STUDIES IN THE SEXUALITY OF THE HETEROBASIDIAE

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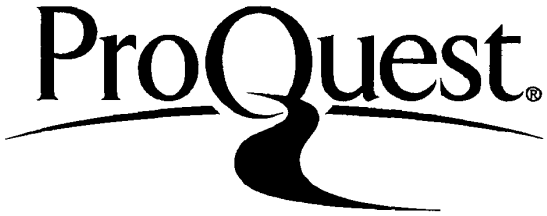

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INTRODUCTION

In the present work a study was made of various species of the orders Auriculariales, Tremellales and Dacryomycetales. However, owing to the absence of clamp connections in the species of Dacryomyces, Guepinia and Calocera that were collected, and owing to the failure to develop a suitable technique for staining their nuclei, the sexuality of the species of this last order was not included in this study. Spore germination was studied in Tremella lutescens, but due to the failure of the cultures to produce extensive mycelium on the various media used no work could be done concerning sexuality. The species from which monospore cultures were obtained and whose sexuality was studied were Auricularia auricula-judae (Fr.) Schröt., Exidia glandulosa (Bull.) Fr., E. ricisa (Ditm.) Fr., E. saccharina Fr. and E. nucleata (Schw.) Burt.

The literature concerning the Heterobasidiae, other than from the taxonomic standpoint, is scarce. Several writers, including Brefeld (4), Dangeard (11), Möller (21), Shear and Dodge (30), Neuhoff (24, 25), Gilbert (14), and Kniep (17), have reported germination of spores in some species of this group, but few of them (Brefeld, Shear and Dodge, Neuhoff, and Kniep) have reported the production of extensive mycelium. Cytological work has been published by Neuhoff (24), Gilbert (14), Kühner (19), Rogers (28, 29) and Whelden (40, 41, 42, 43).

MATERIALS AND METHODS

Collections of Species Studied

The collections of the species of Auricularia and Exidia used in cultural work and from which monospore cultures were obtained are given in Table 1.

Table 1

Data Concerning the Collections of Auricularia and Exidia

Species	Collection number	Place of Collection	Date	Host	Spore size
A. auricula-judae	I	Adirondacks, N. Y.[1]	8/23/35	Fir?	10.5-(14.6)-17.5x 4.8-(5.4)-7
"	III	Lincoln Nebr.[2]	Received 11/7/35	Deciduous	11.8-(13.5)-15.5x 4.8-(5.2)-5.7
"	IV	Chapel Hill N. C.[3]	7/6/36	Oak	8.8-(11.9)-14.8x 5.3-(5.6)-6.6
"	V	Lincoln, Nebr.[2]	Received 10/28/35	Deciduous	8.8-(12.4)-14.4x 4.4-(5.2)-6
"	VI	Iowa City Iowa.[4]	9/2/36	"	10.5-(13.1)-15.2x 4.8-(5.2)-5.7
"	VII	" "[4]	9/3/36	Ash	11.3-(13.3)-14x 4.8-(5.5)-6.2
"	VIII	Ft.Collins Colo.[5]	Received 1/5/37	Fir	13.2-(15)-17.5x 4.4-(5.4)-6.6
E. glandulosa	I	E. Lansing Mich.	11/4/34	Hickory	
"	II	" "	4/29/36	"	
"	V	Lyons, Mich.	5/30/36	Oak	
E. recisa	I	E. Lansing Mich.	3/28/36	Hickory	
"	II	Lyons, Mich	5/30/36	Oak	
"	III	E.Lansing Mich.	6/4/36	Hickory	
E. saccharina	I	Grayling, Mich.	9/10/36	White Pine	
E. nucleata	I	E.Lansing, Mich.	7/9/35	Maple	

1. Collected by Dr. E. A. Bessey
2. By courtesy of Dr. Leva B. Walker
3. By courtesy of Dr. W. C. Coker
4. By courtesy of Dr. G. W. Martin
5. By courtesy of Prof. J. L. Forsberg

All other specimens were collected by the author

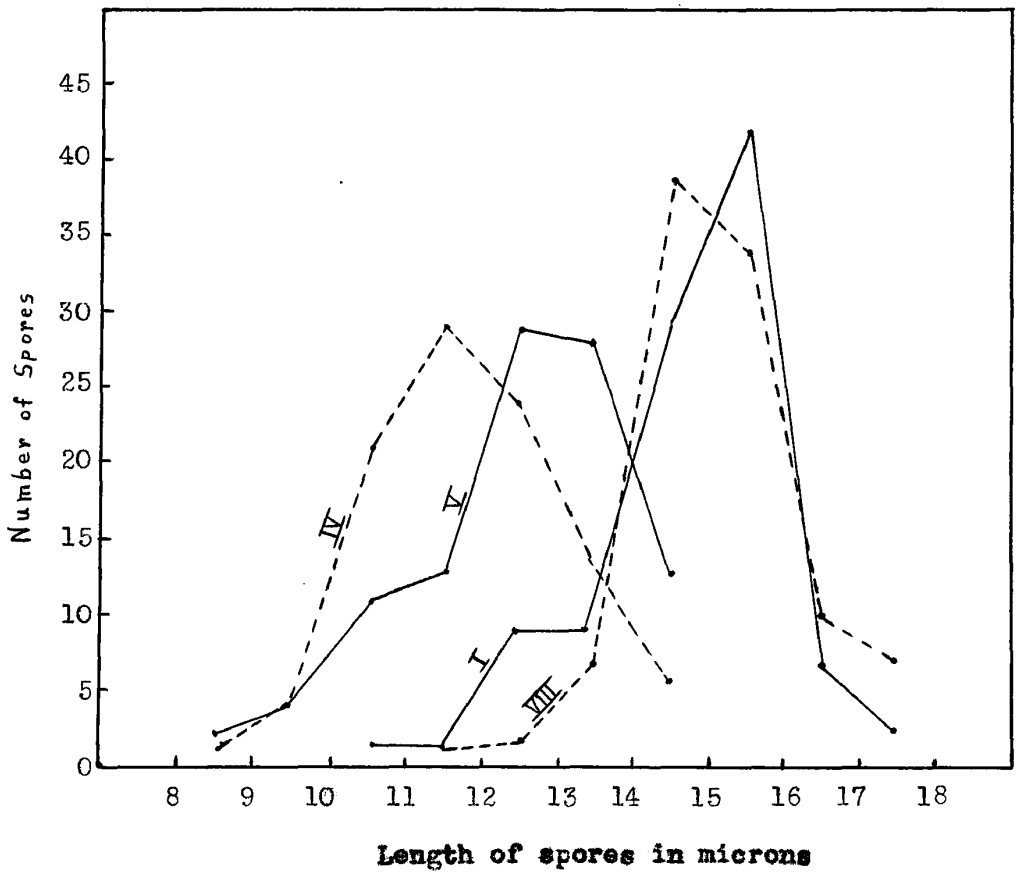
In the records of spore sizes, given in Table 1, the first and last numbers in each case represent the extremes and the numbers in parentheses are the means. One hundred spores from each of collections I, IV, V, and VIII were measured, while 20 spores each of collections III, VI and VII were measured.

It is interesting to note the differences in the sizes of spores of the collections of A. auricula-judae. The width varied only slightly, but in length the spores varied considerably. Collections I and VIII had the longest spores with respective mean lengths of 14.6μ and 15.0μ. The mean lengths of spores from other collections ranged from 11.9μ to 13.5μ. A better comparison between the spores of certain collections is seen when the distribution of spore lengths is given in the form of a graph (Text Fig. 1).

Two fruit bodies were selected from collection V of A. auricula-judae and designated as Va and Vb. These had already been removed from the host wood and the distance apart could not be determined. From collection II of E. glandulosa two fruit bodies (IIa and IIb) were chosen from the same log only two inches apart. The only collection of E. saccharina was found growing on a stick about 12 inches long. Two fruit bodies were selected and designated as Ia and Ib. Only one fruit body was selected from each of the other collections.

Method of Securing Cultures

When fresh material was not available the dried fruit bodies were moistened with distilled water and placed on moist paper toweling in a petri dish. After a few hours the fruit bodies were examined with low power of the compound microscope to be sure that they were producing an abundance of spores. At first a Chamberlain micromanipulator was



Text fig. 1. Graph showing distribution of lengths of 100 spores each of four collections of A. auricula-judae.

used to pick the spores from the basidia. However, this method soon proved to be unsatisfactory from the standpoint of time and because of other discharging spores falling on the needle of the manipulator before a single spore could be picked off. After this the spores were picked out from spore deposits on agar plates.

The most satisfactory method of obtaining spore deposits was similar to the method used by Mounce (22). A portion of a fruit body was fastened to the cover of a petri dish by means of a drop of thick Canada balsam. This permitted the later addition of a small drop of water to the piece of fruit body without destroying the adhesive property of the balsam. The petri dish cover with the piece of fruit body was then placed over an agar plate and the spores were allowed to fall upon the agar. By slow rotation of the cover a more or less even distribution of spores was obtained in a circular area. Spores from fruit bodies produced in culture were suspended in sterile distilled water which was then poured over agar plates.

Multispore cultures were obtained by cutting out blocks of agar containing many spores and transferring them to test tubes of agar. For monospore cultures viable spores were insured by allowing them to germinate before they were picked out. A tool for picking out single spores was fashioned from a small sewing needle with a very small rounded eye. The metal around the eye was ground down somewhat and sharpened making a cutting edge. This end of the needle was then bent at an angle of about 45° and the pointed end inserted into a metal holder. This made a very convenient tool which could be sterilized in a flame and, when not allowed to become red hot, one such tool lasted a long time.

The agar plate with the spore deposit was placed upon the stage of a binocular dissecting microscope and the germinated spores located by use of the high power objective (at a magnification of about 96X). The eye of the specially prepared needle was pressed down around a well-isolated germinated spore, cutting out a small area of agar to the bottom of the plate. At this stage the spore could be observed in the area cut out by the needle. This piece of agar bearing the spore was lifted out and with the point of a second sterile needle the top portion of the agar block was picked off and transferred to a tube of agar. The use of the second needle in removing only the upper portion of the agar block reduced the possibility of transferring other spores which might stick to other parts of the first needle. The cultures were then placed under bell jars in a moist atmosphere and incubated at room temperature.

Culture Media Used

During the first part of the investigations potato-dextrose agar was used exclusively. Later, various modifications of this and several other media were tried in order to determine the media best suited for vegetative growth and for the production of fruit bodies. The following media were tested: potato-dextrose agar with and without peptone, malt agar with and without peptone, dextro-maltose agar, prune extract-corn meal agar, Czapek's medium, powdered wood-malt extract-peptone medium as described by Arnold (1), moistened powdered wood of maple, hickory and oak, and autoclaved sticks of basswood, maple, hickory and oak. For the last medium, living sticks of wood about $\frac{1}{2}$ to 1 inch in diameter were chosen. The bark was removed from one side of the sticks which were placed in large test tubes containing about two inches of distilled water and sterilized at 15 pounds pressure. This furnished

a constantly moist atmosphere in the tubes and a range in the amount of moisture in the wood.

The best media for vegetative growth of all species were potato-dextrose agar (broth of 200 gm. potatoes, 20 gm. dextrose, 15 gm. agar, enough distilled water to make 1 liter), and malt extract agar (15 gm. desiccated malt extract, 15 gm. agar, 1 liter of distilled water). Little difference was seen between these two media. The second medium was used almost exclusively thruout the latter part of the work and for all of the pairings of monospore cultures. The presence of 1 gm. of peptone per liter in each of the media had no apparent effect on the vegetative growth. The poorest vegetative growth was produced on dextro-maltose agar, Czapek's medium and on wood media. For the formation of fruit bodies the best media were potato-dextrose agar with 1 gm. of peptone per liter, malt extract agar and prune-corn meal agar in the order named. The most typically shaped fruit bodies of A. auricula-judae were produced on moist sticks of basswood and hickory.

RESULTS OF EXPERIMENTS

Germination of Basidiospores

A. auricula-judae. When dried fruit bodies were revived, spore discharge began as early as five hours after they had resumed their normal shape and size. The first spores, discharged from the fruit bodies, germinated very poorly, only about 1-4% after four days on agar. Spores discharged later showed almost 100% germination after four days on agar at room temperature. Most of the spores germinated within 12 to 48 hours after being discharged. Spores from a fruit body which had been in a dry condition for almost nine months germinated poorly. At the time of discharge the spores were single celled, but before

germination they often, but not always, become two or three-celled, each cell containing a single nucleus (Pl. I, 1).

On agar the spores germinated by means of one or rarely by two long germ tubes. The germ tube usually arose from the apicular end of the spore (Pl. I, 4), but some arose from the other end or from the convex side of the spore (Pl. I, 3). The germ tubes often remained unbranched for a distance of 75 to 100μ, after which they showed frequent branching. Some of the branches soon penetrated downward into the agar while others grew on the surface or turned upward to produce aerial hyphae. After the germ tube had reached some length the contents in the spore passed out into the tube, revealing clearly the septa in the spore and older portion of the tube (Pl. I, 4). Growth and branching continued until abundant white mycelium was produced. Basidiospores which had fallen on moist paper toweling were examined after two days. Only about 10% had germinated and these had produced coarse germ tubes similar to the germination of spores on agar.

Some of the spores discharged from a fruit body fell back and collected in a mass on the fruit body itself. These were examined and several showed germination by the production of a single, slightly curved, secondary spore on a slender germ tube (Pl. IV, 1). Basidiospores in distilled water were examined after two days. The percentage of germination was high and most of the spores were distinctly two-celled. A few spores produced stout germ tubes, but in most cases germination was by means of a fine germ tube about 1μ wide and 8-15μ long, from each cell of the spore. Each germ tube bore one or more sickle-shaped oidia at its tip (Pl. I, 2). Similar germination of spores and production of oidia in water and in nutrient solution were

described by Brefeld (4) for A. sambucina. Measured along the chord connecting the tips the oidia (Pl. IV, 2) were 2-3μ long, although they were actually 4-5μ in length. The thickness at the middle was 1.5μ, while the curvature was such that the distance from the chord to the outer convex side was 2-3μ.

E. glandulosa. Spores discharged from fresh fruit bodies germinated very readily. Germ tubes as long as 60μ were produced within 12 hours after falling on agar. The spores were single celled at the time of discharge but before germination some became two or rarely three-celled (Pl. I, 10). Most of the spores produced two coarse germ tubes, one at either end of the spore (Pl. I, 12), or one often arose from the convex side of the spore (Pl. I, 13). Some of the germ tubes grew to a length of 100μ before branching and then branched frequently. One collection was revived after being in a dried condition in the laboratory for eleven months. These fruit bodies began to discharge spores after ten hours. Germination of spores was poor and much slower than those from freshly collected fruit bodies. The mycelia produced, however, were just as vigorous in growth as those obtained from fresh fruit bodies.

When spores from a fresh fruit body were placed in distilled water three methods of germination were observed after two days. Some produced long germ tubes very similar to those on agar. A few produced a short slender germ tube bearing a single secondary spore (Pl. I, 11). Many of the spores germinated by means of several (as many as eight) short slender germ tubes (Pl. I, 9A, B). At the tips of these germ tubes one or more sickle-shaped oidia (Pl. I, 9C) were borne. These measured 3.5μ along the chord connecting the tips, with the actual length about 5μ, and in thickness about 1μ. Only a few oidia were seen attached to the germ

tubes. Similar production of oidia by germinating spores of E. glandulosa was described and illustrated by Brefeld (4). Spores fallen in a mass on moist wood germinated poorly. Some did so by means of secondary spores and others by long coarse germ tubes.

E. recisa. Spores from fresh fruit bodies germinated within 12 hours after falling on agar. Germination was almost 100% after 36 hours. The spores were single-celled when discharged and some remained so, while others became two-celled before germination (Pl. II, 1). Germination was by means of one or two coarse germ tubes, one at either end of the spore (Pl. II, 2, 3). About two or three days after germination many of the young hyphae produced small straight or slightly curved oidia, which were 3.5-4μ by 1.6μ in size. These were borne in small clusters at the tips of the hyphae or on short lateral branches (Pl. II, 3). Some short peg-like branches bearing oidia were produced from the main body of the spore. The production of oidia did not prevent the growth of the hyphae nor the subsequent formation of extensive mycelium. Some hyphae bore no oidia (Pl. II, 2) but continued growth to form mycelia. After 36 hours in distilled water very few spores showed any signs of germination. These few produced coarse germ tubes.

E. saccharina. Spores from fresh fruit bodies germinated readily on agar and after 12 hours had formed short coarse germ tubes. One to three germ tubes grew from each spore. Some spores were two-celled at the time of germination. About 30 hours after the spores had fallen on agar, abundant small rod-shaped oidia were observed on the young hyphae (Pl. V, 1). They were borne in the same manner as those of E. recisa. The oidia measured 3.5-7μ x 1.5-2μ. Brefeld (4) has described and illustrated similar production of oidia in E. saccharina var. foliacea.

E. nucleata. Spores collected from fresh fruit bodies showed less than 1% germination on agar in all cases, and those from some fresh fruit bodies failed to germinate at all. However, spores from fruit bodies grown in culture germinated readily on agar with the production of long germ tubes. In distilled water some spores germinated by means of a single secondary spore on a slender germ tube, (Pl. VI, 2), and others by means of one (Pl. VI, 3) or two germ tubes similar to those formed by spores on agar.

Spores which had fallen on moist wood around a freshly collected fruit body germinated poorly. Some produced secondary spores and others bore small clusters of sickle-shaped oidia at the tips of short slender germ tubes (Pl. VI, 1). Only one such germ tube was formed by each spore. These oidia measured 2.5-3.5 μ along the chord between the tips, about 4-4.5 μ in actual length and about 1.5 μ in thickness. Basidiospores in a 2% dextrose solution produced secondary spores.

Other Species. On agar the germination of basidiospores was very similar in Dacryomyces minor Pk., Calocera cornea (Batsch) Fr. and Guepinia spathularia (Schw.) Fr. Spores of all three species were one-celled when discharged from the fruit body but they all became septate before germination. Those of C. cornea (Pl. III, 6) and G. spathularia became two-celled and those of D. minor formed four or more cells before germination (Pl. III, 7). Germination began after about 12 hours on agar. One or more coarse germ tubes were formed from each cell of the spore. These are illustrated for C. cornea (Pl. III, 1, 2) and for D. minor (Pl. III, 9). The germ tubes soon branched abundantly and mycelium was formed directly. Similar formation of mycelium in D. deliquescens was described by Gilbert (14). In distilled water a

few spores of D. minor produced coarse germ tubes while others bore small spherical oidia on short sterigma-like structures (Pl. III, 8). Spores which had fallen in a mass on moist wood produced oidia in the same manner. Such production of oidia was described by Brefeld (4) for species of Dacryomyces.

Spores of Tremella lutescens Pers. germinated very readily on agar by repeated budding (Pl. III, 12) which continued until there was produced a shiny opaque mass of buds, appearing much like a bacterial colony. Some of these buds germinated and produced short hyphae, a part of which bore ovoid buds or oidia, but for some reason yet unknown no definite mycelial cultures were formed. Basidiospores which had fallen back onto the fruit body germinated by means of secondary spores (Pl. III, 11).

Macroscopic Appearance of Cultures

No macroscopic difference was noted between the monocaryon and dicaryon mycelia of any species. The mycelia of A. auricula-judae, E. glandulosa and E. recisa were so nearly alike that in most cases it was impossible to distinguish between the three species by their appearance. The mycelia of all three species were pure while, or brownish in some older cultures of A. auricula-judae and E. recisa, with abundant aerial growth. The mycelium of E. recisa was more variable in appearance than that of any other species. In several of the cultures, both dicaryon and monocaryon, there appeared small, raised dark brown gelatinous bodies about 2-5 mm. in diameter (Pl. V, 5). These were considered possibly to be sterile or abortive fruit bodies and will be discussed later.

An interesting situation was found in the macroscopic appearance

of cultures of E. glandulosa. After the monospore mycelia had been in culture for almost a year, during which time frequent transfers had been made, it was observed that cultures 23, 24 and 50 had ceased to produce an abundance of white aerial growth. Most of the hyphae were confined to the surface or under the surface of the agar. Only cultures 23 and 24 are illustrated (Pl. VII, 1A, B), but culture 50 was very similar in appearance. Pairings were made between cultures of different types, using 21 (Pl. VII, 1C) and 51 as examples of cultures with much fluffy aerial mycelium. The dicaryon mycelium produced from the mating 24 x 50 grew close to the agar (Pl. VII, 4), much in the same manner as did each of the two monocaryon mycelia. The dicaryon mycelium which resulted from the pairing 21 x 51 was fluffy with considerable aerial growth (Pl. VII, 2). When 21 was mated with 50 the resulting dicaryon mycelium was somewhat intermediate in its manner of growth (Pl. VIII, 3). Although no definite conclusions can be drawn from so few results, they do seem to indicate that the changes in the type of growth of the monocaryon mycelia influence the type of growth of the dicaryon mycelium produced when these cultures were mated with others.

The mycelium of E. nucleata was white and grew very slowly. The growth for the most part was low and close to the agar or was bunched up in a mass with very few aerial hyphae. The monospore cultures all produced the same type of growth at first. About six or seven months after they were started, it was noticed that a few of these cultures had produced sectors which were more fluffy and with more aerial growth. When such a sector was transferred the fluffy condition of the mycelium persisted. Such changes in mycelial growth occured in five monospore cultures, numbers 3, 17, 22, 25 and 26. This change was not correlated

with any other perceptible character of the mycelium nor with either sexual phase. Unfortunately, the cultures were lost due to loss of vigor and it was not determined whether the fluffy character was influenced by a genetical factor or whether the change was due merely to prolonged growth or artificial media.

The dicaryon mycelium of E. saccharina was very similar to that of E. recisa but produced a more spreading growth and was closer to the agar. The monospore cultures varied considerably in appearance, some being more fluffy than others (Pl. V, 3). The masses of oidia produced in culture appeared as raised, shiny, opaque areas which were white at first but later turned dark brown (Pl. V, 2).

The mycelia of Dacryomyces minor, Calocera cornea and Guepinia spathularia grew very slowly, being quite close to the agar at the edge and more bunched up and compact in the center of the culture. The mycelia of all three species varied from bright orange to yellow in color, and appeared more faded when grown in the dark. Thick, rope-like, branching upright strands were produced in dicaryon cultures of C. cornea. When first formed they looked very much like young fruit bodies but no spores were produced by them.

Microscopic Appearance of Mycelia

Abundant clamp connections were produced by dicaryon mycelia of A. auricula-judae (Pl. I, 7), E. glandulosa (Pl. I, 14), E. recisa (Pl. II, 5), E. saccharina (Pl. II, 6), and E. nucleata (Pl. II, 13). No clamp connections were present in multispore cultures of D. minor, C. cornea and G. spathularia. The mycelia of all species branched frequently, either acutely or at right angles to the main hyphae. The hyphae of all species were quite narrow, ranging in most cases from

1-4 μ in width, with a few as wide as 5 μ. In general the dicaryon hyphae were somewhat wider than the monocaryon but the difference was not distinct. The hyphae of A. auricula-judae were slightly wider than those of other species. In all species they were composed of long cells.

Production of Oidia

A. auricula-judae. The formation of sickle-shaped oidia on germinating basidiospoes in water has been described above. Oidia similar in shape but slightly larger were borne in clusters on monocaryon mycelia (Pl. I, 6). They measured 3.5-5.5 μ along the chord from tip to tip, about 4-6.5 μ in actual length and about 1.7 μ in thickness. They were rarely found in old cultures but were most abundant in young cultures about one or two weeks after the spores had germinated. No oidia were observed on dicaryon mycelia.

E. glandulosa. Sickle-shaped oidia in very small numbers were observed a few times in the examination of monospore cultures, especially in number 50. They were first observed in this culture about six months after it was started and frequent transfers had been made in the meantime. The oidia were similar in size and shape to those described above in the germination of basidiospores in water. No oidia were produced on dicaryon mycelia.

E. recisa. The production of oidia on very young monocaryon mycelia, shortly after the germination of basidiospores, has been described above. Oidia were seen but rarely in older monocaryon mycelia and never on dicaryon mycelia.

E. nucleata. Sickle-shaped oidia measuring 3.8-6 μ along the chord from tip to tip, about 4.5-7.5 μ in actual length and 1.4 μ in the thick-

ness were found in both young and old monospore cultures. The were produced in small clusters at the tips of branches (Pl. VI, 4). Some cultures produced a greater number than others. The monospore cultures which changed from a low growing type of mycelium to a fluffy type apparently also lost the ability to produce oidia. Oidia were also abundant in dicaryon cultures, but when examined microscopically none were found on hyphae which bore clamp connections. It is thought that the oidia were borne only on monocaryon hyphae.

E. saccharina. The formation of straight rod-shaped oidia on germ tubes of germinating basidiospores has been described above. When the monospore cultures were about three weeks old most of them showed the presence of whitish shiny opaque masses (Pl. V, 2). Microscopic examination revealed the presence of very great numbers of straight rod-shaped oidia measuring 7-12μx 2-3μ. Staining with gentian violet showed that they were uninucleate (Pl. II, 8). In these cultures some of the oidia were borne in loose clusters at the tips of hyphae (Pl. II, 9) while others were apparently cut off singly. Dicaryon cultures also produced abundant oidia which were of the same shape but more uniform in size, measuring 10-14μ(mostly 10-12μ) x 2-3μ. These oidia were observed to be borne directly on hyphae with clamp connections (Pl. II, 6) and apparently were cut off singly. Staining with gentian violet revealed that these oidia were binucleate (Pl. II, 7).

The production of binucleate oidia on dicaryon mycelia has been described by Brodie (5) for Collybia velutipes, by Vandendries (36, 37, 38) for Pholiota aurivella, Trametes cinnabarina and Pleurotus pinsitis, and by Dangeard (11) for Dacryomyces deliquescens. In the last species the binucleate oidia were borne on young fruit bodies but they each

divided to form two uninucleate oidia before germinating.

In Calocera cornea and Dacryomyces minor oidia were produced in both multispore and monospore cultures. They were mostly ovoid or elliptical and measured 3-5 x 1-3μ. They were borne singly or in small clusters on very small peg-like, side branches of the hyphae (Pl. III, 4, 10). The nuclear number of the oidia was not determined. Martens and Vandendries (20) give a review of the presence of oidia in several Basidiomycetes.

Germination of Oidia

A few oidia of A. auricula-judae showed germination in the culture in which they were produced and also in a 2% dextrose solution. Germination was by means of a fine germ tube about 4-6μ long and 1μ thick (Pl. I, 5). They failed to germinate in water and on agar. Germination of oidia of E. glandulosa (Pl. I, 15) was very similar to that of A. auricula-judae. In E. nucleata slight germination of oidia was seen in the culture where they were produced, in water and on moist agar. Germination was by means of a single fine germ tube 10μ long and 1μ thick (Pl. II, 12). In these three species only the first stages in germination were observed and no mycelium was produced.

The oidia of E. recisa germinated readily on agar (Pl. V, 4) and produced either secondary oidia or branching normal monocaryon mycelium. The uninucleate oidia of E. saccharina which were borne on monocaryon mycelia germinated readily on agar to produce normal monocaryon mycelia. The binucleate oidia, borne on dicaryon mycelia, also germinated readily on agar and produced normal dicaryon mycelia with clamp connections. The clamps were often formed on the germ tube near the oidium (Pl. II, 11). Binucleate oidia were picked out and 37 single-oidial cultures were obtained. When examined all of them showed the presence of clamp connec-

tions. This indicates that the two nuclei present in each oidium represented two compatible sexual phases.

Oidia of Calocera cornea (Pl. III, 3) and Dacryomyces minor also germinated readily on agar to produce normal mycelia.

Production of Fruit Bodies in Culture

A. auricula-judae. Dicaryon cultures were grown on various media and under various conditions of temperature and light, but mature fertile fruit bodies were produced in only one culture of collection III and transfers from it. This culture was originally started for a monospore culture, but soon afterward microscopic examination of mycelium revealed the presence of clamp connections. It is assumed that two spores belonging to opposite sexual phases had been picked out by mistake. About six months after the culture was started, a few small, rounded, light brown, gelatinous fruit bodies appeared in a one-month-old transfer. When examined, many young basidia were observed, some of which bore four epibasidia (Pl. I, 8). The basidia were typical in appearance but some were slightly curved. They were produced in a definite layer but did not form so compact a hymenium as in a normal fruit body. A comparatively small amount of gelatinous material was present, and the fruit bodies were less tough than those growing under natural conditions.

Transfers from one of these fruit bodies continued to produce basidia while transfers from the mycelium began to fruit after three to four weeks on agar. In a flask of potato-dextrose-poptone agar the fruit bodies first appeared as small, separate, raised bodies which later coalesced and formed a convoluted gelatinous body about 1 cm. in diameter (Pl. IV, 4). The fruiting structure then continued growth

and after some time it had extended to the side of a 5 cm. flask (Pl. IV, 5). The later growth was somewhat raised but was not so convoluted as the earlier growth. The basidiospores were normal in size and shape. Both monospore and multispore cultures were obtained from these spores, but no fruit bodies were formed in any of the multispore cultures nor in the dicaryon cultures produced by pairing two compatible monospore cultures. Transfers from a fruiting culture were made to sterilized sticks of basswood, hickory, oak, ash and maple. About four months later a culture on basswood produced small but typically shaped fruit bodies (Pl. IV, 6). About a month later similar fruit bodies were formed on two different sticks of hickory. No spores or basidia were found on any of the fruit bodies on wood.

E. glandulosa. No signs of fruit body production were seen in any of the dicaryon cultures. A few dark gray gelatinous bodies 1-2 mm. in diameter appeared in monospore culture number 5. They were examined and many young basidia were seen. Some of these were septate, mostly by a single oblique septum but a very few showed two oblique septa forming a four-celled basidium. The basidia were mostly atypical in shape and measured 14-20 x 7-9μ. A few of the two-celled basidia showed two short epibasidia (Pl. I, 16) but no basidia produced four epibasidia. A few typical basidiospores measuring 9-12 x 4-5.5μ were seen (Pl. I, 10). No cultures were obtained from these spores. The mycelium of culture 5 was examined carefully for the presence of clamp connections but none were found. Nuclear stains were used but all cells observed were uninucleate. Some transfers from this culture produced a few small, gray, gelatinous bodies but no basidia were found in any of them. Monospore culture 85 also formed a few small gelatinous bodies

in which a great number of young basidia were present, but no spores were seen. These appear to be cases where fertile but quite atypical and weak fruit bodies were produced by monocaryon mycelia.

E. recisa. A few typical four-celled basidia with long epibasidia (Pl. II, 4) were observed in one multispore culture on potato-dextrose-peptone agar. No basidiospores were seen. The fruiting body in this case was merely a brown somewhat gelatinous layer on the agar and was partially overgrown by dull brown hyphae.

In many of the dicaryon cultures and also in several of the monocaryon cultures, there appeared rounded, dark brown, gelatinous bodies (Pl. V, 5) closely resembling normal young fruit bodies. They ranged from 2-9 mm. in diameter, and were usually larger in the dicaryon cultures. When examined they were found to be rather tough in texture, much like that of a normal fruit body. Among the slender hyphae present, there were a number of round or oblong cells which resembled young basidia, but none were ever seen to be septate. It was thought that these gelatinous bodies represented abortive fruit bodies which remained sterile, probably because of unfavorable conditions. Such cultures were subjected to various environmental conditions but no recognizable basidia were observed.

E. nucleata. A multispore culture showed several small fruit bodies after about four months on prune-corn meal agar. These were clear, cushion-like bodies about 1-3 mm. in diameter. Examination of these showed a large number of typical basidiospores. Many young club-shaped basidia and a smaller number of mature four-celled basidia with four long epibasidia were present (Pl. II, 14, 15). In most cases the epibasidia were longer than those present in wild fruit bodies and

frequently the four on a basidium were unequal in length. The longer epibasidia were often once or twice septate.

Transfers of mycelium from the fruiting cultures produced fruit bodies with mature basidia within 14 days on potato-dextrose-peptone agar. Fruit bodies were also produced on potato-dextrose agar (without peptone) and on malt agar. Monospore cultures were started from the spores borne in culture, and out of the 13 compatible pairings between the monospore cultures seven produced fruit bodies. No sign of any central crystal structure, characteristic of the wild fruit bodies, was seen in any of the fruit bodies in culture.

No fruit bodies were produced in any cultures of E. saccharina.

In each of two multispore cultures of Calocera cornea a small yellow, rounded gelatinous body was formed. These were very atypical for fruit bodies but both produced forked basidia (Pl. III, 5) and spores.

Growth Rates of Cultures

Duplicate malt agar plates of multispore cultures of the different species were made and inoubated at room temperature (ranging from 21-24° C). A second set of duplicate cultures were placed in the ice box where the temperature ranged from 10° to 13° C. The results of this experiment are shown in Table 2.

Table 2

Radial Growth Rates of Dicaryon Mycelia

		Radial growth in 15 days		Radial growth rate per day	
Species	no. of collection	21°-24° C	10°-13°C	21°-24°C	10°-15°C
A. auricula-judae	I	20 mm.	6 mm.	1.3 mm.	0.3 mm.
E. glandulosa	I	44 mm.	5 mm.	2.9 mm.	0.3 mm.
E. rocisa	I	22 mm.	slight	1.5 mm.	
E. mucosata	I	slight			

No great difference was found in general between growth rates of monocaryon and dicaryon mycelia of any species, but in most cases the growth of dicaryon mycelia was somewhat faster than that of monocaryon mycelia. The growth rates of some of the monocaryon cultures are given in Table 3. Duplicate plates were grown at room temperature on malt extract agar. There were, however, considerable difference between growth rates of different monocaryon cultures. This is noticeable when a comparison is made between cultures 7 and 9 of A. auricula-judae, cultures 50 and 23 of E. glandulosa and cultures 256 and 263 (Pl. V, 3D, B) of E. saccharina.

Union of Hyphae and Production of Clamp Connections

In E. saccharina one fusion between young hyphae from germinating basidiospores (Pl. II, 10) was seen 30 hours after the spores had fallen on agar, or about 20 hours after germination. In E. recisa one such union between young hyphae was seen 48 hours after the spores fell on agar, and in E. glandulosa (Pl. I, 17) one was observed four days after the discharge of the spores. In these three species clamp connections were formed within a few hours after two compatible monocaryon mycelia met. These observations were made on young mycelia from germinating spores.

The production of clamp connections in A. auricula-judae seems to be delayed somewhat, for in certain multispore cultures clamps were first observed 11 to 18 days after the spores had germinated. It was also observed that in a pairing between two compatible monocaryon mycelia clamp connections were not found until the two mycelia had intermingled quite well.

Table 3

Radial Growth Rates of Monocaryon Cultures

Species	no. of culture	Radial Growth rate after 7 days	Radial Growth Rate per day
A. auricula-judae	4	9 mm.	1.3 mm.
"	7	11 "	1.6 "
"	9	6 "	0.9 "
"	101	10 "	1.4 "
E. glandulosa	23	16 "	2.3 "
"	28	12 "	1.7 "
"	30	13 "	1.9 "
"	50	8.5 "	1.2 "
"	53	13 "	1.9 "
"	55	14 "	2.0 "
"	52	14 "	2.0 "
"	57	13 "	1.9 "
E. recisa	4	7 "	1.0 "
"	5	7 "	1.0 "
E. saccharina	256	9 "	1.3 "
"	257	14 "	2.0 "
"	263	15 "	2.1 "
"	265	10 "	1.4 "

Sexuality

It is still a question as to what characteristics are best to use as a standard in determining the true dicaryon mycelium in the Basidiomycetes. Bose (3) quotes Oort (27) as follows: "As a criterion for diploid mycelia the occurrence of clamp connections can only be of a restricted use. Used in connection with the characteristic

diploid habit and the diploid fruit bodies it remains, of course, a valuable criterion". Because the species studied in the present work showed no other distinguishable difference between dicaryon and monocaryon mycelia, and because the production of fruit bodies was rare, the presence of clamp connections alone was taken as an indication of true dicaryon mycelium. By staining mycelia, both those with and without clamp connections, early in the course of this investigation the correctness of this assumption was determined for the species in question.

Dried fruit bodies were revived, or fresh ones were used when available. Spores were collected on agar plates and single germinated spores picked out in the manner described above. After the cultures had grown sufficiently in test tubes they were examined microscopically for the presence of clamp connections. The monospore cultures were numbered consecutively, giving those from each fruit body a different set of Arabic numbers. The number of each fruit body corresponds to the number of the collection of that species and is indicated by a Roman numeral. Rarely a culture became contaminated and was discarded. This fact accounts for some of the missing cultures in a set.

The monospore cultures from each fruit body were then paired in all possible combinations. This was accomplished by placing bits of mycelia of two cultures at the sides of an agar slant in a 15 mm. test tube. The pairings were then placed under bell-jars in a moist atmosphere and left at room temperature. After about two weeks the two mycelia had intermingled thoroughly and at this time a sample was removed from the line of contact of the two mycelia. A little dilute aqueous safranin was usually added to make the hyphae show more clearly and the samples were then examined for the presence of clamp connections

by use of the oil immersion lens.

The presence of clamp connections in such a pairing was taken to indicate that the two monocaryon mycelia were compatible, or that they belonged to different sexual phases. Since only two sexual phases were found in each fruit body, the absence of clamp connections was taken, in general, to mean that the two mycelia belonged to the same sexual phase. However, other factors may be present which influence the compatibility of two monocaryon mycelia. In several cases short branches were seen at septa and these may have been abortive clamp connections, but unless complete and definite clamps were seen the results were considered as negative. When irregular results were obtained the pairings were repeated, but when the results were regular only one mating was made between any two monospore cultures. In all tables the + sign indicates the presence of clamp connections, or that the two cultures were compatible, and the - sign indicates the absence of clamp connections. No special work was done with the phenomenon of aversion but mention is made where it was definitely observed. The presence of aversion is indicated in the tables by a dot (•).

A. auricula-judae

Pairings of Monospore Cultures from the Same Fruit Body

Twenty-two monospore cultures were obtained from fruit body I. These were paired in all possible combinations and the results are given in Table 4. It is observed that the monospore cultures fell into two groups, based upon the production of clamp connections in certain combinations. Throughout this paper these groups are called "sexual phases". These two sexual phases were designated genetically as A and a. When members of the same sexual phase were paired none produced

Table 4

A. auricula-judae. Results of pairing 22 monospore cultures of fruit body I.

		A							a												
		1	3	7	18	20	21	23	25	4	5	6	8	9	10	12	13	15	16	17	19	22	24
A	1		−	−	−	−	−	−	−	+	+	+	+	+	−	+	−	+	+	+	+	−	+
	3			−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+
	7				−	−	−	−	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+
	18					−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	20						−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	21							−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
	23								−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	25									+	+	+	+	+	+	+	+	+	+	+	+	+	+
a	4										−	−	−	−	−	−	−	−	−	−	−	−	−
	5											−	−	−	−	−	−	−	−	−	−	−	−
	6												−	−	−	−	−	−	−	−	−	−	−
	8													−	−	−	−	−	−	−	−	−	−
	9														−	−	−	−	−	−	−	−	−
	10															−	−	−	−	−	−	−	−
	12																−	−	−	−	−	−	−
	13																	−	−	−	−	−	−
	15																		−	−	−	−	−
	16																			−	−	−	−
	17																				−	−	−
	19																					−	−
	22																						−
	24																						

clamp connections. When members of A were paired with a, clamp connections were found in all but six of the 112 pairings.

Nineteen monospore cultures were obtained from fruit body III and were paired in all combinations. The results are given in Table 5. The fruit bodies produced by dicaryon mycelium grown from spores of fruit body III were designated as $IIIF_1$. Several monospore cultures were obtained from them and ten were paired in all combinations. The results are shown in Table 6. Aversion was present in some pairings but little correlation is seen between its presence and the absence of clamp connections.

Eleven monospore cultures of fruit body IV were paired in all combinations and the results are shown in Table 7. It was observed that several of the pairings showed rather distinct aversion. In these pairings three rather distinct types of pairings were observed. (1) 22 pairings showed a space of aversion between the two mycelia (Pl. IV, 3A, B). (2) two showed aversion in which the gap was filled with a growth of dicaryon mycelium (Pl. IV, 3D). (3) 31 pairings showed even intermingling of the two mycelia (Pl. IV, 3C). Clamp connections were found in only one culture of (1), in both cultures of (2) and in all but two cultures of (3). The results indicate that in these pairings there was a rather close correlation between the presence of aversion and incompatibility of the cultures.

Table 8 shows the results obtained when 11 monospore cultures of fruit body Va were paired in all combinations. Slight aversion was seen only in some of the incompatible pairings. The results of pairing seven monospore cultures of Vb are given in Table 9. Table 10

Table 5

A. auricula-judae. Results of pairing 19 monospore cultures of fruit body III.

		A_1											a_1							
		101	103	105	107	109	110	111	113	114	117	118	104	112	115	116	120	121	122	125
A_1	101		−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	103			−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	105				−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	107					−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	109						−	−	−	−	−	−	+	+	+	+	+	+	+	+
	110							−	−	−	−	−	+	+	+	+	+	+	+	+
	111								−	−	−	−	+	+	+	+	+	+	+	+
	113									−	−	−	+	+	+	+	+	+	+	+
	114										−	−	+	+	+	+	+	+	+	+
	117											−	+	+	+	+	+	+	+	+
	118												+	+	+	+	+	+	+	+
a_1	104													−	−	−	−	−	−	−
	112														−	−	−	−	−	−
	115															−	−	−	−	−
	116																−	−	−	−
	120																	−	−	−
	121																		−	−
	122																			−
	125																			

Table 6

A. auricula-judae. Results of pairing ten monospore cultures of fruit body III F_1. +.= more or less aversion present.

		A_1						a_1			
		301	302	303	306	307	309	304	305	308	310
A_1	301		−.	−.	−.	−	−	+	+.	+	+
	302			−.	−.	−	−.	+	+	+.	+.
	303				−.	−.	−.	+	+	+	+
	306					−	—	+	+	+	+
	307						−	+	+	+	+
	309							+	+.	+	+.
a_1	304								−.	−	—
	305									−	−.
	308										−
	310										

Table 7

A. auriclua-judae. Results of pairing 11 monospore cultures of fruit body IV. +.= more or less aversion present.

		A_2						a_2				
		151	152	155	157	158	161	153	154	156	159	160
A_2	151		−.	−.	−.	−	−	+	+	+	+	+
	152			−.	−.	−.	−.	+	+.	+	+	+
	155				−.	−.	−.	+	+	+	+	+
	157					−.	−.	+	+.	+	+	+
	158						−.	+	+	+.	+	+
	161							+	+	+	+	+
a_2	153								−.	−.	−.	−.
	154									−.	−.	−.
	156										−.	−.
	159											−.
	160											

Table 8

A. auricula-judae. Results of pairing 11 monospore cultures of fruit body Va.

		A_3						a_3				
		201	205	207	208	209	210	202	203	204	206	211
A_3	201		−.	−	−	−.	−	+	+	+	+	+
	205			−.	−.	−	−.	+	+	+	+	+
	207				−	−.	−	+	+	+	+	+
	208					−.	−	+	+	+	+	+
	209						−.	+	+	+	+	+
	210							+	+	+	+	+
a_3	202								−	−.	−	−.
	203									−	−.	−
	204										−.	−
	206											−
	211											

Table 9

A. auricula-judae. Results of pairing seven monospore cultures of fruit body Vb.

		A_4			a_4			
		401	403	405	402	404	406	407
A_4	401		−	−	+	+	+	+
	403			−	+	+	+	+
	405				+	+	+	+
a_4	402					−	−	−
	404						−	−
	406							−
	407							

Table 10

A. auricula-judae. Results of pairing nine monospore cultures of fruit body VI.

		A_5						a_5		
		251	253	254	255	257	259	252	256	258
A_5	251		−	−	−	−	−	+	+	+
	253			−	−	−	−	+	+	+
	254				−	−	−	+	+	+
	255					−	−	+	+	+
	257						−	+	+	+
	259							+	+	+
a_5	252								−	−
	256									−
	258									

Table 11

A. auricula-judae. Results of pairing six monospore cultures of fruit body VII.

		A_6			a_6		
		351	354	355	352	353	356
A_6	351		−	−	+	+	+
	354			−	+	+	+
	355				+	+	+
a_6	352					−	−
	353						−
	356						

gives the results obtained when nine monospore cultures of fruit body VI were paired, and Table 11 shows the results of pairing six cultures of fruit body VII. Nine monospore cultures of fruit body VIII were paired and the results, given in Table 12, showed that clamp connections were present in only one pairing (502 x 505). The pairings were repeated and clamp connections were found only in the same pairing. A culture arising from a mass of spores showed no clamp connections present, but they were present in fruit body VIII.

From the foregoing results it will be noted that the compatibility between monospore cultures from the same fruit body of A. auricula-judae was, in general, quite regular. All fruit bodies, except VIII, showed distinctly the presence of two sexual phases, and all of these, except I, showed normal regular compability between the two sexual phases.. Such results indicate that the factors for compatibility are located on a single pair of chromosomes and that A. auricula-judae is typically "heterothallic" and bipolar. The results obtained when monospore cultures of fruit body VIII were paired among themselves are quite puzzling. The fact that one of the pairings produced clamp connections would indicate that both sexual phases were represented in the nine monospore cultures used. However, if this is true, there must exist some condition or factor which causes a very low degree of compatibility between the two sexual phases. Such low compatibility is further indicated by the fact that no clamp connections were present on mycelium arising from a mass of many spores. These results are similar to the condition described by Kniep (17) for A. mesenterica. He reported finding a fruit body in which clamp connections were present but mycelium grown from a mass of spores produced no clamps.

Table 12

A. auricula-judae. Results of pairing nine monospore cultures of fruit body VIII.

	501	502	503	504	505	506	507	508	509
501		−	−	−	−	−	−	−	−
502			−	−	+	−	−	−	−
503				−	−	−	−	−	−
504					−	−	−	−	−
505						−	−	−	−
506							−	−	−
507								−	−
508									−
509									

Table 13

A. auricula-judae. Results of pairings between fruit bodies I and III.

		I: A	I: A	I: a	I: a
		3	7	4	6
III: A_1	101	−	−	−	−
III: A_1	105	−	−	−	−
III: a_1	104	−	−	−	−
III: a_1	112	−	−	−	−

Table14

A. auricula-judae. Results of pairings between fruit bodies I and IV .

		I: A	I: A	I: a	I: a
		3	7	4	6
IV: A_2	151	−	−	−	−
IV: A_2	152	−	−	−	−
IV: a_2	153	−	−	−	−
IV: a_2	154	−	−	−	−

Pairings of Monospore Cultures from Different Fruit Bodies

In most cases two monospore cultures were selected from each sexual phase of each fruit body and paired with the two cultures from each sexual phase of the other fruit bodies. When pairings were made between I and Va, the results were irregular, They were then repeated, using eight cultures from each fruit body.

Monospore cultures of I were paired with cultures of III, IV, Va, VI, VII and VIII. The results showed that fruit body I was completely incompatible with III, IV, VI and VII; ie., no clamp connections were produced in any of the pairings. These results are shown in Tables 13, 14, 17 and 18 respectively. In these tables the brackets connect the cultures of the same sexual phase. In I x Va, only 11 out of the 64 pairings were compatible (Table 15). Even in these the clamp connections were few and often poorly formed. In most of these pairings the mycelia did not intermingle readily and all but five of them showed the presence of a dark brown line where the two mycelia met below the surface of the agar. When examined microscopically this line was found to consist of dark brown hyphae much like the normal white hyphae in other respects. Clamp connections were found in only one of the pairings between I and Vb (Table 16). Only three of the 20 pairings between I and VIII failed to produce clamp connections and all these involved culture 3. The results of these pairings are given in Table 19.

The four monospore cultures of fruit body III were compatible with all four cultures of IV, Va, VI and VII. The results are given in Tables 20, 21, 22 and 23 respectively. When six monospore cultures from III were paired with six of $IIIF_1$, clamp connections were found

Table 15

A. auricula-judae. Results of pairings between fruit bodies I and Va.

		I: A				I: a			
		1	3	7	18	4	6	8	9
Va: A	201	−	−	−	−	−	−	−	−
	205	−	−	−	−	−	−	−	−
	207	−	+	−	−	−	−	−	−
	208	−	+	−	−	−	+	−	−
Va: a	203	−	−	−	−	+	+	+	−
	204	−	−	−	+	−	+	−	−
	206	−	−	+	−	−	−	−	−
	211	−	−	−	+	−	−	−	−

Table 16

A. auricula-judae. Results of pairings between fruit bodies I and Vb.

		I: A		I: a	
		3	7	4	6
Vb: A_4	401	−	−	−	−
	402	+	−	−	−
Vb: a_4	403	−	−	−	−
	405	−	−	−	−

Table 17

A. auricula-judae. Results of pairings between fruit bodies I and VI.

		I: A		I: a	
		3	7	4	6
VI: A_5	251	−	−	−	−
	253	−	−	−	−
VI: a_5	252	−	−	−	−
	256	−	−	−	−

Table 18

A. auricula-judae. Results of pairings between fruit bodies I and VII.

		I: A 3	I: A 7	I: a 4	I: a 6
VII: A_6	351	−	−	−	−
	352	−	−	−	−
VII: a_6	354	−	−	−	−
	356	−	−	−	−

Table 19

A. auricula-judae. Results of pairings between fruit bodies I and VIII.

		I: A 3	I: A 7	I: a 4	I: a 6
VIII	501	−	+	+	+
	502	+	+	+	+
	503	+	+	+	+
	504	−	+	+	+
	505	−	+	+	+

Table 20

A. auricula-judae. Results of pairings between fruit bodies III and IV.

		III: A_1 101	III: A_1 105	III: a_1 104	III: a_1 112
IV: A_2	151	+	+	+	+
	152	+	+	+	+
IV: a_2	153	+	+	+	+
	154	+	+	+	+

Table 21

A. auricula-judae. Results of pairings between fruit bodies III and Va.

		III: A_1 161	III: A_1 105	III: a_1 104	III: a_1 112
Va: A_3	201	+	+	+	+
	205	+	+	+	+
Va: a_3	203	+	+	+	+
	204	+	+	+	+

Table 22

A. auricula-judae. Results of pairings between fruit bodies III and VI.

		III A_1 101	III A_1 105	III a_1 104	III a_1 112
VI A_5	251	+	+	+	+
VI A_5	253	+	+	+	+
VI a_5	252	+	+	+	+
VI a_5	256	+	+	+	+

Table 23

A. auricula-judae. Results of pairings between fruit bodies III and VII.

		III A_1 101	III A_1 105	III a_1 104	III a_1 112
VII A_6	351	+	+	+	+
VII A_6	352	+	+	+	+
VII a_6	354	+	+	+	+
VII a_6	356	+	+	+	+

Table 24

A. auricula-judae. Results of pairings between fruit bodies III and III F_1.

		III A_1 101	III A_1 103	III A_1 105	III a_1 104	III a_1 112	III a_1 115
III F_1 A_1	301	−	−	−	+	+	+
III F_1 A_1	302	−	−	−	+	+	+
III F_1 A_1	303	−	−	−	+	+	+
III F_1 a_1	304	+	+	+	−	−	−
III F_1 a_1	305	+	+	+	−	−	−
III F_1 a_1	308	+	+	+	−	−	−

Table 25

A. auricula-judae. Results of pairings between fruit bodies III and VIII.

		III A_1 101	III A_1 105	III a_1 104	III a_1 112
VIII	501	+	+	+	+
VIII	502	−	−	−	−
VIII	503	+	+	+	+
VIII	504	+	+	+	+
VIII	505	−	−	−	−

in 18 of the 36 pairings in a regular manner, as shown in Table 24. The results show that the two sexual phases of $IIIF_1$ were identical with those of fruit body III. The results of pairing monospore cultures between fruit bodies III and VIII (Table 25) were irregular. Clamp connections were present in some and absent in others.

Monospore cultures of fruit body IV showed compatibility in all pairings with monospore cultures of Va, VI and VII. The results are given in Tables 26, 27 and 28 respectively. In the pairings between IV and VIII only one was compatible. Table 29 gives these results. Fruit body Va was also completely compatible with Vb, VI and VII. These results are given in Tables 30, 31 and 32 respectively. Only six of the 20 pairings between Va and VIII (Table 33) were compatible. Complete compatibility was found between fruit bodies VI and VII, and between VII and VIII. The results are given in Tables 34 and 35 respectively.

A summary of all pairings of monospore cultures is given in Table 36. The + sign indicates the presence of clamp connections in every pairing, while the - sign indicates their absence in every pairing. The $\pm$ sign indicates irregular results; i.e., some pairings produced clamps and others did not.

It has already been observed that when pairings were made between monospore cultures of different fruit bodies, four different types of results were obtained. (1) In the majority of cases clamp connections were present in every pairing. (2) In some crosses involving fruit body I, none of the pairings produced clamp connections. (3) In some crosses involving fruit body I or VIII, clamp connections were found in only a part of the pairings, and their presence apparently did not correspond

Table 26

A. auricula-judae. Results of pairings between fruit bodies IV and Va.

		IV A_2 151	152	IV a_2 153	154
Va A_3	201	+	+	+	+
	205	+	+	+	+
Va a_3	203	+	+	+	+
	204	+	+	+	+

Table 27

A. auricula-judae. Results of pairings between fruit bodies IV and VI.

		IV A_2 151	152	IV a_2 153	154
VI A_5	251	+	+	+	+
	253	+	+	+	+
VI a_5	252	+	+	+	+
	256	+	+	+	+

Table 28

A. auricula-judae. Results of pairings between fruit bodies IV and VII.

		IV A_2 151	152	IV a_2 153	154
VII A_6	351	+	+	+	+
	352	+	+	+	+
VII a_6	354	+	+	+	+
	356	+	+	+	+

Table 29

A. auricula-judae. Results of pairings between fruit bodies IV and VIII.

	IV A_2 151	152	IV a_2 153	154
501	−	−	−	−
502	−	−	−	−
VIII 503	−	−	+	−
504	−	−	−	−
505	−	−	−	−

Table 30

A. auricula-judae. Results of pairings between fruit bodies Va and Vb.

		Va: A_3 201	A_3 205	a_3 203	a_3 204
Vb: A_4	401	+	+	+	+
	402	+	+	+	+
a_4	403	+	+	+	+
	405	+	+	+	+

Table 31

A. auricula-judae. Results of pairings between fruit bodies Va and VI.

		Va: A_3 201	A_3 205	a_3 203	a_3 204
VI: A_5	251	+	+	+	+
	253	+	+	+	+
a_5	252	+	+	+	+
	256	+	+	+	+

Table 32

A. auricula-judae. Results of pairings between fruit bodies Va and VII.

		Va: A_3 201	A_3 205	a_3 203	a_3 204
VII: A_6	351	+	+	+	+
	352	+	+	+	+
a_6	354	+	+	+	+
	356	+	+	+	+

Table 33

A. auricula-judae. Results of pairings between fruit bodies Va and VIII.

	Va: A_3 201	A_3 205	a_3 203	a_3 204
501	−	−	+	−
502	−	−	−	+
VIII 503	−	+	+	−
504	−	−	−	+
505	−	−	−	+

Table 34

A. auricula-judae. Results of pairings between fruit bodies VII and VI.

VII \ VI		A_5 251	253	a_5 252	256
A_6	351	+	+	+	+
	354	+	+	+	+
a_6	352	+	+	+	+
	356	+	+	+	+

Table 35

A. auricula-judae. Results of pairings between fruit bodies VII and VIII.

VIII \ VII		A_6 351	354	a_6 352	356
	501	+	+	+	+
	502	+	+	+	+
	503	+	+	+	+
	504	+	+	+	+
	505	+	+	+	+

Table 36

A. auricula-judae. Summary of results of all pairings of monospore cultures.

		I		III		$III F_1$		IV		Va		Vb		VI		VII		VIII
		A	a	A_1	a_1	A_1	a_1	A_2	a_2	A_3	a_3	A_4	a_4	A_5	a_5	A_6	a_6	
I	A	−	+	−	−			−	−	±	±	±	−	−	−	−	−	±
	a		−	−	−			−	−	±	±	−	−	−	−	−	−	+
III	A_1			−	+	−	+	+	+	+	+			+	+	+	+	±
	a_1				−	+	−	+	+	+	+			+	+	+	+	±
III F_1	A_1					−	+											
	a_1						−											
IV	A_2							−	+	+	+			+	+	+	+	—
	a_2								−	+	+			+	+	+	+	±
Va	A_3									−	+	+	+	+	+	+	+	±
	a_3										−	+	+	+	+	+	+	±
Vb	A_4											−	+					
	a_4												−					
VI	A_5													−	+	+	+	
	a_5														−	+	+	
VII	A_6															−	+	+
	a_6																−	+
VIII																		±

to any certain combination of sexual phases. (4) In the cross III x $IIIF_1$, clamp connections were found in just half of the pairings, or in certain combinations of sexual phases, showing that the two sexual phases of $IIIF_1$ were identical with those of III. In the first, second and third types the results show that no two sexual phases involved were identical. This implies the conception that within this species several or many sexual phases are present, only two such sexual phases being present in the same fruit body, and that only one pair of chromosomes is involved. This view supposes the presence of the compatibility factors as multiple allelomorphs. In the tables the genetical designation of the sexual phases (A, a, A_1 a_1, etc.) is entirely arbitrary. The letters with subscripts indicate allelomorphs of A and a. Following Kniep (17), it is assumed that the presence of two identical factors causes incompatibility and that compatibility results, i.e., clamp connections are formed, when two somewhat different factors of the allelomorphic series are present. It is further assumed that incompatibility also results if there is too great a difference between the two factors present. This latter assumption may serve as a partial explanation of the low degree of compatibility between certain fruit bodies, but it also seems quite likely that some other factors or conditions are present which influence the production of clamp connections.

E. glandulosa

Pairings of Monospore Cultures from the Same Fruit Body.

Fifteen monospore cultures were obtained from fruit body I and paired in all possible combinations. The cultures fell into two sexual phases designated as A and a. The results, given in Table 35,

Table 37

E. glandulosa. Results of pairing 15 monospore cultures of fruit body I.

		A						a								
		1	2	4	6	8	13	3	5	7	9	10	11	12	14	15
A	1		−	−	−	−	−	+	+	+	+	+	+	+	+	+
	2			−	−	−	−	+	+	+	+	+	+	+	+	+
	4				−	−	−	+	+	+	+	+	+	+	+	+
	6					−	−	+	+	+	+	+	+	+	+	+
	8						−	+	+	+	+	+	+	+	+	+
	13							+	+	+	+	+	+	+	+	+
a	3								−	−	−	−	−	−	−	−
	5									−	−	−	−	−	−	−
	7										−	−	−	−	−	−
	9											−	−	−	−	−
	10												−	−	−	−
	11													−	−	−
	12														−	−
	14															−
	15															

Table 38

E. glandulosa. Results of pairing nine monospore cultures of fruit body IIa.

		A_1					a_1			
		21	23	24	25	29	22	27	28	30
A_1	21		−	−	−	−	+	+	+	+
	23			−	−	−	+	+	+	+
	24				−	−	+	+	+	+
	25					−	+	+	+	+
	29						+	+	+	+
a_1	22							−	−	−
	27								−	−
	28									−
	30									

Table 39

E. glandulosa. Results of pairing ten monospore cultures of fruit body IIb.

		A_2							a_2		
		50	53	55	56	58	59	60	51	52	57
A_2	50		−	−	−	−	−	−	+	+	+
	53			−	−	−	−	−	+	+	+
	55				−	−	−	−	+	+	+
	56					−	−	−	+	+	+
	58						−	−	+	+	+
	59							−	+	+	+
	60								+	+	+
a_2	51									−	−
	52										−
	57										

Table 40

E. glandulosa. Results of pairing ten monospore cultures of fruit body V.

		A_3					a_3				
		101	102	104	108	110	103	105	106	107	109
A_3	101		−	−	−	−	+	+	+	+	+
	102			−	−	−	+	+	+	+	+
	104				−	−	+	+	+	+	+
	108					−	+	+	+	+	+
	110						+	+	+	+	+
a_3	103							−	−	−	−
	105								−	−	−
	106									−	−
	107										−
	109										

Table 41

E. glandulosa. Results of pairings between fruit bodies I and IIa.

		A	I	a	
		1	2	5	7
IIa A_1	21	+	+	+	+
	23	+	+	+	+
IIa a_1	27	+	+	+	+
	28	+	+	+	+

Table 42

E. glandulosa. Results of pairings between fruit bodies I and IIb.

		A	I	a	
		1	2	5	7
IIb A_2	50	+	+	+	+
	55	+	+	+	+
IIb a_2	52	+	+	+	+
	57	+	+	+	+

were very regular and indicate normal bipolarity.

From collection II two different fruit bodies, or "lobes" from a confluent fruiting structure on bark, were selected at a distance of 2 inches apart. These were designated as IIa and IIb. Nine monospore cultures from IIa were paired in all combinations, as were ten monospore cultures from IIb. The results are given in Tables 38 and 39 respectively. Table 40 shows the results of pairing ten monospore cultures of fruit body V.

Pairings of Monospore Cultures between Different Fruit Bodies

Four monospore cultures were selected (two from each sexual phase) from each fruit body and paired with those of the other fruit bodies. The results of pairings of I with IIa, I with IIb, and I with V are shown in Tables 41, 42 and 43 respectively.

The results of the pairings between IIa and V are shown in Table 45. As the fruit bodies IIa and IIb were growing so close together, the pairings between them were repeated, using eight monospore cultures of IIa and all ten cultures of IIb. These results are given in Table 44. In all the pairings between monospore cultures from two fruit bodies, the results were very regular. Clamp connections were present in every pairing, showing that no two of the sexual phases involved were identical. As in A. auricula-judae, it is considered that the compatibility factors in E. glandulosa exist as multiple allelomorphs.

The complete compatibility between fruit bodies IIa and IIb was unexpected, because they grew so close together. It would seem probable that the two fruit bodies originated from the same dicaryon mycelium, but on the other hand, it is quite possible that they grew from two different dicaryon mycelia growing on the same log. Whatever

Table 43

E. glandulosa. Results of pairings between fruit bodies I and V.

V \ I	A		a	
	1	2	5	7
A_3 101	+	+	+	+
A_3 102	+	+	+	+
a_3 103	+	+	+	+
a_3 105	+	+	+	+

Table 45

E. glandulosa. Results of pairings between fruit bodies IIa and V.

V \ IIa	A_1		a_1	
	21	23	27	28
A_3 101	+	+	+	+
A_3 102	+	+	+	+
a_3 103	+	+	+	+
a_3 105	+	+	+	+

Table 44

E. glandulosa. Results of pairings between fruit bodies IIa and IIb.

IIb \ IIa	A_2							a_2		
	50	53	55	56	58	59	60	51	52	57
A_1 21	+	+	+	+	+	+	+	+	+	+
A_1 23	+	+	+	+	+	+	+	+	+	+
A_1 24	+	+	+	+	+	+	+	+	+	+
A_1 25	+	+	+	+	+	+	+	+	+	+
a_1 22	+	+	+	+	+	+	+	+	+	+
a_1 27	+	+	+	+	+	+	+	+	+	+
a_1 28	+	+	+	+	+	+	+	+	+	+
a_1 30	+	+	+	+	+	+	+	+	+	+

the origin of the two fruit bodies may have been, the fact remains that the two sexual phases of IIb were both unlike the two sexual phases of IIa.

The genetical designations applied to the sexual phases are given in the tables.

When the pairings between fruit bodies IIa and IIb were examined it was noticed that abundant clamp connections were present in some, while in others they were present in much fewer numbers. Preliminary experiments showed that the low numbers of clamp connections were limited to pairings between certain definite sexual phases, namely $A_1 \times A_2$ and $a_1 \times a_2$.

To determine the quantitative differences in the numbers of clamp connections, pairings were made between all combinations of sexual phases, using three monospore cultures from each. These cultures were the following: 21, 23, 24, 27, 28 and 30 from IIa, and 50, 53, 55, 51, 52 and 57 from IIb. Bits of the two monocaryon mycelia were placed on malt agar plates at a distance of about 1 cm. apart and incubated at room temperature. In order to make the conditions uniform, it was necessary to know the exact time at which the two mycelia came in contact. This was accomplished by placing sterilized halves of cover glasses into the agar in an upright position between the two mycelia of a pairing. The mycelia were allowed to grow up against the cover glass which was then removed. Care was taken not to disturb the mycelia any more than necessary.

At the end of 48 hours after the removal of the intervening cover glasses, the pairings were examined for clamp connections. The agar plate containing a pairing was placed on the stage of the microscope

and examined with the oil immersion lens at the line of contact between the two monocaryon mycelia. A drop of aqueous safranin was usually added to make the hyphae more distinct. Areas in which the hyphae could be seen clearly and in which the number of hyphae was about the average were chosen and counts were made of the clamp connections seen in the entire field, both on the surface of the agar and below the surface as far as the focus of the lens would reach. Four counts were made from one set of pairings and six were made from a later set. The results are shown in Table 46. The numbers in the table represent the average of the ten counts. In Table 47, which is a summary of Table 46, the averages for each combination of sexual phases are given. The numbers in the table represent the average number of clamp connections per microscopic field for nine pairings.

Certain distinct differences between the sexual phases are evident. The combinations $A_1 \times a_1$, $A_2 \times a_2$ and $A_2 \times a_1$ showed approximately the same number of clamp connections, averaging 13.6, 13.0 and 13.0 respectively. The combinations $A_1 \times A_2$ and $a_1 \times a_2$ showed a decidedly smaller number of clamps, averaging 6.4 and 5.5 respectively. The number found in pairings of $A_1 \times a_2$ was intermediate, averaging 10.3.

Statistical data comparing the different combinations of sexual phases are given in Table 48. Statistically significant differences were found between the following pairs of combinations: $A_1 \times A_2$ and $A_1 \times a_2$, $A_1 \times A_2$ and $A_1 \times a_1$, $A_1 \times A_2$ and $a_1 \times A_2$, $A_1 \times A_2$ and $A_2 \times a_2$, $a_1 \times A_2$ and $a_1 \times a_2$, $A_1 \times a_2$ and $a_1 \times a_2$, and $A_1 \times a_2$ and $A_1 \times a_1$. No significant differences were found between $A_1 \times A_2$ and $a_1 \times a_2$, $A_2 \times a_1$ and $A_2 \times a_2$, and $A_1 \times a_1$ and $A_2 \times a_2$.

Table 46

E. glandulosa. Average number of clamp connections (for ten counts) per microscopic field in pairings between fruit bodies IIa and IIb.

		A_1			a_1			a_2		
		21	23	24	27	28	30	51	52	57
A_2	50	5.5	5.1	7.5	11.8	13.1	13.1	13.9	14.3	12.4
	53	7.3	6.1	7.1	14.6	16.4	11.4	14.7	13.5	14.6
	55	4.8	7.9	6.7	11.3	12.6	13.1	12.7	12.0	8.8
a_2	51	11.6	12.8	9.2	5.2	7.5	5.0			
	52	10.0	10.5	8.8	6.5	5.0	5.0			
	57	9.8	10.5	9.6	5.3	4.9	5.3			
a_1	27	15.3	17.1	10.9						
	28	11.9	13.2	12.2						
	30	12.3	16.9	12.5						

Table 47

E. glandulosa. Summary of Table 46.

	A_1	a_1	a_2
A_2	6.4	13.0	13.0
a_2	10.3	5.5	
a_1	13.6		

Table 48

Statistical Significance of the Differences between the Means of Numbers of Clamp Connections in Various Combinations of Sexual Phases of Fruit Bodies IIa and IIb.

Combinations of Sexual Phases	σ	Actual Difference	$\frac{\text{Actual difference}}{\sigma}$
$A_1 \times A_2$ and $A_1 \times a_2$	0.525	3.9	7.43
$A_1 \times A_2$ and $A_1 \times a_1$	0.795	7.2	9.06
$A_1 \times A_2$ and $a_1 \times A_2$	0.614	6.6	10.75
$A_1 \times A_2$ and $A_2 \times a_2$	0.698	6.6	9.46
$A_1 \times A_2$ and $a_1 \times a_2$	0.449	0.9	2.00
$a_1 \times A_2$ and $a_1 \times a_2$	0.576	7.5	13.02
$A_1 \times a_2$ and $a_1 \times a_2$	0.480	4.8	10.00
$A_1 \times a_2$ and $A_1 \times a_1$	0.879	3.3	3.75
$a_1 \times A_2$ and $A_2 \times a_2$	0.785	0	
$A_1 \times a_1$ and $A_2 \times a_2$	0.927	0.6	0.06

σ = Standard Error of Difference.

Attempts were made to determine exactly where the difference in the numbers of clamp connections lay. The number of clamps in relation to the number of septa was counted on dicaryon mycelia. It was found that the number of septa without clamp connections was very small and no distinct difference was noted between any of the dicaryon mycelia observed. It was also thought possible that a difference might exist in the rate of diploidization of one monospore culture by another. The method employed to determine this was similar to that used by Buller (3). Monospore cultures of 53, 55, 52 and 57 were grown

Table 49

B. glandulosa. Presence of clamp connections after seven days at given distances from the first point of diploidization and growth rates of dicaryon mycelia.

Cultures diploidized by	Presence of clamps at given distance from first point of diploidization				Radial growth of dicaryon mycelium in seven days
	1 cm.	2 cm.	3 cm.	4 cm.	
53 by 28	+	+	—	—	12 mm.
28 by 53	+	—	—	—	
53 by 30	+	—	—	—	12 mm.
30 by 53	+	—	—	—	
55 by 28	+	—	—	—	15 mm.
28 by 55	+	+	+	—	
55 by 30	+	—	—	—	14 mm.
30 by 55	+	—	—	—	
52 by 28	+	—	—	—	13 mm.
28 by 52	+	—	—	—	
52 by 30	+	—	—	—	14 mm.
30 by 52	+	—	—	—	
57 by 28	+	—	—	—	12 mm.
28 by 57	+	+	+	—	
57 by 30	+	—	—	—	16 mm.
30 by 57	+	—	—	—	

on agar for 13 days and at this time one set was diploidized by placing bits of culture 28 at their edges. A second set of cultures was diploidized by culture 30. Conversely, bits of cultures 53, 55, 52 and 57 were used to diploidize 13-day cultures of 28 and 30. Seven days later small samples of mycelium were taken at distances of 1, 2, 3 and 4 cm. from the point of first diploidization and placed on fresh agar plates. After new growth had started they were examined for clamp connections and the results are recorded in Table 49. It is seen at once that in every case clamp connections were present at a distance of 1 cm. This distance, however, is short enough that their presence can be accounted for in every case by the radial growth of the dicaryon mycelia. These growth rates are also given in Table 49. It is also evident that there were only slight differences between the growth rates of the dicaryon mycelia. In only three cases were clamp connections found at a distance greater than 1 cm. These were 53 diploidized by 28, which showed clamp connections at a distance of 2 cm., 28 diploidized by 55 and 28 by 57, both of which showed clamps at a distance of 3 cm. from the first point of diploidization. At present no explanation can be given for these differences.

It was also thought that the quantitative difference found in the numbers of clamp connections might be due to a difference in the exact time of the first union of the two mycelia after the intervening cover glass was removed. It seems that there may be a greater attraction between certain cultures (if such an attration exists at all) and as a result the union of mycelia may take place sooner than if a lesser attration is present. Consequently, a greater number of clamp connections would be formed within the 48 hours that elapsed between the

removal of the cover glass and the counting of the clamps. This, however, is purely suppositional and is offered merely as a possible explanation for the differences found in the numbers of clamps described above. As yet, no successful attempts have been made to demonstrate the presence of such an attraction between compatible mycelia.

E. recisa

Pairings of Monospore Cultures from the Same Fruit Body

Twenty monospore cultures of fruit body I were paired in all combinations and the results are given in Table 50. Ten monospore cultures from fruit body II were paired, as were ten cultures from fruit body III. The results are shown in Tables 51 and 52 respectively. In all fruit bodies the results of pairings were very regular. Each showed the presence of two distinct sexual phases, showing that E. recisa is typically bipolar.

Results of Pairing Monospore Cultures from Different Fruit Bodies

Four monospore cultures of fruit body I was paired with four cultures from each of fruit bodies II and III. The results, which are shown in Tables 53 and 54 respectively, showed the presence of clamp connections in every pairing. In the pairings between cultures of II and III (Table 55), clamp connections were again found in every pairing. Such complete compatibility between the monospore cultures of different fruit bodies shows that no two of the sexual phases were identical. As in A. auricula-judae and in E. glandulosa the compatibility factors are considered to exist as multiple allelomorphs. The genetical designations of the sexual phases are given in the tables.

Table 50

E. recisa. Results of pairing 20 monospore cultures of fruit body I.

		A												a						
		1	2	5	8	9	10	12	16	18	20	21	22	24	4	11	15	17	19	23	25
A	1		−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	2			−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	5				−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	8					−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	9						−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	10							−	−	−	−	−	−	−	+	+	+	+	+	+	+
	12								−	−	−	−	−	−	+	+	+	+	+	+	+
	16									−	−	−	−	−	+	+	+	+	+	+	+
	18										−	−	−	−	+	+	+	+	+	+	+
	20											−	−	−	+	+	+	+	+	+	+
	21												−	−	+	+	+	+	+	+	+
	22													−	+	+	+	+	+	+	+
	24														+	+	+	+	+	+	+
a	4															−	−	−	−	−	−
	11																−	−	−	−	−
	15																	−	−	−	−
	17																		−	−	−
	19																			−	−
	23																				−
	25																				

Table 51

E. recisa. Results of pairing ten monospore cultures of fruit body II.

		A_1					a_1				
		51	52	55	56	62	53	54	58	59	60
A_1	51		−	−	−	−	+	+	+	+	+
	52			−	−	−	+	+	+	+	+
	55				−	−	+	+	+	+	+
	56					−	+	+	+	+	+
	62						+	+	+	+	+
a_1	53							−	−	−	−
	54								−	−	−
	58									−	−
	59										−
	60										

Table 52

E. recisa. Results of pairing ten monospore cultures of fruit body III.

		A_2			a_2						
		101	106	109	102	103	104	105	107	108	110
A_2	101		−	−	+	+	+	+	+	+	+
	106			−	+	+	+	+	+	+	+
	109				+	+	+	+	+	+	+
a_2	102					−	−	−	−	−	−
	103						−	−	−	−	−
	104							−	−	−	−
	105								−	−	−
	107									−	−
	108										−
	110										

Table 53

E. recisa. Results of pairings between fruit bodies I and II.

		I: A		I: a	
		1	5	4	11
II: A_1	51	+	+	+	+
	55	+	+	+	+
II: a_1	53	+	+	+	+
	54	+	+	+	+

Table 54

E. recisa. Results of pairings between fruit bodies I and III.

		I: A		I: a	
		1	5	4	11
III: A_2	101	+	+	+	+
	106	+	+	+	+
III: a_2	102	+	+	+	+
	103	+	+	+	+

Table 55

E. recisa. Results of pairings between fruit bodies II and III.

		II: A_1		II: a_1	
		51	55	53	54
III: A_2	101	+	+	+	+
	106	+	+	+	+
III: a_2	102	+	+	+	+
	103	+	+	+	+

E. saccharina

Two fruit bodies of the only collection were chosen and designated as Ia and Ib. Seven monospore cultures of Ia were paired in all combinations and the results are given in Table 56. The results of pairing 16 monospore cultures of Ib are given in Table 57. The monospore cultures of each fruit body fell into two sexual phases and showed normal bipolarity.

Four monospore cultures (two from each sexual phase) of Ia were paired with four cultures of Ib. Clamp connections were found in every pairing. The results are shown in Table 58. Again, as in E. glandulosa, complete compatibility was found to exist between monospore cultures from two fruit bodies growing very close to each other.

E. nucleata

A few multispore cultures were obtained from one wild fruit body, but owing to the very poor germination of the spores no monospore cultures were secured at this time. Monospore cultures, however, were obtained from spores borne on fruit bodies in culture. These cultures grew very slowly and several of them either became contaminated or died out because of loss of vigor. Eleven cultures were paired in all combinations (with the exception of a few pairings) and the results are given in Table 59. The blanks in the table represent no pairing due to loss of cultures. Cultures 3, 14, 15, 17, 26 and 27 fell into one sexual phase, while 12, 13 and 22 fell into a second sexual phase. All members of the first phase were compatible with all members of the second. Cultures 25 and 30, however, were exceptions to the normal polarity in their reactions. They were incompatible with all other

Table 56

E. saccharina. Results of pairing seven monospore cultures of fruit body Ia.

		A			a			
		201	202	205	203	204	207	208
A	201		−	−	+	+	+	+
	202			−	+	+	+	+
	205				+	+	+	+
a	203					−	−	−
	204						−	−
	207							−
	208							

Table 58

E. saccharina. Results of pairings between fruit bodies Ia and Ib.

		A Ia		a	
		201	202	203	204
A_1	251	+	+	+	+
	252	+	+	+	+
Ib					
a_1	256	+	+	+	+
	260	+	+	+	+

Table 57

E. saccharina. Results of pairing 15 monospore cultures of fruit body Ib.

		A_1										a_1				
		251	252	253	254	255	257	258	259	264	265	256	260	262	263	266
A_1	251		−	−	−	−	−	−	−	−	−	+	+	+	+	+
	252			−	−	−	−	−	−	−	−	+	+	+	+	+
	253				−	−	−	−	−	−	−	+	+	+	+	+
	254					−	−	−	−	−	−	+	+	+	+	+
	255						−	−	−	−	−	+	+	+	+	+
	257							−	−	−	−	+	+	+	+	+
	258								−	−	−	+	+	+	+	+
	259									−	−	+	+	+	+	+
	264										−	+	+	+	+	+
	265											+	+	+	+	+
a_1	256												−	−	−	−
	260													−	−	−
	262														−	−
	263															−
	266															

Table 59

E. nucleata. Results of pairing 11 monospore cultures. ⊕ = mature fruit bodies and basidiospores.

	3	14	15	17	26	27	12	13	22	25	30
3		−	−	−		−	+	⊕	+	−	−
14			−	−	−	−		⊕		−	−
15					−	−	+	⊕	⊕	−	−
17					−	−	+	+	⊕	−	−
26						−	+	⊕	+	−	−
27							+		⊕	−	
12									−	−	−
13									−	−	−
22										−	−
25											−
30											

Table 60

Results of pairings between E. glandulosa and E. recisa.

		E. recisa A		E. recisa a	
		5	10	4	11
E. glandulosa A	1	−	−	−	−
	2	−	−	−	−
E. glandulosa a	3	−	−	−	−
	5	−	−	−	−

Table 61

Results of pairings between E. recisa and E. saccharina.

		E. recisa A		E. recisa a	
		1	5	4	11
E. saccharina A	201	−	−	−	−
	202	−	−	−	−
E. saccharina a	203	−	−	−	−
	204	−	−	−	−

monospore cultures and also with each other. It is not possible to tell from these results whether these groups represent three sexual phases of a quadripolar species, or whether E. nucleata is typically bipolar and that cultures 25 and 30 were merely exceptions to the normal polarity. The latter view seems the more probable. In Table 59, the ⊕ sign represents the production of fruit bodies with mature basidia and basidiospores. It is interesting to note that none of the pairings in which culture 12 was involved produced fruit bodies.

Attempts to Hybridize Species of Exidia

Four monospore cultures of E. glandulosa I were paired with four monospore cultures of E. recisa I. No clamp connections were found in any pairing. The results are given in Table 60. Pairings were also made between cultures of E. saccharina Ia and E. recisa I. The results are given in Table 61. Again no clamp connections were found in any of the pairings. No indications were observed of the presence of fruit bodies or any kind of gelatinous bodies.

DISCUSSION

The terms used by different authors in describing the sexuality of the Basidiomycetes vary to a certain extent. The term "sexes" has been used by a number of investigators to apply to the different groups into which the monospore cultures fall, based upon the production of clamp connections when they are paired with other monospore cultures of the same fruit body. "Sex", in its fundamental sense, seems to imply the fusion of nuclei at some stage in the life of the fungus. There is some evidence to indicate that the union of nuclei and the production of basidia do not always follow the union of mycelia and the formation of clamp connections. Brunswik (6) found that certain

dicaryon mycelia of Coprinus fricsii arising from the union of two monocaryon mycelia produced fruit bodies with mature basidia and spores while under the same conditions other dicaryon mycelia did not. Dickson (12) found the same conditions in C. sphaerosporus. A similar situation was found in E. nucleata in the present work.

Considering the fungi in general, the term "sexes" is most commonly used to distinguish two individuals, one of which acts as male and the other as female. This conception includes the passage of nuclei from only one individual (male) to the other (female). In many fungi, however, this is not the case. Buller (9) has shown that, in Coprinus lagopus, two compatible mycelia may unite and there would follow an exchange of nuclei, so that each monocaryon mycelia becomes diploidized by the other. It seems more reasonable to consider that in such fungi, sexuality is present but with no distinction between maleness and femaleness.

For these reasons, "sexes" does not seem the most appropriate term to use in referring to the different groups of monocaryon mycelia. Other terms have been used for these same groups. Brodie uses the term "sexual groups", Dickson (12) uses "pairing groups", while Bessey (2) uses the term "sexual phases". This latter term, "sexual phases" is used throughout the present paper in the same sense that "sexes" has been applied to many of the Basidiomycetes.

Many investigators have used the term "fertile" to describe the presence of clamp connections in a pairing of two monocaryon mycelia and the term "sterile" when no clamp connections were produced in such a pairing. The term "fertile", however, seems to apply more appropriately to the production of spores, or at least to the formation of fruit

bodies. Following Smith and Brodie (31) and Bessey (2), in the present paper the term "compatible" is used to describe two monocaryon mycelia which unite, with subsequent production of dicaryon mycelium (indicated) by the presence of clamp connections). The term "incompatible" is used to describe monocaryon mycelia, which when paired, do not produce dicaryon mycelium.

The results obtained from pairings of monospore cultures of the same fruit body of each species indicate that A. auricula-judae, E. glandulosa, E. recisa and E. saccharina are typically bipolar. The pairings of monospore cultures of one fruit body of E. nucleata did not show normal bipolarity and it is not known whether the species is normally bipolar or quadripolar.

The falling of the monospore cultures of a fungus into a certain number of sexual phases based upon compatibility is considered to be due to the presence of one or two pairs of factors which follow the simple Mendelian ratios. Kniep (17) has called these "copulation factors". Butler (10) quotes Brunswik (7) as follows: "Brunswik has interpreted identical phenomena [referring to the presence of Kniep's copulation factors] in his experimental material by assuming the operation of sterility factors. He assumed that autogamy is the fundamental process in both monoecious and dioecious fungi; that is, both have the same genotypic constitution as far as sex is concerned. Heterothallism is determined by the addition of inhibiting or sterility factors, and mutability and allelomorphism are linked with these rather than with the real sex factors". Whether the condition of sexual phases is to be considered as due to the presence of "sterility factors" or due to the absence of factors which may be called "copulation

factors" or "compatibility factors", seems to be mainly one of terminology.

Scattered irregularities were found in the results of the pairings of monospore cultures from fruit bodies I and VIII of A. auricula-judae. These were represented by constant incompatibility between certain monospore cultures belonging to different sexual phases. Such irregularities or deviations from the normal polarity are similar to conditions reported by Vandendries (35, 36) for Panaeolus campanulatus, P. separatus, Coprinus micaceus and Leptoporus inberbis; by Kniep (17, 18) for Schizophyllum commune; by Brunswik (6) for Coprinus picaceus; by Dickson (12) for C. sphaerosporus; and by Zattler (44) for Collybia velutipes.

Concerning the pairing of monocaryon mycelia from the same fruit body of C. sphaerosporus, Dickson (12) says, "It is considered that the two pairing groups are due to a single factor difference, but the evidence is not sufficient to show whether the various degrees of sterility exhibited are due to the presence of incompatibility factors and, if so, how many such factors are concerned". Vandendries (36) states that the tendency toward "sterility" between the two "sexes" of Leptoporus inberbis is probably due to "une déficience des réalisateurs sexueles". Kniep (17) attributes such deviations from normal polarity to quantitative gene changes.

It has been observed in the present paper that within the same species complete compatibility was found between the three collections of E. glandulosa, the three collections of E. recisa and between five of the seven collections of A. auricula-judae (III, IV, V, VI, VIII). Two of the collections of A. auricula-judae were from Nebraska, two

from Iowa and one from North Carolina. In addition to the compatibility between collections, complete compatibility was found between two fruit bodies of the same collection of A. auricula-judae (Va and Vb). This was also true for two fruit bodies of E. saccharina (Ia and Ib) growing on the same stick within 12 inches of each other, and also for two fruit bodies of E. glandulosa (IIa and IIb) growing but two inches apart.

Complete compatibility between two or more fruit bodies growing either at some distance apart or near one another has been reported by Kniep (17) for Schizophyllum commune, Aleurodiscus polygonius, Collybia velutipes, C. conigena, C. cirrhata, Armellaria mellea and Coprinus fimetarius; by Brunswik (6) for C. fimetarius, C. comatus, C. niveus, C. picaceus, C. lagopus and C. friesii; by Hanna (15) for C. lagopus; by Newton (26) for C. rostrupianus; by Vandendries (32, 33, 34, 36, 39) for C. radians, C. micaceus, Panaeolus campanulatus, Trametes suaveolens and Hypholoma sublateritium; by Mounce and Macrae (23) for Lenzites saepiaria, L. trabea and Tremetes americana; by Mounce (22) for Fomes pinicola; and by Arnold (1) for Marasmius elongatipes. Vandendries (34) concluded from his work with Coprinus micaceus that pairings between collections of the same region were compatible and that, in general, pairings between very distant collections were incompatible. Several exceptions were found to this general rule.

Complete incompatibility was found in A. auricula-judae when pairings were made between fruit body I and each of fruit bodies III, IV, VI and VII. Partial incompatibility was found between fruit bodies I and Va, I and Vb, I and VIII, III and VIII, IV and VIII, and Va and VIII. Fruit bodies I and VIII were collected from coniferous hosts

and had longer spores than the other fruit bodies, which were collected from deciduous hosts. It is suggested that the variation in the spore lengths of the different fruit bodies, or the difference in the hosts, or both, may be associated with genetic differences in the fruit bodies great enough to influence the compatibility of the monocaryon mycelia. Similar cases of complete or partial incompatibility between fruit bodies of the same species have been reported by Kniep (17) for Schizophyllum commune and Collybia cirrhata; by Brunswik (6) for Coprinus comatus; by Vandendries (34) for C. micaceus; and by Mounce (23) for Fomes pinicola.

Several different theories have been advanced in an attempt to explain the sexual phenomena in the Basidiomycetes. Among them are the theory of sexual mutations, the theory of relative sexuality advanced by Hartmann (16), the theory of multiple "sexes", and the theory of multiple allelomorphs advanced by Kniep. Hanna (15) states that "the sex factors for a given species may be undergoing frequent mutations with the result that new sexual strains are continually appearing". In speaking of the "complete interfertility" between different geographic races of Coprinus rostrupianus, Newton (26) says, "..... while each strain is bisexual, the species as a whole must be regarded as multisexual". Kniep (17) considered that the complete intercompatibility between two fruit bodies was due to quantitative gene changes and that multiple allelomorphs were involved. He also stated that incompatibility between fruit bodies of the same species may be caused by too great a quantitative difference in the genes, or may be due to secondary factors. Kniep's theory of multiple allelomorphs seems to be the best for interpreting the results obtained

in the present work.

Considering that the compatibility factors exist as multiple allelomorphs, the question arises whether certain pairs of factors produce a stronger degree of compatibility than others. An opportunity to study this question arose when all monospore cultures of E. glandulosa IIa were found to be compatible with all monospore cultures of IIb and a greater number of clamp connections were found in certain combinations than in others. The assumption is made in these experiments that the greater number of clamp connections indicates a stronger degree of compatibility between the two monospore cultures involved. The results given in Table 45 would then indicate a much stronger degree of compatibility between A_1 and a_1, A_2 and a_2, A_1 and a_2, and a_1 and A_2, than existed between A_1 and A_2, and a_1 and a_2. If the numbers of clamp connections can be considered, in this case, as representing differences between members of an allelomorphic series, it is found that a_1 and a_2 are the most similar, with A_1 and A_2 only slightly less similar. The greatest difference would be between A_1 and a_1, with A_2 equally different from a_1 and a_2. It may, therefore, be concluded that in E. glandulosa the compatibility factors may show varying degrees in the strength of compatibility when they are paired.

Diploidization experiments with monospore cultures of E. glandulosa showed that in only three out of 16 cases was the diploidization rate distinctly greater than the growth rate of the dicaryon mycelium in the same pairing. The differences in the numbers of clamp connections produced in various pairings, therefore, cannot be due (at least in all

cases) to a differential diploidization rate. It is suggested that there may be a greater attraction between certain monocaryon mycelia than between others, but as yet, no means have been found to demonstrate this.

SUMMARY

1. A. auricula-judae, E. glandulosa, E. recisa and E. nucleata were found to be "heterothallic" and bipolar.

2. Pairings between monospore cultures from seven fruit bodies of A. auricula-judae showed that five of them were completely compatible with each other. Two fruit bodies showed varying degrees of incompatibility with the others and between themselves.

3. Complete compatibility was found between four fruit bodies of E. glandulosa, even between two which were growing only two inches apart.

4. A difference was found in the number of clamp connections formed in pairings involving certain combinations of sexual phases of two completely compatible fruit bodies of E. glandulosa.

5. Complete compatibility was found between three fruit bodies of E. recisa.

6. Complete compatibility was found between two fruit bodies of E. saccharina growing within 12 inches of one another.

7. The theory of multiple allelomorphs seems to serve as the best explanation of the results obtained.

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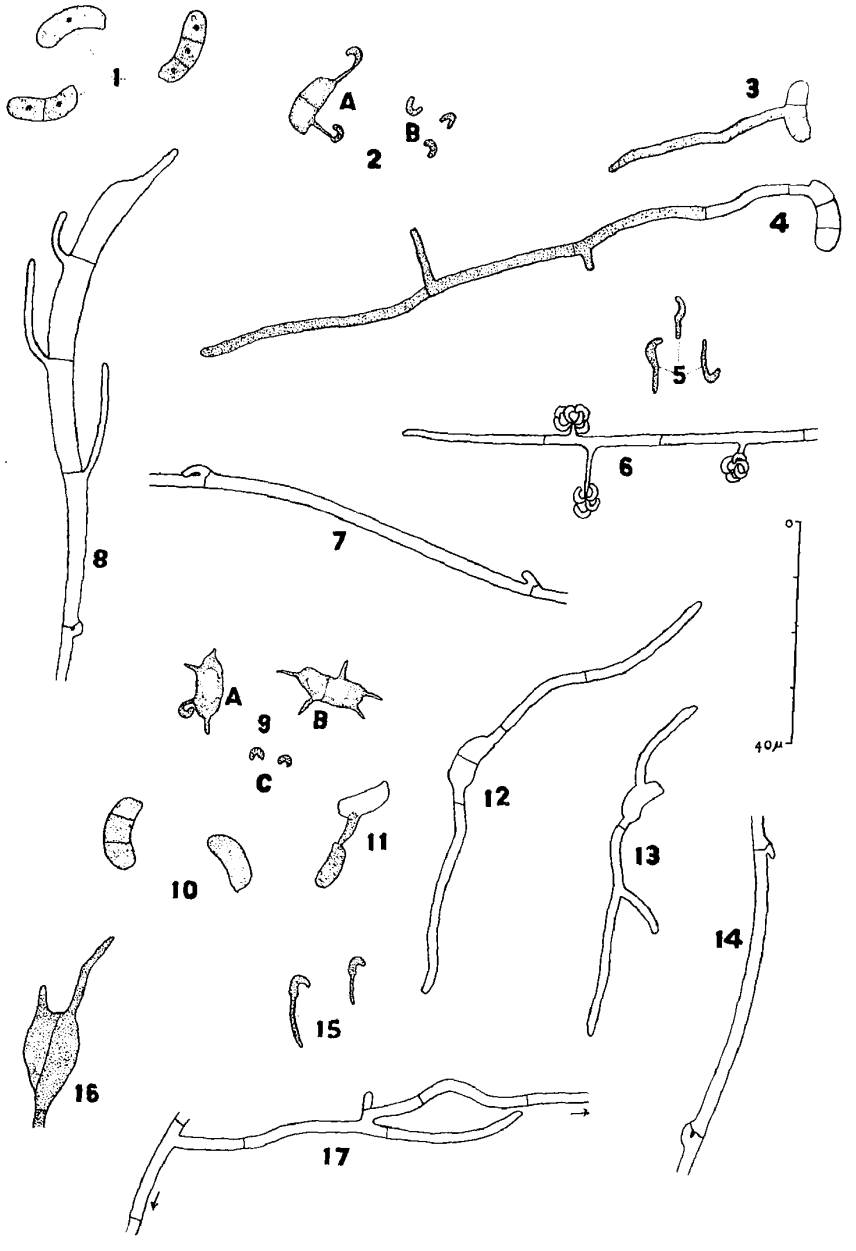
Explanation of Figures in Plate I

Figs. 1-8, A. auricula-judae. 1, basidiospores; 2, A, basidiospore germinating in water by means of oidia, B, oidia produced by spores in water; 3, 4, basidiospores germinating on agar; 5, germinating oidia; 6, monocaryon hyphae bearing oidia; 7, dicaryon hypha showing clamp connections; 8, basidium from fruit body in culture. Figs. 9-17, E. glandulosa. 9, A, B, basidiespores germinating in water, C, oidia produced by spores in water; 10, basidiospores; 11, basidiospore producing secondary spore; 12, 13, basidiospores germinating on agar; 14, dicaryon hypha showing clamp connections; 15, germinat ng oidia; 16, two-celled basidium with two epibasidia, produced by monocaryon mycelium; 17, union of two young hyphae from germinating basidiospores, arrows point toward spores.

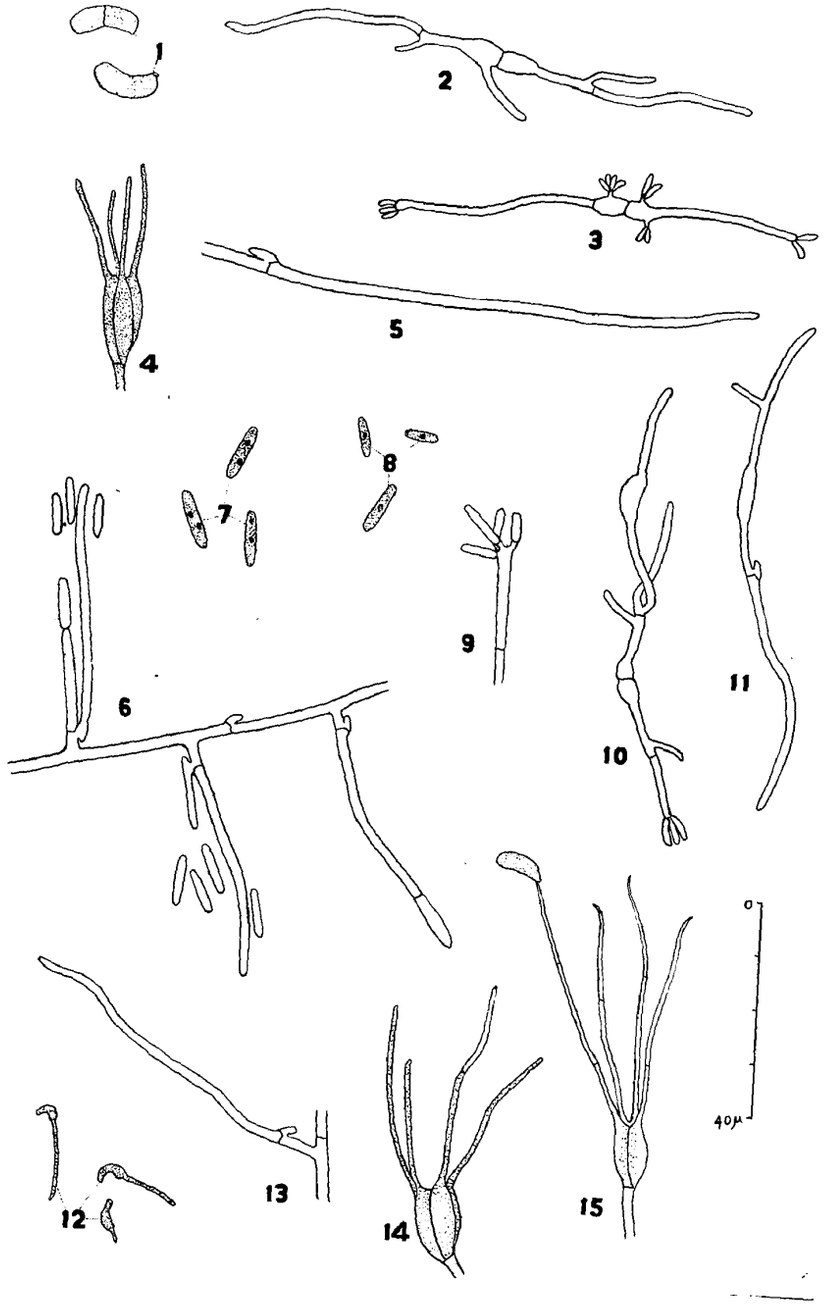
PLATE I



Explanation of Figures in Plate II

Figs. 1-5, E. recisa. 1, basidiospores; 2, basidiospore germinating on agar; 3, same as 2 but showing production of oidia on young hyphae; 4, four-celled basidium produced by dicaryon mycelium; 5, dicaryon hypha showing clamp connection. Figs. 6-11, E. saccharina. 6, dicaryon bearing oidia, also showing clamp connections; 7, binucleate oidia from dicaryon mycelium; 8, unimucleate oidia from monocaryon mycelium; 9, monocaryon hypha bearing oidia; 10, union of young hyphae from two basidiospores; 11, binucleate oidium germinating on agar showing clamp connection on the germ tube. Figs. 12-15, E. nucleata. 12, germinating oidia; 13, dicaryon hypha showing clamp connection; 14, 15, four-celled basidia showing septate epibasidia from fruit body in culture.

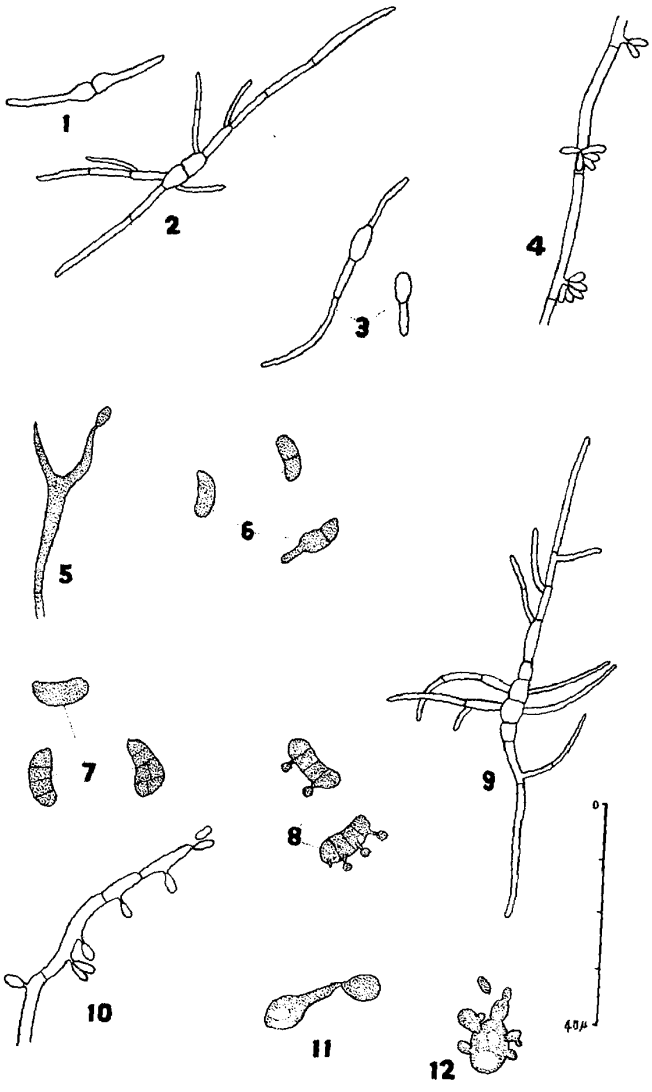
PLATE II



Explanation of Figures in Plate III

Figs. 1-6, Calocera cornea. 1, 2, basidiospores germinating on agar; 3, oidia germinating on agar,; 4, hypha from monospore culture producing oidia; 5, basidium from fruit body in culture; 6, basidiospores from fruit body in culture. Figs. 7-10, Dacryomyces minor. 7, basidiospores; 8, basidiospores producing oidia in water; 9, basidiospore germinating on agar; 10, hypha from monospore culture producing oidia. Figs. 11-12, Tremella lutescens. 11, basidiospore germinating by means of secondary spore; 12, basidiospore germinating on agar by budding.

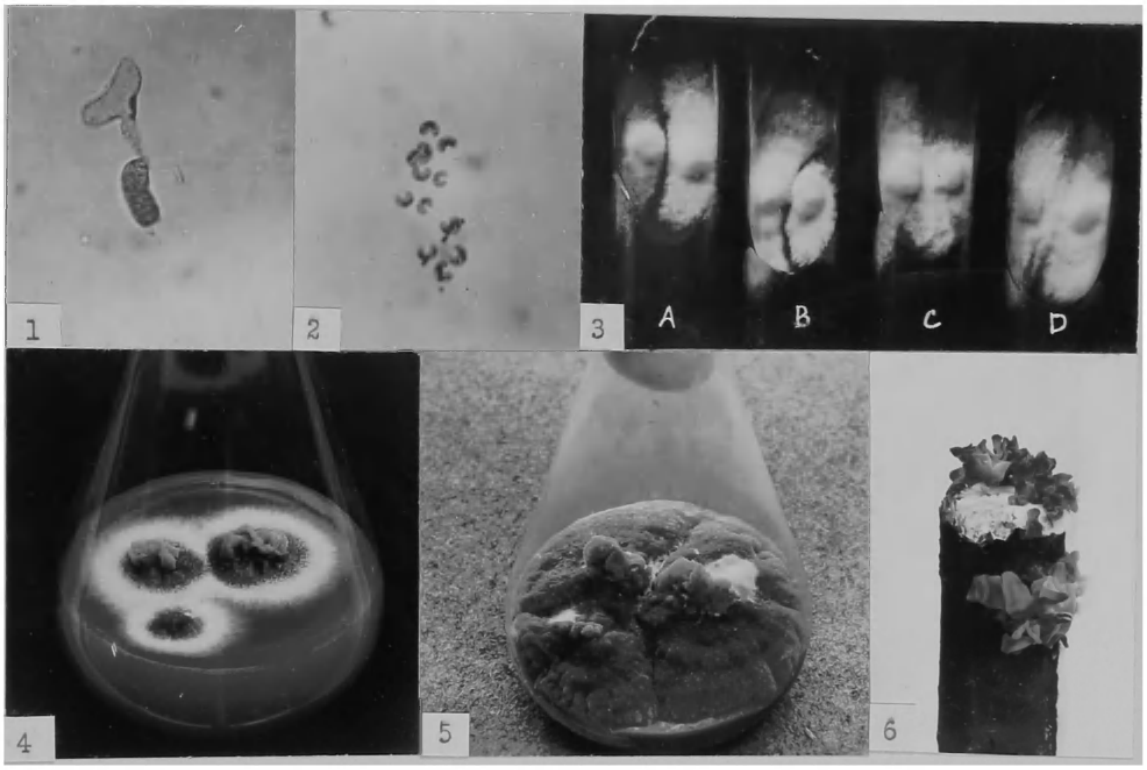
PLATE III



Explanation of Figures in Plate IV

All figures A. auricula-judae. 1, basidiospore producing secondary spore, X750; 2, oidia produced by germinating basidiospores in water, X750; 3, pairings of monospore cultures from fruit body IV, X7/8; A, aversion, 155 x 157, B, aversion, 157 x 161, C, even intermingling of mycelia, 157 x 160, D, aversion with gap filled with dicaryon mycelium, 154 x 157; 4, young fruit bodies in 12-day agar culture, X4/5; 5, same culture as 4, three weeks later, X4/5; 6, fruit bodies growing on basswood stick, X4/5.

PLATE IV

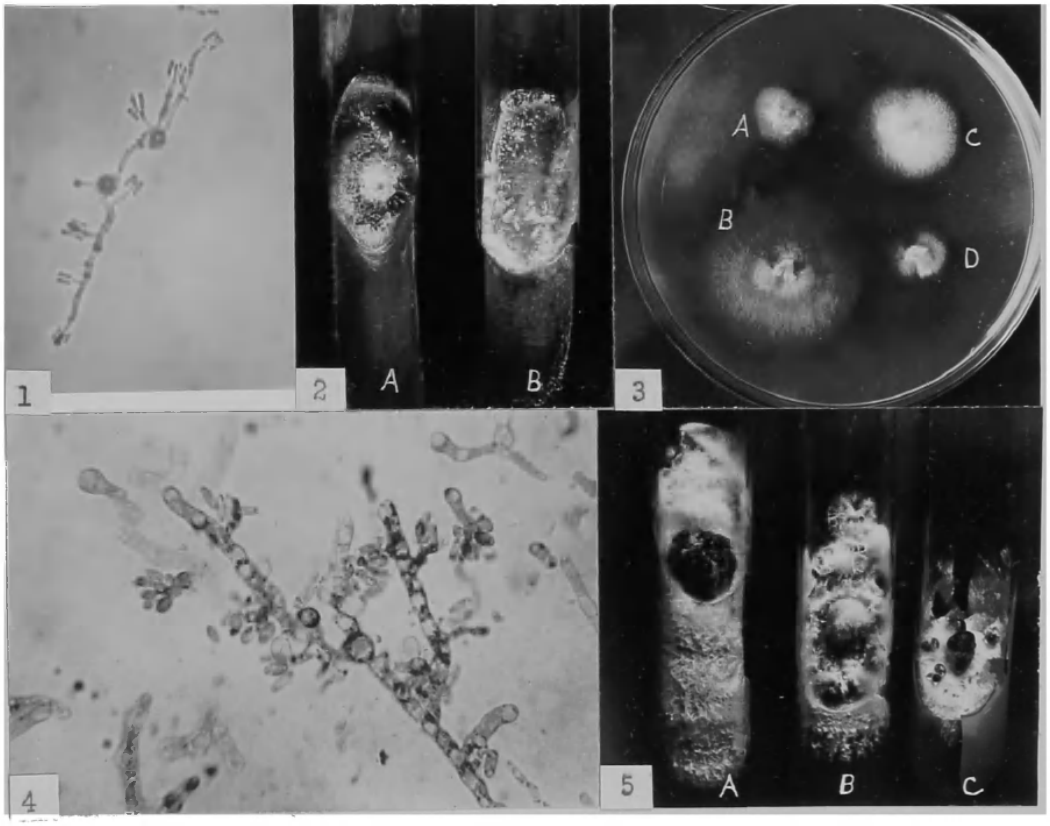


Explanation of Figures in Plate V

Figs. 1-3. E. saccharina. 1, basidiospore germinating on agar and producing oidia, X400; 2, monocaryon cultures showing shiny masses of oidia, A, culture five weeks old, B, culture three weeks old, X7/8; 3, monocaryon mycelia, A, 265, B, 263, C, 257, D, 256, X½.

Figs. 4, 5, E. recisa. 4, oidia germinating on agar, some producing mycelium and others producing secondary oidia, X750; 5, sterile gelatinous bodies produced by, A, dicaryon mycelium, B, C, monocaryon mycelia, X7/8.

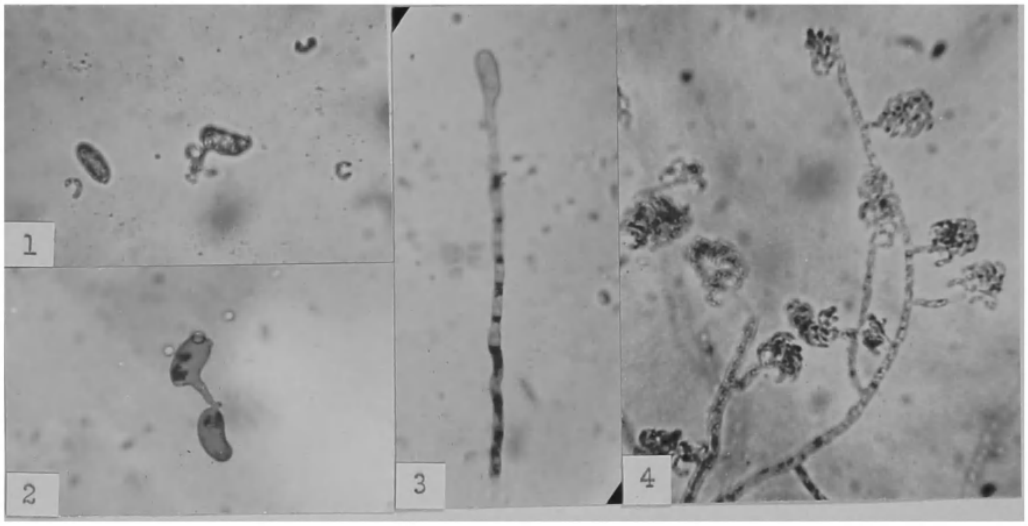
PLATE V



Explanation of Figures in Plate VI

All figures *E. mucleata*. 1, basidiospore germinating on moist wood and producing oidia, X700; 2, basidiospore forming secondary spore in water, X750; 3, basidiospore forming long germ tube in water, X750; 4, monocaryon hyphae producing clusters of oidia, X750.

PLATE VI



Explanation of Figures in Plate VII

All figures E. glandulosa. 1, monospore cultures, A, 23, B, 24, C, 21, X½; 2, dicaryon mycelium formed by pairing monospore cultures 21 and 51, X4/5; 3, dicaryon mycelium formed by pairing monospore cultures 21 and 50, X4/5; 4, dicaryon mycelium formed by pairing monospore cultures 24 and 50, X4/5.

PLATE VII