KCl	0.5 gm.
$FeSO_4$	trace
Agar	15.0 gm.
Distilled water	1 liter

(53) Modification of corn-meal starch sawdust medium (61) (June, 1937)

Corn meal	48.0 gm.
Corn starch	16.0 gm.
Sawdust	8.0 gm.
Agar	20.0 gm.
Distilled water	1 liter

(54) Modification of Etter's (25) Medium (June, 1938)

Corn starch	16.0 gm.
Corn meal	50.0 gm.
Sawdust	8.0 gm.

The sawdust was a mixture of several woods. The above quantities of the constituents were placed in 500 ml. Erlenmeyer flasks and saturated with a solution of 25 gm. of malt-extract in 1 liter of distilled water. Flasks were then sterilized. When fruiting-bodies began to appear, more of the malt-extract solution was added.

(55) Prune agar (October, 1936; February, 1937)
120 gm. of pitted prunes were steamed in 1 liter of distilled water for one-half hour. The filtrate was made up to volume and to it were added 18 gm. agar.

(56) Prune corn-meal agar (October, 1936; February, 1937)
An extract of prunes was prepared as in the prune agar medium (number 55). To 1 liter of the extract were added the following:

Corn-meal	20.0 gm.
Agar	15.0 gm.

(57) Oat agar (July, 1938)
100 gm. of rolled oats were steamed for one-half hour in 1 liter of distilled water. To the filtrate were added 18 gm. agar.

(58) Modification of Tubeuf's (61) Medium (June, 1937)

NH_4NO_3	10.0 gm.
K_2HPO_4	5.0 gm.
$MgSO_4 \cdot 7H_2O$	1.0 gm.
85 per-cent lactic acid	2.0 ml.
Agar	20.0 gm.
Distilled water	1 liter

C. Addition of poisons or weakly antiseptic substances

(59) Phenol potato malt agar (June, 1937)
The following solutions of phenol were made up: 100 per-cent (saturated water solution), 50 per-cent, 10 per-cent, 1 per-cent, 0.5 per-cent. One drop of one of these solutions was added by means of a sterile pipette to a test tube containing 10 ml. of sterile potato malt agar of standard strength. The tube was then slanted.

(60) $CuSO_4$ potato malt agar (June, 1937)
The following solutions of $CuSO_4$ were made up: 2 N (1 N was considered to be 159.63 gm. of $CuSO_4$ per liter of solution), 1 N, 0.5 N, 0.1 N., 0.01 N. These solutions were sterilized at 15 pounds for 30 minutes. The rest of the procedure was the same as for medium number 59.

(61) HCl potato malt agar (June, 1937)
The solutions were made up as follows: 1 N, 0.5 N, 0.1 N, 0.01 N. The rest of the procedure was the same as for medium number 59.

(62) $KMnO_4$ potato malt agar (June, 1937)
The following solutions were made up: 1N (1N was considered to be 31.6 gm. of $KMNO_4$ per liter of solution since the medium was acidic), 0.5 N, 0.1 N, 0.01 N. The solutions were sterilized at 15 pounds for one-half hour. The rest of the procedure was the same as for medium number 59.

(63) $AlCl_3$ potato malt agar (July, 1937)
The following solutions were made up: 5 per-cent, 1 1 per-cent, 0.1 per-cent, 0.01 per-cent. Petri dishes, each containing one Van Tieghem ring, were sterilized. Each dish was then maneuvered in such a manner that the ring came to lie at a distance from the edge of the dish equal to about one-fourth of the dish's diameter. In each ring were placed a few drops of one of the solutions. Standard potato malt agar was slowly poured into the dish without disturbing the ring. When the

agar was firm the fungus was planted about one-fourth of the distance across the dish on the side of the dish opposite to the ring. It was thought that as the solution in the ring gradually diffused into the agar it would come into contact with the mycelium and would perhaps induce the formation of fruiting-bodies.

(64) $ZnSO_4$ potato malt agar (July, 1937)
Solutions were made up as follows: 5 per-cent, 1 per-cent, 0.1 per-cent, 0.01 per-cent. The rest of the method was the same as for medium number 63.

(65) $AgNO_3$ potato malt agar (July, 1937)
Solutions were made up as follows: 2 per-cent, 1 per-cent, 0.1 per-cent, 0.01 per-cent. The rest of the method was the same as for medium number 63.

(66) Crystal violet potato malt agar (July, 1937)
The following solutions were made up: 1:2,000; 1:50,000; 1:100,000; 1:500,000. The solutions were not sterilized. Otherwise, the remainder of the procedure was the same as for medium number 63.

(67) Brilliant green malt agar (July, 1937)
Solutions were made up as follows: 1:2,000; 1:50,000; 1:100,000; 1:500,000. The solutions were not sterilized. Otherwise, the procedure was the same as for medium number 63.

(68) Urine potato malt agar (July, 1937)
Human urine was placed in the Van Tieghem rings. It was not diluted or sterilized. Otherwise, the remainder of the procedure was the same as for medium number 63.

(69) Boric acid potato malt agar (August, 1937)
H_3BO_3 ---------- 1.0 gm.
Standard potato malt agar ---------- 1 liter

D. Addition of "growth - stimulating" substances.

(70) 3-indolepropionic acid potato malt agar (June, 1937)
A stock solution was made by using 0.1 gm. of the acid in 1 liter of distilled water to give a solution with a strength of 500,000 A. E. per ml. Dilutions were made to give solutions with strengths of 50,000; 12,500; 6,250; 1,500; 500 A. E. per ml. Each of these solutions was filtered through a Berkefeld filter. One drop of a solution was added to 10 ml. of potato malt agar in test tubes. This resulted in a final dilution within the tubes to give solution of the following strengths: 250, 62.5, 31, 7.5 and 2.5 A. E. per ml. of medium. The test tubes of medium were then slanted.

(71) 3-indolebutyric acid potato malt agar (February, 1938)
0.1 ml. of indole butyric acid ("auxiline" manufactured by the Pennsylvania Chemical Corporation by the use of 3.5514 mg. of indole butyric acid per ml. of 40 per-cent alcohol) was added aseptically to a tube containing potato malt agar. This gave a solution of 0.0355 mg. of the acid per ml. of medium.

(72) 3-indolebutyric acid treatment (February, 1938)
1.0 ml. of indole butyric acid was added to 350 ml. of sterile distilled water to give a solution containing 0.0124 mg. of the acid per ml. of medium. This solution was filtered through a Berkefeld filter and then tubed aseptically. The cultures of different fungi were left in different tubes of the solution for 22 hours and were then transferred to slants of standard potato malt agar.

(73) Vitamin B_1 potato malt agar (April, 1938)
The vitamin B_1 had been prepared by the Williams and Cline Method by Merck and Company, Rahway, New Jersey.
(a) Malt extract ---------- 3.5 gm.
Vitamin B_1 ---------- 0.0001 gm.
Agar ---------- 3.5 gm.
Standard potato extract ---- 300.0 ml.
Tubed, sterilized and slanted.
(b) Prepared as part (a) except that 0.005 gm. of vitamin B_1 was used.

(74) Theelin potato malt agar (April, 1938)
The theelin or esta-triene - ol - 3 - one - 17 (67) was secured from G. F. Cartland (the serial number was (Lot no. I - 35 G F C 15) 12 - 11 - 36). The following solutions were made up:
(1) 1 mouse unit per ml. of medium
(0.14 ml. of the theelin solution was added to 72 ml. of potato malt agar)
(2) 5 mouse units per ml. of medium
(0.72 ml. of the solution of theelin was added to 72 ml. of the medium)
(3) 25 mouse units per ml. of medium
(3.5 ml. of the solution of theelin were added to 72 ml. of potato malt agar)
All of these solutions were tubed and sterilized.

E. Modifications of Conditions of Growth

(75) Additional light (September, 1936 - June, 1937)
During the first part of the experiment the cultures were kept by a window on the west side of the laboratory. Supplementary light was afforded at night by means of a 60 watt electric light bulb that was suspended about 3 feet above the cultures.

(76) Change in H^+ ion concentration (October, 1937)
By the addition of HCl or NaOH the H^+ ion concentration of various lots of potato malt agar was adjusted (with the aid of the La Motte colorimeter) so as to give the following pH values for the media: ph 3.6, pH 5.8 (the usual pH of standard potato malt agar) and pH 8.4.

(77) Increase of food material (February, 1938)
(a) 20-25 ml. of standard potato malt agar were poured into sterile Petri dishes and the fungi planted on that medium.
(b) About 50-60 ml. of standard potato malt agar were put into tubes 30 cm. long and 3.5 cm. wide and were then sterilized.

(78) Transfer of cultures to a second medium (June, 1937)
Fungi were grown for three weeks in tubes of liquefied potato malt agar at pH of 4. Each fungous mat was then washed aseptically in an individual Petri dish containing sterile distilled water. Following this the cultures were transferred to slants of standard potato malt agar.

(79) Water potato malt agar (June, 1937)
After the cultures had grown for 18 days on slants of potato malt agar 1 ml. of sterile distilled water was added aseptically to each tube.

(80) Pottery potato malt agar (June, 1937)
To each tube of standard potato malt agar were added a few pieces of clay pottery. The tubes were then sterilized. It was thought that the pottery might serve as a foundation for the formation of fruiting-bodies.

(81) Repeated subculturing (May, 1937 - July, 1937)
As soon as the culture had covered the surface of the slant of potato malt agar a transfer was made to a fresh tube of the same kind of medium. This was repeated a number of times. The final sub-culture was planted on potato malt agar in a Petri dish and allowed to grow for several weeks.

(82) Ultra violet (July, 1937)
This test was performed with only two species: Coprinus comatus and C. sp.
(a) Cultures of the fungi were grown on potato malt agar in Petri dishes for a week and were then exposed (with the Petri dish cover removed) to ultra violet rays from a mercury-vapor lamp. The exposures were made at a distance of about 8 cm. for 5 minute, 15 minute and 30 minute intervals. Immediately after the exposures the treated fungi were subcultured on fresh potato malt agar in Petri dishes.

(b) Freshly poured plates of potato malt agar were irradiated under the conditions outlined in part (a). The fungi were then transferred to these irradiated plates of medium from cultures that had not been treated.

(83) Heat treatment (August, 1937)
Cultures of a number of the fungi were grown in replicate for 14 days in tubes of slanted potato malt agar. One tube of each fungus was heated for 10 minutes in water held at the following temperatures: 40°C., 55°C., 70°C., 80°C. At the end of the 10-minute period the tubes were placed in cold water so that the temperature of the tubes was quickly reduced to room temperature. The cultures were then left at room temperature to continue growth.

(84) Scarified potato malt agar (August, 1937)
Slants of potato malt agar were scarified thoroughly by means of the transfer needle. The cultures were then planted on the medium.

(85) Scarified cultures (August, 1937)
Cultures were grown on slants of potato malt agar for 20 days and were then lacerated by means of the transfer needle either at one place or over the entire culture.

(86) Transfer of cultures to sterilized cultures (August, 1937)
Cultures that had been scarified were sterilized and the medium again slanted. Onto these slants were planted transfers of other species of mushrooms.

(87) Revival of cultures (October, 1937 and June, 1939)
Cultures were started in December, 1936, of a number of the fungi. They were kept in the laboratory until October, 1937. At that time about 8 ml. of potato malt agar were aseptically added to each dried culture in an attempt to revive the fungi and perhaps cause fructification.

Another series was started in November, 1937, and the medium was added to the tubes in June, 1939.

Results

The chief interest in this part of the study was the value of the various media for the production of typical fruiting-bodies. Of secondary interest were the variations in rate and manner of growth of the various species on the different media.

Since most of the media were in test-tubes when tested, only the relative amount of growth (good or poor as compared to the growth on standard potato malt agar) and the presence or absence of fruiting-bodies were usually recorded. If sporophores did develop, careful note was made of their appearance and growth.

The words "good" and "poor" as applied to the growth of the mycelium are very general terms to indicate the relative amount of growth of most of the species on a medium. In referring to the value of a medium for supporting the growth of the fungi all of the species that were tested were considered, although not all of them were identified.

The term "typical," when applied to fruiting-bodies, means that the sporophores looked very much like the wild specimens that the writer has seen. This resemblance was in regard to the gross morphology and color of the sporophore and the discharge of the spores.

It should be pointed out again that some of the media were tested with only a few species. A wider range of tests might have given different results. The media upon which only a few (less than ten) species were tried are as follows: phenol potato malt agar, ultra violet, straw potato malt extract and potatoes.

Growth of fungi on various media.

Growth of the fungi was good upon the following media: straw, straw malt-extract, straw potato malt, bread, milk malt agar, standard potato malt agar, twice standard potato malt agar, potato malt agar - Czapek, yeast extract potato malt agar, leaf mold potato malt agar, soil extract potato malt agar, phenol potato malt agar (except in the 100 and 50 and to some extent the 10 per-cent solutions), $CuSO_4$ potato malt agar, HCl potato malt agar, $KMnO_4$ potato malt agar, $AlCl_3$ potato malt agar, $ZnSO_4$ potato malt agar (except in the stronger concentrations), $AgNO_3$ (except in the stronger concentrations), crystal violet and brilliant green potato malt agars (except in the stronger concentrations), boric acid potato malt agar, urine potato malt agar, 3-indolepropionic acid potato malt agar, indole butyric acid potato malt agar, 3-indolebutyric acid treatment, Vitamin B_1 potato malt agar, theelin potato malt agar (the use of 25 mouse units per ml. of medium gave an increase in growth), additional light, alteration in pH from 3.6 to 8.4, increase of food material, transfer of cultures to a second medium, water potato malt agar, repeated subculturing, ultra-violet, heat treatment, revival of cultures (for some species; others did not revive).

Some media supported good growth of about one-half of the species and poor growth for the remainder of the species. These media are as follows: glucose agar, potato glucose agar, stronger potato glucose agar, glucose corn-meal agar, peptone potato glucose agar, yeast potato malt agar, dung extract malt agar, soil dung yeast potato malt agar, soil dung potato malt agar, yeast grass malt agar, live yeast

potato malt agar, mineral malt peptone agar, modification of meal starch sawdust medium, mineral potato malt agar, lactose milk agar, xylose potato agar, cellobiose potato agar, mannitol potato agar, modification of Etter's medium.

Growth was poor or entirely lacking on the following media: decayed wood, sawdust, horse-dung, grass, horse-dung straw, sand horse-dung decayed cottonwood, horse-dung agar, potatoes, malt peptone agar, dung potato malt agar, modification of Claussen's medium, modification of Leonian's agar medium, modification of Piefer, Humphrey and Acree's medium, modification of Tubeuf's medium, potato raffinose agar, modification of Barnes's medium, potato mannose agar, half standard potato malt agar, Duggar's medium, sucrose corn-starch agar, Czapek's glycerine agar, oatmeal agar, pottery potato malt agar, transfer of cultures to sterilized lacerated cultures, scarified potato malt agar.

Production of fruiting-bodies on various media.

Typical fruiting-bodies formed on the following media: Straw malt, straw potato malt, standard potato malt agar, twice standard potato malt agar, potato glucose agar, strong potato glucose agar, peptone potato glucose agar.

The modification of Etter's medium was good for the production of fruiting-bodies only of Coprinus quadrifidus. Dung-extract malt agar was good only for C. radians, C. semilantanus, Panaeolus campanulatus and Stropharia semiglobata.

Abnormal sporophores of Coprinus radians developed on potatoes.

These will be discussed later.

All of the media that have not been mentioned specifically in regard to the production of fruiting-bodies were very poor for this purpose. Either no sporophores of any species formed or the fruiting-bodies were quite small and abortive and did not develop.

The following species did not form fruiting-bodies on any of the media upon which they were grown: Coprinus atramentarius, C. comatus, C. plicatilis, Galera crispa, G. tenera, Psathyrella disseminata, and Psilocybe subviscida.

Stropharia semiglobata formed fruiting-bodies only on dung extract malt agar. Coprinus quadrifidus formed fruiting-bodies only on the modified Etter's medium. Coprinus fimetarius macrorhizus formed fruiting-bodies only on bread, straw potato malt and potatoes. Panaeolus campanulatus, P. retirugis and P. solidipes formed typical fruiting-bodies on only a few of the media (horse-dung, straw and straw potato malt extract), but small sporophores were formed on several other media.

Coprinus radiatus formed typical sporophores on several media.

Coprinus semilantanus produced typical fruiting-bodies on standard potato malt agar, twice standard potato malt agar, strong potato glucose agar, and on horse-dung (plate 1, fig. 1). On a number of other media this fungus formed only rudimentary fruiting-bodies.

Coprinus radians developed fruiting-bodies on a number of media and on many more formed small but fairly typical sporophores.

When Coprinus radians was grown on potatoes the first fruiting-bodies that developed were typical of the species except that only one of them shed spores and deliquesced. A little later, however, there

developed fruiting-bodies that were quite different from the normal specimens. They were as large as the more typical specimens but differed in that the caps were distinctly yellow with a brownish shade only at the top. The scales on the cap were slightly paler than in the normal specimens and seemed to be more pointed. The gills were crisped perpendicular to the long axis. Within the next few days more of these aberrant fruiting-bodies developed. In these the pilei were more brownish than yellow, but the gills were crisped. Moreover, the gills were dry and remained united side by side. The edge of the cap was white and was inrolled. The stipes of these as well as the first specimens were coarsely striate. Spores were not shed from any of these sporophores, and there was no deliquescence of the gills.

Tissue cultures from one of these unusual sporophores were placed on standard potato malt agar, straw malt and horse-dung straw. On all of these the fruiting-bodies that developed were nearly typical as regards color of the pileus, but they were abnormal in that the gills were crisped. Moreover, there was considerable variation in the formation of spores, their coloration, their discharge and the deliquescence of the gills. In no case was there a normal coloration and discharge of spores with accompanying deliquescence.

About one-half of the cap of each sporophore that formed on the potato malt agar had colorless or very pale-brown gills. This was due in some instances to failure of the spores to develop. In other fruiting-bodies the spores developed but were only weakly pigmented. Some of the fruiting-bodies discharged the spores that were most deeply colored, whereas others did not. The size of the spores was normal.

The sporophores that formed on the straw malt medium were like those on the potato malt agar except that the gills were more crisped, there was no discharge of spores and the deliquescence resulted in a somewhat dry and rather gummy mass. The spores were normal in size and shape but were either hyaline or pale brown. Germination of the spores was nearly 100 per-cent.

The mycelia of all of the transfers of these aberrant fungi were very light brown or whitish as compared with the rich brown color of the typical Coprinus radians.

Some of the cultures of Coprinus micaceus formed fruiting-bodies on standard potato malt agar and twice standard potato malt agar.

General observations.

It was observed in most of the species that did form fruiting-bodies that the sporophores that were first formed after the fungus was cultured were most typical. After that the sporophores were abnormal in some way, and this tendency to form rudimentary fruiting-bodies continued to become more pronounced until finally only primordia might be formed, or perhaps there would be no indication whatsoever of the formation of any fruiting-bodies.

Coprinus micaceus produced fruiting-bodies only immediately after it was cultured. The same was true of Stropharia semiglobata. The three species of Panaeolus soon lost the ability to form fruiting-bodies. Coprinus semilantanus gradually lost the ability of forming sporophores, but the culture of that species that had been left for over a year in the laboratory and was then revived immediately formed typical fruiting-bodies. Coprinus radians did not entirely lose the

ability to form fruiting-bodies, but the sporophores were not as large as they were when the fungus was first cultured. Coprinus quadrifidus did not form any fruiting-bodies until it was grown upon Etter's medium.

An interesting observation is the fact that in all of the fungi, with, perhaps, the exception of Coprinus radians, the production of fruiting-bodies was greater and more typical during the months of April to November.

On a number of the media the mycelia of at least some of the fungi behaved in some unusual manner, i.e. a change of color (from white to pink, yellow, brown or black) or a change in the habit of growth.

Discussion and Conclusions

Of the species that did not fruit, Coprinus atramentarius (47), C. comatus (50) and C. plicatilis (46) appear to be the only ones that have fruited for other workers. However, the following species that produced fruiting-bodies in pure culture seem not to have produced fruiting-bodies in culture before: Coprinus quadrifidus, C. semilantanus, Panaeolus retirugis, P. solidipes and Stropharia semiglobata.

One outstanding fact about the results is that so few media were valuable for the production of fruiting-bodies. The modification of Etter's medium was good for only one species, and dung-extract agar was of value for only four species. Seven other media were suitable for a number of fungi. This means that nearly 80 of the 87 media tried were of no value for the production of fruit-bodies.

An interesting feature is the fact that the media that were useful were straw, the sawdust-corn meal-corn starch-malt extract medium of Etter, dung-extract agar and an artificial medium consisting of potato extract and either malt extract or glucose. Except for the fact that the horse-dung was of so little value, these results are in agreement with what has been found by most workers.

The reasons for the lack of fruiting-bodies on more of the natural media are unknown. It may have been due to the particular fungi that were being used. It might be due to insufficient experimentation with each medium to determine the best conditions for its use. The possibility of the effect of light must not be overlooked, but the fungi probably were exposed to as much illumination as was necessary. It is quite possible that the poor results were due to a lack of abundant, readily-available food in a porous medium as emphasized by Etter (25) and implied by Humphrey (38) and Mounce (50).

The lack of fruiting-bodies on the artificial media, even on those on which the mycelia grew abundantly, indicates that much more must be learned concerning the chemical (sources of carbon and nitrogen, kind and amount of mineral salts needed) and physical factors needed for the production of fruiting-bodies. A glance at the results will show that the fungi grew well only on some of the natural media or on potato malt agar alone or potato malt agar to which were added various substances. Some of the artificial media allowed some of the fungi to grow rapidly, but most of these media were poor for growth and of no value for the formation of fruiting-bodies.

It has been mentioned before that a number of workers reported that malt extract was very good for the growth of the Agarics that were studied, and LaFuze (45) has shown that polysaccharides were best for the growth of certain wood-rotting fungi. The reasons for the value of malt extract for growth and fruit-body production are unknown.

It was thought that the use of poisonous or "growth-promoting" substances might induce the fungi to form sporophores. Failure to do so must mean that the production of fruiting-bodies depends upon more than the stimulus given by the substances as they were used in the experiment.

The reasons for the gradual decrease or entire loss of the ability to form fruiting-bodies can only be guessed at. The reasons for the decrease in fruit-body production during the winter months may be related to the length of day.

In any work of this nature it should be kept in mind that the fungi may be changing as the experiment proceeds. It has been shown that there may be changes in the fungi that are interpreted as due to changes in the genes. If the production of fruiting-bodies depends

upon one gene (as Gilmore (32) and Zattler (90) claim for certain species) or upon several (as Dickson (24) claims), and if these genes are capable of changing, then the problem is further complicated by the fact that one may be working with a culture that would not produce fruiting-bodies under any condition.

GENETICAL STUDIES

Review of literature

Kniep (42, 43, 44) had been studying for several years the formation of clamp-connections in certain Agarics, but Bensaude (5) was the first to discover in the Basidiomycetes the phenomenon that has been called "heterothallism." She found in Coprinus fimetarius Fr. that the dicaryon condition of the hyphae and the presence of clamp-connections could be brought about only when the mycelia derived from individual spores of a sporophore were grown together in certain combinations.

Many individuals in various parts of the world, chiefly in Europe and North America, have investigated the behavior of the mycelia derived from the basidiospores of many species of Hymenomycetes. Most of this work has been done with members of the Agaricaceae, Polyporaceae and Thelephoraceae.

A species, or the spore from a fruit-body of that species, will show one of two general types of behavior. It may be "homothallic" or "heterothallic." Most writers today appear to be using the term "heterothallic" in the sense that Nobles (55) used it. She says that this term means, " ... that the thalli which react together to produce a clamp-bearing mycelium contain nuclei of different genetic constitution as regards the factors which determine for or against this reaction." This definition, however, excludes those species that are not homothallic and that do not form clamp-connections (the heterothallic species without clamp-connections).

If the four spores on the typical basidium differ in only one such factor, there will be two groups of spores as regards their reactions when paired with one another. If, however, there are two such factors located on different chromosome pairs, then there would be the possibility

of the spores' falling into either two or four reaction groups (or "sexual phases" or "sexual groups"), depending upon the time of disjunction of the pairs of chromosomes during meiosis in the basidium.

The term "homothallic" now has come to mean that the mycelium that develops from the spore rather soon becomes dicaryon without being paired with any other mycelium; that is, pairing of two mycelia is unnecessary.

The definitions that have just been given are not the same as those originally applied to the terms. When "homothallism" was first used in the Phycomycetes it meant that all of the mycelia of a particular species were bisexual and any male and female nuclei could unite, whether they were from the same mycelium or not. "Heterothallism" was the term applied to those species some mycelia of which produced male organs and some female. In such species the male cells had to come from a mycelium other than the female.

When the Ascomycetes were studied it was found in some (as Neurospora and Pleurage) that all of the mycelia from the uninucleate spores had both male and female organs, as did the homothallic Phycomycetes, but that the male cells would function only when they were applied to the female organs of another mycelium. There is a similar situation in the Basidiomycetes except that there are no sex organs (unless one wishes to consider the oidia as male gametes).

These phenomena could have been designated as examples of "hermaphroditism with factors for self-sterility but mutual cross-fertility" or as "heterothallism" with a change in the meaning of the word "heterothallism." Most of the workers have chosen to use the

latter term.

As mentioned previously, the species that are "heterothallic" may show two groups of spores or mycelia. The members of each group, when paired among themselves on a medium, will not become dicaryon. However, when any member of one group is paired with any member of the other group the mycelium that develops will be dicaryon. Such a species is said to be bipolar. The two groups are designated as A and a. These two letters are also used to indicate the two allelomorphic genes that are assumed to exist in order to explain the results.

In other "heterothallic" species there are four groups of spores. In normal cases the members of any one group will give rise to a dicaryon mycelium only when they are paired with the members of one of the other groups. A species the spores of which behave in this manner should be designated as quadripolar. It is believed that the quadripolar species have two different pairs of factors located on two pairs of chromosomes. The four groups of spores, and also the genes, are labeled as AB, Ab, aB and ab.

Disjunction of both pairs of factors in the second division of meiosis could result in either bipolar or quadripolar results. Disjunction of one pair in the first division and of the other pair in the second division would give only quadripolar results. Disjunction of both pairs in the first division would result in bipolarity. Newton (53), Hanna (33), Quintanilha (60), Bohn (7) and Brunswik (16) (and Kniep, according to the latter) have studied the distribution of the factors by isolation of the tetrads of spores of individual basidia.

During the first years of study of these phenomena the investigators emphasized the importance of the production of fruiting-bodies in their pairing of monosporous mycelia. If a sporophore developed from a particular pairing, the two mycelia were considered to be "fertile." However, it soon became apparent that this criterion could not be considered in at least some cases because monocaryon mycelia of some species produce sporophores and because not all dicaryon mycelia would form fruiting-bodies. At present, the emphasis is placed upon the presence of clamp-connections as indicative of the dicaryon state of the mycelium. In almost all cases this is satisfactory because the clamp-connections appear only when the cells have two nuclei. Buller (18) and Newton (53) have also found that a branch of a dicaryon hypha forms a much more acute angle than does a branch of a monocaryon hypha. It has also been observed that the dicaryon mycelia usually grow more rapidly than do the monocaryon, and sometimes the dicaryon mycelium assumes an appearance quite distinct from that of the monocaryon. There are instances in which oidia are produced by the monocaryon hyphae but not by the dicaryon (Buller (18), Hanna (33), Newton (53) and Vandendries (75)). These characters have been used to identify the diploidized (dicaryon) mycelium. Some workers, as Quintanilha (60) have attacked the problem by staining the nuclei.

The development of the clamp-connections and the conjugate divisions have been worked out by Bensaude (5), Buller (18), Kniep (42, 43, 44), Noble (54) and Quintanilha (60).

Discussions of the manner in which diploidization is accomplished have been given by Buller (18), Noble (54) and Quintanilha (60).

In some homothallic species the hyphae develop clamp-connections. In others no such structures are produced. In the latter case most workers have assumed that a species is homothallic if the mycelia do not develop clamp-connections when grown in contact with a number of different mycelia. Some have stained the nuclei in order to be sure that there are two nuclei in each cell of the hyphae.

Some of the workers in this field are the following: Arnold (2), Bohn (7), Brodie (12, 13, 14), Brunswik (15, 16), Buller (17, 18, 19), Chow (21), Dickson (22, 23, 24), Gilmore (32), Hanna (33, 34, 35), Heldmaier (36), Mounce (49, 50, 51), Newton (53), Oort (56), Quintanilha (59, 60), Smith and Brodie (66), Vandendries (69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89) and Zattler (90).

The exceptions to the scheme as outlined are numerous. That an apparently normally homothallic species may have a few spores that are not homothallic and which are said to be heterothallic has been shown by Sass (62) to be the case in a two-spored form of Coprinus ephemerus.

There are a number of fungi that have been recorded as homothallic by one worker and heterothallic by another: Mounce (49), Vandendries (73). The sudden change of a monocaryon mycelium to the dicaryon state has been reported by several workers: Chow (21), Dickson (22), Hanna (33, 34), Newton (53) and Vandendries (72, 78).

Irregularities in the pairing reaction between spores of different sexual phases have been found by various investigators for several species. Some of these workers are the following: Brunswik (15),

Heldmaier (36), Oort (56) and Vandendries (78).

Some workers have reported that the monosporous mycelia of certain species have remained the same in their reaction capacities for many years: Hanna (34) and Mounce and Macrae (51). Vandendries (84), however, has reported a number of species in which the mycelia changed their reaction capacities, and any of the cases of the change of a monocaryon mycelium to a dicaryon would come under this class of abnormalities.

In quadripolar species the only combinations that will result in the diploidization of the mycelium and the formation of true clamp-connections are as follows: AB x ab and Ab x aB. Buller (18), however, found that certain dicaryon mycelia would diploidize monocaryon mycelia which, on theoretical grounds, they should not diploidize (as "(AB) x (Ab) ≠ (aB) or (Ab) x (AB) ≠ (ab)"). He called these "illegitimate combinations" and considered that they were at least quite similar to the "Durchbrechungskopulationen" that Brunswik (15) and Oort (56) had found earlier.

Quintanilha (60), working with Coprinus fimetarius, was the first to study these illegitimate combinations extensively and to use cytological methods in his investigation. He found that diploidization occurred but that after the dicaryon condition was established it persisted only in the apical cell. This was due to the fact that the clamp-connection never fused with the penultimate cell. The result was that one of the daughter nuclei produced by the conjugate division of the two nuclei in the terminal cell was isolated from the hypha, and the penultimate cell came to have only one nucleus. The formation of these false clamp-connections occurred only when the following

mycelia were paired: AB x aB and Ab x ab. The formation of false clamp-connections seems to depend upon the possession by the two mycelia of factors B or b in common at the same time that the factors A and a are different. Quintanilha (60) has also reported a mutation in which the monocaryon mycelium bears false clamp-connections and has two nuclei in the apical cell.

In 1933 Vandendries and Brodie (87) reported a phenomenon that they called "barrage sexuel" and which since then has usually been called "barrage." They found that in certain combinations of the mycelia of quadripolar species the aerial hyphae of the two mycelia would grow towards each other until a space of only 3-5 mm. separated them. The hyphae would then turn backward and would not occupy the space between the two masses of mycelia. The submerged mycelia would approach each other until a space of about one mm. separated them. The force causing this mutual repulsion was not stopped by lead, paraffin, celluloid, mica, glass and a number of other substances unless they were quite thick. Vandendries (86) later reported that this barrage would not occur if the pairing was done in a closed container. Because strips of these materials placed between the two mycelia merely decreased the intensity of the reaction and because a water-tight partition of glass had no effect, the authors suggested that the reaction might be due to some sort of radiation between the two mycelia.

The barrage phenomenon was found to appear when the mycelia ab x ab' or the mycelia a'b x a'b' were paired. Brodie (14) later found that the barrage also appeared temporarily between the mycelia that would diploidize each other (as AB x ab and Ab x aB). Apparently barrage appears in some species whenever recently isolated mycelia that

are genetically different for B and b are paired. Many workers report that they do not find this barrage.

Another interesting feature that has been found to exist in almost every species that has been studied with this point in mind is the existence of what have been called "geographic races." Bauch (4), working with Ustilago longissima, has found that within a limited area it is likely that various collections will be partially compatible. This appears to be due to slight changes in one or both of the allelomorphic genes involved. Bauch has found that there are a number of multiple allelomorphs of each of the two allelomorphic genes that were first found. As the distance between the collections is increased the collections are likely to be completely compatible.

On the basis of his work and that of others, Vandendries (81) formulated a law to the effect that all of the spores of a fruiting-body of a heterothallic species would form clamp-connections when grown as mycelia in contact with the mycelia from spores of another fruit-body, if the two sporophores were collected in the same general locality. However, if the spores came from fruiting-bodies that were widely separated (as America and Europe), then the mycelia would remain monocaryon. This reaction has been used by Buller and Newton (19) to identify a certain Coprinus.

In spite of the fact that sometimes the mycelia from one sporophore will not form clamp-connections when paired with mycelia from another fruit-body that was collected in the immediate vicinity as has been found by Vandendries (79, 89), and in spite of the fact that Mounce and Macrae (51) and Vandendries (77, 79, 89) found that mycelia from widely-separated fruiting-bodies sometimes did form clamp-connections, this rule is true for most of the species that have been studied.

This behavior of the mycelia brings up the question of how to explain all of the divergent results that have been obtained. At first, the factors A, a, B and b were called sexual factors.

According to Butler (20), Kniep later adopted the term "copulation factors" and assumed that the presence of different copulation factors in the two mycelia was necessary for the diploidization process to occur.

Brunswik, according to Butler (20), attempted to explain results by assuming that homothallic and heterothallic species had the same genotype except for the introduction or appearance in the heterothallic species of one or two pairs of self-sterility factors. Quintanilha (59) has recently re-emphasized this theory.

Hartmann, according to Bose (10), thinks that every cell of the mycelium is potentially sexual and that the normally dominant sex is determined by some internal factor. However, if this factor is weak, then some external factor, such as the proximity of another gamete of stronger sexual tendency, will determine the behavior. This theory would explain the results as being due to the relative sexuality of the two mycelia concerned.

In all of these theories it is assumed that mutations of the various factors concerned would explain the abnormal (or unexpected) reactions that sometimes occur, the changes in reaction-capacity of some mycelia after a length of time, the change from the monocaryon to the dicaryon state and the existence of geographic races.

Probably most workers have explained their results by the assumption of the existence of multiple allelomorphs. If the genetic constitution of a mycelium is very similar to or very unlike that of another mycelium (whether it be from the same fruiting-body or from one some distance away), clamp-connections will not form. However, if the

mycelia are different but not too dissimilar, then clamp-connections will form when the mycelia are paired.

A suggestion made by Vandendries (81) on the basis of his work with Coprinus micaceus seems worthy of consideration in attempting to explain the geographic races. He assumes the existence of at least two hereditary, genetically dominant factors within the members of a species. The spores must possess the same factor before it is possible for them to form clamp-connections. Then, and only then, will the ordinary factors (A, a, B and b) come into play. However, if the mycelia possessed different dominant factors, it would be impossible for the two to form clamps.

In order to explain the partially fertile (compatible) reactions between certain geographic races of Coprinus micaceus (a suggestion which would also be applicable to the results obtained by Mounce and Macrae (51) with Fomes pinicola) Vandendries further assumed that the two or more dominant factors (which may be called X and Y) could mutate in such a manner that they would gradually grade into each other. In this group of specimens possessing a factor that is neither X nor Y clamp-connections would form if the mycelia were of the proper genetic constitution.

Chow (21) seemed to think that the idea of hermaphroditic self-sterility elaborated by Ames (1) could be properly applied to the Basidiomycetes. According to this theory the cells of all mycelia are alike in respect to their "sexual" factors but possess factors that make each mycelium self-sterile but cross-fertile (with another particular mycelium).

It is customary to think of the basidiospores in the Agarics when the term "spore" is used, but it has been found that there are other spores in a great many of the mushrooms. They are classified as oidia, conidia and chlamydospores. There are numerous references in the literature to their occurrence and method of formation (Bensaude (5), Brodie (12), Brunswik (15), Gilmore (32), Martens (48) and Vandendries and Martens (88)).

It is quite evident, however, that there has been confusion in the literature because not all of the workers have used the term "oidium" in the same sense. This has been pointed out in a recent paper by Brodie (13) who characterizes the oidia as " ... short, hyaline, rod-shaped cells with thin walls and dense protoplasmic contents" which are " ... formed by the breaking up of mycelium into segments" usually without the formation of transverse walls and which are produced endogenously, not exogenously as the conidia are.

Brodie (13) believes that the oidia are most important as a means of diploidization of monocaryon mycelium but admits that in some cases they may serve as a means of vegetatively propagating the fungus.

From a review of the literature Brodie (13) came to the conclusion that monocaryon mycelia of most heterothallic species develop oidia but that, as far as is known, homothallic species do not produce oidia. He also decided that dicaryon mycelia rarely produce oidia and then the oidia are often uninucleate.

The occurrence of uninucleate conidia on monocaryon mycelia and usually dicaryon conidia on dicaryon mycelia have been reported by Kaufert (40) for Pleurotus corticatus and by Nobles (55) for Peniophora allescheri. Probably many of the references in the literature to

oidia should be considered as references to conidia.

Usually the conidia are borne on short sterigma-like branches, but Nobles (55) reported an oedocephaloid type of conidiophore in Peniophora allescheri, and Kaufert (40) reported coremia for Pleurotus corticatus.

It has been found by many of the persons listed in the preceding paragraphs that the asexual spores developed on either the monocaryon or dicaryon mycelia behave in the same manner as did the parent hyphae as regard the pairing reaction.

There have been few attempts to hybridize species or varieties of Agarics. Vandendries (84) paired monocaryon mycelia of Hypholoma Candolleanum with H. velantinum; Pholiota aurivella with P. mutabilis and Pleurotus columbinus with P. nidulans. No clamp-connections were formed. However, crosses (70) between three unnamed species of Panaeolus formed a dicaryon mycelium and also a rudimentary sporophore. A cross by the same worker (86) between the normal black form of Omphalia maura and an albino form that he discovered developed clamp-connections but no sporophore.

Brunswik (15) crossed Panaeolus fimicola with P. campanulatus. In one of the pairings he found clamp-connections.

Arnold (2) paired Collybia cirrata, C. cirrata var. cookei and C. tuberosa in all combinations but did not find clamp-connections.

Materials and methods

The species that were used in this phase of the study are as follows[1]: Anellaria separata (Fr.) Karst., Coprinus fimetarius macrorhizus Fr., C. micaceus Fr., C. plicatilis Fr., C. radians Fr., C. semilantanus Peck, Naucoria semiorbicularis (Fr.) Quelét, Panaeolus papilionaceus (Fr.) Quelét, P. retirugis (Fr.) Quelét, Psathyrella disseminata (Fr.) Quelét, Psilocybe foenisecii (Fr.) Quelét, P. subviscida Peck.

The culture of Coprinus radians was a tissue culture from a specimen secured on the campus of Northwestern University, Evanston, Illinois, in June, 1935. Monosporous cultures of Panaeolus papilionaceus and of P. retirugis and some of Coprinus micaceus were secured from the Centraalbureau voor Schimmelcultures, Baarn, Holland. The two monosporous strains of opposite sexual phase of Panaeolus papilionaceus were sent to Baarn by R. Vandendries and had been obtained in Vienna in 1930. The two monospore cultures of P. retirugis were sent to Baarn in 1934 from Paris by A. Quintanilha. The two monospore cultures of Coprinus micaceus were sent to Baarn in 1927 from Belgium by Vandendries.

The other species were collected either in the city of East Lansing, Michigan, or within a mile of that place.

The monospore cultures of Coprinus radians and C. semilantanus were secured through the use of fruiting-bodies that appeared in test tubes or flasks of media in the laboratory. Each of these had been in culture for about two and one-half years when the monospore cultures were obtained. Single-spore cultures of the other species were obtained from

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[1] The species named by Peck as well as Coprinus fimetarius macrorhizus are listed as they are given by Kauffman (41). For the correct author citation of the others the works of the following men were consulted: Fries (26, 27, 28, 29, 30), Gillet (31), Karsten (39) and Quelét (58).

the specimens that were collected.

The method of obtaining the spore cultures was essentially the same as the method employed by Barnett (3). A small portion of the pileus was fastened, gills down, to the inner surface of the lid of a Petri dish by means of glue. The lid was placed over the lower part of the dish that contained hardened potato malt agar and was rotated. There was thus an even distribution of the spores that were discharged from the basidia. It could be determined by examination of the surface of the medium through the agar from the bottom of the dish by means of a binocular microscope whether or not enough spores had fallen. If there were sufficient spores on the agar, the Petri dish was set aside in a moist chamber until, by means of the microscope, one could see that the spores had begun to germinate.

The removal of the germinating spores to tubes of the medium was accomplished in the following manner. The lid of the Petri dish was removed and the spores located under high power magnification of the dissecting microscope. While observing the spores through the microscope, a spore and a small amount of surrounding agar were removed on the sharpened tip of an ordinary sewing needle that was held in the end of a short holder for transfer needles. The tip of the needle was then gently stroked along the surface of an agar slant. At least 30 monospore isolations were made for each species. The needle was never flamed between separate spore isolation but was merely cleaned with a piece of lens paper. Sometimes even this was omitted.

For work of such a nature that one does not need to know what spores came from the same basidium this method is quick, easy and efficient. The amount of contamination is practically zero. There

is only one disadvantage that the writer encountered, and it may not have been due to the method itself. Sometimes only a very small number of the young hyphae that were transferred will continue to grow in the tubes of medium. If one does not remove a great many more spores than he intends to use, he may not have as many cultures as would be desirable.

A different accession number was given to each sporophore from which monospore isolations were made. The various single-spore cultures from any fruiting-body were designated as spores 1, 2, 3, etc. by placing that number under a horizontal line above which was the accession number.

The mycelium of each monospore culture was examined to determine whether oidia were produced, whether the hyphae bore clamp-connections and whether there were any features of the hyphae that would distinguish them from hyphae of other species. These observations are included in the results for each species.

The medium used almost exclusively for spore germination was the standard potato malt agar. This was suitable for many of the species whose spores did germinate, but the spores of some species would not germinate on this medium. In those cases the following modifications were tried: dung-extract agar, acidified (pH 4) potato malt agar, potato malt agar to which a few drops of the juice of a lemon or a third of a drop of 3-indolebutyric acid had been added, and the placing of a small amount of the mycelium (tissue culture) of the fungus concerned on the plates near the spores.

The monospore cultures were maintained on potato malt agar, and all of the pairings of these cultures were made on that medium. Before pairing of the cultures they were retransferred to different Petri

dishes and allowed to grow for more than a week in order to ensure that they would be growing vigorously. The pairings were made in test-tubes using transfers from the edges of the colonies on the Petri dishes.

The mated mycelia were allowed to grow for from two to four weeks before they were examined for the presence of clamp-connections. In making the examination a small amount of mycelium was removed from the edge of the growth in the tube at a position that would correspond to a line drawn midway between the two paired cultures. The mycelium was studied under the high power or oil immersion lens of the microscope. In most species the hyphae were so wide that the use of the high power lens was sufficient.

In the case of one fungus (Coprinus plicatilis) the mycelia that appeared to have clamp-connections were studied further by growing the hyphae on a nutrient medium in a manner suggested by Newcomer and KenKnight[1]. The method as used by the writer was as follows: a nutrient medium was made up using the formula outlined below:

glucose	1.0 gm.
agar	1.2 gm.
gelatin	2.0 gm.
distilled water	100 ml.

The ingredients were dissolved in the water, and the medium was then sterilized at 15 pounds pressure for 30 minutes.

Into the lower half of a number of Petri dishes was placed one watch glass and, resting on top of the glass, one microscope slide. The Petri dishes were then sterilized.

About two ml. of sterile distilled water were aseptically placed in each watch glass under the microscope slide to furnish a humid

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[1] Their paper is in press.

atmosphere. A small drop of the liquefied medium was smeared aseptically on the slide by means of a bent glass rod that had been dipped in alcohol and then flamed. When this had solidified a bit of the mycelium of a culture was planted on this medium. The dishes were kept in a moist chamber for two to three days before examination. In order to study the cultures a drop of water was placed on the mycelium and the cover slip placed on top of the water.

In recording the results of the search for clamp-connections in any of the fungi a - sign means that none were found in that particular mating. A + sign means that clamp-connections were found.

In most of the + reactions the clamp-connections were so large and so numerous that a brief survey of the preparation on the slide was sufficient. When the clamps were small or very infrequent it was necessary to spend several minutes in study of the hyphae. After all of the matings for a species had been studied and the reactions recorded, a table was prepared to show how many sexual phases there were and to which one each of the monospore cultures belonged. If the reaction of any particular pairing did not agree with the theoretical reaction, the pairing was examined again very carefully. In the case of Psilocybe foenisecii and P. subviscida the writer failed to reexamine doubtful results.

The term "conidium" has been used when definite conidiophores could be found. The term "oidium" was used when the asexual spores were small, rod-shaped, hyaline and with dense protoplasm, although in no case did the author see just how they were formed.

Results

Spore germination

The spores of the following species germinated in 12 to 48 hours on standard potato malt agar: Coprinus fimetarius macrorhizus, C. micaceus, C. plicatilis, C. quadrifidus (one collection), C. radians, C. semilantanus, Naucoria semiorbicularis, Psathyrella disseminata, Psilocybe foenisecii, and P. subviscida (one collection). The germinated spores of C. quadrifidus did not survive the transfer to tubes of the medium. Spores of Anellaria separata germinated on dung-extract agar.

Spores of the following species did not germinate on any of the media that were used: Coprinus quadrifidus (several collections), C. radiatus, Galera crispa, G. tenera, Lepiota Friesii Lasch., Panaeolus campanulatus, Psilocybe foenisecii (several collections) and Stropharia semiglobata (several collections).

Polarity

Anellaria separata

In June, 1938, the monospore cultures of this species (culture number 124) were secured. The mycelium in all of the cultures was appressed, and in some this was so extreme that the hyphae appeared to be water-soaked. Most of the mycelia were white, but a few were brownish. Growth was rather vigorous. The larger hyphae were 4 - 5 μ in diameter. On some of the hyphae there were large, chlamydospore-like cells (plate I, fig. 3). No oidia or conidia were found.

The results of the pairing are shown in Table 1. Clamp-connections were large and numerous. They were never seen on the enlarged cells or on the thick hyphae that formed the chlamydospore-like cells. No fruiting bodies developed, and oidia did not form on the dicaryon

cultures. Attempts were made to demonstrate barrage by growing a few of the proper mycelia in Petri dishes, but no aversion was observed.

Table 1. Results of pairing of eighteen monosporous cultures of Anellaria separata (culture 124). Paired March, 1939

		AB			Ab						aB					ab			
		1	12	29	6	7	8	19	27	28	11	13	17	25	30	9	10	14	23
AB	1															+	+	+	+
	12															+	+	+	+
	29															+	+	+	+
Ab	6										+	+	+	+	+				
	7										+	+	+	+	+				
	8										+	+	+	+	+				
	19										+	+	+	+	+				
	27										+	+	+	+	+				
	28										+	+	+	+	+				
aB	11				+	+	+	+	+	+									
	13				+	+	+	+	+	+									
	17				+	+	+	+	+	+									
	25				+	+	+	+	+	+									
	30				+	+	+	+	+	+									
ab	9	+	+	+															
	10	+	+	+															
	14	+	+	+															
	23	+	+	+															

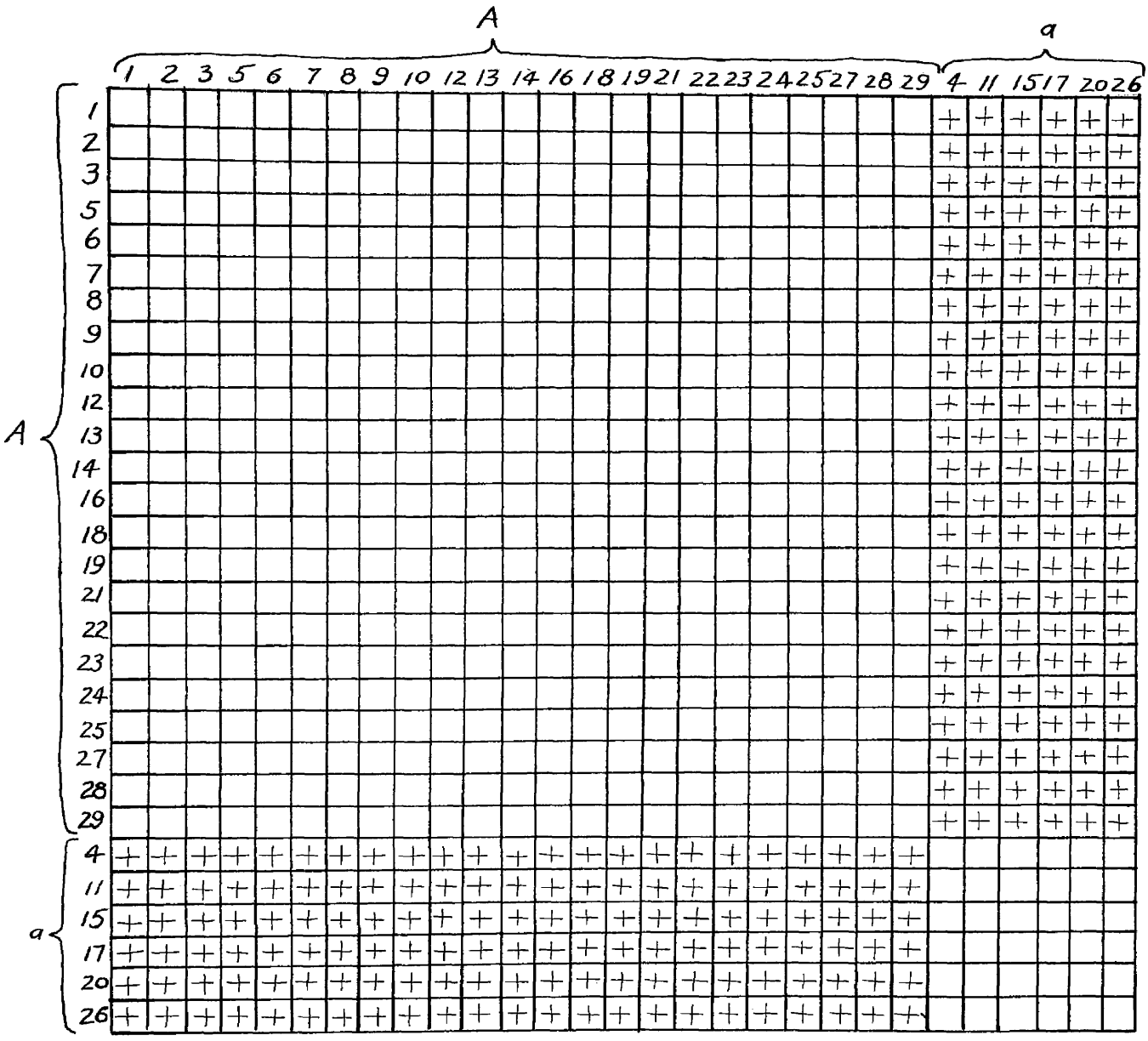
Coprinus fimetarius macrorhizus

Monospore cultures of this species (culture number 150) were obtained in August, 1938. All of the cultures grew very vigorously with the production of a great amount of white, aerial mycelium. In all of the mycelia many, very small (50 - 100 μ in width) sclerotia were found. The hyphae were 4 - 5 μ in width. Abundant oidia were produced (plate I, fig. 4).

The results of the pairing appear in Table 2.

On about one-half of the dicaryon mycelia typical fruiting-bodies developed. Rudimentary sporophores formed on a number of other dicaryon and on many monocaryon pairings.

Table 2. Results of pairing of twenty-nine monosporous cultures of Coprinus fimetarius macrorhizus (culture 150). Paired in November, 1938.



Coprinus micaceus

Monospore cultures of this species were secured from several sources. Cultures designated as 16a were obtained from a fruiting-body that developed near the laboratory in September, 1936. Cultures numbered 127 were obtained from a sporophore that developed about 300 yards from where the sporophore for culture 16a appeared. Cultures of this sporophore were secured July, 1938. Culture 82 was a haploid strain that was secured from Centraalbureau voor Schimmelcultures in October, 1938. Culture 83 was the opposite sexual strain and was secured also from the Bureau. Both cultures had been sent to the Centraalbureau in 1927 from Belgium by Vandendries.

The mycelia of culture 16a when first isolated grew vigorously and produced a dense, white mass of hyphae. Oidia similar to those of C. fimetarius macrorhizus were produced.

Some of the cultures were paired soon after they were obtained. Clamp-connections formed on some of the pairings, but no record was kept of this experiment. On one of the dicaryon mycelia typical sporophores were formed (plate I, fig. 2).

When the cultures were used for pairing reactions in April, 1939, they had changed in appearance and in action. Most of the cultures were producing a pink color along the edge of the slant and to some extent in the mycelium that did not touch the sides of the container. At this time no oidia were seen. However, there were present in great numbers some elliptical, somewhat pointed, hyaline spores (plate I, fig. 7). These conidia developed on many of the hyphae at the tips of usually club-shaped, sometimes constricted and often branching conidiophores (plate I, figs. 5 and 6). These conidia were present on all of

the monocaryon mycelia. Each culture also caused the formation of some substance that had an odor very similar to that of the flesh of ripe peaches.

The presence of the color, the presence of a new and quite different type of asexual spore, the production of a distinctive odor and the pairing reactions would indicate that the cultures had become contaminated. However, the fact that the color did not develop in all of the cultures; that some of the cultures were powdery on the surface and that some were not and the fact that contamination of all of the nine cultures by the same fungus does not seem likely might indicate that the differences in the cultures may not have been due to contamination but to a change in the fungus itself.

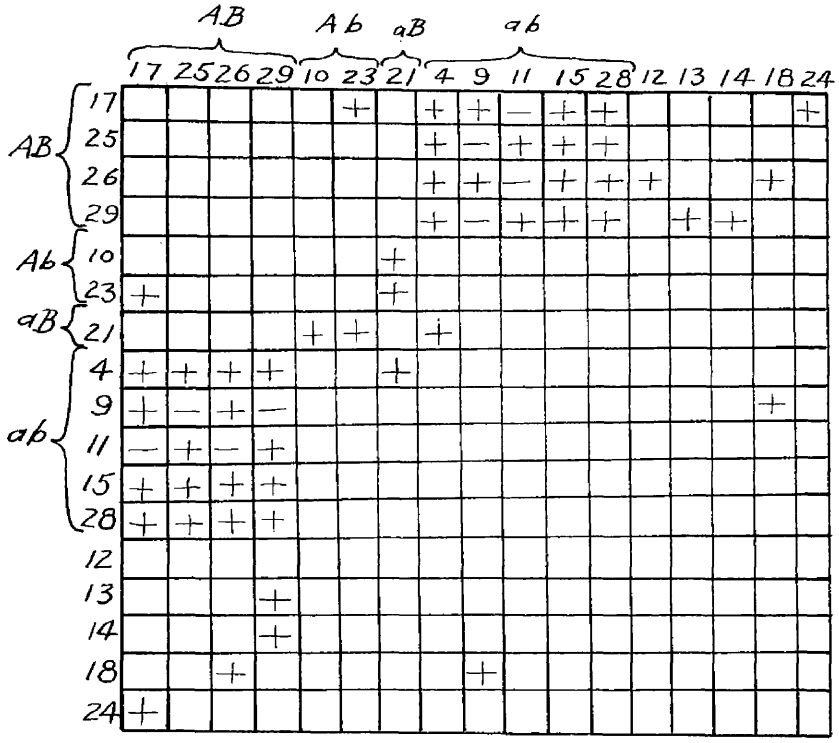
Dilution plates were poured of all of the cultures in an attempt to obtain cultures that had been derived from single spores. Most of these cultures that came (theoretically) from single spores also developed a pink color and all produced the spores and the "fruity" odor.

The nine monospore cultures of 16a were paired in April, 1939, in all possible combinations. In no pairing did clamp-connections develop.

Monosporous cultures of culture 127 grew vigorously with the production of a dense mass of white, aerial hyphae. In many of the cultures very slender, brown, rhizomorph-like strands of hyphae were developed where the mycelium came into contact with the sides of the test-tube. The rather wide hyphae developed oidia in abundance.

The results of the pairing of the mycelia are shown in Table 3. In many cases the dicaryon hyphae were wider than the monocaryon, and the clamps could be easily seen. In others it was difficult to find the clamp-connections because they were infrequent and were small. No fruiting-bodies formed.

Table 3. Results of pairing of monosporous cultures of C. micaceus (culture 127). Paired April, 1939.



Culture 82 looked very much like cultures of 127 in growth and color. Culture 83, however, did not grow as rapidly, and it showed a profusion of wide hyphae that had yellowish contents. There was some development of oidia on both cultures.

Cultures 82 and 83 were paired in April, 1939. No clamp-connections were found on the mycelium that developed.

Coprinus plicatilis

Monospore cultures of this species (culture 109) were obtained in June, 1938. The mycelia were appressed and white, and they grew rapidly. Chlamydospore-like cells were found on some of the hyphae. On the

mycelia were found some structures that resembled oidia (plate II, fig. 2) and others that resembled conidia (plate II, fig. 2).

Sixteen cultures that were devoid of clamp-connections were crossed in all combinations. At first examination it was thought that the species was bipolar, but a careful study of the structures that looked like clamp-connections revealed that in some of the apparently dicaryoh hyphae the short branch that turned backward from the tip cell in the formation of a clamp did not fuse with the main hypha. In order to study this process better the mycelia that appeared to have clamp-connections were grown on agar that had been placed on sterile glass slides (as described in the discussion of methods). By this method it could clearly be seen what mycelia had the typical clamp-connections (plate I, fig. 9) and what ones had the incomplete or false clamps (plate I, fig. 10) characteristic in some species of illegitimate crosses.

The results of the pairing of the mycelia are given in Table 4. Since the illegitimate combinations could be identified by the presence of the false clamp-connections, it was possible to assign each culture to a particular sexual phase in the manner that Quintanilha (60) has done in C. fimetarius. No fruiting-bodies developed.

Table 4. Results of pairing sixteen monosporous cultures of C. plicatilis (culture 109). Paired in March, 1939. ⊥ indicates an illegitimate cross.

		AB				Ab		aB					ab				
		4	5	14	28	7	15	6	16	21	24	29	9	17	19	20	26
AB	4							⊥	⊥	⊥	⊥	⊥	+	+	+	+	+
	5							⊥	⊥	⊥	⊥	⊥	+	+	+	+	+
	14							⊥	⊥	⊥	⊥	⊥	+	+	+	+	+
	28							⊥	⊥	⊥	⊥	⊥	+	+	+	+	+
Ab	7							+	+	+	+	+	⊥	⊥	⊥	⊥	⊥
	15							+	+	+	+	+	⊥	⊥	⊥	⊥	⊥
aB	6	⊥	⊥	⊥	⊥	+	+										
	16	⊥	⊥	⊥	⊥	+	+										
	21	⊥	⊥	⊥	⊥	+	+										
	24	⊥	⊥	⊥	⊥	+	+										
	29	⊥	⊥	⊥	⊥	+	+										
ab	9	+	+	+	+	⊥	⊥										
	17	+	+	+	+	⊥	⊥										
	19	+	+	+	+	⊥	⊥										
	20	+	+	+	+	⊥	⊥										
	26	+	+	+	+	⊥	⊥										

Coprinus radians

Monosporous cultures of this species (culture 10) were secured in December, 1937, from a fruiting-body that developed on a tissue culture of the fungus. The spores began to germinate in seven hours. Most of the cultures were appressed with only a little aerial mycelium of powdery appearance. The cultures were whitish or very pale brown. The powdery appearance was due to the presence of many conidia (plate II, fig. 3). No fruiting-bodies developed on the mycelia.

A few of the cultures possessed much aerial mycelium that was dark brown in color and that grew more rapidly than did the cultures mentioned above. On some of these mycelia fruiting-bodies developed. The

resemblance of these cultures to the tissue cultures used in the first part of the experiments was so great that it seems probable that these cultures just described were dicaryon. The presence of clamps could not be considered in settling the question because the tissue cultures did not show any clamp-connections.

Monosporous cultures were paired in all possible combinations. The examination of the pairings did not reveal any clamp-connections.

Spores from the aberrant fruit-body (culture number 69a) of this species that developed on the potatoes and which has been discussed previously were germinated. The appearance of the mycelia was quite similar to that of the monosporous mycelia of culture 10.

The monosporous mycelia of culture 69a were also paired in all combinations, but no clamp-connections were found.

Coprinus semilantanus

Monospore cultures of this species (culture 21 Rev.) were obtained in March, 1939, from a fruiting-body that developed in pure culture. Two of the cultures formed a deeply wrinkled or convoluted, yellowish colony that grew slowly. The colonies could easily be disorganized by disturbance with the transfer needle; the colonies crumbled apart. The mycelium above the surface of the agar consisted of chlamydospore-like cells; the cells are illustrated by fig. 4 on plate II. The hyphae were filled with dense protoplasm. The hyphae in the substratum were pale but contained very large granules.

Two other cultures began to develop in the same manner as just described but soon formed one or more small patches of typical, white, aerial mycelium.

The remaining ten cultures began as did the others but very quickly formed a large mass of white, aerial mycelium over the small, rugose

colony. Further growth was limited to this fluffy mycelium.

Oidia similar to those of C. fimetarius macrorhizus were produced on the aerial mycelium.

The results of the pairing are given in Table 5. The clamp-connections were in all cases quite large and distinct. They were never found on the wide hyphae that made up the rugose colonies that were used in some of the pairings. No oidia were produced on the dicaryon mycelium. A few small but fairly typical fruiting-bodies developed on some of the dicaryon mycelia, but fruiting-body primordia were formed on both the dicaryon and monocaryon mycelia that were examined in the pairings.

Table 5. Results of pairing of fourteen monosporous cultures of C. semilantanus (culture 21 Rev.) Paired May, 1939.

		AB						Ab		aB			ab		
		3	5	7	8	9	13	1	2	4	6	11	10	12	14
AB	3											+	−	+	+
	5												+	+	+
	7												+	+	+
	8												+	+	+
	9												+	+	+
	13												+	+	+
Ab	1									+	+	+			
	2									−	+	+		+	
aB	4							+	−						
	6							+	+						
	11	+						+	+						
ab	10	−	+	+	+	+	+								
	12	+	+	+	+	+	+		+						
	14	+	+	+	+	+	+								

Naucoria semiorbicularis

Monosporous cultures of this species were secured from two fruiting-bodies. One of these (culture 106) sporophores was found on a lawn in June, 1938. The other (culture 112) was obtained in June, 1938, in a meadow about one and one-half miles from the place of collection of culture number 106.

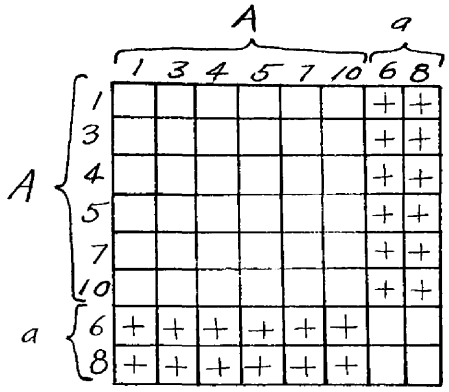
The mycelia were appressed but formed a thin felt. There was very little aerial mycelium. All of the mycelia grew vigorously. No oidia were seen, but chlamydospore-like cells were present.

The results of the pairings are given in Tables 6 and 7.

Table 6. Results of pairing of seventeen monosporous mycelia of N. semiorbicularis (culture 106). Paired in January, 1939.

		A									a							
		4	12	15	16	20	23	24	26	28	5	11	14	17	18	22	25	27
A	4										+	+	+	+	+	+	+	+
	12										+	+	+	+	+	+	+	+
	15										+	+	+	+	+	+	+	+
	16										+	+	+	+	+	+	+	+
	20										+	+	+	+	+	+	+	+
	23										+	+	+	+	+	+	+	+
	24										+	+	+	+	+	+	+	+
	26										+	+	+	+	+	+	+	+
	28										+	+	+	+	+	+	+	+
a	5	+	+	+	+	+	+	+	+	+								
	11	+	+	+	+	+	+	+	+	+								
	14	+	+	+	+	+	+	+	+	+								
	17	+	+	+	+	+	+	+	+	+								
	18	+	+	+	+	+	+	+	+	+								
	22	+	+	+	+	+	+	+	+	+								
	25	+	+	+	+	+	+	+	+	+								
	27	+	+	+	+	+	+	+	+	+								

Table 7. Results of pairing eight monosporous cultures of N. semiorbicularis (culture 112). Paired January, 1939.



Psathyrella disseminata

The monospore cultures of this species (culture 138) were obtained in July, 1938, at the base of a stump in a wooded area. All of the mycelia were white, and all of the mycelia grew very vigorously. When the cultures were grown on agar in Petri dishes there was considerable variation in the appearance of the various mycelia. About one-half of the mycelia formed an aerial mass. In some this consisted of loosely interwoven hyphae, whereas in others the aerial portion was very dense and compact.

The remainder of the mycelia were much more appressed with a scant amount of aerial hyphae. In some of these cultures the hyphae were so appressed that the colony appeared water-soaked. In several cultures there were tufts of hyphae that protruded from the surface of the colony at a 45 degree angle. Several colonies were of a powdery appearance. In one of the cultures with appressed hyphae the hyphae grew to the left; in another culture the hyphae grew to the right.

The hyphae were either quite wide or quite narrow. The wide hyphae

were often compacted into small rhizomorph-like strands.

Seventeen monosporous mycelia were paired in all combinations in December, 1938.

The pairings of three of the mycelia with the other mycelia were carefully examined. Failure to find clamp-connections in any of these combinations seemed to indicate that no clamp-connections would be found. Therefore, the pairings were not studied further. No fruiting-bodies developed.

Psilocybe foenisecii

Monospore cultures of this species (culture 136) were obtained in July, 1938. The cultures were mostly appressed with only a small amount of white, aerial mycelium. In each culture there were many small, irregularly-shaped, cream-colored knots of hyphae. All of the cultures grew slowly. Chlamydospore-like cells were rather abundant. No oidia were found.

The results of the pairing are given in Table 8. Failure to find clamp-connections wherever spore number 5 was involved may have been due to the fact that the clamps were on very narrow hyphae and were infrequent. No fruiting-bodies developed.

Table 8. Results of pairing of nine monosporous mycelia of Psilocybe foenisecii (culture 136). Paired August, 1938.

		AB		Ab			aB		ab		
		7	4	6	8	2	9	1	3	5
AB	7							+	+	
Ab	4					+	+		+	
	6					+	+			
	8					−	+			
aB	2		+	+	−					
	9		+	+	+					
ab	1	+								
	3	+	+							
	5									

Psilocybe subviscida

Monospore cultures of this species (culture 116) were obtained in June, 1938. The cultures were very similar in appearance and growth to those of Naucoria semiorbicularis. There were no asexual spores.

The results of the pairings are given in Table 9. In almost all of these pairings the hyphae had to be examined by means of oil immersion lens because they were so narrow. Another difficulty in this species was the fact that the clamp-connections were often infrequent in various pairings. In some of the pairings marked as having clamps only two or three such structures were seen in the entire preparation.

Table 9. Results of pairing of seventeen monosporous mycelia of P. subviscida (culture 116). Paired in July, 1938).

		A					a											
		7	14	16	18	6	15	25	1	2	5	8	9	11	12	21	22	23
A	7						+	−	+	+	−	+	+	+	+	+	+	+
	14						−	−	+	+	+	+	+	+	+	+	+	+
	16						+	+	+	+	−	−	−	+	−	−	+	+
	18						+	+	+	+	−	+	+	+	+	−	+	−
	6						+	−	−	−	−	−	−	−	−	−	−	−
a	15	+	−	+	+	+			+	+					+	+	+	
	25	−	−	+	+	−												
	1	+	+	+	+	−	+											
	2	+	+	+	+	−	+											
	5	−	+	−	−	−												
	8	+	+	−	+	−												
	9	+	+	−	+	−												
	11	+	+	−	+	−												
	12	+	+	−	+	−	+											
	21	+	+	−	−	−	+											
	22	+	+	+	+	−	+											
	23	+	+	+	−	−												

Geographic races

Coprinus micaceus

Five mycelia of culture 16a were paired in all combinations with ten mycelia of culture 127 that represented all sexual phases. No clamp-connections were found in any of the pairings.

Cultures 82 and 83 (two compatible mycelia) were paired in all combinations with nine mycelia of culture 16a. No clamp-connections were found.

Cultures 82 and 83 were paired in all combinations with ten mycelia of culture 127. No clamp-connections were found.

Naucoria semiorbicularis

Eight mycelia of culture 112 were paired in all combinations with eight mycelia of culture 106. The results are given in Table 10.

Table 10. Results of pairing of mycelia of N. semiorbicularis (culture 112) with mycelia of culture 106 of the same species.

112 \ 106	4	5	11	15	16	17	20	27
1	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+
7	+	+	+	−	+	+	+	−
8	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+

Interspecific pairings

Coprinus fimetarius macrorhizus x C. semilantanus

Four mycelia of C. semilantanus (culture 21 Rev.), representing all sexual phases, were paired in all combinations with eight mycelia

of C. fimetarius macrorhizus (culture 150) that represented all sexual phases. No clamp-connections were formed. There was no sign of aversion between the mycelia. In most of the pairings C. semilantanus was overgrown by the other fungus, but in a number of such cases it still formed primordia of fruiting-bodies.

C. fimetarius macrorhizus x C. micaceus

Four mycelia of C. micaceus (culture 16a) were paired with eight mycelia of C. fimetarius macrorhizus (culture 150) in all combinations. No clamp-connections developed. There was no sign of aversion.

Four mycelia of C. micaceus (culture 127) were paired in all combinations with eight mycelia of C. fimetarius macrorhizus (culture 150). No clamp-connections were formed. There was no sign of aversion.

C. micaceus x C.plicatilis

Four mycelia of C. micaceus (culture 127), representing all sexual phases, were paired with eight mycelia of C. plicatilis (culture 109). No clamp-connections were found. There was no sign of aversion.

Panaeolus papilionaceus x P. retirugis

Cultures 85 and 86 (two compatible mycelia of P. papilionaceus) were paired with cultures 88 and 89 (two compatible mycelia of P. retirugis). No clamp-connections were found. There was no sign of aversion.

Intergeneric pairings

Anellaria separata x Naucoria semiorbicularis

Three mycelia of A. separata (culture 124) were paired with eight mycelia of N. semiorbicularis in all combinations. In all of the

pairings the cultures of Naucoria grew the more rapidly. This fungus often completely covered the mycelium of Anellaria. In ~~any~~ the + preparations the number of clamp-connections varied from one to three. Since they were so infrequent the pairings were studied a second time. That time no clamp-connections were found in any of the pairings that at first were recorded as positive. One of the clamp-connections that was most clear is illustrated by plate II, fig. 5. The results that are given in Table 11 are the results obtained the first time that the pairings were observed. There was no sign of aversion.

Table 11. Results of pairing of three monosporous mycelia of Anellaria separata with eight mycelia of Naucoria semiorbicularis.

124	112	1	3	4	5	6	7	8	10
	6	−	−	−	+	+	−	−	−
	11	−	−	−	−	−	−	−	−
	17	−	+	−	+	−	−	−	−

Anellaria separata x Panaeolus retirugis

Cultures 88 and 89 (compatible mycelia of P. retirugis) were paired with eight mycelia of A. separata in all combinations. There was seen only one structure that resembled a clamp-connection. It is illustrated by plate II, fig. 6. There was no sign of aversion.

Anellaria separata x Panaeolus papilionaceus

Cultures 85 and 86 (compatible mycelia of P. papilionaceus) were paired in all combinations with eight mycelia of A. separata. No clamp-connections were found. There was no sign of aversion.

Naucoria semiorbicularis x Panaeolus papilionaceus

Cultures 85 and 86 (compatible mycelia of P. papilionaceus) were paired with six mycelia of N. semiorbicularis (culture 106). No clamp-

connections were found. There was no sign of aversion.

Naucoria semiorbicularis x Panaeolus retirugis

Cultures 88 and 89 (compatible mycelia of P. retirugis) were paired in all combinations with six mycelia of N. semiorbicularis (culture 106). No clamp-connections were found. There was no sign of aversion.

Coprinus plicatilis x Psathyrella disseminata

Four mycelia of Psathyrella disseminata (culture 138) were paired with eight mycelia of Coprinus plicatilis (culture 109). No clamp-connections were found. There was no sign of aversion.

Discussion and conclusions

The presence of oidia or conidia and the variation between monosporous mycelia of some species is in agreement with what other workers have found.

Coprinus radians was found to be either homothallic or heterothallic. On the basis of clamp-connections alone it could not be decided into which class it fell. Vandendries (71), working with a fungus that he called C. radians Desm., found that clamp-connections were produced and that the species was bipolar. According to Vandendries (74), Brunswik never found clamp-connections in this species but proved it to be bipolar by observing what pairings produced fruiting-bodies. This method might have yielded definite results if it had been used for this species by the author. Chow (21), using six mycelia, also reported that the species is bipolar.

Psathyrella disseminata, also, since it did not produce clamp-connections or fruiting-bodies, may be either homothallic or heterothallic. Vandendries (80, 82), on the basis of the distribution of clamp-connections, has stated that this species is bipolar. He also found that there were various irregularities in the pairing reactions.

Coprinus fimetarius macrorhizus (C. macrorhizus that Buller (18) speaks of) was found to be bipolar but with so many mycelia in one of the groups that one might suspect that there really are four sexual phases. The absence of barrage, however, would indicate that the species is not quadripolar. Buller (18) reports that Newton found this species to be heterothallic.

Since Coprinus fimetarius (or C. lagopus, according to Buller (18) and Quintanilha (59)) is quadripolar, it is interesting that this variety (or species) is bipolar. There immediately arises the question

whether the variety has only one allelomorphic pair of genes affecting the polarity or whether there are two pairs. If there are two pairs, then the disjunction of these pairs must be of such a nature that the spores are usually bipolar.

Naucoria semiorbicularis was found to be bipolar. Sass (63) suggested on the basis of cytological study that N. semiorbicularis form bispora was probably homothallic. He found that some of the spores were apparently uninucleate and that others were binucleate. Genetical study of this variety would probably help explain the relation of the distribution of nuclei to the spores and the polarity of the fungus.

Coprinus plicatilis was found to be quadripolar and to form false clamp-connections in certain "illegitimate" crosses.

Coprinus semilantanus was found to be quadripolar.

Anellaria separata was found to be quadripolar.

Psilocybe foenisecii, on the basis of study of nine mycelia, was found to be irregularly quadripolar.

When Coprinus micaceus (culture 16a) was first cultured it was found that it was heterothallic, but the type of polarity was not determined. After the change in the cultures appeared, it was impossible to work out the polarity because no clamp-connections were produced.

Coprinus micaceus (culture 127) might be interpreted by some as being bipolar with many irregularities. Others might consider it to be quadripolar with numerous irregularities. Since the species has been found by Vandendries (76) to be quadripolar and to show numerous abnormalities in some cases (81), one is prejudiced in favor of the thought that the specimen that was studied was quadripolar. More mycelia should have been used in the pairings.

Psilocybe subviscida has been shown in the results (Table 9) to be bipolar but with many irregularities. It is possible, however, to arrange the results in such a manner that the species would be thought to be quadripolar. It can not be stated definitely which type of polarity the species possesses.

No irregularities were observed in the pairing of mycelia of the following species: Anellaria separata, Coprinus fimetarius macrorhizus, Coprinus plicatilis and Naucoria semiorbicularis.

There were a few irregularities in Coprinus semilantanus and Psilocybe foenisecii and numerous abnormalities (or perhaps failure in some cases to find the clamps?) in C. micaceus (culture 127) and Psilocybe subviscida.

Throughout the paper the terms "homothallic" and "heterothallic" have been used in the sense that other workers appear to be using them, although the author thinks that perhaps other terms would be better to indicate that the phenomena appear to be due to the existence of one or two pairs of self-sterility factors. That is, the two terms as used here do not mean the same as they do when used to explain conditions in the Phycomycetes.

In no species was there any evidence of barrage such as found by Vandendries and Brodie (87) in some fungi.

False clamp-connections were found only in Coprinus plicatilis.

In regard to geographic races it was found in Naucoria semiorbicularis that spores from two fruiting-bodies about one and one-half miles apart were almost completely compatible in all combinations.

In Coprinus micaceus the cultures 16a and 127, separated by about 300 yards, did not produce clamps in any of the pairings. This was

only to be expected, however, since none of the mycelia of 16a would form clamp-connections when paired among themselves in all combinations.

Mycelia of 16a also failed to form clamp-connections when paired with two mycelia (cultures 82 and 83) from Belgium.

Mycelia of culture 127 of C. micaceus from East Lansing failed to form clamp-connections when paired with the two mycelia (82 and 83) from Belgium.

These results are in agreement with those of other workers in that the spores from sporophores that are not distant one from another are usually nearly entirely compatible (as in Naucoria semiorbicularis), whereas spores from fruiting-bodies that are far apart (Coprinus micaceus) are usually incompatible in all combinations. It should be pointed out here, and there appears to be no reference to this in the literature, that confusion may arise from the fact that workers do not explain what they mean by such terms as "near" and "far" in relation to these geographic races.

For the present, at least, the theory of multiple allelomorphs of the self-sterility factors appears to explain the results of study of geographic races. The author is inclined to favor the suggestion that Vandendries (81) made in regard to the existence of dominant, hereditary factors that determine primarily whether or not two mycelia from different fruiting-bodies could form clamp-connections.

Of the five interspecific crosses that were attempted, clamp-connections were not found in any of the pairings. This is in general agreement with the results of other workers who have found clamps to be very few or lacking in such crosses.

In the six intergeneric crosses that were attempted, ~~there were~~

~~attempted~~, there were no clamp-connections found in the pairings of Anellaria separata and Panaeolus papilionaceus, Naucoria semiorbicularis and P. papilionaceus, N. semiorbicularis and P. retirugis and Coprinus plicatilis and Psathyrella disseminata.

In the pairings of Anellaria separata and Naucoria semiorbicularis a very few clamp-connections were found in four of the twenty-four matings. In the pairings of A. separata and Panaeolus retirugis only one structure that resembled a clamp-connection was found.

It should be emphasized that most of the previous work dealing with interspecific crosses in the Agarics involved only a few spores. The writer also did not use many spores, but the number was greater than most workers have employed. It seems to the author that many interspecific and intergeneric crosses could be obtained if large numbers of mycelia were used. In the higher plants the number of pollinations that are made in order to secure crosses, even between species, is large. Crosses have been obtained between the following genera in the higher plants: Zea x Tripsacum, Zea x Euchlaena, Triticum x Secale, Triticum x Agropyron, Triticum x Aegilops; in the Rosaceae successful crosses have been made between Sorbus x Aronia, Pyrus x Sorbus and Cydonia x Pyrus, and in the Orchidaceae the crosses of Laelia x Cattleya are numerous. It is quite possible that crosses between many genera of the Agaricaceae could also be obtained, if one were to work with numbers as great as were used in securing the crosses mentioned above.

SUMMARY

1. Seventeen species of the Agaricaceae were grown on eighty-seven media, chiefly in test-tubes, in an affort to find a medium that would allow the production of fruiting-bodies. On most of the media nearly all of the seventeen species were used. On many of the media a number of other unidentified species were also used.

2. The following species did not form fruiting-bodies on any of the media that were tried: Coprinus atramentarius, C. comatus, C. plicatilis, Galera crispa, G. tenera, Psathyrella disseminata and Psilocybe subviscida.

3. Coprinus radians, C. radiatus and C. semilantanus formed fruiting-bodies on a number of media. C. fimetarius macrorhizus, C. micaceus, Panaeolus campanulatus, P. retirugis and P. solidipes formed sporophores on only a few media. C. quadrifidus formed fruiting-bodies only on a modification of Etter's medium. Stropharia semi-globata formed fruiting-bodies only on dung-extract malt agar.

4. The media that were most valuable for sporophore production were straw with water or malt extract, modification of Etter's medium, dung-extract agar, potato malt agar and potato glucose agar.

5. Oidia, conidia or chlamydospore-like cells were found on the monocaryon mycelia of most of the species, but none were found on mycelia that were known to be dicaryon. If the tissue cultures of Coprinus radians are dicaryon, then that species produces conidia on dicaryon mycelium.

6. Coprinus fimentarius macrorhizus and Naucoria semiorbicularis were found to be bipolar.

7. Coprinus plicatilis, C. semilantanus, Anellaria separata and

Psilocybe foenisecii were found to be quadripolar.

8. There were so many irregularities in the pairing reactions of Coprinus micaceus (one fruiting-body) and of Psilocybe subviscida that it can not be stated definitely whether they were bipolar or quadripolar. Probably the former is quadripolar and the latter bipolar.

9. Since Coprinus radians and Psathyrella disseminata did not produce clamp-connections in any of the pairings, it can not be decided whether they are homothallic or heterothallic. The same applies to a culture of Coprinus micaceus which at first was heterothallic. After about two and one-half years in culture the fungus became so changed (or contaminated?) that no clamp-connections would form when the mycelia were paired.

10. The existence of two geographic races was demonstrated in Naucoria semiorbicularis. The two fruiting-bodies were collected about one and one-half miles apart.

No compatibility was found between mycelia of two fruiting-bodies of Coprinus micaceus that were collected about 300 yards apart. One of these cultures was the one that had changed during its period of culture on artificial media. No compatibility was found between mycelia of C. micaceus secured in East Lansing, Michigan, and mycelia secured in Belgium.

11. Barrage was not found in any of the species.

12. False clamp-connections were found in certain combinations of the mycelia of Coprinus plicatilis.

13. Of the five interspecific crosses that were attempted, clamp-connections were not found in any of the pairings.

14. Of the six intergeneric crosses that were attempted, a few

clamp-connections were found in certain of the pairings of Anellaria separata and Naucoria semiorbicularis, and a structure that might be considered to be a clamp was found in one pairing of A. separata and Panaeolus retirugis.

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Plate I

Fig. 1. Sporophores of Coprinus semilantanus on horse dung

Fig. 2. Sporophores of C. micaceus on potato malt agar; these are the product of the pairing of two monosporous mycelia of culture 16a soon after the mycelia were secured.

Fig. 3. Chlamydospore-like cells of Anellaria separata.

Fig. 4. Oidia of Coprinus fimetarius macrorhizus.

Fig. 5. Conidia and conidiophores of C. micaceus (culture 16a). These are typical of conidia that developed on a film of agar on a slide.

Fig. 6. Conidia and conidiophores of C. micaceus (culture 16a) that were mounted in water for examination.

Fig. 7. Conidia of C. micaceus (culture 16a) in a water-mount.

Fig. 8. Stages in germination of conidia of C. micaceus (culture 16a) growing on a film of agar on a slide.

Fig. 9. True clamp-connections of C. plicatilis.

Fig. 10. False clamp-connections of C. plicatilis.

PLATE I

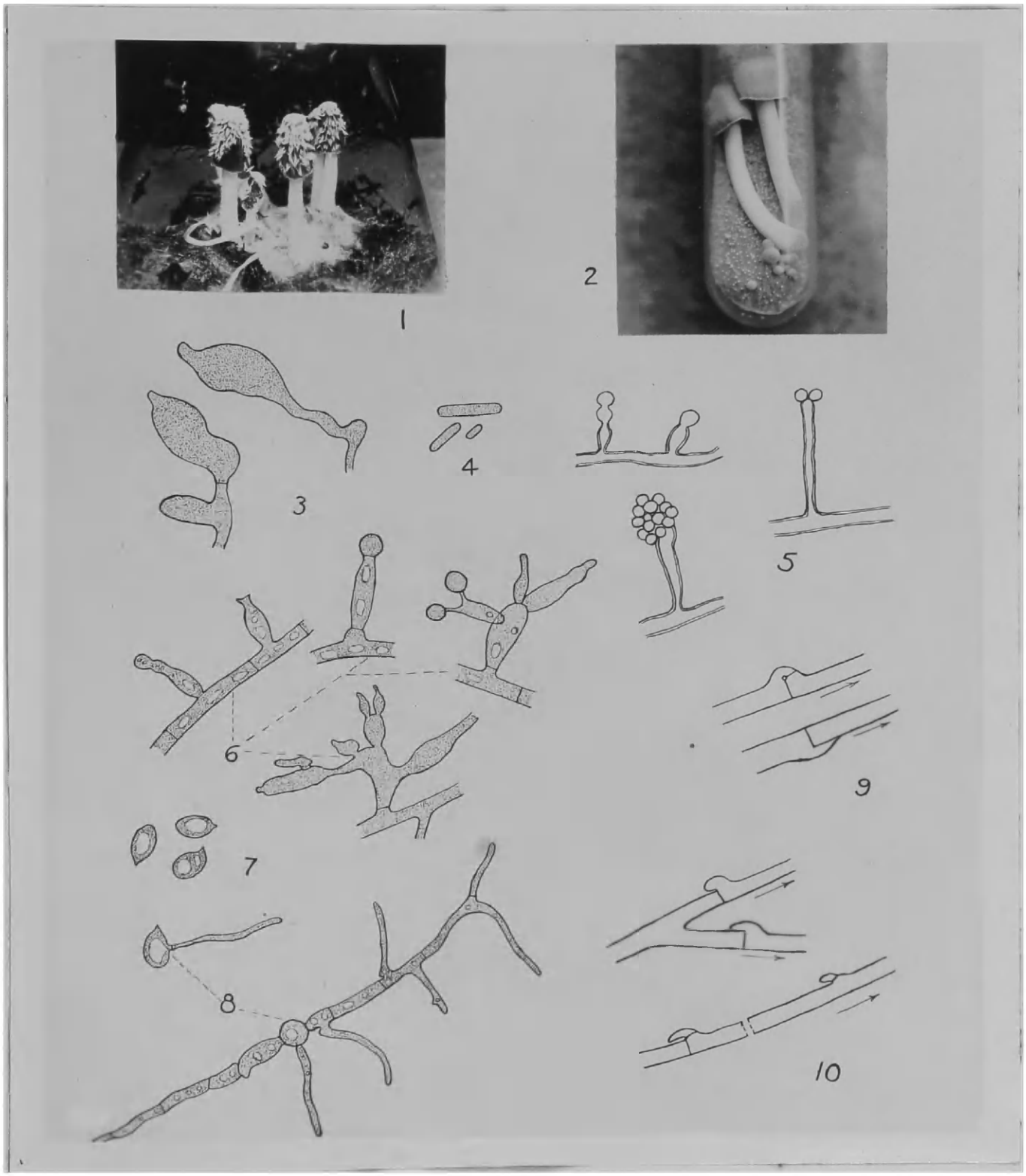

Plate II

Fig. 1. *Coprinus plicatilis*. A portion of a mycelium bearing false clamp-connections.

Fig. 2. Conidia and oidia-like cells of *C. plicatilis*.

Fig. 3. Conidia and conidiophores of *C. radians*.

Fig. 4. Chlamydospore-like cells from a rugose colony of *C. semilantanus*.

Fig. 5. Clamp-connection found in one of the pairings of *Anellaria separata* with *Naucoria semiorbicularis*.

Fig. 6. Clamp-like structure found in one pairing of *Anellaria separata* with *Panaeolus retirugis*.

PLATE II