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Tetrapolar Heterothallism in the Basidiomycete, *Lentodium Squamulosum*

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Abstract: *Lentodium squamulosum* has been shown to be tetrapolarly heterothallic. The 4 predicted mating reactions can be recognized by gross characters on Potato dextrose agar, but not on other media tested. Three sectors were found, 2 of which do not seem to dikaryotize with any of the 4 established mating types.

Malt extract would appear to yield more consistent fruiting than Oat flake agar.

Lentodium squamulosum is a species of Basidiomycetes occurring on decorticated logs of deciduous species. It is commonly collected in Iowa and has been routinely collected in the Iowa City area during each of the past 3 years. It is curious in being very similar to *Lentinus tigrinus*, a typical member of the Agricaceae, in both habit and morphology except that *L. squamulosum* produces gills which anastomose very early in development, giving rise to enclosed locules which bear basidia and basidiospores. For a detailed account of development of *L. squamulosum* the reader is referred to Lyman (1907) and Bobbitt (1965).

Singer (1949) considers *L. squamulosum* to be merely a sterile form of *L. tigrinus*. Martin (1956) takes issue with Singer emphasizing that typical basidia and basidiospores are produced on sporophores of *L. squamulosum* and concludes that "It is not a teratological form, still less an abnormality or monstrosity, but a common, easily recognized, constant entity, with a wide distribution extending at least from Massachusetts to Iowa. In south-western Ohio and in eastern Iowa it is more abundant than *L. tigrinus*, from which it is supposed, with good reason, to have derived." Martin considers the species to be an excellent example of an agaric showing a distinct approach to a Gasteromycete habit. Bobbitt (1965) carried out a developmental study of basidiocarps of *L. squamulosum* under pure culture conditions on agar. This work expanded on the previous development study by Lyman (1907) and the observations by Martin (1956) in showing that under the more moist conditions existing on agar media in test tubes, gill structure was readily apparent and that spores were discharged externally. Bobbitt found that the tendency toward gill production in culture was extremely variable. Though he worked with only 2 different dikaryons and though he observed a wide range of variability in the tendency toward gills, it seemed that some work should be done to determine how genetic variability might influence sporophore morphology as well as to attempt crosses between *L. squamulosum* and *L. tigrinus*.

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L. tigrinus is known to be tetrapolarly heterothallic (Quintanilha et. al. 1941) and any likelihood of mating between the 2 species would undoubtedly depend upon their both having the same basic compatibility structure. Since preliminary work by one of us had indicated that *L. squamulosum* is heterothallic, it was deemed advisable to determine at the outset if *L. squamulosum* is also tetrapolarly heterothallic. The question of tetrapolar heterolthallism is the principal subject of this paper.

Methods and Materials

Fruiting bodies were obtained in pure culture on Oat flake agar (see Martin, 1956) from a compatible cross of 2 presumed monokaryotic mycelia, each of which had been derived from single basidospores obtained from sporophore collected in 1962. Potato dextrose agar (Difco) was routinely used for germinating the spores and for making the initial crosses. Two other media, which were used by Raper and San Antonio (1954) in their work on *Schizophyllum commune,* were used later in crosses for comparative purposes. These media are quite similar except that one contains 3 g yeast extract in addition to the following basic formula: 20.0 g glucose, 2.0 g "Bacto-peptone", 0.46 g KH_2PO_4, 1 g K_2HPO_4, 0.5 g $MgSO_4$, trace of $FeCl_3$ and 20.0 g "Bacto Agar" to 1 liter of distilled water. The medium with the yeast extract added was used by Raper and San Antonio to obtain fruits of *S. commune.* In all cases the sterile media were poured into sterile petri dishes to a depth of 2-3 mm.

The presence of clamp connections indicates compatible matings. To detect the clamp connections, samples of mycelia were mounted and stained with cotton blue in lacto-phenol.

To isolate single spore colonies, a mature basidocarp was transferred aseptically to a 125 ml erlenmeyer flask containing sterile distilled water. The pileus was then cut into several pieces by reaching into the flask with a sterile scalpel. The flask and contents were agitated, until the water became cloudy with the liberated spores. Ten ml of this suspension was used to obtain a dilution series of 1:10, 1:100, 1:1000 etc, up to 1:1,000,000,000 in distilled water. One ml samples from each of the above dilutions were placed aseptically in separate sterile petri dishes and mixed with warm, melted agar. The plates were swirled to distribute the spores evenly. After 2 days the spores had germinated and had produced hyphal colonies which could be seen under a dissecting microscope. Discreet colonies were removed with a sterile dissecting needle and transferred to fresh plates of Potato dextrose agar. Particular care was taken to select only those colonies which had not touched other colonies. The monospore isolates thus obtained were labeled 101-114. One isolate, 102, was later discarded as it was suspected of having been dikaryotized. The crosses were made by placing 5 mm diameter discs of agar and mycelia from two different isolates on fresh agar media in perti dishes. The 2 plugs were placed 1 cm apart. The 13 isolates were paired with each other in all possible combinations, and consequently 78 different crosses were made.

Results and Discussions

After 10 days, examination of hyphae from the zone of contact between certain of the matings showed numerous and conspicuous clamps. Fig. 1 shows all the possible combinations between the 13 isolates and which combinations produced clamps. Fig. 2 shows a separation of the four groups obtained on the basis of their mating reactions with all other strains. The only discrepancies (101 X 112 and 103 X 108) are cases where clamps were not seen and should have been. A repeat of these doubtful crosses yielded clamps.

On the basis of these observations, it seems clear that *L. squamulosum* fits Whitehouse's (1949) concept of a tetrapolarly heterothallic species in which compatibility is dependent upon heterozygosity at 2 independent loci, and for this reason the 4 mating types herein described are designated AB, Ab, aB, and ab.

Since some workers studying tetrapolar heterothallism in other species of Basidiomycetes have been able to distinguish between the 4 mating types on the mode of interaction between them (Papazian, 1950), and since we had previously observed that compatible matings in *L. squamulosum* produced a dikaryotic mycelium conspicuously different in appearance from the mycelia developed in non-compatible crosses, we attempted to determine differences between the appearance of mycelia in the various incompatible combinations. Mycelia produced from incompatible combinations have been described as "flat" (homozygous at the "a" locus) "barrage" (homozygous at the "b" locus) and "overgrowth" (common factors[1] at both "a" and "b" loci) by Papazian for *S. commune.*

By referring to fig. 2, it can be seen that numbering the groups from top to bottom as first, second, etc. that the first group is compatible with the third and that the second group is compatible with the fourth. We arbitrarily assigned group 1 as ab, and therefore group 3 as AB. In order to determine which of the mating interactions might correspond to Papazian's "common a" and which might correspond to "common b", we re-examined some of the matings for the presence of incomplete clamp connections, "pseudoclamps". Some workers (see Fincham and Day 1963) have shown that pseudoclamps occur in the common b heterokaryon but not in the common a heterokaryon. Pseudoclamps were observed in crosses between groups 1 and 2 and between groups 3 and 4. On this basis group 2 was disignated as Ab and group 4 as aB.

[1] Mycelia having both compatibility factors the same do not usually anastomose, and hence no heterokaryon is formed.

	103	104	105	106	107	108	109	110	111	112	113	114
101	-	-	+	-	-	-	-	-	-	-	-	-
103		-	-	+	-	-	-	+	-	-	-	-
104			+	-	-	-	-	-	-	+	-	-
105				-	+	-	+	-	-	-	-	+
106					-	-	-	-	+	-	+	-
107						-	-	-	-	+	-	-
108							-	-	+	-	+	-
109								-	-	+	-	-
110									+	-	+	-
111										-	-	-
112											-	+
113												-

All possible matings of single spore isolates. + indicates presence of clamp connections.

(1)

		101	103	104	105	106	107	108	109	110	111	112	113	114
	101	-	-	-	+	-	-	-	-	-	-	-	-	-
	104	-	-	-	+	-	-	-	-	-	-	+	-	-
ab	107	-	-	-	+	-	-	-	-	-	-	+	-	-
	109	-	-	-	+	-	-	-	-	-	-	+	-	-
	114	-	-	-	+	-	-	-	-	-	-	+	-	-
	103	-	-	-	-	+	-	-	-	+	-	-	-	-
Ab	111	-	-	-	-	+	-	+	-	+	-	-	-	-
	113	-	-	-	-	+	-	+	-	+	-	-	-	-
AB	105	+	-	+	-	-	+	-	+	-	-	-	-	+
	112	-	-	+	-	-	+	-	+	-	-	-	-	+
	106	-	+	-	-	-	-	-	-	-	+	-	+	-
aB	108	-	-	-	-	-	-	-	-	-	+	-	+	-
	110	-	+	-	-	-	-	-	-	-	+	-	+	-

(2) Four mating types. + indicates presence of clamp connections.

With the mating types so designated, the macroscopic detection of different mating reactions could be attempted. Representatives of each mating reaction are shown in fig. 3-6. The

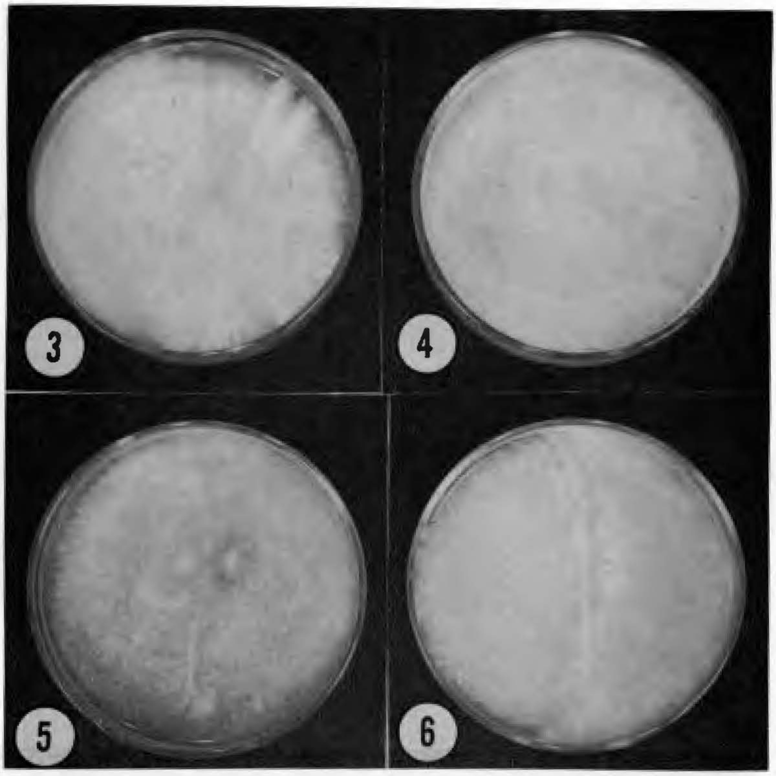

Fig. 3-6. Four mating reactions on Potato dextrose agar. Fig. 3. compatible reaction. Fig. 4. reaction where both a and bfactors common. Fig. 5. common a. Fig. 6. common b.

compatible reaction results in a streaky appearance with the streaks radiating from the inoculation points (fig. 3). Fig. 4 is the reaction between mating types where both the a and b factors are common (Ab X Ab etc.). It is characterized by the lack of a distinct reaction line coupled with a uniformly aerial mat of mycelium. Both common a reactions (AB X Ab and aB X ab) are characterized by depressed flat growth and a thin line of aerial mycelium along the line of original contact between the two parent mycelia (fig. 5). The common b reactions (AB X aB and Ab X ab) show a marked line of aerial mycelium at the point of juncture of the two parental strains with a narrow depressed area on either side (fig. 6). The main body of the mycelium however remains fluffy.

Our next step was to determine whether these reactions varied greatly on different kinds of media. The two media used by Raper and San Antonio (1954) were selcted for this test. Again all possible combinations were tried using all 13 isolates. None of

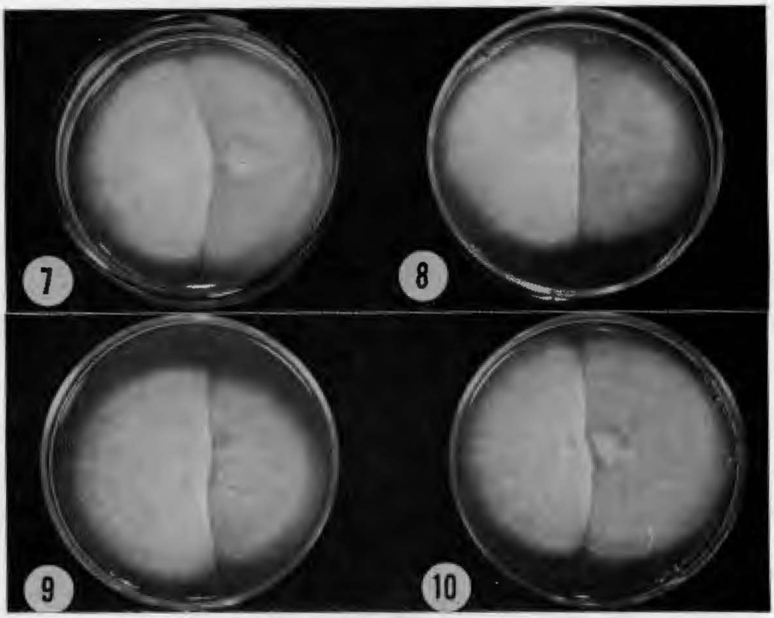

Fig. 7-10. Matings of sector isolated from the 105 side of (105 x 114) with a representative from each of the 4 established mating types on Potato dextrose agar. In all cases the sector is on the left. Fig. 7. with ab. Fig. 8. with Ab. Fig. 9. with AB. Fig. 10. with aB.

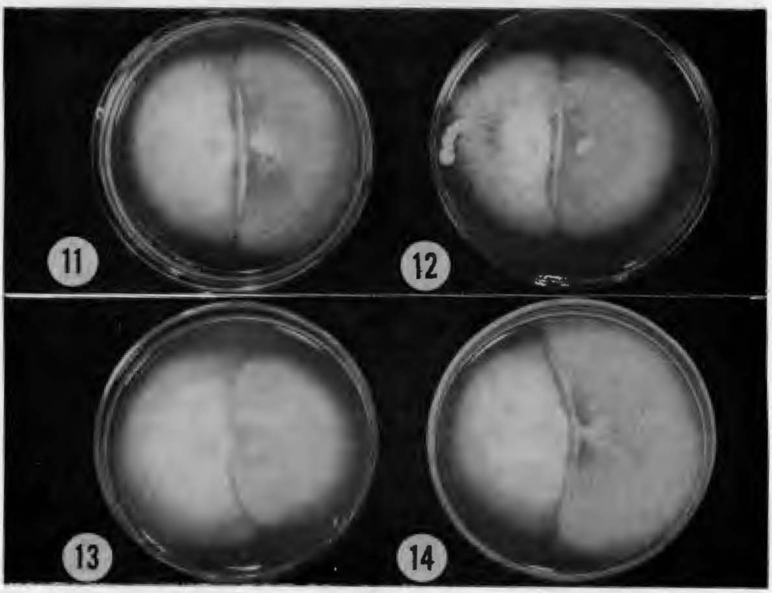

Fig. 11-14. Matings of sector isolated from the midline of (106 x 113) with a representative from each of the four establishtd mating types on Potato dextrose agar. In all cases the sector is on the left. Fig. 11. with a b.Fig. 12, with AB. Fig. 13. with Ab. Fig. 14. with aB.

the anticipated reactions were observed on these 2 media though they were again apparent on a Potato dextrose agar control.

The basal medium with yeast extract added did produce 3 interesting sectors from 3 different matings. Two of these matings were compatible and 1 was common b. One sector which came from the 105 side of a cross between 105 and 114 (compatible) was isolated and then mated with a representative of each of the 4 mating types. These 4 test matings were done on Potato dextrose agar in hopes that they would yield reactions to indicate the mating type of the sector. Figs. 7-10 show these 4 matings. No differences between the 4 combinations are observable, and the reaction line is different from anything seen previously. An examination for clamps or pseudoclamps proved negative in all 4 combinations. The sector side of the crosses developed a mound of white smooth mycelium along the reaction line. This mound was very tough, making it difficult to remove a sample for micrscopic examination. A second sector from the reaction line of 106 X 113 (compatible) was isolated and mated with all 4 of the mating types on Potato dextrose agar. These reactions are shown in figs. 11-14. Again no differences are observable between the 4 test matings. The reaction of this sector, however, is different from that previously mentioned, as is the morphology of the sector itself. This sector has a "powdery" appearance. As before, no clamps were observable in any of the 4 combinations. Preliminary experiments with the third sector which originated on the 103 side of a 103 X 109 mating indicates that it acts as a donor in crosses with the 4 mating types, dikaryotizing unilaterally. Observations on the nature of these sectors, mating type, effect on sporophore production, and ability to recombine and to segregate with other factors are presently being planned.

In addition, we have preliminary evidence that 2% malt extract agar is a better medium for consistent fruiting than is Oat flake agar. Since it is desirable from the standpoint of studying genetic variability to have a medium which can be uniformly produced and which will consistently produce basidiocarps, this would represent an important contribution to the continued study of the genetics of *L. squamulosum.* We are currently evaluating various media and their effect on fruiting.

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The Vascular Plants of Berry Woods

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Abstract. A list of the genera and species of vascular plants occurring in Berry Woods is presented. This 40 acre mixed hardwood forest, located in central Warren County, Iowa, is owned and maintained as a natural preserve by The Nature Conservancy.

Berry Woods, a 40 acre mixed hardwood forest, is located about two miles northwest of Indianola, Iowa in Warren County, (T-76N, R-24W, S.W. section 2). It was given to The Nature Conservancy in 1961 by Mr. and Mrs. Don L. Berry of Indianola as a memorial to Captain B. C. Berry and Senator W. H. Berry.

The woods covers a portion of the bluffs overlooking the flood plain of Middle River, in an area of Shelby loam with some Tama loam soils along the western edges. A small creek runs diagonally through the woods dividing it into predominantly eastern and western slopes. The area is completely fenced and is bordered on the west by a county road, on the south and east by agricultural land and on the north by cut-over woodland which is rapidly being cleared.

Since the woodland is now preserved for scientific study and teaching it was deemed advisable to make a thorough survey of the vascular flora for future references. Voucher collections made in Berry Woods during the past four years are maintained in the herbarium of Simpson College. Identification and nomenclature has followed the manuals of Gleason and Cronquist (1963) and Steyermark (1963). A total of 105 genera and 134 species of vascular plants have been collected and identified from this area.

The dominant tree species include *Quercus alba, Tilia americana, Carya ovata and Ostrya virginiana.* A typical prevernal flora is present during April and May and includes such forms as *Dicentra cucullaria, Claytonia virginica, Sanguinaria canadensis Asarum canadense, Hepatica acutiloba, Dentaria laciniata, Erythronium albidum, Isopyrum biternatum* and *Uvularia grandiflora.*

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